Mimicking the Tribo-Mechanical Performance of Human Skin:

a Scale-Dependent Approach based on
Poly (vinyl alcohol) Hydrogel

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PERFORMANCE OF HUMAN SKIN:
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DISSERTATION

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DEDICATED TO MY PARENTS,
WHO TAUGHT ME WHAT
COMMITMENT AND
HUMILITY
IS

Summary

Sliding contact between the human skin and product-interfaces is experienced in many situations of the daily life. Consumer products, medical devices, sportswear or sports equipment, cosmetics or healthcare products are some of the applications which can benefit from a broad analysis on skin-related interactions. In addition, dermatological problems which have their origin in a mechanical contact against the skin can also be analysed within the scope of skin tribology. As a result, the functionality, comfort and perception of products in contact with the skin can be enhanced.

The design and optimization of products in contact with the skin requires experimental testing prior to commercialization to ensure their quality. Thus, a thorough knowledge of the influence of skin's structure, composition and mechanical properties under different environmental and operational conditions is crucial for a complete understanding of the interactions "skin-materials" during motion. However, the analysis of the frictional performance of *in vivo* or *ex vivo* skin is complicated due to ethical arrangements and protocols which imply a limitation at industrial scale. Cadaveric skin could be a source of samples but, the use of chemicals to prevent the decomposition of the tissue alter the properties and performance of the skin. Moreover, the great variability of the results obtained on *in vivo* skin support the development of a more reproducible alternative to standardize results. Therefore, a mechanical skin substitute with a similar mechanical and frictional performance as human skin would be of interest for testing applications at industrial scale.

In line with this, a two-layered synthetic skin model which takes into account the surface properties and mechanical performance of the skin is presented in this thesis. The physic-chemical properties of the two layers were determined as well as their surface properties and tribo-mechanical performance. In addition, to ensure the suitability of this physical model, the length scale dependence of the elastic modulus and a frictional performance, variable with hydration, was achieved. The validation of this physical model was conducted in comparison to *ex vivo* skin results and research on *in vivo* skin presented in literature.

The developed model is divided in two layers with different surface properties and elastic behaviour to resemble the layered structure of the skin. The top layer consists of a thin film between 150-200 μm synthesized from a mixture of poly (vinyl alcohol) (PVA) and rapeseed oil crosslinked with glutaraldehyde. The bottom part is a thicker layer of around 1.2 mm obtained from an aqueous solution of PVA crosslinked via freezing/thawing cycles with ultra-soft mechanical properties. This composition of the model based on PVA hydrogel ensures the possibility of modifications of its surface and

corresponding mechanical properties in the presence of moist or under wet conditions, such a variations in the work of adhesion and the elastic modulus.

This thesis contains also a predictive friction model based on experimental results obtained with *ex vivo* skin. The predictive model for the frictional behaviour of the skin takes into account the response of the different skin layers depending on the applied forces. Further, the role of the capillary forces was considered under wet conditions as the applied forces are in the range of the magnitude of the capillary forces. Thus, the predictive model accounts for the length scale dependence of the human skin and provides insight to the mechanisms influencing the frictional performance of the skin. This theoretical analysis is performed with a different skin structure at the meso and the macro scales since the layers involved in the overall skin response are different depending on the applied force. At the meso scale the influence of the stratum corneum and dermis is crucial to understand the skin's response whereas at the macro scale, the influence of the muscle needs to be implemented. Moreover, the contact parameters are calculated based on Hertz's contact model and the JKR approach with consideration of the effect of the capillary forces acting on the skin's surface, especially at wet conditions.

The length scale dependence implemented in the predictive model was validated by a physical model which elastic modulus indicated a power law decrease with the increase of normal force. As a result, the frictional performance of the physical model showed a length scale dependence with resulted varying from the meso to the macro scale, due to the relative contribution of the adhesive forces and elastic modulus to the total friction force. Moreover, the surface properties of the top layer of the physical model resembled the properties of the human skin, especially in wet conditions. The increase of friction observed at low normal forces predicted by the theoretical model was also observed for the physical model. At higher normal forces, the physical model showed a nearly constant coefficient of friction as predicted by the theoretical model.

Samenvatting

Nabootsing van het tribo-mechanisch gedrag van de menselijke huid: een schaal-afhankelijke benadering gebaseerd op poly (vinyl alcohol) hydrogel

In het dagelijks leven zijn er vele situaties waarin het glijdend contact tussen de menselijke huid en product interfaces wordt beleefd. Consumentenproducten, medische apparaten, sportkleding of sportuitrustingen, cosmetica of gezondheidszorgproducten zijn enkele van de toepassingen die profijt kunnen hebben van een brede analyse op het gebied van huid-gerelateerde interacties. Daarbij komt dat dermatologische problemen die hun oorsprong vinden in een mechanisch contact tegen de huid ook in het licht van de huidtribologie geanalyseerd kunnen worden. Met als gevolg een mogelijke verbetering van de functionaliteit, het comfort en de perceptie van producten in contact met de huid.

Het ontwerp en de optimalisatie van producten in contact met de huid vereist experimenteel onderzoek voorafgaand aan de commercialisatie, om zo de kwaliteit van de producten te garanderen. Grondige kennis van de invloed van de structuur van de huid, de samenstelling en mechanische eigenschappen voor verschillende milieus en operationele condities is cruciaal voor een volledig begrip van “huid-materialen” interacties gedurende een beweging. De analyse van het wrijvingsgedrag van *in vivo* of *ex vivo* huid is echter gecompliceerd door ethische afspraken en protocollen, wat tot beperkingen leidt op industriële schaal. Huid van kadavers zou een bron van proefstukken kunnen zijn ware het niet dat de chemicaliën die nodig zijn om ontleding tegen te gaan de eigenschappen en het gedrag van de huid veranderen. De grote variabiliteit van de resultaten verkregen met *in vivo* huid ondersteunen de noodzaak van de ontwikkeling van een reproduceerbaar alternatief om zo de resultaten te standaardiseren. Een mechanisch huidalternatief met vergelijkbare mechanische en wrijvingseigenschappen als de menselijke huid zou daarom interessant zijn voor meettoepassingen op industriële schaal.

In het verlengde hiervan wordt in dit proefschrift een twee-laags synthetisch huidmodel gepresenteerd dat rekening houdt met de oppervlakte-eigenschappen en het mechanisch gedrag van de huid. De fysisch-chemische eigenschappen van de twee lagen zijn bepaald, net als de oppervlakte-eigenschappen en het tribo-mechanisch gedrag. Om de geschiktheid van het fysieke model te garanderen is aanvullend bereikt dat het wrijvingsgedrag en de lengteschaal afhankelijkheid van de elasticiteitsmodulus variëren met hydratatie. Het fysieke model is gevalideerd door de resultaten te vergelijken met *ex vivo* huid resultaten en te vergelijken met onderzoeksresultaten aan *in vivo* huid uit de literatuur.

Het ontwikkelde fysieke model is verdeeld in twee lagen met verschillend oppervlakte-eigenschappen en elastisch gedrag om zo de gelaagde structuur van de huid te benaderen. De bovenste laag bestaat uit een dunne film van 150 – 200 μm gesynthetiseerd vanuit een mengsel van poly (vinyl alcohol) (PVA) en raapzaadolie, vernet met glutaaraldehyde. Het onderste gedeelte is een dikkere laag van zo'n 1.2 mm met ultra zachte mechanische eigenschappen, verkregen uit een waterige oplossing van PVA vernet via vries / dooi cycli. Deze samenstelling van het fysieke model, gebaseerd op PVA hydrogel, garandeert dat het mogelijk is om het oppervlak en de daarmee samenhangende mechanische eigenschappen zoals adhesie-arbeid of elasticiteitsmodulus, te laten variëren met de aanwezigheid van vocht of natte condities.

Dit proefschrift bevat een voorspellend wrijvingsmodel, gebaseerd op resultaten van experimenten met *ex vivo* huid. Het voorspellende model voor het wrijvingsgedrag van de huid houdt rekening met de response van de verschillende huidlagen, afhankelijk van de toegepaste krachten. Verder wordt de rol van de capillaire krachten in beschouwing genomen onder natte condities omdat daar de toegepaste krachten van dezelfde orde grootte zijn als de capillaire krachten. Op deze manier houdt het voorspellende model rekening met de lengteschaal afhankelijkheid van de menselijke huid en geeft het inzicht in de mechanismen die van invloed zijn op het wrijvingsgedrag van de huid. Deze theoretische analyse is uitgevoerd met verschillende huidstructuren op de meso- en op de macroschaal omdat de lagen die van invloed zijn op de totale huid response verschillen met de toegepaste kracht. Op de mesoschaal is de invloed van het stratum corneum en dermis cruciaal voor het begrijpen van de response van de huid, terwijl op de macroschaal de invloed van de spieren meegenomen moet worden. Tevens zijn de contactparameters berekend op basis van het contactmodel van Hertz en de JKR benadering, met aandacht voor het effect van de capillaire krachten die werken op het huidoppervlak, met name voor natte condities.

De lengteschaal afhankelijkheid die verwerkt is in het voorspellende model, is gevalideerd met het fysieke model waaruit bleek dat de elasticiteitsmodulus van het fysieke model daalde met een machtsfunctie met toenemende normaalkracht. Het resultaat hiervan was dat het wrijvingsgedrag van het fysieke model een lengteschaal afhankelijkheid liet zien variërend van de meso- tot de macroschaal, door de relatieve bijdrage van de adhesieve krachten en van de elasticiteitsmodulus aan de totale wrijvingskracht. Tevens bleek dat de oppervlakte-eigenschappen van de toplaag van het fysieke model de eigenschappen van de menselijke huid benaderde, met name onder natte condities. De toename van de wrijving, zoals geobserveerd bij lage normaalkrachten en voorspeld bij het theoretische model, is ook zo geobserveerd voor het fysieke model. Bij hoge normaalkrachten liet het fysieke model een constante wrijvingscoëfficiënt zien, zoals voorspeld door het theoretische model.

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Nomenclature

Roman symbols

a	Contact radius	[m]
A	Contact area	[m ²]
A_{real}	Real contact area	[m ²]
E^*	Combined elastic modulus of skin	[Pa]
E_1	Asymptotic elastic modulus of the material according to the SLS model	[Pa]
E_2	Material constant of spring 2 in the SLS model	[Pa]
E_{eff}^*	Combined effective elastic modulus	[Pa]
E_H	Elastic modulus of the skin based on the Hertzian approach	[Pa]
E_{H+CAP}	Elastic modulus of the skin based on the Hertzian approach with capillary effect	[Pa]
E_i	Elastic modulus of body i	[Pa]
E_{JKR}	Elastic modulus of the skin based on the JKR approach	[Pa]
$E_{JKR+CAP}$	Elastic modulus of the skin based on the JKR approach with capillary effect	[Pa]
F	Normal force	[N]
F_{adh}	Adhesion force	[N]
F_{app}	Apparent normal load	[N]
F_{cap}	Capillary force	[N]
$F_{\mu,def}$	Deformation component of the friction force	[N]
$F_{\mu,adh}$	Adhesion component of the friction force	[N]
F_H^{cap}	Normal force in the case of Hertzian contact with capillary effect	[N]
F_{JKR}^{cap}	Normal force in the case of JKR contact with capillary effect	[N]
P_{cap}	Capillary pressure	[Pa]
R	Radius of curvature of the indenter	[m]
S	Stiffness of the material	[N/m]
t_i	Thickness of the i^{th} layer	[m]
T_g	Glass transition temperature	[° C]
T_m	Melting temperature	[° C]
W_{12}	Work of adhesion	[N/m]

Greek symbols

β	Viscoelastic loss fraction	[-]
δ	Indentation depth	[m]
ε	Strain	[-]
γ_L	Surface tension of the liquid	[N/m]
η	Viscosity of the damper in the SLS model	[Pa · s]
φ [t]	Creep compliance	[Pa ⁻¹]
μ	Coefficient of friction	[-]
ν	Poisson's ratio	[-]
θ	Contact angle of the	[rad]
σ	Applied stress	[Pa]
τ	Interfacial shear strength	[Pa]
ψ [t]	Stress relaxation	[Pa]

Abbreviations

2LSM	2-Layered skin model
BSSM	Bi-stratified skin model
COF	Coefficient of friction
DW	Deionized water
EGF	Epidermal growth factor
EMC	Extra cellular matrix
ESE	Epidermal skin equivalent
EWC	Equilibrium water content
GA	Glutaraldehyde
GAG	Glycosaminoglycan
HA	Hyaluronic acid
HE	Hematoxylin-eosin
JKR	Johnson, Kendall and Roberts model
NBR	Acrylonitrile-Butadiene rubber
NMF	Natural moisturizing factor
-OH	Hydroxyl group
PA	Polyamide
PDMS	Polydimethylsiloxane
PE	Polyethylene
PG	Proteoglycan
PLGA	Poly (lactic-co-glycolic acid)
PVA	Poly (vinyl alcohol)
RH	Relative humidity
Ro	Rapeseed oil
SBR	Styrene-Butadiene rubber
SC	Stratum corneum

SDS	Sodium Dodecyl Sulphate
SE	Standard error
SLS	Standard linear solid model
SSC	Synthetic stratum corneum
SSLF	Skin surface lipid film
TEWL	Trans epidermal water loss
VAFT	Vacuum Adhesive and Friction Tester

Chapter 1

1. Introduction

1.1 Tribology & bio-tribological systems

Tribology comes from the Greek word “tribo”, which means “rub”, and refers to the interaction between 2 body-surfaces in relative motion. Tribological applications already arose in the ancient Egypt, back to the 15th century BC, when olive oil was used to reduce the friction while pulling large stones [1] on the ground. Centuries later, Leonardo Da Vinci (1452-1519) realized about the importance of friction in working machines. By that time, he noted that the area of contact did not affect friction and understood the phenomena that, later would address to the formulation of the first Amontons’ law: the friction force is proportional to the applied load. Nowadays, the evolution of a tribological system implies a broad analysis of the mechanical and surface properties of the contacting materials as well as, of the operational and environmental conditions during their interaction [2]. A better understanding of the mechanisms that govern the contact between the two bodies conducts towards a reduction of the energy consumption, the improvement of the system performance and elongation of its lifetime.

Besides the technological applications of tribology, its principles and innovations can also be extended to living systems, such as those which are part of the human body (see Fig. 1). As a consequence of the principle of minimum energy, biological systems also minimize the energy consumption to enlarge their lifetime. However, the tribological analysis of biological structures is hindered due to the complexity of these structures and their interactions.

In bio-tribology at least one of the two interacting surfaces is a living material. Thus, the specific structure, composition, mechanical and surface properties of the contacting materials need to be examined for a better understanding of the overall performance of the system.

Figure 1. Examples of bio-tribological systems in the human body: (a) ocular tissue in contact with lenses [3]; (b) articular damage due to cartilage-cartilage contact [4].

1.2 Human skin as a part of a bio-tribological system

Human skin is the interface of the body against the fluctuations and circumstances of the environment. As a consequence, it is permanently exposed to disruptions of its performance due to chemical, biological or mechanical damage. During the daily life human skin is in touch with different materials, at different environmental conditions and subjected to diverse stresses. The study of these interactions, which corresponds to Skin Tribology [2, 5-7], is connected to the optimization of product's design to improve the comfortability of the skin. Hence, a better understanding of skin tribology can address to the improvement of those applications in which skin interacts with other surfaces. The design of sportswear and sport accessories, tools, biomedical devices and prosthetics, healthcare and lifestyle products or cosmetics can benefit from the advances in skin tribology [8 - 14]. Moreover, the improvements in this area can also address to a better comprehension of dermatological problems related to mechanical damage such as pressure ulcers [15].

Figure 2. Schematic illustration of the tribological system formed between the skin and other counter surface.

When a loaded surface contacts the skin and a certain velocity is applied in the tangential direction of the contact, a resistive force opposing the movement emerges [16, 17]. This force is the friction force, F_f , and its magnitude depends on the mechanical and surface properties of the contacting materials as well as on the applied load, velocity and environmental conditions, see Fig. 2. Commonly, the frictional behaviour of a systems is described by means of the coefficient of friction, COF or μ , which can be obtained as the quotient between the friction force, F_f , and the normal force, F_N , as indicated in Figure 2.

It is possible to investigate skin friction by means of *in vivo* or *ex vivo* skin. In the former case, friction tests are performed on volunteers whereas in the last case, a piece of skin tissue previously removed from an individual is evaluated. The manipulation of alive tissue demands series of the ethical

procedures and protocols that hinder their analysis and imply a limitation, especially at an industrial scale. Moreover, the availability of *ex vivo* samples either from cadaveric or surgical source is limited and the accessible body sites as well. Measurements on *in vivo* skin show inter and intra-individual variability and frequently, instrumental limitations. Thus, attempts to develop a mechanical skin model to simulate the tribo-mechanical performance of the skin have been performed. Most of the available skin substitutes are used for the treatment of dermatological wounds or cosmetic testing so that, their composition and characteristics focus on the biological properties of human skin. Commonly, these surrogates are based on biocompatible and non-toxic materials in combination with biological components and/or cultured cells. Some commercially available examples are Alloderm, Dermagraft, Integra, LaserSkin, ApliGraft, Epicel or Oasis wound matrix among other. In general, the main goal of these surrogates is to protect the injured tissue against the environment and at the same time, they can carry drugs to assist the healing process. The mechanical and surface properties of these materials are not suitable to use them in tribological studies. Few synthetic materials have been proposed as mechanical skin substitutes. They are mainly based on silicone elastomers or polyurethanes, non-mixed or combined with fibers. Silicone Skin L7350, Lorica and other materials based on polyurethanes are commonly used examples of skin substitutes in tribological testing [18 - 25]. Although these materials display mechanical properties similar to the human skin, their tribological performance does not fully simulate the one of the human skin, especially at hydrated or wet conditions. The main disadvantage of these materials is related to the hydrophobic properties they display, a consequence of their chemical composition primarily based on carbon, hydrogen, oxygen or silicon [24]. Thus, due to their inability to absorb/desorb water they cannot properly simulate the tribological performance of the skin at other conditions than dry or normal. There are several aspects that a mechanical skin model for tribo-testing applications should comply to. Hydration is one of the main aspects influencing skin friction, so that the capability of a skin surrogate to interact with water and moisture is essential to properly simulate the tribological performance of the skin. The most important aspects influencing to the tribo-mechanical performance of the skin and consequently, of vital importance to implement in a novel mechanical model are described in the following section.

1.3 Overview of the frictional performance of the skin

The frictional performance of the skin is influenced by mechanical and surface properties of the skin itself and the contacting materials as well as by the operational conditions. The application of substances, such as creams, moisturizers, cosmetics, the effect of sweat or the amount of sebum modifies the surface condition of the skin and consequently, its frictional performance. In addition, hydration significantly affects the elastic properties of the skin and, subsequently its frictional behaviour [24, 26-29]. Modifications of the composition and concentration of the surface lipids with age are connected to

an increase of the water loss through the skin and changes on friction [27 - 32]. Lastly, the alterations on elasticity and hydration have an effect on the surface roughness of the skin [33–35]. These modifications have also an impact on the coefficient of friction as a consequence of the changes in the contact area. A brief summary of the main influencing factors in the tribo-mechanical performance of the skin is presented below.

○ *Roughness*

From a general perspective, the coefficient of friction between a hard surface and the skin decreases with the increase of the material's roughness due to the subsequent decrease in the contact area. However, the influence of roughness in skin friction requires a wider analysis of the surface properties of the contacting materials and the specific configuration of the tribological system. For instance, in tactile friction the finger pad typically applies pressure to another surface. The surface topography of this anatomical region, based on a pattern of ridges and furrows, plays a crucial role in the resulting frictional performance [36]. Thus, when analysing texturized surfaces in contact with the fingertip is necessary to consider the surface roughness of the skin and the counter surface to properly understand the overall system's performance [37 - 39]. In general, the smoother the contact surface, the higher the friction due to the larger area of contact [40, 41]. For example, the frictional performance of the skin against several hard materials with different surface roughness pointed out a decrease of the coefficient of friction with increasing roughness [40]. An increase of the hard material's roughness raises the ploughing component with a consequent increase of the hysteresis and a large effect on friction [42, 43].

Conversely, when a smooth-hard surface applied pressure to the skin, the contacts situation and hence, the tribological system changes. In this case, already at very low forces the true area of contact reaches the nominal area of contact because of the differences in elasticity between the counter surface and the skin.

○ *Sebum*

The frictional behaviour of the skin depends also on the presence of substances at the interface with the counter surface [2]. The effect of the surface properties of the skin on friction is largely commanded by a top film composed of sebum and water from the sweat, the Skin Surface Lipid Film (SSLF). Consequently, the coefficient of friction is determined by means of the adhesive properties between the two surfaces with additional effects of roughness. For normal conditions, that is "untreated skin", the top layer of the skin is covered by this hydrophilic film which consists of a mixture of lipids and water from the sweat [44 - 46]. The composition of this layer is known yet, the specific proportion of constituents' changes among individuals and also differs with the

body site [50, 51]. This film has an essential role in the control of skin's hydration [44, 47, 48] and it characterizes the adhesive properties of the skin. The importance of the SSLF in the adhesive properties of the skin was demonstrated by Pailler-Mattei et al. [49] by a decrease of the adhesive force after lipid removal. Thus, modifications in the concentration or chemical composition of the SSLF's constituents result in further variations of the mechanical and interfacial properties of the skin and, consequently, on its frictional performance [49 - 53], particularly in the case of sliding friction.

○ *Elasticity*

Human skin is a multi-layered structure with viscoelastic properties similar to those of elastomers [7, 54]. Thus, the response of the skin to stress results on the overall contribution of the individual layers. The outermost layer of the skin consists of a hard thin film composed mainly by keratin. Water is present in the inner layers of the skin and it has an important role in its viscoelastic response. The elasticity of the skin is also attributed to a network of elastic polymer chains of collagen and elastin in a matrix which contains water [54, 55].

The elastic modulus of the human skin has been analysed by different techniques showing results in a range of several orders of magnitude depending on the anatomical region, hydration level, age and the operational conditions [56 - 61]. These results show a decrease of the elastic modulus with the indentation depth due to the layered structure of the skin and the softness of the inner layers. Whereas the top layer indicates elastic moduli in the range of few MPa to GPa, the inner layers show values not higher than a few kPa. Additionally, the outermost layer of the skin has indicated an elastic modulus which tends to decrease with increasing hydration [62 - 65]. The elastic modulus of the outermost layer of the skin have been reported to decrease from 2 GPa at 30 % relative humidity (RH) to 3 MPa at 100 RH. Rheological analysis confirms that hydration has an important effect on the viscoelastic properties of this layer due to a transition from glassy to rubbery state [63, 64]. At low RH conditions, the movements of the polymeric chains contained in the skin are restricted; at higher RH levels, the presence of hydroxyl bonds new conformational changes that increase the viscoelasticity.

○ *Hydration*

Hydration is known to cause an increase of the skin's friction as it has been extensively observed in other research [7, 26, 28, 41, 43, 66 - 69]. Under normal conditions, the skin displays a COF typically between 0.25 and 0.5 depending on the individual's age, body location, contacting material and experimental parameters applied. As the moisture rises, the coefficient of friction undergoes also an increase up to a maximum when the skin is wet or subjected to very humid

climate. At that point, the coefficient of friction can reach values of 2 or even 4 times higher than at dry conditions [5]. The increase of friction at hydrated or wet conditions has been pointed as a consequence of the softening effect of the stratum corneum and the increase of adhesion due to the capillary forces [7, 28, 66, 69, 70].

The effect of creams and moisturizers has a similar influence on the frictional performance of the skin with an alteration of the elastic and adhesive properties of the skin which typically, results in a consequent increase of friction [71]. Additionally, an evaluation of the influence of cream on untreated skin at the nano and the macro scale have indicated a different tribological performance depending on the scale. Therefore, at the nano scale, the frictional behaviour seems to be essentially due to viscous friction because of the surrounding liquid. Yet, at the macro scale, it depends essentially on the contact area [71, 72].

Possible deviations related to the influence of the previous aspects on friction can be also found as a consequence of spatial or temporal effects. The measuring technique as well as the shape, material and surface properties of the contacting material have also showed an influence on the output. This fact is also observed in previous measurements on *in vivo* skin by using different probes yet, in some cases the differences found in each study-case are attributed to the intrinsic variability among individuals or anatomical place. Typically, dry skin presents a COF around 0.45 whilst under wet conditions is commonly above 1. To broadly summarize the frictional performance of the skin as a function of the normal force, Figure 3 collects results from *in vivo* skin from other research [8]. Even considering the deviations resulting from inter and intra-individual variability, it is clear that water has an effect on the tribo-mechanical properties. As it can be seen, the COF at dry conditions remains not higher than 0.7 whereas under wet conditions, the COF ranges from 0.6 to 2.6.

Figure 3. Coefficient of friction of *in vivo* human skin as a function of the normal force at dry and wet conditions. The results are taken from reference [8]. The red and blue areas separate the dry and wet friction zones, respectively.

1.4 Aim of the research

A suitable mechanical skin substitute is desirable for tribological testing applications. The analysis of previous results on skin friction address to consider the variation of the mechanical behaviour of skin with depth, its specific surface properties and hydration characteristics as major aspects influencing the frictional performance.

This research aims to develop a skin substitute with a similar tribo-mechanical performance as the human skin at dry, normal and hydrated or wet conditions. To be able to mimic the tribo-mechanical performance of the human skin, the length scale dependence of its elastic modulus has to be implemented in the physical model. Thus, the model requires also a layered-structure with individual mechanical properties. The overall elastic modulus of the model has to be within the range of the values for human skin. Further, the mechanical and surface properties of the skin surface should also be taken into account specially in the case of sliding friction. Thus, the top layer of the model must be harder than the bulk and the adhesive properties typical of the skin also need to be resembled. Additionally, to cover the changes in the frictional performance of the skin under wet or hydrated conditions, a moisture-sensitive skin substitute with ability to adapt to the environmental conditions is essential. This research focuses on the use and analysis of water absorbent polymers, specifically poly (vinyl alcohol) hydrogel. The properties of these hydrogels are well known as well as the crosslinking approaches to produce them. Moreover, their mechanical performance can be adjusted by varying several aspects related to their production, such as polymer concentration or crosslinking agent. Due to their intrinsic properties, the structure and elasticity of these materials can adapt to the environmental changes in temperature and humidity by affecting their tribo-mechanical performance.

No *in vivo* measurements were performed in this research so that, the parameters considered for the development of a mechanical skin substitute, such as the elastic modulus, were based on results on *in vivo* skin from the literature. Moreover, no roughness effects are considered for the theoretical analysis of the frictional performance of the skin in this thesis. Thus, the real contact area is implemented as the 80 % of the nominal area of contact in the contact model. The *ex vivo* skin measurements presented in this work were specifically performed to evaluate the role of adhesion in the frictional performance of the skin.

1.5 Outline of the thesis

This thesis focuses on the development of a layered mechanical skin substitute with a tribological performance similar to that of the human skin under different conditions. Several options based on the use of hydrogels are investigated to achieve the development of a layered structure with a similar scale dependent elastic modulus as human skin. The suitability of the elastic modulus of the model with

respect to human skin is examined by means of indentation tests. Moreover, the surface properties of the skin are also simulated by the use of oil compounds on the top layer of the model. The suitability of the developed substitute was evaluated by friction measurements at the meso and the macro scales in comparison to *ex vivo* human skin results. Additionally, the tribo-mechanical results are also contrasted with previous observations on *in vivo* skin. Lastly, the role of the surface properties of the skin on its frictional performance is analysed by means of a model which accounts for role of the adhesive and capillary forces on the contact.

The thesis is divided in 2 parts with a total of 11 chapters. **Chapter I** includes the aim of the thesis as well as a brief introduction of the main aspects influencing skin friction. **Chapter II** provides the background of this dissertation and it explores the structure and composition of the human skin, as well as the variability of its tribo-mechanical performance based on previous research on *in vivo* or in *ex vivo* skin. **Chapter III**, contains a review of hydrogels, their physical and chemical properties as well as their mechanical performance.

From Chapter IV onwards, part 2 of the thesis begins. In this part, each chapter contains a scientific article which describes a different phase of the research goal. The initial steps addressed towards the development of a skin mechanical model. The last chapters focus on the definition of a theoretical skin friction model based on the length scale dependence of the elastic modulus and the adhesive properties of the skin.

Specifically, **Chapter IV, Paper 1** reports the fabrication of a novel Epidermal Skin Equivalent (ESE) based on the combination of a hydrophobic silicone and a hydrophilic hydrogel. The physico-chemical and surface properties of the ESE as well as its mechanical performance are described in the chapter in comparison to *in vivo* results from the literature. In **Chapter V, Paper 2** the frictional performance of several synthetic skin models in comparison with the ESE and *ex vivo* skin results is analysed. During this measurements, the materials components of artificial turf are used as a counter surface against the skin. Thus, the results of this research helped to evaluate the influence of the material properties in the frictional performance of the skin. In **Chapter VI, Paper 3** the adhesive properties and frictional performance of *ex vivo* skin untreated and treated with water and rapeseed oil are investigated. The differences in the frictional performance, especially in the case of wet skin, are suggested to be influenced by the capillary forces at the contact. **Chapter VII, Paper 4** presents a description of raw PVA blocks and in combination with cellulose fibers obtained by freezing/thawing cycles. The physico-chemical properties and tribo-mechanical performance of the blocks is examined to evaluate their suitability as skin mechanical substitutes. Moreover, **Chapter VIII, Paper 5** presents an alternative synthetic stratum corneum with improved the interfacial shear properties of the PVA hydrogel. The composition of the material, its adhesive and hydration properties are described in the chapter as well as its viscoelastic properties from tensile and stress relaxation measurements. **Chapter IX, Paper 6** evaluates the elastic

modulus of a Bi-Stratified Skin model (BSSM) as a function of the normal force in comparison with the theoretical results of a multi-layer model which accounts for the length scale dependence of human skin. In **Chapter X, Paper 7** a comparison of the frictional performance of the BSSM and *ex vivo* skin at dry and wet conditions is presented. The friction measurements were performed at the meso and the macro scale to account for the length scale dependence of the skin as a function of the indentation depth. Based on the frictional results of *ex vivo* skin, a theoretical Skin Friction Model (SFM) is described based on the length scale dependence of the elastic modulus and the effect of the capillary and adhesive forces in the contact situation. Finally, in **Chapter XI** the conclusions of this thesis and recommendations for future research are presented.

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Chapter 2

2. Structure and tribo-mechanical properties of human skin

The influence of skin's structure, composition and mechanical properties need to be analysed for a better comprehension of the frictional performance of the skin. In the next section a description of the relevant skin properties and the factors influencing its tribo-mechanical behaviour are presented.

2.1 General properties & composition of human skin

Human skin is the largest organ of the human body, the so-called integumentary system. It constitutes an interface between the inside body and the environment which covers an anatomical surface of 1.5 – 2.0 m² [1]. Skin's function is to protect the human body against the external changes, such as continuous desiccation, pathogens, impacts, chemicals, ultraviolet light or changes in temperature [2]. Moreover, it contains receptors which transfer the external touch stimuli to the brain where, once processed, they provide the touch perception. The sensory receptors distributed through the skin experience the mechanical, thermal, nociceptive and epicritic changes of the body under a specific external situation [3 - 7]. These receptors forward electrical signals to the brain informing of changes in pressure, UV intensity, temperature, softness, hardness or roughness. After processing this information, the brain elaborates a response to accommodate the body to the actual environment. In addition, the skin has an important hormonal role and contains sebaceous and sweat glands which act in response of motor stimuli [8].

The human skin is divided in several layers with different composition and properties. From the top to the bottom, there are the stratum corneum, viable epidermis, dermis, and hypodermis. In Fig. 1 is illustrated a cross section of the human skin with indication of the main structural layers. Each skin layer has a different biological composition, thickness and hydration degree which results in different mechanical and frictional properties.

Figure 1. Human skin structure with indication of the main layers and epidermis sub-layers [based on an image from *Skin Care and Cosmetic Ingredients Dictionary* by Varinia and Di Nardo. Copyright © 2014 edition; adapted from ref. [9]].

○ Epidermis

The outermost layer of skin is epidermis which is composed of five different sub layers: stratum corneum, stratum granulosum, stratum lucidum, stratum spinosum and basal membrane as indicated also in Fig. 1. Each of these layers conform a compact structure of cells, the keranocytes, which origin is in the basal stratum. Due to the differentiation process the keranocytes transform into corneocytes, flattered cells without nucleus, which define the outermost layer of the skin, the so-called stratum corneum (SC) [10]. The intermediate layers correspond to different stages of the cellular differentiation process [10].

The composition and properties of the stratum corneum significantly affect the tribo-mechanical performance of the human skin. Besides, a well-functioning skin depends in the barrier capabilities of the SC to prevent the dehydration due to trans epidermal water loss. The stratum corneum comprises a bi-phasic assembly of corneocytes surrounded by an organized periodic matrix of fatty acids, ceramides, cholesterol and water domains [11 - 13]. It is a 25 to 200 μm thick layer which characteristic structure and composition of keratin, cholesterol and ceramides provides rigidity to the membrane and prevents the skin dehydration [14 - 16]. Corneocytes are based on a cornified enveloped with a matrix made primarily of keratin and a natural moisturizing factor (NMF) which contributes to the skin's hydration [17]. Keratin plays also a vital role in the barrier properties of the skin to control hydration and it influences its elasticity and mechanical performance [17, 18]. The cornified envelops are connected to each other by intercellular junctions called desmosomes and they

are all surrounded of lipids which conform the mortar phase. A schematic image of the main components of the stratum corneum is presented in Fig. 2.

Figure 2. Structure of the stratum corneum (above). An illustration of the main components of the brick (left) and mortar (right) is also presented below [based on an image from *In Vitro Percutaneous Absorption: Principles, Fundamentals, and Applications* by Bronaugh and Maibach. Copyright © 1991]. Adapted from ref. [9]

The stratum corneum is covered by a lipid film, the Skin Surface Lipid Film (SSLF), a mixture of sebum secreted by the sebaceous glands, lipids from the keratinocytes and water from the sweat. This layer plays also an important role in the hydration and the adhesive properties of the skin and, in consequence it affects its frictional performance [19 - 21]. The SSLF is basically composed of triglycerides, squalene, wax esters and ceramides [12, 14, 22] with variations depending on the body site and from human to human. Modifications of the composition or amount of the lipid constituents in the SSLF have been related to an increase of the trans epidermal water loss (TEWL), dehydration and dermatological problems [14 - 16, 19 - 27].

○ Dermis

Crossing the dermal-epidermal junction, a physical barrier which protects the inside body against possible hazards, there is the Dermis. It is a supportive cellular matrix, the so-called Extra Cellular Matrix (ECM), based on proteoglycans (PGs), glycosaminoglycans (GAGs) ions and an important amount of water (see Figure 3). This matrix conforms a soft tissue which strength and extensibility is given by some embedded collagen and elastin fibers [28, 29]. Dermis has a variable thickness between 1 - 4 mm depending on the body place and it is the place where the sensory receptors, hair follicles, sweat and sebaceous glands are located [30]. Within the soft matrix of PGs, GAGs and water, an

entangle network of collagen, elastin and reticulin fibers form a space where cells, mainly fibroblasts, are embedded. The specific composition of the dermis is responsible for the viscoelastic performance and resilience to the skin [30 - 32]. In consequence, the elastic response of this layer when the skin is under stress has an important influence on its tribological performance.

Figure 3. Schematic illustration of the human dermis. The main constituents of dermis are mentioned [adapted from an image of Cummings, Pearson Education, Inc. Copyright © 2006]. Taken from ref. [9].

○ Hypodermis

Hypodermis is the innermost layer of the skin and its main function is to serve as energy reservoir. It is also involved in the thermoregulation of the body and it has a hormonal role. Hypodermis provides mobility to the skin and it protects the inner organs against mechanical damage due to impact. It is the last layer of the integumentary system and it comprises a physical barrier which separates the skin from the muscles [1]. This layer is extremely soft and viscous due to a composition basically formed by adipocytes with some fibers of collagen and elastin which connect to dermis and fascia. Although hypodermis has a protective role against mechanical contact, its influence in the frictional performance of the skin has been neglected [33, 34].

○ Muscle

The muscle does not conform a part of the integumentary system. It is separated from the skin by the fascia a dense connective tissue based on collagen and elastin yet, its mechanical properties might influence the frictional performance of the skin at large applied forces.

2.2 Mechanical properties of the human skin

Human skin has been characterized as a complex soft structure with a non-linear elastic, non-homogeneous, anisotropic and viscoelastic behaviour [35, 36]. The mechanical properties of the skin strongly influence the contact parameters when the skin is subjected to tangential stresses by lastly affecting its frictional performance. Since the skin presents a different layer composition and thickness, its overall response under stresses depends on the mechanical properties of the individual layers involved. Thus, depending on the magnitude of the applied forces it might be that also the underlying tissue, such as the muscle and the bone, has an impact in the mechanical response. In addition, the mechanical properties are spread over a wide range of results with an elastic modulus depending on the age, hydration degree, anatomical region or applied technique [28, 33, 37 – 40]. Therefore, the results obtained on *in vivo* skin from previous work are difficult to compare in many cases. A general overview of the mechanical properties of each layer is presented below.

○ *Stratum corneum*

The mechanical behaviour of the stratum corneum is characterized by tensile tests at constant elongation rate as a function of the relative humidity [28, 29] as indicated in Figure 4.

Figure 4. Elongation curves of the stratum corneum at different relative humidity. Adapted from reference [61, 67].

These results show a variable performance depending on the elongation with three clearly defined phases which correspond to the mechanical response of different SC constituents. Thus, at elongations lower than 10% the response of the SC is mainly given by the arrangements of keratin fibrous but without substantial structural changes. In this stage, the mechanical response of the SC

can be considered purely elastic. The influence of hydration at this elongation rate results in a lower elastic modulus of the SC as the humidity increases. At elongations ranging between 20 and 100% structural changes of the keratin filaments occur with the consequent structural modifications of the SC. In addition, water causes chemical and structural modification of keratin fibers which may reduce the inter-chain interactions by increasing the mobility of the structure and decreasing its stiffness (see Fig. 5). Thus, as humidity increases the keratin filaments become less stiff but, at the same time their toughness arises. At this stage, the effect of water in the overall mechanical properties of the SC results in a viscoelastic response. Thus, it is observed a plateau zone after the yielding point which corresponds to the stress relaxation of the hydrated SC just before rupture. At fully hydrated conditions, three different regions can be observed. The first corresponds to the elastic performance which, at high humidity levels, implies a low elastic modulus. The second step, the yielding region, is a plateau zone which characterizes the viscoelastic behaviour of the SC. Lastly, a post-yield region appears as a consequence of structural modifications of the keratin filaments which address to an increase of the strength before rupture [41]. Consequently, the mechanical performance of the SC is characterized by a non-linear behaviour only at high strains so that, at low deformations it can still be considered fully elastic.

Figure 5. Schematic diagram for the interaction “keratin - water” according with reference [41].

The elastic modulus of *in vivo* and *ex vivo* SC has been reported by using different techniques such as torsion, extension, suction or indentation [29, 37, 39, 40, 42, 43] albeit the results are difficult to compare. Levi [44] measured the elastic modulus of the isolated SC by micro-tensile tests over a range of RH environments. These results indicate a decrease of the elastic modulus with increasing humidity as expected from the effect of water in keratin. A similar trend is reported by other research [29, 45, 46] and the given elastic moduli are presented in Figure 6 as a function of the RH.

Figure 6. Elastic modulus of isolated stratum corneum obtained from tensile tests as a function of the relative humidity. Data taken from [29, 45, 46].

○ Dermis

The viscoelasticity characteristic of human skin derives principally from the mechanical performance of dermis. Collagen and elastic fibers combine their mechanical properties to provide strength and elasticity to the skin [36, 47]. Further, the viscoelastic behaviour of skin arises from the interstitial fluids and water within the ECM [47, 48].

When the skin is subjected to stresses which involve the dermis response, the initial deformation is elastic and it is controlled by the elastin fibers. If the stress ceases, the elastin fibers return to the original shape and the skin recovers its elasticity [49 - 51]. On the contrary, if the stress continues, the collagen fibers progressively straighten out so that, the load-carrying role is transfer from the elastin to the collagen which results in an increase of stiffness. At a certain point further deformation of the collagen fibers is not possible and the rupture of the fibers occurs. Furthermore, the viscoelasticity characteristic of the skin results of the mobility of the ECM constituents within a watery environment as it has been described by creep compliance and stress relaxation measurements [48, 52, 53]. Due to the viscoelastic character of the dermis, the mechanical properties of the skin become dependent on the time and hydration [54 - 57]. Figure 7 (a) and (b) displays the elastic and viscoelastic performance typical of the dermis given by the response of the collagen-elastin fibers and the ECM, respectively.

The mechanical performance of dermis has been characterized by a nonlinear elastic stress-strain behaviour associated to the properties of the network collagen-elastin. The nonlinear behaviour of dermis is similar to that of rubbers [58] which undergo large strains with small applied stresses. Dermis show a non-constant elasticity with an initial elastic modulus, E_0 , that decreases with the strain in an initial stage but, increases considerably at very large deformations due to the stiffness of the collagen fibres. Thus, Hooke's law is inadequate to describe its mechanical performance.

Figure 7. Mechanical properties of the main components of dermis: a) stress-strain curve of elastin and collagen; b) typical performance of a viscoelastic material, such as dermis under deformation as a function of time. Adapted from ref. [57].

Additionally, the viscoelastic behaviour (time dependent) of the skin is due to the viscous resistance of the ECM components and its water content as well as, due to the viscoelastic properties of collagen and elastin. The viscoelastic properties of the skin can be predicted by purely phenomenological models consisting on springs and dashpots connected in series and/or parallel. One of these models, the so-called Standard Linear Solid model (SLS), gives a proper description of the stress and creep behaviour of viscoelastic materials. Based on the SLS (see Figure 8), the instantaneous and a delayed elastic deformation of the skin can be calculated from stress relaxation or creep measurements. The parameters of the SLS model can be obtained from tensile measurements by applying a constant stress, σ , or strain, ε ; that is, by creep or stress relaxation tests, respectively.

Figure 8. Schematic representation of the SLS model.

According to the SLS model the generalized Hook's equation can be written as:

$$\eta \cdot E_1 \cdot \dot{\varepsilon} + E_1 \cdot E_2 \cdot \varepsilon = \eta \cdot \dot{\sigma} + (E_1 + E_2) \cdot \sigma \quad (1)$$

With E_1 , E_2 the elasticity of the springs and η the viscosity of the dashpot; ε and σ refer to the strain and stress, respectively.

Thus, if the strain is kept constant and equal to ε_0 , the stress relaxation function is given by:

$$\psi(t) = \frac{\sigma(t)}{\varepsilon_0} = \frac{E_1}{E_1 + E_2} \cdot [E_2 + E_1 \cdot e^{-(t/\tau)}] \quad (2)$$

Where τ is the relaxation time given by:

$$\tau = \frac{\eta}{(E_1 + E_2)} \quad (3)$$

2.3 Length scale dependence of the elastic modulus

As indicated above, human skin is an intricate structure with a mechanical performance influenced by different layers and may be an influence of the underlying tissue as well. The mechanical properties of the skin vary depending on the anatomical site, hydration of the skin, age and between individuals [28, 33, 37 - 40]. Indeed, according to the literature the elastic modulus of *in vivo* skin shows a deviation between 4 and 5 orders of magnitude with values fluctuating from the kPa range to MPa. Although the elastic modulus of *in vivo* skin has been determined by different approaches, such as torsion, extension or suction, indentation tests are a preferred method from a tribological point of view. This method is simple and provides the elasticity in terms of the Effective Elastic Modulus, E^* , a combination of the elastic modulus of the contacting surfaces which is required in the contact models [37 - 39, 59] as indicated in Eq. 4.

$$\frac{1}{E^*} = \frac{1 - \nu_{skin}^2}{E_{skin}^*} + \frac{1 - \nu_2^2}{E_2^*} \quad (4)$$

With ν_{skin} and ν_2 the Poisson's ratios of the skin and the counter-surface, respectively.

During an indentation test, a rigid tip (the indenter) measures continuously the force and displacement applied to the studied material. Therefore, the stiffness, S , can be calculated as the initial slope of the unloading part of the load-displacement curve as described in Figure 9, according to Oliver and Pharr [60]. From the stiffness, S , the Effective Elastic Modulus, E^* , can be calculated according to equation (5).

$$S = \frac{dF}{d\delta} = \frac{2}{\sqrt{\pi}} E^* \sqrt{A} \quad (5)$$

Figure 9. Schematic representation of an indentation measurements with indication of the loading and unloading curves. The tangent of the unloading curve is denoted in green with the initial part of the curve in bright green to point the data from which the stiffness is calculated according to Equation (12).

The elastic properties of *in vivo* and *ex vivo* skin have been analysed by this technique at different normal loads, speeds and indenter geometries. The results show an elastic modulus variable with the indentation depth. Thus, at the length scale of the skin's surface roughness, the mechanical properties of the skin are determined by the stratum corneum with values ranging from 1 GPa to 3.44 MPa with increasing indentation under dry conditions [28, 33, 61, 62]. Additionally, humidity and hydration cause a considerable decrease of the elasticity of the SC as it has been previously discussed (see Fig. 6). Indentation tests on wet SC show an elastic modulus decreasing from 50 to 10 MPa in a range of indentation between 0.2 and 2 μm [61]. Further, with increasing applied forces lower values for the elastic modulus of *in vivo* skin are reported. This indicates that at larger length scales, i.e. millimetres, inner layers contribute to the overall elasticity of the skin. Pailler-Mattei et al. [19] carried out indentation tests on the inner forearm of ten women after tape-stripping measurements with an indenter of 6.35 mm radius. These results indicated an average elastic modulus between 7-8 kPa independently of the SC thickness removed which confirmed that at large scales, the influence of the SC in the overall elastic modulus is minor. Then, the role of the bulk properties of the skin becomes more significant. Similar results were obtained by Zahouani et al. [38] on the forearm of 20 volunteers measured up to an indentation depth of 1000 μm with a resulting elastic modulus of 8.3 ± 2.1 kPa. Moreover, indentation tests on the forearm and facial skin of ten individuals were performed by Jachowicz et al. [37] with an indenter diameter of 5/16 in. Elastic modulus of 7 kPa and 33 kPa were found for the forearm and face, respectively. These results indicate an elastic modulus for dermis in

the range of few kPa so that, it can be classified as ultra-soft material in contrast to the hard elastic modulus of isolated SC.

To account for the layered structure of the skin when evaluating the elastic modulus, van Kuilenburg et al. [33] suggested a multi-layered model which considers the mechanical properties and thicknesses of the individual layers of the skin. According to this model, the global elastic modulus of the skin can be calculated as:

$$\frac{1}{E_{eff}} = 2 \sum_{i=1}^{n-1} \frac{t_i}{f_i(a)\pi a E_i} + \frac{1}{f_n(a)E_n} \quad (6)$$

t_i , E_i , the thickness and the elastic modulus of each individual layer and f_n , E_n referring to the properties of the underlying tissue.

The polynomial functions f_i and f_n are defined below to ensure the correct boundary conditions:

$$f_1(a) = 1 + \frac{2t_1}{\pi a} \quad (7)$$

$$f_i(a) = \left(1 + \frac{2}{\pi a} \sum_{j=1}^{i-1} t_j\right) * \left(1 + \frac{2}{\pi a} \sum_{j=1}^i t_j\right) \quad \text{for } i = 2 \dots, n-1 \quad (8)$$

$$f_n(a) = \left(1 + \frac{2}{\pi a} \sum_{j=1}^{n-1} t_j\right) \quad (9)$$

The subscript i refers to the skin layers whereas the subscript n concerns the underlying tissue.

In this thesis, the length scale dependence of the skin was implemented based on the previous equations to obtain the overall elastic modulus at two different range of forces. Due to the relative influence of the skin layers in the mechanical properties of skin is clear that, the dermis has a main role in determining the overall elastic modulus whereas surface related phenomena are influenced by epidermis, particularly by the stratum corneum. Therefore, to evaluate the elastic modulus of the skin in the range of mN, in this thesis referred as the meso scale, the individual elastic modulus and thickness of the stratum corneum, viable epidermis and dermis is implemented in Eq. 6. At forces of few N, the macro scale range, the overall elastic modulus is calculated by the properties of stratum corneum, dermis and the muscle as underlying tissue. Table 1 collects the input parameters used to calculate the overall elastic modulus at both scales. Further details are included in Chapter 10, Paper 7.

Table 1. Summary of the individual thicknesses and elastic moduli of the layers implemented in the model to evaluate the length scale dependence of the skin. Partially adapted from ref. [33].

MESO SCALE				
<i>i</i>	Skin layer		Elastic modulus <i>E_i</i> (MPa)	Thickness <i>t_i</i> (mm)
1	Stratum Corneum	dry	100	0.02
		wet	10	
2	Viable Epidermis		1	0.04
3	Dermis		0.05	1.1
Underlying tissue	Hypodermis		2·10 ⁻³	-

MACRO SCALE				
<i>i</i>	Skin layer		Elastic modulus <i>E_i</i> (MPa)	Thickness <i>t_i</i> (mm)
1	Stratum Corneum	dry	50	0.02
		wet	5	
2	Dermis		0.06	1100
Underlying tissue	Muscle		0.25	1.1

To evaluate the length scale dependence of the skin, the contact area needs to be introduced as an input parameter. Thus, a more complete description of the influence of the specific contact situation in the overall elastic modulus of the skin and, subsequently in its frictional performance is presented in the next section.

2.4 Requirements for a suitable tribo-mechanical skin substitute

Based on the structure, composition and mechanical properties of the skin, the development of a suitable skin substitute for friction testing applications requires to consider several aspects. An essential subject to have into account is the length scale dependence of the elastic modulus due to the layered structure of the skin. The other is related to differences in the frictional performance of the skin as a consequence of the effect of water. Therefore, to mimic the tribo-mechanical performance of the human skin, a physical model divided in several layers with different mechanical properties needs to be produced. The overall elastic modulus of the model has to be within the average range of the values given by the literature for the human skin. In addition, the response of the skin to hydration should also be reflected in the model since it affects considerably its frictional performance. Thus, a water- responsive material with hydration properties is essential to resemble the modifications of the mechanical and adhesive properties of the skin when contacting water. Furthermore, the surface properties of the skin, such as the roughness and, especially the adhesive forces acting between the skin and the counter surface need to be mimicked to properly asses the frictional performance of the skin, especially at low applied forces under wet conditions.

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Chapter 3

3. Properties and mechanical performance of hydrogels

Hydrophilic gels (or hydrogels) are polymeric materials with an important water uptake capacity and promising properties for a wide range of fields as pharmacy, medicine, industrial engineering or agriculture [1 - 5]. The ability to swell in water and adapt to the environmental changes of temperature and moisture make them potential materials for biomedical applications as bio absorbable sutures, bio adhesive, in contact lenses, drugs delivery systems, or biosensors [1 - 3, 6]. Hydrogels are studied with great interest in tissue engineering applications where bio compatible materials with water absorption capabilities are required to simulate the characteristic environment of the Extra Cellular Matrix (ECM) [4, 5]. Thus, they are applied as matrices for the regeneration of biological tissues, such as artificial cartilage, artificial esophagus, artificial skin or other artificial organs [7 - 12].

In this chapter, a description of the physico-chemical and mechanical properties of hydrogels is presented in connection to the development of a skin mechanical model.

3.1 Physico-chemical properties of hydrogels

Hydrogels are three-dimensional networks of physically or chemically crosslinked polymers, copolymers or other complex macromolecules with functional groups, such as amide, hydroxyl, carboxyl or sulfonic acids among others [13]. The existence of hydrophilic groups in the system enables the formation of hydrogen bonds in the presence of water by forming an interconnected system able to absorb water without dissolving in it. The stability of this reticulate is associated to the resistance of the bonds between polymer chains against the solvation process initiated by water molecules [14]. Hydrogels behave as a single molecule so that, conformational transitions of the polymer chains due for instance, to the interaction with water, become visible at the macro scale. Hydrogels are not solid neither liquid materials but combine the properties of these two states [15 - 16]. They look like solid materials under certain conditions and they present typical properties of the solid state. Nevertheless, the physico-chemical and mechanical properties are significantly conditioned by the water content.

The structure and properties of a hydrogel are definitively influenced by the synthetic methods and reagents used during the synthesis [17]. As indicated before, the ability of hydrogels to absorb water arises from the hydrophilic groups of the polymer backbone, while their resistance is associated to the strength of the crosslinks between the polymer chains via physical or chemical entanglements.

A proper understanding of the properties and performance of hydrogels involves the exploration of the physic-chemical properties of this supramolecular structure and its interaction with water.

3.1.1 Physical properties of polymers: background

Polymer chains present rotational motion around the main axis of the molecular backbone yet, this rotation is limited by the nature of the steric groups in the chain, their size and other boundary conditions the polymer is subjected to. The spatial configuration of an entanglement of polymer chains tends to decrease the repulsive interactions and minimize the energy state of the macromolecule to adapt to the most stable configuration (see Fig. 1). A similar situation happens between the polymer chains that confirm a hydrogel with an extra effect of the molecular interactions due to the water presence. These interactions are ultimately responsible for the macroscopic properties and performance of hydrogels. Thus, the hydrogel behaves as a mobile structure whose properties are function of short range effects (as the bond angles between atoms in the main chain or the interactions between the stearic groups) and long range effects (thermodynamic changes of the environment, such as those due to the interactions with water) [18 - 20].

Figure 1. Hydrogel reticule which is originated in the swollen state with water phases next to the chains and other of free water.

The molecular conformations within the hydrogel address a particular macroscopic performance which is a function of the temperature and the swollen conditions. Thus, to understand the physic-chemical and mechanical performance of hydrogels it is needed to refer to the physical parameters that influence their microstructure.

- **Glass transition temperature (T_g)**

The glass transition temperature (T_g) is related to the properties of polymers when subjected to temperature changes. It defines the limit between the vitreous and the rubbery state of the hydrogel as a result of the mobility of the polymer chains due to internal energy modifications [18]. Thus, above the glass transition temperature the polymer chains intensify their movements so that, the macroscopic behaviour of the hydrogel seems liquid although, it is still a solid material. Instead, below the glass transition temperature, the polymer chains loss kinematic energy and then, their random or oriented spatial distribution leads to an amorphous or semi crystalline material, respectively (see Fig. 2).

Figure 2. Hydrogel reticule which is originated in the swollen state with water phases next to the chains and other of free water.

At temperatures below T_g the mobility of the polymer chains and their steric groups are limited and they are practically immobilized. As temperature rises, the increase of the molecular energy causes an intensification of the rotations in the main chain as well as an increase of the mobility of the functional groups. Thus, depending on the glass transition temperature, hydrogels exhibit a variable degree of cluttering in the microscopic structure which, subsequently affects its properties and performance.

In an upper organizational level, polymers are either entirely amorphous or semi crystalline with a structure formed by alternating layers of lamellae and amorphous phases as indicates in Fig. 3. Due to the biphasic structure of semi crystalline polymers, in addition to the glass transition temperature, a melting temperature, T_m , associated with the crystalline phase appears. This is responsible for the structural changes in the vitreous phase, such as the change condition from solid to liquid [18].

Figure 3. Hydrogel reticule which is originated in the swollen state with water phases next to the chains and other of free water.

○ Equilibrium Water Content (EWC)

The amount of water content in the hydrogel also influences its properties and performance [21]. This is a consequence of the changes in the mobility of the polymer chains resulting of the interactions with water molecules. The water uptake of a hydrogels is commonly expressed in terms of Equilibrium Water Content (EWC) as the quotient between the weight of water in the hydrogel, W_w , and its total weight, W_t .

$$EWC = \frac{W_w}{W_t} \cdot 100 \quad (1)$$

With the weight of water calculated as the difference between the total weight of the hydrogel, W_t , minus the dry weight, W_d .

$$W_w = W_t - W_d \quad (2)$$

The EWC depends on the polymer nature related to the amount of available hydrophilic groups as well as on the crosslinking degree between the polymer chains in the net. The greater the entanglement between molecules is, the more compact the polymeric structure results. Consequently, the possibility of spatial arrangements to locate water molecules is more limited [22 - 25].

Water is distributed within the hydrogel in two different ways [23, 26 - 29]. An amount of water is juxtaposed to the polymer chains as little drops linked to the hydrophilic groups of the molecular chains. As the water uptake increases, hydrophobic groups are exposed. Thus, in an attempt to avoid an interaction with water, the mobility of the chains increased to provide space for the water molecules. Consequently, the joint of water molecules in the same space ends up by forming larger domains within the network as indicates in Fig. 4.

Figure 4. Hydrogel reticule which is originated in the swollen state with water phases next to the chains and other of free water.

As it was indicated above, the glass transition temperature is an essential parameter to understand the properties and performance of hydrogels. In addition, the presence of water also modifies the thermodynamics of the supramolecular structure with important variations of physical properties such as the glass transition. Thus, in swollen hydrogels it is necessary to consider the water uptake to understand the differences in the physical and mechanical properties of these materials. In general, the glass transition temperature decreases with the water uptake [30 - 34]; that is, the glass transition temperature of a dry polymeric network is higher than in the swollen state. Consequently, to understand the mechanical performance of hydrogels under hydrated conditions it is necessary to account for the effects of the water uptake in its physical and microstructural properties.

3.2 Synthetic strategies

Hydrogels can be synthesized through different approaches which influence the physico-chemical properties and mechanical performance of the resulting system. In general, hydrogels present soft mechanical properties which are insufficient for different applications, especially as a consequence of the softening effect of water in the system [23, 29, 30, 35 - 37]. Consequently, several approaches have been indicated to improve the stability of the network and to adapt their mechanical performance to the requirements of each application.

Chemical strategies imply changes in the monomer composition, polymer concentration, crosslinking approach and/or cross linker density [20 - 22, 25, 26, 28]. For instance, changes in the monomer composition, such as a decrease in the content of hydrophilic monomers (or increase in the hydrophobic segments) have been suggested to decrease the degree of swelling and increase in the mechanical strength of the hydrogel [38]. The effect of polymer concentration has also been related to a decrease in the equilibrium water content (EWC) with a subsequent increase of the mechanical properties [38, 39]. Additional changes include to modify the density of crosslinking agents to improve

the mechanical strength of the hydrogel [22]. Other strategies combine different polymeric networks in an attempt to enhance the stiffness of the hydrogel. The incorporation of particles and/or fibers as a reinforcement has also been suggested in some research as an option to improve the mechanical strength of hydrogels [40 - 42].

In addition to the strategies described above which involve the use of a crosslinking agent, physical methods have also demonstrated to be efficient for the development of hydrogels with different properties. Physical methods used to synthesize hydrogels without chemical reactions include photopolymerization or successive cycles of freezing/thawing [34, 43, 44]. A great advantage of these methods with respect to the chemical strategies is related to the absence of toxic agent which ensures their biocompatibility.

Figure 5 shows the differences in the supramolecular structure of a physically crosslinked hydrogel (left) based on chain's entanglements and a hydrogel crosslinked gel by a chemical agent (right).

Figure 5. Schematic representation of a physically and a chemically crosslinked hydrogel (a) and (b) images, respectively.

3.3 Mechanical performance of hydrogels

The polymeric network of a hydrogel presents certain mobility due to the amorphous or semi crystalline structure which leads to a rubbery-like behaviour. Moreover, the presence of water increases the flexibility of the system by providing a higher mobility for the chains. Thus, water molecules diffuse freely through a network which acts as a water sponge matrix. As a consequence of the interactions between this two phases, the macroscopic behaviour of hydrogels is characterized by a time independent and time dependent performance. The elastic performance can be explained according to the assumptions of the rubber elasticity theory based on classical thermodynamics and phenomenological approaches. The time dependent response of hydrogels can be analysed by means of

empirical methods in comparison to theoretical linear or non-linear models formed by a combination of springs and dashpots to represent elastic and viscous components, respectively.

3.3.1 Elastic performance

The elastic properties of hydrogels, such as the elastic modulus, yield strength or ultimate strength, can be analysed by tensile, compression or indentation tests [45 - 48]. Rubbery-like materials present a high extensibility under low stresses which can be complete or partially recovered depending on the specific mechanical properties of the material and the applied load. In general, hydrogels present a low modulus of elasticity in the range of kPa that is, the ultra-soft materials. Nevertheless, in some cases the crosslinking and reinforcement strategies lead to significantly stiffer structures with elastic modulus in the range of MPa [49, 50].

Rubbers are considered isotropic, nonlinear and practically incompressible materials [51]. The rubber elasticity theory is related to the statistical thermodynamics theory so that, the mechanical behaviour of the hydrogel can be related to the number of network chains and other internal properties dependent on the mobility of the supramolecular structure [51 - 53]. Temperature is one of the most important parameters affecting the mechanical response of rubbers. Figure 6 displays the typical mechanical performance of a rubber as a function of temperature. At lower temperatures than the glass transition, the mobility of the polymer chains is restricted due to the absence of thermal activation so that, the polymer displays the high elastic modulus.

Figure 6. Schematic representation of the mechanical performance of a polymer with indication of the different stages of the material as a function of the temperature.

As the temperature raises, the thermal energy causes an activation of the polymer chains translated into a decrease of the mechanical properties which become similar to the performance of leather materials. During a plateau zone the polymer exhibits steady mechanical properties but, a progressive increase of temperature addresses to a state change in the material from solid to liquid when the melting temperature is crossed. Consequently, above the glass transition temperature, the elastic modulus of the polymer decreases and the failure strain is larger; on the contrary, below the glass transition the elastic modulus increases and the failure strain decreases due to the more likely glassy properties.

Further, the presence of water in the hydrogel implies modifications of the mechanical performance with respect to the typical behaviour of a rubber material. Structural and thermal modifications of the polymeric network as a consequence of the water presence cause a decrease of the glass transition temperature [54]. Consequently, the water content in the polymeric network addresses to a reduction of its elastic modulus with an increase of the deformation and the viscoelastic behaviour of the hydrogel [17, 23, 28, 29]. In the presence of water within the polymeric network the polymer chains acquire more mobility so that, the material intensifies its viscoelastic performance specially at temperatures around the glass transition. The changes in the mechanical performance of the hydrogel are related to the amount of water content in the structure according to previous research as indicates in Fig. 8.

Figure 7. Changes of the glass transition temperature as a function of the hydrogel water content. Taken from reference [54].

3.3.2 Viscoelastic performance

Due to the interactions between the molecular flow of the polymeric network and the free water content in the matrix, hydrogels present a mechanical response under stress dependent on time [51]. This is due to the arrangements of the polymeric chains and the water domains within the system when subjected to stress. The viscoelastic properties are also function of the specific composition of the

network, such as nature of the polymer chains, molecular weight, concentration of the polymer or crosslinking degree. Besides, temperature and water content within the structure affect also the glass transition temperature as seen before and, subsequently the viscoelastic performance of the hydrogel. External factors such as the magnitude of the stress imposed and the velocity of the applied force have also a strong influence in the viscoelastic response. The viscoelastic properties of hydrogels can be assessed by applying a constant stress or strain over a certain period of time. At short periods of time, in general no changes are observable at the macroscopic level but, at large periods of time, the time dependence of hydrogels is evidenced by stress relaxation and creep compliance behaviour.

○ Creep compliance

In a creep tests a constant stress is applied to the hydrogel and the evolution of the strain in time is measured as indicates in Figure 9. The first part of the strain curve as a function of the time corresponds to the instantaneous elastic deformation, followed by the creep section, an elastic recovery and a residual deformation. Moreover, the ratio between the time dependent strain and the applied stress is known as the creep compliance:

$$\varphi(t) = \frac{\varepsilon(t)}{\sigma_0} \quad (8)$$

Figure 8. Example of a creep measurement after applying a certain stress.

○ Stress relaxation

During a stress relaxation measurement, a constant displacement is applied to the material and the evolution of the stress in time is measured as displays Figure 10. In this case, the stress relaxation modulus, $\psi(t)$, is calculated as the measured stress in time divided by the applied strain, ε_0 :

$$\psi(t) = \frac{\sigma(t)}{\varepsilon_0} \quad (9)$$

Figure 9. *Example of a stress relaxation measurements after applying a certain strain.*

The viscoelastic properties of hydrogels can be obtained by implementing the experimental results of stress relaxation or creep tests in phenomenological models consisting of springs and dashpots. One of these models, the Standard Linear Solid model (SLS) was described in Chapter 2.1 and it gives a proper description of both stress relaxation and creep behaviour. Thus, the viscoelastic properties of hydrogels, such as the elasticity of the springs and the viscosity of the dashpot can be predicted according to this model.

3.4 Hydrogels for a synthetic skin model

Hydrogels are investigated since some decades ago as potential alternative for the development of artificial tissue in biomedical applications. PolyHEMA-based hydrogels crosslinked by UV-radiation have been manufactured to produce artificial skin substitutes to improve the healing of burned skin [55]. Monodispersive nanoparticles of polystyrene were introduced in a matrix based on polyacrylamide hydrogel to study the adhesive and mechanical properties of the composite as pressure-sensitive adhesives for skin contact applications [56]. Among the most accepted hydrogels Poly(vinyl) alcohol (PVA) is selected due to its biocompatibility, suitable mechanical properties and ease of manufacturing. PVA hydrogels present interesting properties for different uses as the development of adhesives, hydrophilic membranes, in the textile or paper industry, and more recently, in pharmaceutical and biomedical applications, alone or in combination with starch, chitosan and other compounds [57-59].

In this thesis, PVA hydrogel is applied for the development of a Bi-Stratified Skin model for tribo-testing applications. Thus, for a better understanding of the properties and performance of PVA hydrogels a brief review is presented below.

3.4.1 General properties of poly(vinyl) alcohol

Polyvinyl alcohol is a biodegradable, biocompatible synthetic polymer with a relatively simple chemical structure which is indicated in Fig. 10. The monomer of PVA presents a hydroxyl group which is responsible for the hydrophilic character of this polymer. Commercial PVA hydrogels present different degree of hydrolysis and polymerization which, in combination with the molecular weight of the chains, affect its solubility in water and the crystallinity of the gel [44, 60, 61].

Figure 10. Skeletal formula of poly (vinyl alcohol).

Depending on the applied route for the hydrogel formation, they can be classified as physical or chemical hydrogels [62]. Polyvinyl alcohol hydrogels can be obtained by the crosslinking reaction between PVA in aqueous solution and a chemical agent [61, 63 - 65]. Depending on the molecular weight of the polymer chains, the concentration of the polymer in the solution, the nature of the crosslinking agent and the boundary conditions of the reaction, the properties of the resulting hydrogel vary. Nevertheless, the crosslinking reaction can also happen via physical methods, such as UV-radiation [62, 66] or freezing and thawing cycles [44, 62, 64] so that, no chemical agents are required to form the gel. Once the crosslinking is finished, whatever the applied method is, the hydrogel does not dissolve in water yet, it swells.

3.4.2 Crosslinking methods

○ Chemical crosslinking

Common crosslinking routes to obtain PVA hydrogels imply the use of glutaraldehyde, formaldehyde, acetaldehyde, aldehyde or other aldehydes. Moreover, when the hydrogel is obtained by a chemical route, the addition of an initiator is required to activate the crosslinking reaction. Usually sulphuric acid or acetic acid are used as initiator by conducting the formation of acetal groups between the PVA polymer chains and the cross linker agent. A frequent route to obtained PVA hydrogels is crosslinking with glutaraldehyde via sulphuric acid catalysis which addresses to the formation of acetal junctions as presented in Figure 11.

Figure 11. Skeletal representation of an acetal junction from the union of poly (vinyl alcohol) and glutaraldehyde. Taken from ref [67].

The type of cross linker and the conditions of the reaction influence many of the hydrogel's properties, such as swelling degree, elasticity and strength or diffusion capabilities. These hydrogels display high strength and large degradation times due to the strong covalent bounds between the polymer chains and the cross linker agent. However, when the hydrogel is obtained by chemical methods, amounts of cross linker and initiator can remain within the matrix with the subsequent disadvantages for the biomedical uses due to the toxicity of these compounds. Thus, to avoid the presence of toxic agents in the hydrogel other procedures such electron beam radiation or physical methods have also been applied for the development of PVA hydrogels.

○ Radiation methods

Electron beam polymerization implies the use of beta irradiation for the formation of a hydrogel without involving any chemical agent [68, 69]. In this method, the formation of hydroxyl radicals due to the radiation initiates a free radical polymerization which ends once the network reaches the gelation point. Electron beam polymerization can be performed at room temperature and physiological pH so that, it implies advantages over chemical methods which can be of interest for many biomedical applications. However, the limitation of the exposure areas due to the small size of the beam slows the polymerization process and it involves a great disadvantage to produce large structures.

Other type of radiation crosslinking which have been also used for the production of PVA hydrogels is γ -radiation [70, 71].

○ Physical crosslinking

Other method to obtain hydrogels is by physical crosslinking via freezing/thawing cycles, reported first time in 1975 by Peppas [71]. This process is widely used in biomedical applications since it does not involve the use of toxic agents. Hydrogels obtained via freezing/thawing cycles of an aqueous solution of PVA polymer are characterized by crystallite formation which consists of semi-permanent entanglements of the polymer chains. The molecular weight, degree of hydrolysis of the polymer and

polymer concentration in the solution influence the size and amount of crystallites and, lastly the mechanical properties of the resulting hydrogel [44, 72 - 75]. In addition, the number of cycles and the freezing/thawing temperatures have also a key role in the crystallites formation [72, 76].

One disadvantage of the hydrogels obtained by this method is related to their integrity when immersed in water over long time periods. Physical crosslinking imply the formation of entanglements between the polymer chains and hydroxyl interactions between the polar groups and also with the water molecules. Thus, water immersion for a long period can address to a progressive solvation of the polymer with a subsequent dissolution of the hydrogel network. The stability of the 3D-network in water depends on chemical aspects of the polymer, such as the degree of hydrolysis or the polymer concentration as well as, the number of cycles and the freezing-thawing temperatures.

3.5 PVA hydrogels crosslinked by freezing/thawing cycles

PVA hydrogels have been synthesized by freezing/thawing cycles from PVA aqueous solutions at concentration from 2.5 up to 35%. The process requires to freeze the solution at around -20 °C and later thaw it back to room temperature leading to the formation of crystallites. Freezing times of at least 24 h are essential to obtain gels with strong mechanical properties [72]. The cold temperatures during the freezing part of a cycle cause a phase separation with regions of high concentration of PVA polymer and domains of water. Due to the interactions between polymer chains and the hydrogen bonds the formation of entanglements between chains occur. When the temperature is raised above 0 °C, these connections remain and the water melts and distributes over the network by forming the crystallites and, subsequently the three-dimensional hydrogel as presented in Figure 12. Previous experimental research have showed an increase of the mechanical strength of these gels with the increase of the polymer concentration [72, 76]. Moreover, the number of freezing-thawing cycles has been related to the number of entanglements between the polymer chains and a subsequent increase of the number of crystallites [72, 76]. Thus, as the number of freezing/thawing cycle increases, the diameter and number of crystallites rises as well, with a following increase of the hydrogel's resistance to the solvation process when immersed in water. Consequently, the mechanical properties of the gel also increase because of the higher ratio of the crystallites. Further, hydrogels with higher strength have been obtained when the molecular weight of the polymer is increased [44, 77]. Thus, gels prepared from high molecular weight polymers were stronger than those prepared from low molecular weight which presented a more rubbery behaviour.

Figure 12. Morphology of a hydrogel from the macro to the micro scale. Image (a) macroscopic photograph of a PVA hydrogel obtained by freezing/thawing cycles; (b) cryoSEM image of the microstructure of the same hydrogel with indication of the water domains and polymer domains; (c) schematic representation of the polymer entanglements in the hydrogel with the dots (•) representing physical entanglements or crystallites (taken from reference [78]).

The properties of the polymer and the specific parameters applied during the freezing/thawing cycles affect also the water uptake of the hydrogels. In fact, there is a relationship between these parameters, the water uptake, the crystallinity and the mechanical response as it can be seen from Figure 13. Gels exposed to a higher number of cycles exhibit lower water uptake and higher crystallinity which lastly addresses to stronger mechanical properties.

Figure 13. Evolution of two hydrogel properties: (a) water uptake as a function of the number of freezing/thawing cycles and the time for each cycle (taken from ref. [72]); (b) crystallinity of the hydrogel as a function of the number of freezing/thawing cycles (taken from reference [79]).

Further, the role of the glass transition temperature is essential to understand the viscoelastic properties of hydrogels which is further influenced by the water content as it was indicated in previous sections. Besides, as the water uptake is highly influenced by the number of freezing/thawing cycles, the glass transition depends also on it. For instance, in PVA hydrogels combined with gelatine a shifting of the glass transition from 39.7 to 45.7 °C was observed when increasing from 1 to 3 the number of cycles

[80]. Hence, the stability of the gel results in the combination of intrinsic properties of the polymer and external factors, such as the temperature.

Further, the melting of a PVA hydrogel can also occur over a range of temperatures depending on the degree of crystallization of the structure which is a function of the polymer properties and applied conditions for the hydrogel formation. A DSC curve typical of a PVA hydrogel obtained by freezing/thawing cycles is presented in Fig. 14 with indication of the glass transition and melting temperatures.

Figure 14. Thermal properties obtained from DSC measurements of a PVA hydrogel obtained by freezing/thawing cycles. Taken from ref. [73].

3.5.1 Viscoelastic performance of PVA hydrogels

The molecular morphology of the polymer chains in the hydrogel is significantly influenced by the specific conditions applied during the freezing/thawing cycles as indicated previously. This affects the stability, strength and other mechanical properties of the network. In the following sections a brief introduction to the mechanical performance of PVA hydrogels obtained by freezing/thawing cycles is presented.

According to the literature, the maximum stress and the elastic modulus of the hydrogel increases with the number of freezing/thawing cycles. On the contrary, the elongation to fracture decreases. This is a consequence of an increase in the number of entanglements between polymer chains due to the higher number of cycles which increases the rigidity of the network [44, 72, 76, 79]. The more stable and higher mechanical properties are related to the intra and inter-molecular hydrogen bonding between hydroxyl groups (-OH) of PVA and water which addresses to an increase of the crystallinity and rigidity

with the number of cycles. As a consequence, a higher number of crystallites over which the mechanical load can be distributed appears.

The maximum stress and elastic modulus of PVA hydrogels has been obtained around 6-7 cycles with almost constant properties afterwards [82]. The polymer concentration influences also the maximum stress and elastic modulus as indicates Figure 15 (b) with increasing mechanical properties of the hydrogel as the concentration raises [83].

Figure 15. Mechanical performance of a PVA hydrogel obtained by freezing/thawing cycles: (a) compressive modulus as a function of the number of freezing/thawing cycles (taken from ref.[82]); (b) ultimate tensile strength as a function of the polymer concentration (taken from ref.[83]);

Temperature affects significantly the mechanical properties of the hydrogel. The elastic modulus decreases as the temperature increases due likely to the melting of the crystallite regions. Thus the network becomes more ductile, the modulus decreases and the elongation increases. The elastic modulus of PVA hydrogels presents a wide range of values due to the differences in the experimental methods to produce the gel. Values have been found in a range between the kPa to the GPa depending on time, temperature and number of cycles implemented. Differences have been also found depending on the type of measurement (tensile, compression, indentation...). Holloway et al. [82] studied the mechanical performance hydrogels made of 10, 20, 30 and 35 % wt. of PVA in aqueous solution mixed with 1% wt. of poly(vinyl pyrrolidone) (PVP). These solutions were subjected to 21 hours of freezing at -20°C and 3 hours of thawing at room temperature for up to ten cycles. The elastic modulus under compression tests showed variations between 1.2 and 850 kPa depending on the polymer concentration and number of cycles as indicates Figure 15 (a). According to the results of Gupta et al. [83] the elastic modulus of PVA hydrogels under tensile tests displayed values between 60 and 90 MPa increasing with the concentration of the polymer as seen in Fig. 15 (b). Additionally, elastic modulus from 0.36 to 0.85 MPa were found by Wong et al. [22] at increasing concentrations between 10 and 20 % wt. of PVA in the hydrogel.

The viscoelastic behaviour of physically crosslinked PVA hydrogels has been demonstrated in previous research. Urushizaki et al. [75] studied the viscoelastic properties of 15 % wt. PVA hydrogels by Dynamic Mechanical Analysis (DMA) in a range of temperatures from 20 to 60 °C. The obtained results of the storage modulus decreased with the temperature between 20 and 3 kPa. A drop of the elastic modulus around 50 °C evidenced the glass transition of the hydrogel. The viscoelastic properties of PVA hydrogels under compressive measurements have been also demonstrated by Stammen et al. [46] by stress relaxation and compressive strain rate dependence measurements. Moreover, the viscoelastic performance of 20 % wt. PVA hydrogels obtained after 11 freezing/thawing cycles was analysed in our previous research [84]. Creep compliance measurements were performed and the viscoelastic parameters of the samples based on the SLS model were obtained. These results pointed out a value of 36 kPa for the elastic modulus of the spring E_t , a viscosity of 300 kPa and a retardation time of 23.3 which indicates the viscoelastic character of the hydrogel. A more detailed description of the viscoelastic behaviour of these hydrogels is presented in Chapter 7, Paper IV.

In summary, it can be concluded that physical crosslinking via freezing/thawing cycles is a versatile method to obtain hydrogels with a mechanical performance adjustable to the requirements of the target applications.

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Chapter 4

Paper I: *A new water absorbable mechanical epidermal skin equivalent: the combination of hydrophobic PDMS and hydrophilic PVA hydrogel*

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Abstract

Research on human skin interactions with healthcare and lifestyle products is a topic continuously attracting scientific studies over the past years. It is possible to evaluate skin mechanical properties based on human or animal experimentation, yet in addition to possible ethical issues, these samples are hard to obtain, expensive and give rise to highly variable results. Therefore, the design of a skin equivalent is essential. This paper describes the design and characterization of a new Epidermal Skin Equivalent (ESE). The material resembles the properties of epidermis and is a first approach to mimic the mechanical properties of the human skin structure, variable with the length scale.

The ESE is based on a mixture of Polydimethyl Siloxane (PDMS) and Polyvinyl Alcohol (PVA) hydrogel cross-linked with Glutaraldehyde (GA). It was chemically characterized by XPS and FTIR measurements and its cross section was observed by macroscopy and cryoSEM.

Confocal Microscope analysis on the surface of the ESE shown an arithmetic roughness (Ra) between 14-16 μm and Contact Angle (CA) values between 50-60°, both of which are close to the values of *in vivo* human skins reported in the literature. The Equilibrium Water Content (ECW) was around 33.8% and Thermo Gravimetric Analysis (TGA) confirmed the composition of the ESE samples. Moreover, the mechanical performance was determined by indentation tests and Dynamo Thermo Mechanical Analysis (DTMA) shear measurements. The indentation results were in good agreement with that of the target epidermis reported in the literature with an elastic modulus between 0.1 – 1.5 MPa and it shown dependency on the water content. According to the DTMA measurements, the ESE exhibits a viscoelastic behaviour, with a shear modulus between 1 – 2.5 MPa variable with temperature, frequency and the hydration of the samples.

4.1 Introduction

Skin is the first barrier of the body against the environment and it frequently interacts with product surfaces during daily activities. These interactions are affected by the mechanical properties of human skin. Thus, comprehensive understanding of the mechanical behaviour of the human skin, can lead to an improvement of industrial applications in which skin plays a role, like the development of healthcare or lifestyle products that interact with the human body to improve skin comfort and prevent skin damage.

Since the human skin is a layered structure with a non-linear, non-homogeneous, anisotropic and viscoelastic behaviour, its effective elastic modulus results from a combination of the effect of the applied stresses in each one of its layers. From the top to the bottom, human skin is composed of epidermis, dermis and hypodermis. Each layer has a different composition and thickness and variable mechanical response to stress. The upper layer, epidermis, has a thickness between 30 - 130 μm depending on the body site. The stratum corneum is the skin's outermost layer and it provides the skin's rigidity. It is also essential for the prevention of water evaporation and the control of skin hydration [1-3] which plays an important role in the mechanical performance of skin. The following layer, dermis, has a thickness in the range of 1 - 4 mm depending on the body location. It is a flexible connective tissue, containing collagen and elastin fibers, proteoglycans (PGs), glycosaminoglycans (GAGs) and ions, all of them involved in the equilibrium of water content in dermis [4]. De facto, PGs and GAGs can tie water molecules and swell up to 1000 times their own volume. This affects the viscoelasticity of dermis, meanwhile collagen and elastin fibers are responsible for maintaining the strength and resilience of this layer [5]. Concurrently, ions regulate the homeostasis of dermis, both responsible of the balance of water in this layer [6]. This specific composition provides viscoelasticity and resilience to the skin and is responsible for its particular mechanical behaviour. The influence of water changes in this layer results in an alteration of, at least, one order of magnitude in the Young's modulus of the skin [7, 8]. At the bottom of the skin structure is the hypodermis whose influence on the mechanical behaviour is typically neglected [9]. In addition, skin structure and properties depend on the age, body location, hydration, use of creams or moisturizers and ambient conditions. Hence, the effective elastic modulus varies also with all these factors [10-15].

Current commercially available skin equivalents are mainly used for healing skin damage, such as burns, scars or chronic ulcers. These replacements are based on soft biocompatible and non-toxic materials, and as well as, on skin autographs (skin samples from the patient), allografts (from other humans) or xenografts (from animal origin). Natural components like cotton, silk, natural collagen or hydrogels, and synthetic materials such as polyamides, polypropylenes, synthetic hyaluronic acid or

chitosan are used to develop new skin composites for biomedical applications. Alloderm (Lifecell Corporation; USA.), Integra (Integra LifeSciences Holdings Corporation; USA), Dermagraft (Organogenesis, U.S.A.), Apligraf (Novartis; Switzerland), Epicel (Genzyme; USA) or Oasis wound matrix (Smith & Nephew; UK) are some of the commercial samples developed for this target. Representative skin mechanical properties are not essential for these applications, since the materials are used as protection against bacteria from the environment or to deliver drugs that help in the healing process. Some polymers have been used as mechanical skin equivalents for tribological applications, for instance, Silicone Skin L7350 used for a FIFA test [16], Lorica used for the development of textiles [17,18] and polyurethanes applied for flap surgery [19]. These materials emulate skin in dry situations, yet interaction with the environment is not taken into account adequately. As skin hydration is one of the main factors influencing the mechanical behaviour, and thus affecting the tribological performance of human skin, it is essential to develop a new water absorbable mechanical skin equivalent.

Among the available alternatives to develop a material for mimicking skin mechanical performance, hydrogels have attracted attention for being water absorbing materials [20] with the ability to swell in water without dissolving. Hydrogels are basic materials for biomedical applications, such as bio absorbable sutures, contact lenses, drugs delivery systems, orthopaedic uses and for tissue regeneration applications [21 - 26]. However, their elastic moduli are low when compared to the mechanical properties of *in vivo* human skin, which are dependent on the combined influence of the layers of human skin. Thus, a scale-dependent model representing better this dependence in a better way is fundamental [27, 28].

Typically, the elastic modulus of hydrogels is no more than a few kPa in both shear and indentation modes. For instance, a shear modulus ranging from 2.25 to 14.70 kPa is obtained for hydrogels of Dimethylaminoethyl Methacrylate (DMAEMA) and Acrylamide (AAm) monomers, depending on the composition and the pH of the reaction [29]. Also, alginate/agarose gels are found to be within the range of 20-30 kPa depending on the polymer concentration [30]. Additionally, the elastic modulus of PVA hydrogels obtained by freezing/thawing cycles are in the range of some MPa [31, 32], depending again on the polymer concentration. However, even though hydrogels obtained by this route have an elastic modulus within the range of the target one, the fabrication process partially crystallizes them by giving up viscoelasticity. As a result, the hydrogel becomes stronger, with a higher elastic modulus, but decreasing the viscoelastic properties and limiting its use as a skin substitute [33 – 35].

On the contrary, the elastic modulus of PDMS is in the range of a few MPa [36] as required for the targeted human skin equivalent, yet the elastic modulus cannot change like the elastic modulus of the human skin with water content [37 – 39]. The micro pores in PDMS materials are able to transport oxygen through the structure. However, the material structure cannot absorb water due to its hydrophobicity which is also an essential issue for the development of a suitable skin equivalent.

Hence, to overcome the hydration problem and produce a more hydrophilic material with an effective elastic modulus within the range of a few MPa, PDMS and PVA were combined to obtain a new Epidermal Skin Equivalent (ESE). The material presented in this work aims at mimicking the viscoelasticity, effective elastic modulus, hydration capabilities and surface properties of *in vivo* human skin under dry and hydrated conditions. Furthermore, the properties of pure PDMS, pure PVA and several commercially available skin equivalents were analysed for comparison.

4.2 Materials & methods

The materials used for the synthesis of the ESE are PDMS from Dow Corning (Sylgard kit 184; USA), PVA (Mw 31,000-50,000) from Sigma Aldrich (USA), Glutaraldehyde (GA) 50% solution reagent grade from AMRESCO (USA), H₂SO₄ 96% solution in water for analysis from Acros Organics (USA), Sodium Chloride (NaCl) from Sigma Aldrich (USA). Three commercial artificial skin samples used in this study were: Lorica from Lorica (Italy), Cutinova Hidro from Smith & Nephew plc (UK) and Silicone Skin L7350 supplied by Maag Technic AG, (Switzerland). In addition, pure PDMS and pure PVA cross-linked with Glutaraldehyde 50% solution reagent from VWR International (USA) were also used for comparison.

4.2.1 Synthetic methods

The Epidermal Skin Substitute was fabricated from two types of polymers: hydrophobic PDMS and PVA hydrogel. Firstly, a solution of 20% (w/v) PVA and 2% wt. in DW water was obtained. This solution was placed in a round bottom flask with a reflux unit, stirred overnight at 90 °C, and then, coded as PVA -1 and stored. For the preparation of PDMS, a 2-component PDMS kit from Dow Corning (Sylgard 184 kit with catalyst) with a 20:1 base to curing agent ratio was used. This product was coded as PDMS – 1.

Further, PVA-1 solution was poured into a beaker and 10% wt. of an acidic solution of H₂SO₄ (aq) (pH = 1), with respect to the PVA amount, was added to initiate the cross-linking of PVA - GA. After stirring for 1 minute, the cross-linker in a ratio of 10:1 PVA to GA was added, and the blend was stirred for 2 more minutes.

Afterwards, a ratio of 7:3 PDMS to PVA-GA solution was mixed to produce the ESE samples. Consecutively, 1% wt. of NaCl salt (with respect to the total weight of the mixture), partially ground, was added to this mixture. The mixture was stirred until the formation of a jelly-white solution with a similar morphology to an emulsion, inasmuch as it is composed of 2 immiscible compounds. Next, the material was poured into a Teflon mould and heated at 45 °C for 24 hours to cure. After one day,

the samples were immersed in DW for another 24 hours at 80 °C while stirring to dissolve and remove the NaCl salt and the possible non-cross-linked traces.

4.2.2 Characterization

The characterization of the material was done under equilibrium conditions at one of the 2 specific environments: laboratory conditions (25 °C and 50% relative humidity) or hydrated conditions. Table 1 shows the conditions under which the material was characterized for each test. The equilibrium at each environment was analysed by measuring the weight mass during 10 days. However, it was observed that, 4 days after the samples were placed in a specific environment, the equilibrium was reached. Hence, to guarantee the equilibrium of the samples during the measurements, they were stabilized after 4 days in the required environment.

Table 1. Summary of the applied conditions for the samples as a function of the test. The samples were measured either at laboratory conditions of 25 °C and 50% relative humidity and/or hydrated conditions.

Test	Sample conditions for the test measurement
Scanning Electron Microscope	Hydrated conditions (after immersion 4 days in water)
Equilibrium Water Content	Immersion 10 days in water; equilibrium reached after 4 days.
Fourier transform infrared spectroscopy	Hydrated conditions (after immersion 4 days in water)
Contact Angle	Laboratory conditions
Surface Roughness	Laboratory conditions
Dynamic mechanical analysis	Hydrated conditions (after immersion 4 days in water)
Indentation tests	Laboratory and hydrated conditions

4.2.2.1 Structure & morphology of the samples

CryoSEM images of the cross section of ESE were collected after the lyophilization to ensure the structure of the material was unmodified during the measurement. Hence, previous to measurement, the samples were frozen with N₂ liq. and lyophilized to remove the content water and preserve the structure. Later, they were covered with a thin coating of gold to increase the conductivity of the samples. These images were obtained with a Scanning Electron Microscope NeoScope JCM-5000 from Nikon with a magnification range of 10x – 20000x at a voltage of 10 kV.

4.2.2.2 Equilibrium water content analysis

The water absorbent capabilities of the material were analysed and the Equilibrium Water Content (EWC) of the ESE was compared to pure PVA and PDMS. The importance of the water absorbance of the ESE is linked to the water contained at epidermis and its scale - dependence on the depth of this

layer. The stratum corneum, the outermost layer of epidermis which properties are critically variable with the water amount, shows a percentage of water content between 15 – 40% as a function of the depth for healthy skin [40].

To calculate the EWC, 6 samples of PDMS, PVA and ESE were immersed in water for 10 days and were later dried in a vacuum at 27 °C for 96 hours. The equilibrium water content of the samples was reached after 4 days of immersion and the amount of water within the bulk material was determined by using the following formula:

$$EWC = \frac{(weight_{swollen} - weight_{dry})}{weight_{dry}}$$

4.2.2.3 *Physico chemical composition*

The chemical composition of the material was determined by X-ray Photoelectron Spectroscopy (XPS) and Fourier Transform Infrared Spectroscopy (FTIR) techniques. The homogeneity and the reproducibility of 4 samples was checked by using XPS at 4 different points on the material cross section. These measurements were performed with a PHI Quantera II Scanning Microprobe from Physical Electronics with a beam of 15 keV. The FTIR measurements were carried out in absorbance mode by using a Spectrometer 100 series from Perkin Elmer (USA) with a resolution of 0.5 cm⁻¹ and a data collection within the range of 7800 - 370 cm⁻¹.

The weight loss and degradation of the samples as a function of the temperature was determined by TGA measurements. TGA curves of 2 different samples were obtained on a TGA Q5000IR from Waters-TA Instruments. The samples were placed in High Temperature Platinum-TGA pans (HT-Pt) which were heated from room temperature to 600 °C at 10 °C/min under nitrogen gas with a flow rate of 25 ml/min.

4.2.2.4 *Surface properties*

The surface of the samples was analysed with a laser confocal microscope VK 9700 from Keyence at a magnification of 10x and z-axis resolution of 1 nm. Surface images of 6 different samples of each material (commercial samples, PVA, PDMS and the ESE) were obtained and the profile roughness parameters determined. However, only the arithmetic mean (Ra) of the roughness distribution of the surfaces is presented and compared to human skin roughness from the literature. Other surface parameters with relevance in aspects related to the interface are not considered for this material characterization.

The contact angles between the samples and water drops were also calculated by using a contact angle device from Dataphysics (Germany) model OCA 20. These results were also compared to *in vivo* human skin values from the literature.

4.2.2.5 *Mechanical properties from indentation and DTMA measurements*

The mechanical performance of 4 samples of each material was analysed by indentation tests using a Micro Hardness Tester Shimadzu, DUH200 from Shimadzu Corp. (Japan) with a steel ball 2 mm in diameter for the indenter. The maximum force applied was between 0.5 – 1 N depending on the sample and the loading rate was 0.5 mN/s. A holding time of 2 seconds was established at the maximum displacement to avoid creep at the long-term response of the material and to ensure a complete elastic recovery during the unloading curve. The effective elastic modulus was calculated by fitting the data of the slope of the unloading curve in the Oliver Pharr model after a holding time of 2 s to avoid the creep.

In addition, shear measurements by 3-point bending configuration were performed by using a dynamic mechanical analyzer VA2000 150N which includes Dynatest software v6.2 from MetraviB (France). A dynamic force of 1 N was applied to 4 different samples to determine the shear at different frequencies from 0.1 Hz to 100 Hz and at a range of temperatures from -40 °C to 80 °C at steps of 20 °C.

4.3 Results & discussion

4.3.1 The synthesis of the material

The ratio PDMS/PVA was observed to be crucial for the complete mixing of both materials and the formation of a well-mixed solution of the 2 immiscible compounds with similar morphology onto an emulsion. Higher percentage of PVA (>30% w/w) resulted in a non-aggregated and extremely weak material. For instance, when using a ratio of 6:4 PDMS to PVA, the high PVA ratio leads to the formation of PDMS drops in the aqueous media, thus preventing PVA from mixing appropriately with the PDMS to form a network. Conversely, increasing the amount of PDMS leads to a material with similar properties as to pure PDMS, so neither the contact angle nor the EWC changes. The pH of the PVA solution was slightly changed by adding a 10% wt. of acidic solution of H₂SO₄ to initiate the cross-linking reaction between PVA and GA.

4.3.2 Structure and morphology of the samples

Macroscopic optical images (Figures 1 and 2) of the material were obtained under laboratory conditions (25 °C and 50% relative humidity). The structure and composition of the epidermis allows water from the environment to diffuse through specific routes from and to inner layers of the skin. Hence, it is essential that this water penetration is emulated in a skin equivalent.

Figure 1. Photographs of the commercial samples, PVA, PDMS and the ESE.

ESE samples, looked at through the macro scope, appeared as a white – porous material, which indicates the possibility of water penetration and diffusion. Indeed, the water presence was obtained via EWC measurements and the results demonstrated that ESE also provides paths for the water to go in and out from the material by mimicking this property of the skin.

Figure 2. Macroscopic images of the cross section of the ESE at a magnification of 8x showing the porosity of the sample.

Furthermore, CryoSEM images of the material at different magnifications are presented in Figure 3, which further show the presence of macro pores within the ESE structure. This random porosity results from the generation of H_2 (gas) during the cross-linking reaction of PDMS, thus the stoichiometry of the reaction determines the generated amount of porosity. For a specific amount of material, stated by the ratio of PDMS and curing agent, the amount of gas is also well defined. Besides, the resulting porosity seemed to be partially responsible for the water absorption and water diffusion, while the presence of PVA hydrogel helped in retaining water. Indeed, FTIR results, presented in Figure 5, show two peaks at 1641.9 and 3347.9 cm^{-1} , corresponding to hydroxyl groups, for both the PVA sample and the ESE material, by confirming the presence of water in the material.

Figure 3. SEM micrographs of the cross section of the ESE after lyophilization process at different magnifications: a) 27x; b) 44x; c) 220x.

4.3.3 The equilibrium water content of the samples

The water absorbent capabilities of the 6 samples of ESE were analysed and compared to those of PVA and PDMS. The results are presented in Figure 4, which shows the intermediate water absorbent capabilities of the ESE with respect to the hydrophobic PDMS and the highly water retentive hydrogel of PVA. The EWC analyses addressed to an average swelling ratio of the ESE of $33 \pm 1\%$ wt., while the value for the PVA samples was $121 \pm 8\%$ wt. and 0% wt. for the PDMS. The EWC values shown the partially hydrophilic behaviour of the ESE, since this material had a lower degree of swelling compared to PVA hydrogel, yet higher than the pure PDMS. This hydrophilicity, and hence the midway water content, is inherent to the amount of hydroxyl groups in the PVA contained in the ESE samples. The porosity of the material allows the water to penetrate and afterwards diffuse through its internal structure.

Figure 4. EWC of the PVA, PDMS and the ESE samples after equilibrium in water for 10 days.

4.3.4 The physical chemical composition

XPS analysis was carried out to identify the amounts of the main elements in the material and confirm the reproducibility of the samples. As shows Table 2, the average weight percentage of the main elements in the material was 44.5 wt. % for Carbon, 30.9 wt. % for Oxygen and 24.6 wt. % for Silicon.

Table 2. Weight percentage of the main elements present in the samples and average $\pm$ SD of the elemental composition of the material. The results correspond to different batches.

	C	O	Si
Batch 1	45.13	30.56	24.31
Batch 2	44.04	31.9	24.06
Batch 3	43.82	30.57	25.61
Batch 4	44.83	30.62	24.55
Average	44.46	30.91	24.63
SD	0.5	0.6	0.6

The values for four different batches were similar, indicating the reproducibility of the process. The chemical composition was further studied by FTIR measurements of the ESE, PDMS and PVA (Figure 5) after 10 days in water. The curve of ESE matched with the main peaks of pure PDMS curve at 788.81, 1010.55, 1258.60 and 2962.32 cm^{-1} , respectively [40]. Additionally, two peaks at 1641.91 and 3347.90 cm^{-1} , corresponding to hydroxyl groups, are present for both, the PVA samples and the ESE material [41, 42].

Figure 5. The FTIR spectra of PDMS, PVA and ESE. The values and the corresponding chemical groups of the main peaks are shown (in the space where the legend is there are artefacts).

TGA results are presented in Figure 6 and Table 3. In Fig.6 the curve of the ESE material has the same behaviour as the PVA sample which confirms the presence of the hydrogel in the ESE. Yet, the decomposition of the ESE material (450 °C) is delayed compared to that of PVA (340 °C). The amount of residue is between that of PVA and PDMS, indicating the presence of inorganic compounds at 600 °C deriving from PDMS, while the organic traces from PVA are oxidized at lower temperatures. It is also possible to elucidate the presence of 2 features related to the TGA analysis for the PVA hydrogel which are also present in the ESE: the first TGA step is most likely related to the water loss in the material when increasing temperature; and the second transition may be attributed to the breaking of the acetal bounds formed after the cross-linking of PVA – GA, and to the loss of terminal groups from the polymeric chain of the hydrogel and also the ESE. The organic decomposition started earlier for PVA ($T_i = 342, 4$; $T_f = 462, 9$ °C) than that of ESE ($T_i = 451, 9$ °C; $T_f = 525, 8$ °C) (Fig. 6, see slope of the curve) due to the oxidation of the organic compounds, in a higher amount in PVA.

Figure 6. TGA curves for PDMS, PVA and ESE after 4 days in water.

PDMS does not show the water desorbing step, supporting the results of FTIR which does not show the presence of hydroxyl groups, thus only a decrease in weight, starting between 220 °C and 600 °C is noticeable, which may be related to the rupture of the cross links between the Siloxane oligomers and the oxidation of the methyl groups within the chain.

Table 3. Summary of the decomposition steps for each sample. The table indicates the step number, the initial and final temperature, mass change of the corresponding process and the residue at 600 °C for each material.

Material	Step	T_i (°C)	T_f (°C)	Δ_m (%)	Process	Residue (%)
PDMS	1	220.0	600.0	42.5	Partial oxidation	57.0
PVA	1	Room T^a	158.3	27.9	Water evaporation	3.2
	2	342.4	462.9	68.3	Oxidation	
ESE	1	Room T^a	139.9	13.2	Water evaporation	16.7
	2	451.7	525.8	62.7	Oxidation	

The weight loss change (Δ_m) for every step is presented in Table 3, together with the initial and final temperatures (T_i, T_f) at which each process happens, for each material. The residue at 600 °C is given for every single material, and this can be correlated to the organic/inorganic ratio of compounds, which for ESE is between that of PDMS and PVA, as expected.

4.3.5 Surface properties

The results of both contact angle and roughness are presented in Table 4 and Figures 7 - 8 for several commercially available samples: PDMS, PVA and ESE.

Table 4. Average contact angles and roughness values (magnification $\times 10$) plus their respective standard deviations for the commercial samples, PDMS, PVA and the ESE. The values for human skin (HS) have been obtained from the literature.

Sample name	Contact angle ($^{\circ}$)	R_a (μm)
Lorica	109 ± 6	18 ± 5
Silicone	111 ± 5	0.55 ± 0.2
Cutinova	15 ± 5	5.4 ± 0.2
PVA	81 ± 4	0.9 ± 0.6
PDMS	90 ± 5	2 ± 1
ESE	$50-60$	13 ± 2

The contact angle was evaluated for water droplets due to the important role of water in the mechanical behaviour of human skin and its role in the lubrication of tribological systems as the cartilage [43, 44]. The measured contact angle of ESE was $53 \pm 6^{\circ}$, comparable to the results found for human skin from the literature, i.e. between 50° and 60° [45 - 48].

Figure 7. Summary of the contact angles for ESE, the commercial samples and the precursors compared to human skin.

Furthermore, the arithmetic roughness (R_a) of the ESE was $13 \pm 2 \mu\text{m}$, showing more resemblance to human skin results than other studies [49 – 52]. Therefore, contact angle and roughness were not influenced by the porosity of the material, since this porosity appeared mainly internally and not on the surface.

Figure 8. Summary of the roughness (R_a) values for ESE, the commercial samples and the precursors compared to human skin.

3.6 Mechanical properties from indentation and DTMA measurements

The viscoelastic performance of the material was analysed by DTMA measurements using a 3-point bending mode. The samples were clamped and measured under laboratory conditions and the shear modulus was analysed within a temperature slope ranging from - 40 to 120 °C with intervals of 20 °C. The results indicated that the shear modulus increased when the temperature decreased as a consequence of the formation of water crystals and the glass transition effect (T_g), with reduction of the energy chains and movements within the network. Figure 9 shows the shear storage and loss modulus as a function of the temperature for a range of frequencies between 0.1 and 100 Hz. The graph elucidates the changes in the mechanical properties of ESE with the time (demonstrated by frequency changes), the effect of the water reinforcement (demonstrated by the high elastic modulus at very low temperatures) and a plateau zone between -25 °C and 100 °C. Starting at 100 °C, a slight drop is observed in both, the storage and the loss modulus, is observed. This is due most likely to the thermodynamic changes at high temperatures, which can lead to the breaking of cross-linking points. However, the applications of the material are located in a range of conditions between 10 and 30 °C,

and never lower than 0° C or higher than 40 °C; thus, the possible changes of the material at higher temperatures can be neglected.

Figure 9. Storage shear modulus (G') and loss shear modulus (G'') of ESE (dots) at a range of different temperatures between -80 °C and 120 °C and frequencies between 0.1 and 10 Hz indicated by different colours.

Additionally, Figure 10 summarizes the changes of the storage shear modulus within the range from -40 to 80 °C and frequencies ranging from 0.1 to 100 Hz. The graph shows the decrease in the storage elastic modulus of PDMS with respect to the ESE at low temperatures. At -40 °C water crystal reinforcement is present in the structure of ESE, but not in PDMS. When the temperature increases, the melting of water crystals reverts this effect and ESE becomes more viscoelastic with a lower shear modulus than PDMS.

Figure 10. Storage shear modulus (G') of pure PDMS (lines) and the ESE (dots) for the ranges 0.1-1, 1-10 and 10-100 Hz at different temperatures.

As regards the frequency effect, at low temperatures the shear modulus of ESE and PDMS increased with frequency increasing as a result of the hysteresis effect, which is typical of viscoelastic materials. This hysteresis is also revealed in the Figure 11, which as an example of an indentation test of ESE shows the different lines for the loading versus unloading curve.

Figure 11. Indentation test curve from an ESE sample. Holding time of 2 seconds to ensure the elasticity of the samples is indicated. The effective elastic modulus is obtained as a derivative of the force respect to the displacement at the 20% of the data points during the unloading part of the curve.

The area between the 2 curves represents the storage energy during the stress application, and is related to the conformational changes in the polymer chains and the restructuration of the internal architecture of the material after stresses.

The mechanical properties of human skin vary over a wide range due to the dependency on factors such as: test method, age, hydration or the position on the body. It may be the reason why the results from Figure 10, with ESE in the order of MPa, did not agree with experimental data from shear measurements of *in vivo* human skin taken from the literature. For instance, Holt et al. [53] carried out rheological measurements on human skin samples composed of epidermis and dermis, and samples in which epidermis was removed. They indicate a shear storage modulus in the range of a few kPa in both cases. In contrast, Sanders [54] performed torsional measurements on *in vivo* forearm human skin of individuals ranging from 6 to 61 years of age. These results are in the range of 20 – 100 MPa, much higher than the previous ones, and shown a tendency to decrease according to age. Finally, DMA shear tests as a function of the temperature were also performed in pig skin to evaluate changes in skin viscoelastic properties [55]. These results, however, cannot be considered as suitable data resource since they are not coming from human skin, state changes in E' due to the release of water, supporting the importance of water in skin viscoelasticity. In addition, the results of the storage

shear modulus of pig back skin show a similar value (around 0.5 MPa) to the results presented in Figure 10 within a range of temperatures between 30 and 45 °C.

Figure 12. Effective elastic modulus of the commercial samples, PDMS, PVA and the ESE obtained from indentation tests at ambient and hydrated conditions (RH = 50%; $T_a = 25$ °C). The shaded surface indicates the range of values for the effective elastic moduli considered in this case based on literature results (see table 5).

On the contrary, the elasticity of the human skin can be measured by indentation tests [27, 56 -59]. Besides the simplicity of the measurement, the effective elastic modulus (E_{eff}) obtained from the indentation tests describes the mechanical properties of skin as a function of the indentation depth. The effective elastic modulus was obtained after applying a force between 0.5 – 1 N at a speed of 0.5 mN/s. At the maximum displacement the force was kept for 2 seconds to ensure that the sample was not affected by the creep, but remaining in the elastic regime. The elastic modulus was calculated by fitting the 20% of the data points from the unloading curve in the Oliver Pharr model adapted for a spherical indenter (Fig. 11) for the commercial samples, the precursors and the ESE. The measurements were carried out under ambient and wet conditions after swelling the samples for 4 days in water. These results are depicted in Figure 12 and the red square indicates the targeted range for the effective elastic modulus [27, 56 – 59] obtained from the references attached to the table 5.

Table 5. Collection of effective elastic moduli values from the literature. The results are in the range of the expected mechanical properties for an epidermal substitute.

Authors	Conditions	Elastic modulus
Geerligs M. [28]	Sapphire sphere indenter 22 °C, 28 % RH Max load = 1 nN Speed = 0.01 nN/s Isolated human skin	1.1 ± 2 MPa
Pailler-Mattei C, Bec S, Zahouani H. [54]	Conical steel indenter 22 – 24 C, 20 – 30% RH Speed = 400 um/s Inner forearm	12.5 kPa
Jachowicz J, McMullen R, Pretty paul D. [58]	Stainless steel spherical indenter 30 – 50% RH Load = 5 – 10 G force Speed = 0.5 mm/s Forearm	7 kPa (forearm) 33 kPa (facial skin)
Zahouani H, Pailler-Mattei C, Sohm B, Vargiolu R, Cenizo V, Debret R. [59]	Steel spherical indenter 22 C, 50% RH Load = 20 mN	8.3 ± 2.1 KPa

The values presented for ESE in Figure 12 indicate a correlation with results from Figure 13 which shows the evolution of skin effective elastic modulus as a function of the length scale [27]. The latest reference includes a description of the mechanical performance of skin at the micro, meso and macro levels, depending on the length scale of the contact. Besides, a compilation of indentation results from *in vivo* and isolated skin as a function of the length scale is presented, and an approximation of the E_{ef} , based on a model which includes the influence of the properties of each layer of skin, is derived [27, 59]. Considering the conditions of the performed indentation tests, it is possible to point out that the results of 0.7 and 0.5 MPa obtained under laboratory and hydrated conditions, respectively, are in good agreement with the results at the meso scale shown by Van Kuilenburg [27,60], and they are indeed, related to the properties of viable epidermis.

In contrast, Figure 12 shows that the elastic moduli of the commercial samples were too high which, together with their inability to interact with water, makes them inappropriate materials. PDMS is close to the targeted range, with an elastic modulus of 2 MPa, but it lacks the water absorption capabilities and its surface properties are not similar to those of the human skin either. Hence, the developed ESE material is the best option for simulating the mechanical properties of human skin in both hydrated and wet conditions within the selected set.

Figure 13. Effective elastic modulus as a function of the length scale adapted from reference [27]. The graph shows the 3 scale-dependent levels of mechanical properties of human skin and the obtained value for ESE fitting with these results.

4. Conclusions

This study describes the procedure for the fabrication of a new Epidermal Skin Equivalent (ESE) composed of hydrophobic PDMS and a hydrogel of PVA as well as its chemical and mechanical characterization. This material is designed for testing applications in a range of temperatures between 10 and 30 °C.

With regard to the fabrication process, reproducibility of the samples is confirmed by XPS and macro and cryoSEM micrographs.

ESE presents a contact angle of $53 \pm 6^\circ$ and an arithmetic roughness of $13 \pm 2 \mu\text{m}$, both close to values of human skin data from the literature. Besides, the material also shows water absorbent capabilities with a water percentage of 33.8%.

The viscoelasticity is demonstrated by DTMA measurements, which also prove the shear storage modulus is in the range of the required values (0.1 – 1.5 MPa). Moreover, the effective elastic modulus of ESE appears also to be in the order of a few MPa, showing different values depending on the conditions: 0.7 MPa and 0.5 MPa under laboratory or fully hydrated conditions, respectively as expected from a soft material simulating epidermis.

Future experimental work is foreseen on developing a multi-layer model able to simulate the scale - dependent mechanical behaviour of human skin both at the micro and macro scale. To this end, other layers of human skin structure need to be incorporated into the Epidermal Skin Equivalent to address the combined mechanical performance of skin at the micro, meso and macro level.

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Chapter 5

Paper II: *Tribological behaviour of skin equivalents and ex-vivo human skin against the material components of artificial turf in sliding contact*

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Abstract

This research aims to analyse the interaction of three artificial skin equivalents and human skin against the main material components of artificial turf. The tribological performance of Lorica, Silicone Skin L7350 and a recently developed Epidermal Skin Equivalent (ESE) were studied and compared to *ex-vivo* human skin samples. The surface of the *ex-vivo* skin was analysed via confocal microscopy and histology. The results indicated a similar performance of the Lorica and ESE samples when compared to the *ex-vivo* skin whereas the Silicone Skin L7350 showed significant differences. The results suggested also a key role of the intrinsic properties of the NBR material to the friction against human skin, compared to PA 6.6 or PE surfaces in a similar sliding contact.

5.1 Introduction

The use of artificial grass for sports pitches has clear ecological and economic benefits [1-3] over the use of natural grass, in countries with an extreme hot or cold climate, or for places with limited rainfall. Moreover, it can be used intensively for almost 24 hours a day and seven days per week [4]. The quality of the turf and the comfort during the game, essential aspects from the users point of view, are currently secured by the FIFA® Quality Concept for Football Turf [4] requirements, which are based on a series of laboratory tests that the manufacturers of artificial grass must perform before the turf can be applied to football pitches. The tests are among other aspects developed to standardize the quality of the turf and guarantee the safety of football players during the interaction with the grass. The turf is tested in terms of durability, climatic resistance and player-to-surface interaction [1, 3, 4]. The latter evaluation involves the assessment of the tribological behaviour of a skin equivalent material in contact with artificial turf, with the aim to examine the risk of skin abrasion and high skin friction e.g. for a sliding tackle. The need to assess this behaviour is clear from the documented incidences of skin injuries and player's discomfort in relation to player's interaction with artificial turf [5-12]. Silicone Skin L7350 is currently used as artificial skin equivalent in the FIFA test [4], most likely based on the overall mechanical properties and availability of the silicone rubber material. As such, new artificial skin equivalents have been developed and used in explorative tribological studies, focussed on simulating the proper frictional performance depending on parameters including age, anatomical region or hydration of the skin [13]. Limitations exists in simulating the tribo-mechanical performance of skin properly over the full range of conditions [13-16]. The recently developed skin equivalent by Nachmann and Franklin [17] shows a good agreement in dry and moist conditions to human skin, but the model needs to be analysed in a broader range of conditions. Currently, commercial available samples are used as skin equivalent for different applications, including soft biocompatible materials [18-20]. Stiffer samples such as Lorica have been used as a skin equivalent to study the interaction against textiles and non-woven fabrics [21-24]. The counter surface, artificial turf, is typically composed of a layer of sand infill to ensure the fibres stand vertical with a top surface of granulated Acrylonitrile-Butadiene rubber (NBR) and Styrene-Butadiene rubber (SBR) to enhance the impact absorbency of the turf. This turf has also longer fibres compared to the previous generations (> 55 mm) and they are usually made of Polyethylene (PE) instead of Polyamide 6.6 (PA 6.6). From this it is clear that there is not just a single artificial turf surface which can be used for pin-on-disk tests for example, but a series of possible surfaces as a function of time and operational conditions. Yet, in all cases there will be an interaction of the skin with fibres and with the infill.

The aim of this work is limited to measuring and understanding the relative contributions to sliding friction of the infill material and of the fibre material separately, with respect to the currently used skin equivalents, a newly developed skin equivalent material and in comparison with *ex vivo* skin. As such, the interactions of three mechanical skin equivalents and excised human skin in contact with the material components of the artificial turf was investigated by pin-on-disk experiments. The specific role of each turf component during the sliding contact was evaluated in terms of the adhesive and the elastic properties of the involved materials. NBR as the main constituent of the infill crumb of artificial turf fields, and PE and PA 6.6, as main component of the fibres of the artificial grass, were used as counter materials. Such a comparison has not been made before and the results could contribute to enhanced turf design [21, 22, 25-28], as data from this work can serve as friction data for a Finite Element based analysis of player – turf interactions during sliding tackles. Surface changes of the *ex vivo* skin samples were analysed by histology and confocal microscope measurements. Additionally, an analysis of the elasticity and the frictional performance of the skin was made based on the two-term friction model and based on the evolution of the elastic modulus with the length scale [29, 30].

5.2 Materials & methods

Three skin equivalents and *ex vivo* human skin samples were studied. The synthetic materials were cut in circular pieces with a diameter of 30 mm and a thickness of 2 mm. Two were commercially available, i.e. Lorica[®], a synthetic leather made of polyamide fleeces and polyurethane applied in the development of textiles (Italy); and Silicone Skin L7350 a silicon-based organic polymer patented by Maag Technic AG, (Switzerland) and utilized for the FIFA test. In addition, a recently developed skin equivalent [19] composed of a mixture of PDMS and PVA hydrogel crosslinked with glutaraldehyde and referred to as Epidermal Skin Equivalent (ESE), was used. Figure 1 shows the artificial skin materials together with their elastic moduli [19]. Temperature and humidity considerably affect the tribo-mechanical performance of the skin, especially its elastic modulus and adhesive properties of the system and, subsequently, the friction coefficient [31- 33]. Hence, the friction behaviour of the synthetic materials was investigated at both standard (25 °C and 50% relative humidity) and extreme (37 °C and 80% relative humidity) climate conditions.

Furthermore, ten *ex vivo* human skin samples from surgical proceedings belonging to the abdomen of one overweight anonymous person were evaluated at standard conditions.

Figure 1. Images of the samples and their elastic modulus obtained from indentation tests [from ref. 28]

No measurements were performed at extreme conditions with the skin samples because once excised from the body they do not react to humidity or temperature changes in the same way as human skin does. The excised samples were donated by the Radboud Hospital of Nijmegen with the consent of the patient, the agreements of the Ethical Committee of the Hospital and the approval of the University of Twente. The use of abdomen skin introduces a simplification as it differs partially from the skin of the leg due to roughness differences and the presence of hair in the latter case. In this case, differences due to the roughness between the skin of the leg and the abdomen are not expected due to the high normal forces applied. It might be that the presence of hair lowers the coefficient of friction however, this is still unclear and the possible differences fall within the likely scatter in performance that exists intra and inter-individual [13, 34].

Figure 2. Schematic illustration of the tribological system.

5.2.1 Tribological measurements

The tribological measurements were performed on a HC 4057 pin-on-disk machine (CSM, Switzerland) placed into a climate chamber with temperature and humidity control. *Ex vivo* skin and artificial skin equivalent samples of 30 mm diameter and 2 mm thickness were prepared and evaluated. The friction measurements were conducted at a distance of 10 mm from the centre of the disk samples, see Fig. 2, at standard and extreme climate conditions. Cylindrical pins of 10 mm, made of NBR, PE, PA 6.6 with a spherical tip of 15 mm radius were used as a counter surface. Figure 3 shows a 3D image of one of the pins, which were all machined in the same way, and the specifications of the surface roughness, similar for each pin and defined by R_a , R_q , R_{pv} and R_{sk} . Normal loads of 2 N and 4 N were applied during the friction tests to ensure similar pressures at the contact to those originated at the FIFA test friction method [4], assuming Hertzian contact conditions. The tests were performed at a velocity of 50 mm/s to avoid the influence of heating.

Figure 3. Detail of the pin: (a) shape and radius; (b) 3D surface of the pin obtained by confocal measurements and indication of the surface roughness.

The skin equivalent samples were placed on a rotary holder and fixated with double-sided tape whereas the human skin samples were mounted on special stainless steel holders (AISI 316) and clamped at cork pieces with pin sticks as shown in Fig. 4 (a). Figure 4 (b) shows the pin-on-disk tester with human skin sample on the holder during one for the measurements.

After the tribological measurements, the skin samples were taken to the Dermatology Department of Radboud University Medical Center Nijmegen, where the histological procedures were performed.

Figure 4. Images of the ex vivo human skin sample tests: a) indicates the way the samples were clamped with stick pins and cork pieces in the special stainless steel holder with indication of the test track; b) illustrates the pin-on-disk tester with a sample to be tested.

5.2.2 Confocal microscope

The surface roughness of the *ex vivo* human skin samples was analysed before and after the tests with a laser confocal microscope VK 9700 from Keyence at a magnification of 10x and z-axis resolution of 1 nm. Although other roughness parameters were also obtained, the peak-to-valley roughness value (R_{pv}) was used to compare and elucidate the possible changes on the human skin surface after the tribological tests. After the friction measurements, the samples were taken to the confocal microscope and the roughness was measured at a distance of 10 mm from the centre of the sample which corresponds to the track of the measurement as indicated in Fig. 4 (a). The peak-to-valley roughness of the excised skin samples was evaluated before and after the tests to examine possible changes on the surface that they were maybe not visible in the histological images.

5.2.3 Histological procedure

In the medical field, histology is used to study the cellular organization of body tissues and organs under a microscope. A small tissue sample is obtained and thereafter chemically treated to ensure the preservation of the cellular structure. Then, the samples are cut with a microtome and stained with hematoxylin-eosin (HE), to colour different cellular structures. The hematoxylin stains structures such as cellular nucleus into a blue-violet colour, whereas eosin turn basic compounds like the cytoplasm into a pink colour. Hence, changes at the cellular level can be visualized when comparing a control tissue with the target tissue.

In this research, after measuring the roughness of the tested skin samples, small pieces of skin, from the track where the friction was measured, were taken for histological analysis. The samples were cut with a biopsy punch of 8 mm diameter. These biopsies were embedded in paraffin after 4 h fixation in

4% formalin and a chemical process. Paraffin sections (6 μm) were processed side-by-side and dewaxed with histosafe (Adamas, Rhenen, The Netherlands) followed by rehydration in decreasing concentrations of alcohol (100-50%) and demineralised water. The sections were hematoxylin-eosin (HE) stained for assessment of histopathological features. After dehydration with 100% ethanol and histosafe they were mounted on a glass with Permount (BDH chemical, Poole, England) [35].

Finally, the sections were photographed at a magnification of 20x using a microscope (Axioskop2 MOT; Zeiss, Jena, Germany), digital camera (AxioCam MRC5; Zeiss) and Axio-Vision software (Zeiss).

5.3 Results

5.3.1 Tribological performance of artificial skin samples and *ex vivo* human skin

The tribological results are represented by the average friction of 5 samples of each material. Figure 5 displays the friction coefficient of a Lorica sample against a NBR pin at standard conditions for a sequence of 5 laps. It is possible to distinguish the point where the static friction approaches the maximum value and, afterwards a decrease of friction when the pin begins to move. At that point the sliding friction or dynamic friction starts.

Figure 5. Coefficient of friction during 5 consecutive laps for a Lorica specimen against a PE pin and at 2 N force of applied force.

A comparison of the dynamic friction coefficient of the skin equivalents and the *ex vivo* skin is presented in Figures 6 and 7 at standard conditions for the applied forces of 2 and 4 N, respectively.

Figure 6. Friction coefficient of different artificial skin equivalents at standard conditions of 25°C and 50 % relative humidity at a force of 2 N.

Figure 7. Friction coefficient of different artificial skin equivalents at standard conditions of 25°C and 50% relative humidity at a force of 4 N.

The surfaces of the artificial and the excised samples were cleaned with a paper wet with demineralised water before the measurement. Later, the samples were dried at room temperature for 40 s to avoid a water lubrication film on top of the samples. The values of friction were calculated as the dynamic friction average of 5 different measurements for each kind of material. Moreover, Figure 8 summarises the friction coefficient of the skin equivalents at the extreme conditions of 37 °C and 80% of relative humidity for the 2 and 4 N of applied force.

Figure 8. Friction coefficient comparison of different artificial skin equivalents at intense conditions of 37°C and 80% of relative humidity at forces of 2 and 4 N.

During the tribological tests, transfer of skin surface material was observed for the *ex vivo* skin samples in contact with all the tested pins: NBR, PE and PA 6.6. Figure 9 presents the surface of the PE pin after a friction test in which sebum and other surface components of the skin surface accumulated at the surface of the pin.

Figure 9. PE pin after a friction test on *ex vivo* human skin; a white mixture of components is observed on top of the pin (red circle).

5.3.2 Histological and surface roughness results

Histological and surface roughness analysis were performed to detect modifications on the surface of the skin samples as a result of the sliding. Ten human skin samples collected and analysed in groups of 3 for each pin. The results of the friction coefficient, confocal microscope and the histology were obtained from the repetition of 3 different measurements with the skin samples for each pin. Figure

10 gives an overview of the histological images comparing the condition of the skin before and after the tests. Abrasive damage is related to surface modifications of the skin no deeper than the epidermis. Therefore, the analysis was focused mainly on the upper layers such as the stratum corneum. Figure 10 (a) shows an example of SC removal after sliding tackle on artificial turf, adapted from ref [35]. The image was used to compare the results obtained in this study with the damage that occurs during sliding on artificial grass. Figure 10 (b) presents a histological cross section of an as-received human skin sample whereas, Figures 10 (c), (d) and (e) show the samples after testing against NBR, PE and PA 6.6, respectively.

Before the histology, a series of roughness measurements were performed. Peak-to-valley roughness of the skin samples was measured before and after the tribological tests, and the results are shown in Fig. 11 with Fig.12.

Figure 10. Histological images of human skin samples: (a) skin after artificial turf testing; (b) control sample; (c), (d), (e) tested against NBR, PE and PA, respectively.

Figure 11. Peak-valley roughness of the human skin samples after the tests performed against NBR, PE and PA and comparison to a control human skin sample.

Figure 12. Overview of the surface morphology of the ex vivo samples: (a) skin control; (b) skin tested against NBR; (c) skin tested against PE; (d) skin tested against PA.

5.4 Discussion

5.4.1 Explaining friction from the “Two-term friction model”

The contact situation that exists during the sliding contact is that of a spherical probe sliding against the skin which can be described well with the two-term friction mode, see e.g. the works of Zahouani and van Kuilenburg [26, 29, 30, 32]. The two-term friction model considers two non-interacting friction terms, i.e. the adhesion term ($F_{\mu,adh}$) and the deformation term ($F_{\mu,def}$), see Eqs. 1, 2, 3.

$$F_{\mu} = F_{\mu,adh} + F_{\mu,def} \quad (1)$$

The deformation term of friction involves the energy loss by viscoelastic hysteresis. It is proportional to the normal force, the viscoelastic loss fraction, β , and to the ratio between the contact radius, a , and the radius of the spherical indenter, R :

$$F_{\mu,def} = \frac{3}{16} \beta \frac{a}{R} F_N \quad (2)$$

The adhesion component of friction also depends on the normal load as it is a function of the true or real area of contact A_{real} , and the interfacial shear strength, τ :

$$F_{\mu,adh} = \tau A_{real} \quad (3)$$

In the case of skin friction, the effect of the normal force is typically described by a power law relationship of the friction force and the normal force (Eq. 4) with values of the α exponent between 0.67 and 1.1 [30].

$$F_{\mu} = F_{\mu,adh} + F_{\mu,def} \approx k F_N^{\alpha} \quad (4)$$

The value of the α exponent depends on the interaction between the probe and the skin, and the relative contribution of the adhesion and the deformation component to the friction force. This exponent is related to the properties of skin, such as to its elastic modulus, E_{eff} . The evaluation of the E_{eff} requires to consider the elastic moduli of the different skin layers. The mechanical properties of the skin have been described [29, 30] as a combination of the properties of the skin layers as indicated in Eq. 5:

$$\frac{1}{E_{eff}} = 2 \sum_{i=1}^{n-1} \frac{t_i}{f_i(a) \pi a E_i} + \frac{1}{f_n(a) E_n} \quad (5)$$

t_i , E_i , the thickness and the elastic modulus of each individual layer, respectively as indicated in Table 1; whereas f_n and E_n refer to the properties of the underlying tissue.

The functions f_i and f_n ensure correct boundary conditions considering the thicknesses of the layers as described in Equations (6), (7) and (8). The subscript n indicates the total number of layers, in this case three plus the underlying tissue; so that, n is equal to 4 and, subsequently, i takes the values 1, 2 and 3.

$$f_1(a) = 1 + \frac{2t_1}{\pi a} \quad (6)$$

$$f_i(a) = \left(1 + \frac{2}{\pi a} \sum_{j=1}^{i-1} t_j\right) * \left(1 + \frac{2}{\pi a} \sum_{j=1}^i t_j\right) \quad (7)$$

$$f_n(a) = \left(1 + \frac{2}{\pi a} \sum_{j=1}^{n-1} t_j\right) \quad (8)$$

As such, the changes of the E_{eff} with the force were analysed in accordance with these equations.

Table 1. Parameters used for calculating the effective elastic modulus of the skin.

i	Skin layer	Elastic modulus, E_i (MPa)	Thickness, t_i (μm)
1	Stratum Corneum	500	20
2	Viable Epidermis	1.5	80
3	Dermis	2×10^{-3}	1000

For the current case, the mechanical properties of the first 3 layers of skin and their thicknesses indicated in Table 1 were taken as input parameters. To start the iteration process, a constant value of 2.5 MPa for the elastic modulus of the skin was introduced in Eq. 9 to determine the E_{eff} . Therefore, an initial contact radius was calculated to start the iterations by using Equation 10.

$$\frac{1}{E_{eff}^*} = \left(\frac{1 - \nu_c^2}{E_c}\right) + \left(\frac{1 - \nu_{skin}^2}{E_{eff}}\right) \quad (9)$$

$$a = \sqrt[3]{\frac{3}{4} \frac{RF}{E_{eff}^*}} \quad (10)$$

ν_{skin} , ν_c and E_{eff} , E_c are the Poisson ratios of the skin and the counter surface, and their elastic moduli, respectively. An overview of these values for the different pins is presented in Table 2 [36-39]. For the skin a Poisson's ratio of 0.5 was considered based on previous research [40].

Table 2. Overview of the elastic modulus and Poisson's ratio of the NBR, PE and PA 6.6 pins.

Material pin	Elastic modulus (MPa)	Poisson's ratio
NBR	2	0.4
PE	1000	0.45
PA 6.6	2000	0.41

The initial contact radius, a , was implemented in an algorithm to determine the E_{eff} as a function of the applied force, as given in Equation 9 and 10. The imposed condition to end the iteration was a minimized sum of the square differences of the calculated $E_{eff,m}$ and $E_{eff,m-1}$ with a minimum error of 10^{-6} . The results presented in Fig. 13 show an E_{eff} in the range of few MPa as a function of the contact radii (dashed line) or the indentation depth (drawn line) for an indenter radius of 15 mm.

Figure 13. Effective elastic modulus of the skin E_{eff} as a function of the length scale: contact radii (drawn line) and indentation depth (dashed line).

Table 3 collects the E_{eff}^* for the artificial skin samples and the skin theoretical model against each counter surface: NBR, PE and PA 6.6. The values of the effective elastic modulus, E_{eff}^* , were calculated based on Equation 9 for the Lorica, ESE and L7350 samples. For the human skin theoretical model Equation 5 was used to consider its length scale dependence. The first 3 rows of Table 3 present the E_{eff}^* of the artificial skin equivalents whereas the last row shows the results for the skin model in a

range of values for the effective elastic modulus. Specifically, the E_{eff}^* for the skin was ranging from 0.35 MPa to 68 kPa when contacting NBR surfaces. The results regarding to the contact with PE and PA 6.6 gave a range of E_{eff}^* from 0.4 MPa to 67 kPa as indicated in Table 3.

Table 3. Calculated effective elastic modulus for each tested sample. The values for the skin depended on the applied force and they were obtained from Eq.6.

Effective elastic modulus (MPa)	NBR	PE	PA 6.6
Lorica	3.4	9.5	9.5
ESE	0.9	1.1	1.1
L7350	3.1	7.5	7.5
$E_{eff}^* = f(a)$	[0.035 – 0.068]	[0.04 – 0.067]	[0.04 – 0.067]

The evolution of the E_{eff}^* as a function of the contact radii was introduced in the Two term friction model to evaluate the role of the deformation and adhesive component of friction in such a structure (Equations 2 and 3). The parameter β in Equation 2 represents the energy dissipated due to viscoelastic effects during sliding friction and is a function of the $\tan(\delta)$ obtained via DMA measurements. In this model a value of 0.26 for β was set based on references from the literature [41]. The interfacial shear strength, τ , was taken based on the results from the literature [31, 42] and, for the model, a value of 5 kPa was used. The results, presented in Fig. 14, indicated a dominant role of the adhesive component at low forces.

Figure 14. Comparison of the friction forces due to adhesion (dashed line) and deformation (dot line) with respect to the total friction force (black line) for an indenter with a radius of 15 mm made of NBR.

With increasing force, the deformation component increased its influence with a contribution of 5% at 4 N. Then, the total friction force has an influence summation of the two components. At forces of 2 and 4 N, both the deformation and the adhesive component define the total frictional force with a relative contribution of each that depends on the contacting material's properties, the contact situation and the boundary conditions.

Based on the Hertzian contact model, the contact radius, a , and the effective elastic modulus, E_{eff}^* , considering a theoretical 3 layer model of skin (input parameters Table 1) were evaluated and the results were correlated to the measured friction coefficient of the *ex vivo* skin samples and the results arising from the “two-term friction model” as indicated in Figure 15. The changes in the E_{eff}^* of the skin model as a function of the applied load for the 3 counter surfaces, NBR, PE and PA 6.6 are presented in Figure 15 (a).

Figure 15. Results of the effective elastic modulus (a) and contact radius (b) based on the theoretical skin model; Plot (c) presents a comparison of the experimental friction coefficient in *ex vivo* human skin samples and the COF obtained from the theoretical model at forces of 2 and 4 N.

The results indicate that the higher the applied load, the lower the E_{eff}^* will be. Since the E_{eff}^* is considerably smaller than NBR, PE and PA 6.6 elastic moduli, the contact radius did not depend on the elastic properties of the pins and, at 2 and 4 N, it remained practically constant for the three cases as is shown in Figure 15 (b). In Figure 15 (c) the experimental results of the COF for the 3 pins are plotted next to the theoretical COF expected from the “Two term friction model” at forces of 2 and 4 N. The fitting parameter in the model was the interfacial shear strength, τ , which was introduced in the model with a value of 5 kPa for the three cases. As seen in the Figure 15 (c), the Hertzian assumptions and the friction model predict a decrease of the COF with the increase of force which is opposed to the experimental results. Thus, the changes in the COF of these tribological systems cannot be based on variations on the contact radii at a certain load. Consequently, two conclusions can be extracted from these results: on the one hand, the differences in the COF observed for each pin

at a certain force can only be justified as a result of differences in the interfacial shear strength between the skin and the counter-surface; on the other hand, the differences from 2 to 4 N can be explained as a consequence of a higher influence of the deformation of the skin or due to a modification of the shear at higher forces.

The affinity of the pin materials for the skin surface compounds influences the specific place where the shearing between the skin and the pin occurs. Therefore, if the contact radio at 2 and 4 N is considered constant with values of 9 and 12 mm, respectively, for the three pins, the only fitting parameter in the model would be the interfacial shear strength. Thus, the variations in the COF from 2 to 4 N for a same pin could be due to modifications in the shear strength which would raise the friction force. It might be that the interfacial shear strength is modified when the SSLF is removed so that, the higher COF is a consequence of the modifications of the surface condition of the skin. When the skin is contacting NBR likely a higher amount of substances from the SSLF would be removed due to its greater attraction for lipid-like components. This conclusion supported by the histological and confocal measurements which show higher modifications on the skin surface after testing against NBR. These modifications address to higher friction likely due to the affinity of elastomers for oils [43]. In conclusion, the results point a likely load dependence of the shear strength due to a surface modification of the skin condition at these loads. To evidence the latest, the changes in the interfacial shear strength were calculated back from the model by fitting the experimental friction coefficients for the 3 systems at forces of 2 and 4 N. These results are collected in Table 4.

Table 4. *Interfacial shear strength calculated by fitting the experimental results obtained for each tribological system.*

		$F = 2N$	$F = 4N$
$\tau_{NBR - skin}$	(kPa)	2.6	4.2
$\tau_{PE - skin}$	(kPa)	1.8	2.1
$\tau_{PA\ 6.6 - skin}$	(kPa)	1.6	3.1

5.4.2 Analysis of the tribological system

With regard to the experimental results, the friction coefficient at standard conditions indicated a better agreement of the ESE and Lorica samples compared to the human skin results at both force of 2 and 4 N (see Fig. 6 and Fig. 7). However, Silicone Skin L7350, used currently in the FIFA test method, presented a limited applicability for the friction and abrasion test of artificial turfs, because its tribological performance at extreme conditions was not the expected to simulate the one of the human skin. The tribological performance of the human skin is substantially affected by the presence of water due to the absorption capabilities of the stratum corneum [13, 27, 44]. As a consequence, the

outermost the outermost layer of the skin softens in the presence of water by decreasing its elastic modulus. Moreover, the interfacial shear strength is also affected due to this “new” condition of the system. Hence, the decrease of the elastic modulus and the changes on the shear strength conduct to an increase of the area of contact and, consequently to an increase of the friction coefficient as it arises from Equations 2, 3 and 10. Due to the hydrophobic and non-water absorbent character of the Silicone Skin L7350, the tribological performance of this samples cannot properly represent the human skin results in the presence of water or high humidity. Conversely, Lorica and ESE samples showed a friction coefficient between 0.35 – 0.8 depending on the normal force, environmental conditions and applied counter-surface, which is in line with the experimental results of the *ex vivo* human skin and other human skin results from the literature [13,15, 16, 24, 30, 31, 34, 45]. Figure 8 presents the friction coefficient of the tested skin equivalents at high temperature and humidity conditions at forces of 2 and 4 N. At those conditions, the friction coefficient of Lorica and ESE increased in parallel with the force for the 3 tested counter-surfaces whereas Silicone Skin L7350 showed the opposite performance with a decrease of friction with increasing load. Only in the case of L7350 samples tested against NBR at extreme conditions an increase of friction appeared when the normal force was increased from 2 to 4 N. However, if the friction values at 2 N for the dry and the wet conditions are compared, it is clear that there is not actually an increase of friction but, the COF decreases from 1.6 to 0.4 at normal and extreme conditions, respectively. The same happens for the applied force of 4 N. The evident increase of friction for the L7350 against NBR from 0.4 to 1.2 for 2 and 4 N, respectively is likely a consequence of the different shearing regime at these 2 cases. So that, even though the moisture lubricating effect is the same for the 2 cases (because the environmental conditions are the same), the shear happens at the different level between the contact “skin – counter surface”. At 2 N the shear possibly occurs at the interfacial layer due to water condensation between the 2 materials whereas, at 4 N the shear might happen already at the contact between the 2 materials without no effect of water lubrication. This would also explain the slip-stick performance of the system observed at higher loads and the large scatter of the friction results for that tribological system. In summary, the friction coefficient of the L7350 decreased when the load was increased at extreme climate conditions opposing the expected performance of human skin due likely to the hydrophobic surface properties of the Silicone Skin L7350. The interaction of the NBR surface with the skin points at stick-slip behaviour which was reflected in a large scatter of the friction results. The results for this material did not show a clear tendency of the friction coefficient with load whereas Lorica and ESE clearly displayed an increase of friction with load for all the pins.

Furthermore, the artificial skin samples and the *ex vivo* skin presented an increased friction coefficient when sliding against NBR balls at both forces of 2 and 4 N as shows Fig. 6 and Fig. 7. The higher COF for an elastomeric NBR pin sliding against *ex vivo* skin exerts more shear stress on the skin and removes more material than the two polymeric pins, PE and PA. This is due to the intrinsic physico-

chemical properties of the pin material and their affinity for the molecules on the surface of the skin. Hence, the intrinsic interfacial shear strength of each system is different and its tribological performance address to a different COF.

Additionally, segregated compounds from the stratum corneum were attached to the surface of the pins right after the friction tests, as indicated Fig 9. Although the composition of this film was not analysed, it was likely a mixture of sebum and lipids present at the stratum corneum [46]. This mixture of sebum and lipids, known as a Skin Surface Lipid Film (SSLF), protects human skin against the Trans Epidermal Water Loss (TEWL) and microorganism invasion [47-51]. Hence, the partial or complete removal of this protective layer during the measurements, modified the chemical constitution of the SC, even though no structural damaged appeared from the histological images. Thus, can affect a modification of the physico-chemical properties of the skin surface to its constitution and normal functioning? In fact, it has been proved that the normal process of proliferation and the differentiation of keratinocytes is inversely proportional to the TEWL [48, 51, 52]. Hence, disruptions of the SSLF due to a frictional contact removal cause an alteration of the TEWL which affects the reproduction and performance of keratinocytes and their organelles, the lamellar bodies [53-55]. Reciprocally, this affects the synthesis of compounds which constitute part of the SSLF with further limitation of the recovering process of the skin surface. As a result, a chain of reactions unleashed after the mechanical contact with the skin comprises the dysfunction of synthesizing essential compounds and difficulties for the restoration of the skin's barrier function [49-55].

It is likely that during the friction tests a "squeezing effect" of lipids and sebum from the stratum corneum occurs at the contact skin – counter surface. This effect can address to a modification of the skin surface which is higher in the case of NBR samples as the histological images and the roughness results (R_{pv} and 3D images) show after comparison with the control sample.

Hence, the combination of the tribological results and the surface analysis revealed that the physico-chemical and mechanical properties of the NBR have a great influence in the tribological response of the skin, not only because of a higher friction was observed but, also because of the surface changes that happened after the skin was in contact with this material as indicated in Figures 9, 10 and 11. Consequently, the decisions to address an improvement in the design of the turf should consider the infill.

5.4.3 Histology and surface roughness analysis

The analysis of the histological images did not show surface damage of the *ex vivo* skin when compared with fresh human skin samples and results from the literature [35] (see the differences in Figure 10 between the image (a) damage, and compare with the images (b) control; (c) NBR tested;

(d) PE tested; (e) PA 6.6 tested). Yet, for the skin samples tested against NBR, the state of the stratum corneum indicated changes at the surface morphology such as those presented in Fig. 10 (c). The latter image shows a surface wrinkling and thinning of the stratum corneum after the tribological tests when compared to the skin tested against PE, see Fig. 10 (d) and PA 6.6, Fig. 10 (e). Regarding to the roughness analysis, the skin samples tested against PE and PA 6.6 did not present any observable difference on the peak-to-valley roughness, R_{pv} , when compared to the *ex vivo* skin control. The R_{pv} values of the *ex vivo* human skin samples tested against NBR, PE, PA 6.6 and a fresh skin sample (control) are presented in Fig. 11. These results showed differences in the roughness of the samples tested against NBR but, similar values for those tested against PE and PA 6.6 when compared to the control. These results are also supported by the 3D images displayed in Fig.12. The characteristic features of the human skin represented in Fig. 12 (a) are not noticeable in the skin sample tested against NBR due to the modification after the test. Otherwise, the skin's samples tested against PE and PA 6.6 though, presented changes on the surface, still remained the morphological features typical of the skin. The image of the skin control correspond to one only sample which was evaluated at 3 different points. For the images of the skin after the friction tests again NBR, PE and PA 6.6 3 different samples were evaluated to analyse the surface modifications. Figure 12 presents a representative example of each case.

5.5 Conclusions

This paper investigates the tribological performance of mechanical skin equivalents and *ex vivo* human skin samples against the material components of the artificial turf. The results clearly revealed the limited suitability of Silicone Skin L7350, used in the FIFA test method as artificial skin equivalent at both, standard and extreme conditions. Especially at extreme conditions, the Silicone Skin L7350 presented a frictional performance opposite to the one of the human skin. This is a consequence of the inability of the silicone based compounds to absorb water so, their elastic modulus does not softens in the presence of water or humidity. Also, because of the hydrophobic properties of this material, the shear strength at the interface “skin – counter surface” differs from that expected for the case of the human skin by decreasing friction due to water lubrication effects. Hence, in the case of the Silicone skin L7350 the water condensation effect decreases the friction coefficient at extreme conditions instead of increasing it as human skin does. Further, the elastic properties of Silicone skin seem also higher than those expected for the human skin. Additionally, a better agreement of Lorica and ESE at standard conditions was observed when the results were compared to those of *ex vivo* human skin samples. At extreme conditions the results were not ideal but, in general, the friction values slightly increased and they showed closer values to those of the human skin. Moreover, an evaluation of the

role of NBR, PE and PA 6.6 in the tribological performance of the skin in sliding contact was conducted. NBR showed a more significant influence in the friction when contacting with skin and skin equivalents materials. Though, the analysis of the *ex vivo* human skin samples showed no damage of the skin due to friction yet, surface changes on the roughness and the morphology of skin were identified after the tests. Wrinkling of the skin surface was observed in the histological images due to deformation whereas the microscopic analysis showed a decrease of the peak-to-valley roughness (R_{pv}) for the samples in contact with NBR. In contrast, samples in contact with PE and PA 6.6 showed a similar roughness as the human skin reference sample. These changes together with the segregation of sebum and surface lipids observed after each test on the pin's surface were correlated to alterations in the TEWL and modification of the stratum corneum. Concerning the theoretical analysis of skin based on a 3 layered skin model, the adhesive component of friction appeared to have a more relevant role on the friction performance due mainly to the pressure dependence of the interfacial shear strength. The deformation component of friction was relevant only at higher forces due to the increase of the area caused by the decrease of the E_{eff} with the length scale. The latter resulted in an increased of the friction coefficient stronger than that would be expected based on the growth of the area of contact only.

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Chapter 6

Paper III: *On the role of adhesive forces in the tribo – mechanical performance of ex vivo human skin*

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Abstract

The frictional performance of the *ex vivo skin* was studied in a range of pressures between 0.5-20 kPa. Modifications in the coefficient of friction at different conditions stressed the important role of adhesion. The friction coefficient of “untreated” skin was obtained between 2.3–0.5, between 5.5–0.65 for wet skin and in a range between 0.65–0.35 for oil-covered skin. The contribution of capillary adhesion at wet conditions was analysed by the Hertzian and JKR contact models. The high coefficient of friction obtained at wet conditions couldn't be explained due to the softening effect of the stratum corneum and capillary adhesion only. The results suggested a further influence of water in the physic-chemical properties of the stratum corneum with a subsequent effect on friction.

6.1 Introduction

Human skin is a multi-layered structure whose outermost layer, the so-called stratum corneum (SC), plays an important role in the adhesive properties of the skin because of its specific composition. The Stratum Corneum is usually referred as “brick and mortar”, a biphasic layer of “brick” regions surrounded by the “mortar”. The “bricks” are tightly packed flattened anucleated cells (corneocytes) full of keratin which is the main responsible for the hydration properties of this layer. The “mortar” is an organized lamellar periodic bilayer of fatty acids, ceramides, cholesterol and water phases [1, 2]. On top of the Stratum Corneum there is a film composed of sebum, secreted by the exocrine glands, and other lipids which conform the Skin Surface Lipid Film (SSLF) [2-4]. This natural lubricant layer is fundamental to understand the tribological performance of the skin at the meso level and it plays an essential role in the hydration control of the skin [3, 5, 6]. The application of creams and moistures, which are emulsions of oil in water or water in oil, affect the adhesive properties of the stratum corneum with a subsequent effect on the frictional response of the skin.

The tribo-mechanical behaviour of the human skin has been extensively reviewed in connection with different applications [7-11]. In previous research the effects of hydration, skin topography and elastic properties as well as the role of sebum or other components on the skin surface have been presented as the most relevant factors influencing skin friction [12-18]. As it has been demonstrated before, adhesion has a major role in the tribological performance of the skin so that, the changes in friction due to the viscoelastic skin deformation can be neglected [11, 12, 14]. When a smooth spherical ball contacts a flat slice of human skin under certain pressure, already at very low forces the true area of contact reaches the nominal one due to the strong differences in the elasticity of the contacting materials. Then, the effect of skin roughness on friction can be ignored and the friction force due to adhesion can be evaluated by means of the surface condition of the skin. If the skin is “untreated”, friction would be commanded mainly by the properties of its Skin Surface Lipid Film (SSLF), whilst if the skin is “treated” the role of the adhesion forces needs to be approached according to the likely physic-chemical changes emerging on the surface. In dry conditions, the skin shows a coefficient of friction typically between 0.25 and 0.5 depending on the body location, the contacting material, age of the individual or indenter size among other [12]. Conversely, in the hydrated case, the coefficient of friction increases as a consequence of the softening effect of the stratum corneum and the changes in adhesion [12, 19-21]. When the skin is subjected to substances such as oils, creams or moisturizers, a similar effect occurs with a subsequent modification of the adhesive properties of the skin. Although, the increase of friction with hydration either by the direct application of water or by the moisturizing

effect of creams has been extensively analysed [14, 21, 22 - 25], the mechanism behind the high friction has not been completely understood yet. For normal skin, Hertz's theory gives a correct approximation to the contact area between the skin and a spherical ball. However, it has been extensively shown in previous research an increase of the friction force at hydrated conditions which cannot be explain based only in the Hertzian approach [26]. Thus, other mechanisms have been proposed to explain the increase of the coefficient of friction. To include the effect of the adhesive forces due to Van der Waal interactions between the contacting materials, a modified Hertz's model was proposed by Johnson, Kendall and Roberts [27]. Further, a hypothesis made by Persson [28, 29] suggested the role of the capillary forces in the contact between a hard surface and a smooth soft face in wet conditions. According to this reference, the softening effect of water causes a decrease of the elastic modulus linked to an increase of the capillary adhesion which addresses to an increase of the area of contact and, therefore, to an increase of the friction force. Capillary adhesion has been suggested in previous research to affect the frictional performance of the skin especially at hydrated conditions [14, 18, 30, 31]. However, its effect has not been yet introduced in the models to predict skin friction. Hence, to enhance the understanding of the mechanisms behind the changes of friction due to application of substances, and particularly in hydrated conditions, an evaluation of the adhesive forces arising on the skin is addressed in this work.

The main focus of this work was to evaluate the role of the adhesive forces in the tribo-mechanical performance in skin – object interactions by means of an experimental and a theoretical approach using untreated, wet and oil-covered *ex vivo* human skin. Three samples belonging to the abdomen of an individual were evaluated at the meso scale (that is, in a range of forces between 1 mN to 80 mN). The measurements were performed on *ex vivo* human skin to avoid both, the effect of pre-stretching the skin and the influence of the underlying tissue of *in vivo* skin in the results. Modifications of the elastic properties of *ex vivo* skin can occur as a consequence of the effect of ageing yet, for fresh *ex vivo* skin these changes should be minimal in comparison with the effect of pre-stretching on *in vivo* skin. Further, although the results on *ex vivo* tissue are subjected to similar variability as *in vivo* skin, no roughness effects on friction were considered since all the samples were belonging to the same individual. Consequently, the number of parameters possibly affecting skin friction were minimized in order to evaluate the role of the adhesive properties of the skin under different conditions.

According to the “Two-term friction model” [14, 22, 33], the friction force is defined by the contribution of two non-interacting parts: the adhesive and the deformation components. Adhesion is generally considered the main constituent of the friction of human skin, whereas the deformation component seems to have a minor influence on it [14, 11]. The deformation component is proportional to the viscoelastic loss fraction, β , due to the viscoelastic behaviour of the skin, the applied force, F , and the relative indentation into the skin, $\frac{a}{R}$:

$$F_{\mu,def} = \frac{3}{16}\beta\frac{a}{R}F \quad (1)$$

The adhesive component of friction is defined as a function of the area of contact, A_{real} , and the interfacial shear strength, τ , between the contacting surfaces:

$$F_{\mu,adh} = \tau A_{real} \quad (2)$$

Commonly, the total friction force is described as a power law of the normal force, as indicates equation (3), with an exponent, m , that varies with the applied conditions. Thus, m includes information about the relative contribution of each component to the total friction.

$$F_{\mu} = k \cdot F^m \quad (3)$$

When m approaches values close to 1, friction is defined according to Amontons' law. Typically, values between 0.6 and 1.1 can be obtained from the literature for the case of the human skin [14, 34-38].

To determine the area of contact, A_0 , for the case of the contact between a spherical ball and a flat surface representing the skin, Hertz's theory can be used [36, 39, 40]. Based on Hertz's theory, the contact radius, a_H , is a function of the applied force, F , the radius of the probe, R , and the Effective Elastic modulus, E^* , a combination of the elasticity of the contacting materials.

$$a_H = \left(\frac{3RF}{4E^*}\right)^{1/3} \quad (4)$$

However, Hertz's theory does not account for the adhesive forces on the contact area [41, 42] due to the attractive forces between solids. Certainly, the adhesive phenomena appears as a consequence of dispersive Van der Waal forces between two contacting surfaces. Thus, to evaluate the adhesive behaviour of the skin it might be required to include these forces in the contact model. Johnson-Kendall-Roberts [27, 43, 44] proposed a modified Hertz model which includes the force of adhesion due to Van der Waals interactions between the contacting materials. Then, the apparent force acting on the material's surface is:

$$F_{app} = F + 2F_{adh} + 2\sqrt{F_{adh}(F + F_{adh})} \quad (5)$$

With F_{adh} the adhesive force defined as:

$$F_{adh} = \frac{3}{2}\pi RW_{12} \quad (6)$$

Where W_{12} is the work of adhesion between the two contacting surfaces, see [41, 45, 46].

Additionally, it has also been proposed the role of capillary forces which act on the contact surface could influence the frictional behaviour of the skin [29]. The capillary force can be derived from the capillary pressure given by the Young-Laplace [44] as indicate equation (7):

$$F_{cap} = -2\pi R\gamma_L(\cos \theta_1 + \cos \theta_2) \quad (7)$$

R , radius of the sphere, γ_L , the surface tension of water and θ_1, θ_2 , the contact angles of the surfaces.

The influence of this force in the frictional performance of the skin has not been analysed yet for object – skin interactions. The introduction of these forces in the friction model would increase the area of contact, especially, at low forces and it might be provide a better approach to describe the frictional performance of the skin. The negative sign of equation (7) shows that the force is attractive, so that, to include the capillary force in the JKR model the sign would be positive and the apparent normal force would be given by:

$$F_{app}^{cap} = (F + F_{cap}) + 2F_{adh} + 2\sqrt{F_{adh}[(F + F_{cap}) + F_{adh}]} \quad (8)$$

6.2 Material and methods

6.2.1 Material and methods

6.2.1.1 *Ex vivo human skin samples*

Ex vivo human skin samples from abdominal surgery of an anonymous donor were used to perform indentation and friction experiments. The samples, provided by the Radboud Hospital of Nijmegen, were taken with the consent of the patient and the corresponding agreements of the Ethical Committees of the Hospital and the University of Twente. The adipose tissue was completely removed from the samples, so that the friction data corresponded to the response of Epidermis and Dermis only. The average thickness of the samples was 1.2 mm. In general, the source of the skin samples is subjected to availability; it is expected that the differences associated to the body place fall within the deviation among donors which according to the results of Lewis et al. [47] are consistent. In this case, all the measurements were performed with abdominal samples from an individual so that, no

roughness effects on friction were considered. In previous research, the contact between the fingertip and a counter surface has been modelled with a ratio 1:1 between the apparent to the real contact area with an apparent contact area between the 45-55 % of the nominal contact area [48, 49]. Therefore, in this research the real area of contact was considered as the 80% of the nominal area of contact with a ratio 1:1 between the apparent and the real contact areas.

6.2.1.2 Experimental methods

a) Surface characterization

The surface of the *ex vivo* skin was analysed with a laser confocal microscope VK 9700 from Keyence at a magnification of 10x and z-axis resolution of 1 nm. The samples were cut in square shape of 20x20 mm and they were glued in a petri dish. The surface characterization of the skin was done as received that is, only in the dry condition. 2D and 3D confocal images of one of the *ex vivo* samples are presented in Figure 1. Additionally, as indicative value, the average surface roughness of the 3 *ex vivo* human skin samples is given in Table 1.

Figure 1. Confocal microscope images of excised human skin. At the left is presented a 2D image of the skin and at the right a 3D image with a depth scale (size: 1mm x 1.4mm).

Table 1. Summary of the average roughness parameters of the excised human skin samples.

$R_{pv} (\mu m)$	$R_a (\mu m)$	$R_q (\mu m)$	$R_{sk} (\mu m)$
74.03 ± 3	46.20 ± 4	57.67 ± 3	-0.53 ± 0.05

b) Tribo-mechanical performance

Series of pull-off and friction measurements were carried out by a Vacuum Adhesive and Friction Tester (VAFT) created at the University of Twente [48]. Indentation tests were performed with a

relatively large indenter made of Chrome Steel AISI-52100 of 6 mm diameter were to ensure that the contact area was larger than the diameter of the individual cells of the stratum corneum [32]. The forces applied during the measurements ranged from 1 up to 50 mN and they were selected to ensure values between 0.5 – 20 kPa that is, within the range of the applications to which our study is focus on [7 - 10]. The indentation measurements were performed at a velocity of 50 $\mu\text{m/s}$ and the results corresponded to indentation depths between 3 and 350 μm , which is in the meso scale, to assess the layered structure of the skin. Thus, with such an indenter the overall performance of the skin could be evaluated and the influence of the holder's material was avoided. The adhesive forces of “untreated” and “treated” skin with water and rapeseed oil, were obtained from pull-off experiments at $z=z_0$ during an indentation test. Then, the work of adhesion was calculated based on the JKR model as defined in equation (6). These measurements were performed on “untreated” skin and “treated” skin with deionized water (DW) and rapeseed oil from Grease Factory of Lanzhou (China). Moreover, the adhesive forces obtained from the experimental results were compared to the capillary forces calculated for the skin treated with water and rapeseed oil.

Furthermore, friction measurements were performed with Vacuum Adhesive and Friction Tester (VAFT) in a load range of 1 to 80 mN at a velocity 10 $\mu\text{m/s}$ and a sliding distance of 400 to 2000 μm . The skin samples were glued inside a petri dish with Loctite (Loctite, Germany). Friction measurements were conducted on untreated skin and after applying between 3 and 4 drops of water and oil on the skin surface. The fluids where spread on the surface and the excess was removed after 2 minutes with a paper tissue to ensure a boundary lubrication regime. Then, the coefficient of friction was obtained and compared to the average friction obtained previously on normal skin.

6.2.1.3 *Theoretical methods*

The results of the coefficient of friction at different conditions were compared to the results obtained from the theoretical models described in the “Theoretical models” section. To better understand the role of the adhesive forces in skin friction, the Hertzian and the JKR models were applied to describe the contact situation of “untreated” and wet skin. Besides, the capillary force has been previously pointed to have a role in the increased coefficient of friction at hydrated conditions yet, it has not been completely proved whether it has a role or not [51]. The effect of the capillary force in the adhesive forces of the skin can cause an increase of the area of contact at low forces that might affects the interfacial shear strength as well, and then, the coefficient of friction. Thus, the effect of the capillary forces was included in both models according to equations (7) and (8) to evaluate its likely effect in skin friction. The resulting contact parameters where later introduced in the “Two-term friction model” to predict the coefficient of friction at each condition. Lastly, the theoretical coefficient of friction was given as a power law function of the applied force and the role of the obtained m

exponents was analysed in relationship with the role of adhesion and capillarity in skin friction. The parameters used to obtain the capillary forces at normal and dry conditions are presented in Table 2.

Table 2. Surface tensions for normal skin, water and rapeseed oil. The contact angles between the skin and sebum, water and oil as well as the corresponding values for steel in contact with those substances are also given in the table.

	Normal skin		Wet skin	
Surface tension (mN/m)	43		73	
Contact angle (rad)	Skin - sebum	1.58	Skin - water	1.55
	Steel - sebum	1.43	Steel - water	0.96

6.3 Results and discussion

6.3.1 Adhesive properties of *ex vivo* human skin

The adhesive properties of the skin samples obtained from pull-off measurements are presented in Figure 2 for a range of forces from 1 to 50 mN.

Figure 2. Snap-off forces obtained after pull-off measurements on human skin: image (a) displays the results of two of the tested samples as a function of the force; image (b) presents the average values for the adhesion at forces of 10 and 50 mN.

From the data of these measurements the work of adhesion was calculated according to Equation (6) based on the JKR model as a function of the applied force. These results presented in Figure 3 point out a lower work of adhesion at low normal forces whereas from 10 mN it rises to values around -60 mN and slightly higher at 50 mN of normal force. This might be due to the increase of the area of

contact at higher forces due to Van der Waals interactions whereas at lower forces the adhesion is likely due only to capillary forces. The higher pull-off forces could also be related to the viscoelastic increase of the contact area due to the increase of load and longer contact times. The average work of adhesion was $47.5 \pm 4 \text{ N}\cdot\text{m}^{-1}$ which is in accordance with previous research of *in vivo* skin [26].

Figure 3. Work of adhesion of human skin as a function of the force for 3 *ex vivo* samples (square markers in red, blue and green); the black line represents the average values of three samples with the corresponding standard deviation at each force.

6.3.2 Frictional performance of *ex vivo* human skin

6.3.2.1 Experimental results

The frictional performance of 3 *ex vivo* skin samples is presented in Figure 4 over a range of forces between 1 to 80 mN. The average friction obtained from the given results is also presented in the figure. The coefficient of friction at 1 mN was about 2.3 ± 1 and it decreased according to a power law to values of 0.5 ± 0.1 from 30 mN onwards. The standard deviation of the average friction was large, mainly at low forces, as a consequence of the variability of the human samples and the low number of samples studied. This fact can be seen as a limitation of the experimental part of this research yet, it reveals also that, the existing differences in the coefficient of friction at low forces cannot be explained only by means of roughness or other intrinsic surface properties of the skin since all the samples belonged to the same individual and the same anatomical location. Thus, external factors might have an influence in the surface properties of the skin which, especially at low applied forces, would explain

the scattered results for the coefficient of friction. In addition, the high standard deviation at low normal forces can be also due to the error introduced by the equipment, much more relevant at low forces.

Figure 4. Evolution of the coefficient of friction of the excised human samples at the meso scale from 1 to 80 mN. The values of three samples are presented with different markers and the average friction is indicated with a red line.

The adhesive properties of the skin in sliding friction have been found to be influenced mainly by the properties of the SSLF or, in general, the condition of the skin's surface [12 - 14, 25, 26, 42, 43, 52, 53]. The frictional results at the meso scale are displayed in Figure 5 as a function of the normal force for "untreated" skin and "treated" skin with water and oil. Wet skin indicated a higher coefficient of friction than "untreated" skin whereas the skin coated with oil presented lower coefficient of friction than "untreated" skin. Further, at low forces, the coefficient of friction presented higher values than those obtained at higher forces for "untreated" and "treated" skin. Since the samples measured were all belonging to the same person, from the same body site and they were not pre-stretched, the role of roughness in the measured friction should have the same impact.

The higher coefficient of friction indicated by the sample treated with water could be explained partially based on the effect of capillary adhesion on the surface and/or a modification of the interfacial shear strength which, according to Persson [28, 29] is also related to the changes due to capillary effects. Besides, the effect of water causes a decrease of the stratum corneum stiffness and a subsequent increase of the area of contact. This interpretation has extensively been used to explain the higher coefficient of friction of the skin at hydrated conditions albeit the larger area of contact cannot solely justify the friction values observed experimentally.

Figure 5. Coefficient of friction of human skin as a function of the applied force for normal skin (circles), wet skin (crosses) and oil-covered skin (stars). The lines correspond to the fit of the experimental data according to a power law of the force as is presented also in the figure: normal skin (salmon dashed line), wet skin (blue dashed line) and oil-covered skin (yellow dashed line).

Therefore, other mechanisms might also contribute to the increase of skin friction at hydrated conditions. In fact, the effect of water causes structural modifications of the stratum corneum, such as replacement of lipid covalent bonds by weak hydrogen bonds [54] with a likely effect on the physico-chemical properties of the skin surface, such as the surface tension or the dielectrical properties. Changes in the resistance and capacitance of the human skin have been measured after the application of water [55] which reveal an effect of water in the electrical properties of the skin. In the same article, a decrease of the output voltage with the time when the skin was immersed in water and, subsequently exposed to environmental conditions, is presented. Thus, the specific composition and surface properties of the skin are altered when contacting with water in a way that, according to these results, the dielectric properties of the stratum corneum are modified [56]. Then, the interaction with a counter surface is might affected by the electrical changes occurring on the skin surface after the application of water. Additionally, the analysis of similar and dissimilar metal contacts in sliding friction have demonstrated the role of ploughing and debris in the frictional performance of these systems [57]. According to the previous reference, a hard metal sliding on a soft metal is influenced by both, the ploughing effect and the generated debris with a subsequent effect in the coefficient of friction. Skin abrasion has been pointed out by frictional and immunohistological analysis when contacting natural and artificial turf surfaces [58]. This debris from stratum corneum might influence also the coefficient of friction as in the case of metals. Moreover, segregation of compounds and

roughness modifications of the stratum corneum were observed in our previous research after sliding contact of several materials on *ex vivo* skin [59]. Consequently, during the sliding contact between the skin and a steel ball a transfer of debris (stratum corneum removal and sebum) from the skin surface to the ball might also cause an increase of friction which, in hydrated conditions, would be higher due to the increase of adhesion. Therefore, the increase of the coefficient of friction at hydrated conditions might be explained based on the sum of several suppositions:

1. *Modification of the adhesive properties of the stratum corneum*
2. *Increase of the area of contact due to a decrease of the stiffness of the stratum corneum*
3. *Capillary effects*
4. *Alteration of the electrical potential*
5. *Increase of debris due to a higher adhesion and sticky performance of the hydrated stratum corneum.*

Therefore the noticeable differences on the coefficient of friction presented in Figure 5 have to be influenced by the aspects listed above.

Additionally, the viscoelastic loss fraction, β , was also obtained from the indentation measurements. The average value was 0.35 ± 0.01 , which showed a good agreement with the values from the literature [22, 35, 60, 61], and it was used to calculate the deformation component of friction. Moreover, the elastic modulus for dry skin was considered 120 kPa whereas for wet skin the modulus was 50 kPa. A summary of the input parameters used in the model is presented in Table 3.

Table 3. *Parameters used in the friction model. The elastic modulus at dry and wet conditions was obtained according to ref. [57] for a skin model composed of stratum corneum, viable epidermis and dermis. The interfacial shear strength, τ , was the fitting parameter in this model.*

	Dry conditions	Wet conditions
Elastic modulus (kPa)	120	50
Interfacial shear strength (kPa)	$E/28$	$E/18$
Viscoelastic loss fraction, β (–)	0.35	

6.3.2.2 Theoretical analysis

A fit of the experimental coefficient of friction of normal, wet and oil-covered skin is presented in Figure 5 as a power law of the normal force. The differences in the m exponent for the three cases revealed the influence of adhesion in the prediction of friction. Further, Figures 6 (a) and (b) present the contact radius obtained as a function of the normal force at dry and wet conditions, respectively according to the theoretical models described in the “Theoretical methods” section.

Figure 6. Evolution of the contact radius at dry (a) and wet (b) conditions as a function of the normal force. The Hertzian case is indicated with a blue line; the JKR case with a green line; the Hertzian case with capillary force is presented with a red dashed-line; and finally, the JKR model with capillary force is indicated by a black dashed-line.

A comparison of the theoretical models with the experimental data at dry and wet conditions was done to evaluate the possible role of the capillary forces in predicting friction, especially at wet conditions.

Figure 7. Coefficient of friction as a function of the normal force for ex vivo skin at normal (a) and wet (b) conditions. The experimental data is presented by black circular markers. The coefficient of friction based on the models is presented with a black line for the Hertzian case, red line for the JKR, green dashed line for the modified Hertzian case and blue dashed line for the modified JKR case.

Therefore, Figure 7 (a) and (b) display the results of the coefficient of friction at both, dry and wet conditions, respectively in comparison with the theoretical results from the Hertzian and the JKR

models and their modified versions which include the capillary effect. From these figures it is noticeable that the coefficient of friction at low forces was only approached by the JKR and the modified JKR model already at dry conditions. Similarly, at wet conditions the results showed the same disposition with coefficient of friction values especially high at low forces, only reached by the mentioned models. To determine which model estimates better the experimental at each condition, the corresponding power law functions were calculated for the cases presented in Figure 7 and they are presented in Table 4.

Table 4. Summary of the coefficient of friction as a power law function of the normal force at dry and wet conditions according to each of the cases investigated: Hertz, JKR and the modified version of those two models.

Dry conditions			Wet conditions		
		SE			SE
Experimental	$\mu = 0.05 \cdot F^{-0.53}$		Experimental	$\mu = 0.01 \cdot F^{-0.87}$	
Hertz	$\mu = 0.11 \cdot F^{-0.32}$	42.6%	Hertz	$\mu = 0.12 \cdot F^{-0.32}$	55.5%
Hertz + cap	$\mu = 0.11 \cdot F^{-0.33}$	42.6%	Hertz + cap	$\mu = 0.09 \cdot F^{-0.39}$	49.7%
JKR	$\mu = 0.06 \cdot F^{-0.51}$	17.3%	JKR	$\mu = 0.06 \cdot F^{-0.57}$	17.3%
JKR + cap	$\mu = 0.06 \cdot F^{-0.52}$	13.3%	JKR + cap	$\mu = 0.05 \cdot F^{-0.62}$	16.2%

In this table, the standard error (SE) when estimating the experimental results is also presented. Thus, according to the results presented above the JKR model which includes the capillary force exhibits the lowest error for both, dry and wet conditions. The error for the best cases is still considerable high since it is of 13% and 16% for the dry and wet conditions, respectively. Therefore, as mentioned previously in the discussion, this suggests the necessity of considering other mechanics to explain the interaction between the human skin and other surfaces, especially at hydrated conditions where the coefficient of friction raises especially high values. Moreover, the differences with respect to the experimental data can also be due to deviations in the values of some applied parameters such as the elastic modulus. Additionally, the theoretical coefficient of friction presented as a function of the normal force decreases according to a power law function whereas at certain load the experimental coefficient of friction keeps steady around 0.4 by suggesting a different regime at higher forces. So that, the error of 13.3% and 16.2 % for the modified JKR model was mainly due to the differences with respect to the experimental data at the highest forces. Furthermore, the m exponent obtained for the experimental data presented high value at both dry and wet conditions which indicated an important contribution of the adhesive forces to the coefficient of friction. A value of -0.53 for the experimental data was properly assessed in dry conditions by the JKR model which presented an m exponent of -0.52. The value of the m exponent for the experimental data indicated an increase of the adhesive forces in wet conditions in comparison to the dry case. In wet conditions, the experimental results addressed to an increase of the m exponent which reached -0.87. The theoretical m exponents for the Hertzian and JKR models were -0.32 and -0.57 which were too low compared to the experimental

result. Thus, the Hertzian and the JKR models were inadequate to simulate the high adhesion displayed on *ex vivo* skin at wet conditions. The comparison of the function fits including the capillary force showed a better agreement of the experimental results with the modified JKR model given by equation (8). The modified version of the JKR model presented an m exponent of -0.62, still lower than that obtained from the experimental results. Consequently, the contribution of the adhesive forces at low normal loads and, especially at wet conditions, require the consideration of adhesive and capillary forces to approach the frictional performance of the skin. Hence, this analysis suggests that, both adhesive and capillary forces need to be considered for the frictional performance of the skin at wet conditions whereas the JKR model represented properly the tribological behaviour of the skin at dry conditions. The increase of the adhesion at the meso scale under wet conditions can be explained in terms of capillary forces due to condensation of water molecules, the effect of creams, moisturizers or other lubricants or as a consequence of modifications of the SSLF of skin.

6.4 Conclusions

The adhesive properties of *ex vivo* human skin have been analysed in relationship with their frictional performance. Pull-off measurements in a range of pressures between 0.5-20 kPa indicated an average work of adhesion of 43 ± 15 mN/m which is consistent with the literature. The high standard deviation of these results is due to the scattered results of the pull-off forces. Further, the analysis of the frictional performance of “untreated” and wet skin showed similar values for the coefficient of friction as those observed in previous research. A considerable increase of friction was observed at wet conditions with a maximum coefficient of friction of 5.5 decreasing to about 1 as the force was raised. For “untreated” skin, a maximum coefficient of friction of 2.5 decreased to around 0.45 as the force increased. Experiments on oily-covered skin indicated a steadier coefficient of friction with values between 0.65 and 0.35 in the range of applied forces. Based on these results, the coefficient of friction was described as a power law of the normal force with variations in the m exponent which confirmed the role of adhesion in skin friction. The analysis of the frictional models indicated a good agreement of the experimental results with the JKR model at dry conditions. The introduction of the capillary forces in the JKR model caused a slight increase of the coefficient of friction which indicated a better agreement with the experimental results at wet conditions. Nevertheless, a larger coefficient of friction needs to be obtained at wet conditions to resemble the experimental results. Thus, the contribution of other mechanisms to the total adhesion force must be considered. The influence of debris from the stratum corneum removal, the ploughing effect, a higher contribution of deformation or an effect of the electrical potential associated to the physic-chemical properties of the contacting materials especially at low forces, can introduce modifications in the total friction force that are not currently

considered. Moreover, modifications of the interfacial shear strength as a function of the force must be also studied especially due to the layered structure of the skin. Besides, changes in the skin surface lipid film (SSLF) could also introduce alterations of in the location of the shear with a subsequent effect on friction.

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Chapter 7

Paper IV: *A tribo-mechanical analysis of PVA-based building blocks for implementation in a 2-layered skin model*

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Abstract

Poly (vinyl) alcohol hydrogel (PVA) is a well-known polymer widely used in the medical field due to its biocompatibility properties and easy manufacturing. In this work, the tribo-mechanical properties of PVA-based blocks are studied to evaluate their suitability as a part of a structure simulating the length scale dependence of human skin. Thus, blocks of pure PVA and PVA mixed with Cellulose (PVA-Cel) were synthesized via freezing/thawing cycles and their mechanical properties were determined by Dynamic Mechanical Analysis (DMA) and creep tests. The dynamic tests addressed to elastic moduli between 38 and 50 kPa for the PVA and PVA-Cel, respectively. The fitting of the creep compliance tests in the SLS model confirmed the viscoelastic behaviour of the samples with retardation times of 23 and 16 seconds for the PVA and PVA-Cel, respectively. Micro indentation tests were also achieved and the results indicated elastic moduli in the same range of the dynamic tests. Specifically, values between 45-55 and 56-81 kPa were obtained for the PVA and PVA-Cel samples, respectively. The tribological results indicated values of 0.55 at low forces for the PVA decreasing to 0.13 at higher forces. The PVA-Cel blocks showed lower friction even at low forces with values between 0.2 and 0.07. The implementation of these building blocks in the design of a 2-layered skin model (2LSM) is also presented in this work. The 2LSM was stamped with four different textures and their surface properties were evaluated. The hydration of the 2LSM was also evaluated with a corneometer and the results indicated a gradient of hydration comparable to the human skin.

7.1 Introduction

Artificial human skin is used in the medical field to assist the recovery process of burns, scars, chronic ulcers, abrasion or other skin injuries due to chemical, biological or mechanical damage. Different natural and synthetic polymers have been used for wound healing purposes, such as collagen dressings, commonly used because of their ability to attract cells which cover the wound and guide the healing process [1, 2]. Oxidized regenerated cellulose membranes have been synthesized for covering wounds [3, 4], and derivatives of Chitin and Chitosan in the form of hydrogels, membranes, scaffolds or sponges are suggested for wound treatment purposes [5]. Silk fibres with epidermal growth factor (EGF) have been manufactured as wound-healing dressings with promising results [6,7] and Hyaluronic acid (HA) hydrogels have been subject of research since their discovery in the 1930s [8] thanks to its healing properties and angiogenesis promotion [9]. Other synthetic hydrogels show good results as artificial skin membranes. Poly (lactic-co-glycolic acid) (PLGA) scaffolds cultured with cells are able to start the healing process and to grow new skin [10]. Also, artificial skin membranes made of polyvinyl Alcohol (PVA) - starch blends show potential for healing wounds [11]. Indeed, the use of PVA hydrogels is enlarged the last decades because of its biocompatibility and ease of manufacturing. PVA can be easily crosslinked via physical routes such as freeze-thawing cycles of aqueous polymer solutions [12-14] or chemically crosslinked with glutaraldehyde to form hydrogels [15, 16]. It has been used for wound dressings [17], cartilage replacement [18, 19], drug delivery systems [20], contact lenses [21] or artificial organs [22]. Furthermore, its hydrophilicity and ability to modify its mechanical properties with the environmental changes, such as temperature and humidity, makes them a suitable choice to simulate the performance of skin [23, 24]. As the current skin substitutes are used for medical applications, such as wound dressing, they generally involve the use of biological compounds to assist the healing process. Subsequently, most of the skin substitutes in the market are based on natural components, like Integra® [25] a mixture of Collagen, Glycosaminoglycans and Chondroitin-6-sulfate. In other occasions these bio composites are cultured with cells, as fibroblasts or keranocytes from autologous (Epitel™, Epidex®) or allogenic origin (Alloderm®, TransCyte®, Apligraf®, Dermagraft®) [26-34]. They mainly act as a barrier against water loss and microorganism infections and they help the skin to recover faster and more efficiently.

The use of skin substitutes in analysing material-skin interactions is however limited by functional and practical aspects, such as inadequate mechanical performance [35] or ethical procedures related to the presence of biological components [36, 37]. Furthermore, these kind of products are easily degradable, difficult to handle and expensive. Hence, from an industrial perspective this is neither viable nor efficient. Moreover, the reproducibility of the tribological results of the skin cannot be

guaranteed when using *ex vivo* or *in vivo* human skin due to the inherent variability of the biological samples according to the source. A few artificial skin models have been developed with synthetic polymers to mimic the tribo-mechanical performance of the human skin in different applications. Silicone Skin L7350 is used by FIFA [38, 39] to evaluate the abrasion and friction during sliding contact with artificial grass to evaluate the adequacy and quality of the turf. The frictional performance of several skin surrogates was studied by Derler et al. [40]. Their results addressed to a good agreement of the friction coefficient between Lorica, a material based on polyamide fleece mixed with polyurethane, and the human skin in dry conditions. However, the frictional performance of human skin in moist conditions has not been yet achieved. The hydration of the skin is one of the main factors influencing its mechanical and frictional behaviour, so that, the presence and the capability to absorb and desorb water should be approached in the new mechanical models of the skin. There are few mechanical models of the skin which aim to mimic its hydration and its frictional performance both conditions. One of those examples was recently presented by Nachman and Franklin [41] who synthesized a moisture-sensitive skin surrogate. Moreover, in our previous work [42] we approached the mechanical properties of the skin by developing a hydrophilic system based on PDMS/PVA mixture. The material showed surface and mechanical properties close to the human skin, yet the length scale dependence was not implemented. As a consequence, the development of a suitable artificial skin substitute for industrial applications [43, 44] is highly desirable as the analysis of the “skin substitute – material” interactions could enhance consumer products in contact with skin in the everyday life [45-47]. Further, may the use of hydrogels in this application can benefit to resemble the frictional performance of the skin at both conditions.

7.1.1 Skin Tribology: theoretical background

The “skin – surface” interactions can be studied using tribology: the science and technology of interacting surfaces in relative motion [48-50]. When two surfaces are in sliding contact under external load and a lateral movement appears, a resistive force, which opposes the movement, emerges. The coefficient of friction (COF) [51, 52] is calculated as the ratio between the tangential force (F_f), opposing the movement, and the perpendicular force at the contact-plane between the two surfaces, (F_N) (Eq. 1).

$$\mu = \frac{F_f}{F_N} \quad (-) \quad (1)$$

The COF (μ) depends on the material parameters of the two contacting surfaces, the micro – geometry in the contact, operational and environmental conditions, as well as interfacial properties like lubricants or oxides at the surface [48, 50, 53, 54]. A first approximation given by Johnson et al. [55] and Adams et al. [50], described the friction force for the case of a contact situation in which a

smooth spherical probe is sliding against the skin. In general, if the skin is considered fully elastic, its indentation performance can be described by Hertz contact theory [56, 57]. This theory, which considers an isotropic and homogeneous material, is valid for relatively small deformations. Hence, the indentation can be described as a function of the contact geometry, applied force and elasticity of the contacting materials [58]. By referring the contact of the skin loaded over a sphere as an elastic half-space, the contact radius can be calculated as a function of the radius of curvature of the sphere, R , applied force, F_N , and elastic properties of the materials in contact, E^* , as shown in Equation (2):

$$a_H = \sqrt[3]{\frac{3RF_N}{4E^*}} \quad (\text{m}) \quad (2)$$

with E^* , the Effective Elastic modulus combination of the mechanical properties of the interacting surfaces in terms of elasticity [59-61], as indicated in Equation (3):

$$\frac{1}{E^*} = \frac{1 - \nu_{skin}^2}{E_{skin}^*} + \frac{1 - \nu_2^2}{E_2^*} \quad (\text{m}^2/\text{N}) \quad (3)$$

As indicated in equations (2) and (3), the elastic modulus of the skin plays a crucial role in the contact model. Although many investigations have tried to approach the elastic modulus of the skin the results are spread in a wide range of magnitude which can go from few kPa for *in vivo* skin to the range of GPa for isolated stratum corneum [57, 62-65]. Van Kuilenburg et al. [66], collected the elastic modulus of human skin obtained by indentation tests previous to other research and they plotted the results in a graph as a function of the length scale. Later, the authors suggested an analytical model to account for the length scale dependence of the elastic modulus due to the multi-layered structure of the skin.

Figure 1. (a) Evolution of the elastic modulus of the skin as a function of the contact radii; (b) evolution of the elastic modulus between 1 to 100 mN according to the model given in ref. [67].

According to this model, for a 3 layered model of the skin, the evolution of the elastic modulus with the contact radio and the applied force varies as indicates Figure 1 (a) and (b), respectively.

Hence, to properly describe the contact situation of a half-space skin contact against a sphere is required to consider the mechanical properties of the different skin layers [66, 67]. As a consequence, a proper skin equivalent must consider the length scale dependence of the skin's elasticity and its variation with hydration which has been widely demonstrated to affect the frictional results of the skin [68-70].

Accordingly, in this work, an analysis of the tribo-mechanical performance of building-blocks based on PVA hydrogel was made to evaluate their suitability in a 2-Layered Skin Equivalent (2LSM). The goal of such a 2-layered structure would be to implement the length scale dependence of the skin and its hydration capabilities. The synthesis of the blocks and the design and manufacturing of a potential skin equivalent is reported. Furthermore, the implementation of different top surfaces was evaluated to mimic the surface properties of human skin including roughness and wettability, based on the references [71-76]. The thermal properties of the blocks were analysed via TGA measurements, as well as their dynamic-mechanical performance, elasticity via indentation and frictional behaviour. The results were analysed and the suitability of the properties of the building-blocks for a 2-Layered skin equivalent was evaluated.

7.2 Material and methods

7.2.1 Synthetic methods

7.2.1.1 Fabrication of the building-blocks

PVA hydrogel (Mw 31,000-50,000) was purchased from Sigma Aldrich (USA) and Lyocell Cellulose fibres of 1.7dtex and 4 mm length were obtained from Tencel (Austria). Solutions of PVA and PVA - Cellulose were obtained by adding PVA polymer in a round flask with deionized water at a concentration of the 20% w/v. The mixture was refluxed overnight at 90 °C to dissolve the polymer. The same procedure was repeated and the resulting solution was mixed with a 2% w/v of the Lyocell Cellulose fibres. The two solutions were coded as "20% w/v PVA" and "20% w/v PVA-Cel" and they were stored in the fridge at 5 °C until use.

To manufacture the building-blocks a freezing/thawing program was implemented. It is well known that the molecular weight, concentration of PVA, number of cycles and cooling/heating temperatures affect the physico-chemical and mechanical properties of the resulting material. It has been

demonstrated that an increase in the molecular weight and the number of freezing/thawing cycles addresses to higher strength in the gels. Fukumori and Nakaoki [77], prepared PVA films from 15 % wt. concentration after 10 freezing/thawing cycles. The analysis of their mechanical properties indicated an improvement due to the higher amount of crystallites obtained with increasing the number of cycles. Further, maximum stresses were also observed for these samples when compared to other PVA films obtained by the same technique with a different number of cycles. The crystallinity of the samples has been related to the molecular weight, the concentration of the solution and the number of freezing/thawing cycles, and all of them appeared to be essential in determining the properties of the obtained gel. In general, a higher ratio of crystallites formation implies a decrease in the swelling ratio of the samples, and consequently an increase of the material stiffness [17, 19, 78, 79, 80]. In this case, the molecular weight of the polymer and the freezing/thawing program was adapted from the literature [17, 19, 78] to get samples with an elastic modulus in the range of kPa or few MPa and ensure a certain water content. Regarding to the concentration, Wong [81] indicates that the increase on the PVA concentration raises the stiffness of the resulting hydrogel with an elastic modulus increasing from 0.36 to 0.85 MPa for the 10% and 20% PVA films. In this case, a maximum concentration of 20% w/v was chosen to prepare the samples. A number of 11 cycles was selected to achieve a Young's modulus within the same range of magnitude to that of the human skin [67, 82, 83].

7.2.1.1 *Implementation of the PVA-based building-blocks*

The previously described PVA-blocks were implemented in the design of a 2-Layered Skin Equivalent (2LSM). The building process of the 2LSM consisted on several steps which required the use of a specific mould to properly assemble the different parts of the physical model. A 3D printed mould was created by the Ultimaker 2, manufactured by Ultimaker B.V. (The Netherlands) at the Robotics and Mechatronics group (RAM, University of Twente). Secondly, textured samples obtained from laser cutting by a Trotec Speedy 300, manufactured by TrotecLaser (USA), were also obtained on acrylate supports. The textures were used to implement several roughness's on the top layer of the 2LSM to resemble the surface properties of the human skin.

The construction of the 2LSM consisted in 2 steps which correspond to the fabrication process of each individual layer. The production was made by using the 3D printed moulds presented in Figure 2. The mould consisted of 4 separated parts of 30 mm diameter: 3 to construct the model (parts (a), (b) and (d) from Figure 2 and 1 more to implement the surface texture (part (c) from Figure 2). Part (c) was exchanged with 4 different texturized parts to compare the adequacy of the obtained patterns with the experimental results of human skin in terms of roughness and contact angle.

Figure 2. Perspective images of the moulds design to manufacture the conceptual 2LSM.

7.2.2 Characterization of the building-blocks

7.2.2.1 Structure and morphology

The micro structure of the PVA and PVA-Cel layers was analysed with CryoSEM. The images were taken from several cross sections of both films after their fabrication via freezing/thawing cycles. To examine the samples with SEM technique, it was required to remove the water content of the samples. Hence, they were frozen with liquid N₂ and lyophilized afterwards to remove the water and to ensure no changes in their structure. Later, the samples were covered with a thin coating of gold to increase their conductivity. CryoSEM images were taken with a Scanning Electron Microscope NeoScope JCM-5000 from Nikon with a magnification range of 10x – 20000x at a voltage of 10 kV.

7.2.2.2 Thermal properties

The water content, weight loss and thermal degradation of the “20% w/v PVA” and “20% w/v PVA-Cel” films were obtained from TGA measurements on a TGA 7 from Perkin Elmer (USA). The samples were placed in High Temperature Platinum-TGA pans (HT-Pt) and subjected to a heat program from room temperature to 400 °C at 10 °C/min under a N₂ flow of 25 ml/min.

7.2.2.3 Surface properties

The surface roughness of 4 different textures implemented on the PVA blocks was examined with a laser confocal microscope VK 9700 from Keyence at a magnification of 10x and z-axis resolution of 1 nm. The arithmetic mean (R_a), root mean square (R_q) and peak-to-valley (R_{pv}) values were determined and compared with human skin results.

The contact angle of this layer was measured by using a contact angle device from Dataphysics (Germany) model OCA 20. The implemented textures were subjected to water drops and their contact angle was evaluated and compared to the one of human skin.

The hydration degree of the top PVA layer was measured with a corneometer MPA5 from Courage+Khazaka Electronic (Germany). The hydration of the samples was evaluated at 25 °C and 30, 50, 80 and 100% relative humidity.

7.2.2.4 Mechanical properties: DMA and VAFT measurements

○ Dynamic Mechanical Analysis (DMA) measurements

The mechanical properties of the PVA and PVA-Cel layers were studied with a Dynamic Mechanical Analyser (DMA) VA2000 from Metravib (France). Two different analyses were investigated based on the results of the DMA tests: creep compliance phenomena and the dynamic performance of the samples in compression mode as a function of the frequency. With creep compliance measurements, a load of 0.5 N was applied to the samples for 300 seconds. The retardation times were determined and the results were correlated to the theoretical response according to the Standard Linear Solid (SLS) model. This model describes the mechanical behaviour of a viscoelastic material as a combination of springs and dashpots as shown in Figure 3.

The SLS model defines the *relaxation time*, τ , as the quotient of the viscosity of the material and the elasticity of the spring, E_2 , according to Figure 3. Thus, the elastic moduli, E_1 and E_2 , and the viscosity, η , of the model were calculated by fitting with the experimental results.

Moreover, the dynamic behaviour of the samples was evaluated with applying a frequency sweep from 1 to 100 Hz with a constant strain of 1%. The storage and the loss moduli were studied in that range of frequencies to evaluate the effect of velocity in the mechanical performance of the samples.

Figure 3. a) Schematic representation of the SLS model; b) creep compliance equation as a function of the elastic moduli of the springs, E_1 and E_2 , and the retardation time, τ (top); equation for the retardation time, τ , as a function of the viscosity of the material and the elastic modulus of the spring, E_2 (bottom).

○ **Vacuum Adhesive and Friction Tester (VAFT) measurements**

Series of indentation and friction tests were performed by using the Vacuum Adhesive and Friction Tester (VAFT) designed at the University of Twente [84] with a spherical indenter of 6 mm diameter made of Chrome Steel AISI-52100. The indentation measurements were performed at 80 mN of applied force and an indentation velocity of 50 $\mu\text{m/s}$. Based on the experimental results from the indentations, the standard Oliver-Pharr method [85 - 87] was used to calculate the elastic moduli of the PVA and PVA-Cel samples. Due to the viscoelastic behaviour of the materials an additional power-law correction [88] was used to compensate for viscoelastic effects. A holding time of 5 seconds was used for correction calculations. At the maximum holding time, dP/dt in combination with the unloading speed dh/dt will lead to a viscous stiffness S_v . With the elastic stiffness S_e , the corrected stiffness S_c is;

$$S_c = S_e - S_v \quad (1)$$

This is similar to the derivations in [88] except for the fact the setup is displacement controlled instead of load controlled.

The contact depth h_c in Oliver-Pharr will become;

$$h_c = h_{max} - \varepsilon P_{max} / S_c \quad (2)$$

The reduced Young's modulus will be:

$$E_r = \sqrt{\pi} S_c / 2\sqrt{A_c} \quad (3)$$

Where A_c is the projected contact area at maximum depth.

Moreover, the tribological properties of the PVA and PVA-Cel films against a spherical indenter of 6 mm diameter AISI-52100 were evaluated in a load range from 1 to 80 mN during 5 reciprocating loops with a sliding velocity of 10 $\mu\text{m/s}$. The measurements were performed at 25 °C and 30% relative humidity.

7.3 Results and discussion

7.3.1 Structure and morphology of the building-blocks

SEM images of the PVA and PVA-Cel layers showed the presence of connected micro pores where water is clustered. This structure is clearly visible in Figures 4 (a) and (b) which represent the cross section of a PVA sample at a magnification of x1.100 and x500. Moreover, Figure 4 (c) and (d) indicate the presence of the Cellulose micro fibres and the micro porosity at both magnifications of x950 and x330.

Figure 4. CryoSEM images the PVA and PVA Cellulose samples: a) and b) correspond to cross sections of the PVA samples at the magnifications of x1100 and x500, respectively; c) and d) show the cross section of the PVA-Cel samples at magnifications of x950 and x330, respectively.

7.3.2 Physic - chemical performance of the building-blocks

The amount of water that both samples contained was extracted from the TGA results. The water evaporation of the PVA and PVA-Cel samples started at room temperature and it lasted until 115 °C as shown in Table 1.

Table 1. TGA data collection of the PVA and PVA-Cel samples. The range of evaporation of water, the degradation temperature of the samples and the residue at 400 °C are indicated for each case.

	PVA	PVA - CELLULOSE
Evaporation of water (°C)	30 – 115	30 - 115
Evaporation of water (°C)	261	256.1
Residue (%)	9.9	10.8

Based on these results a water content of $73.8 \pm 0.4\%$ and $71.5 \pm 0.6\%$ was found for the PVA and PVA-Cel samples, respectively. From Figure 5 it can also be seen that the evaporation of water lasts until approximately 140 °C for both samples. Since the boiling point of water is 100 °C, this indicates that water is present in different forms inside the material and to completely dry the hydrogels higher temperature and heating exposure times were needed. Thus, water would be present in the samples as free water molecules in the bulk or linked molecules to either Cellulose or the PVA chains.

Figure 5. TGA curves of the PVA and PVA-Cel. The graph indicates the remaining mass (round black and red markers) and the derivative of the weight (full black and red lines).

Highly noticeable is the increase of the water loss rate ($\%/^{\circ}\text{C}$) at 140°C which is likely due to the definite evaporation of the bounded water molecules. The degradation peak of the pure PVA samples was around 261°C whereas for the PVA-Cel samples the corresponding degradation peak appeared with some delay with respect pure PVA at around 256.1°C . Conversely, the residue of the PVA-Cel fibres was 10.8%, i.e. 0.9% more than the residue of the pure PVA samples. Apparently the Cellulose fibres did not reinforce the PVA structure from a thermal point of view yet, they interlocked with the PVA chains and weaken the network, as it can be extracted from the decrease in the degradation temperature of the samples. This decrease in the degradation temperature of the PVA Cellulose samples in respect to the pure PVA indicates that the PVA was more easily decomposed in the presence of fibres. The relative increase off the differential residue between the two samples was due to the presence of fibres in the PVA-Cel samples.

7.3.3 Mechanical properties from DMA of the building-blocks

The viscoelastic performance of the material was analysed by DMA compression measurements by applying a force of 0.5 N in a range of frequencies from 1 to 100 Hz. The samples were clamped and measured under laboratory conditions of 25°C and 50% relative humidity. The storage and loss modulus of the PVA and PVA-Cel films were evaluated from the dynamic tests. Moreover, creep compliance tests were accomplished to evaluate the properties of the samples based on the SLS model. The *relaxation time*, τ , the elastic moduli, E_1 and E_2 , and the viscosity, η , (see Figure 3) of this model were calculated from the creep experiments. The dynamic measurements revealed a storage modulus between 30-35 kPa for the pure PVA samples whereas the PVA-Cel was between 50-70 kPa as shown in Figure 6.

Figure 6. Storage moduli of the PVA and PVA-Cel samples as a function of the frequency obtained by DMA measurements.

The increase of the storage modulus of the PVA-Cel samples has already been demonstrated and it proves the role of the cellulose in the improvement of the mechanical strength of the hydrogels [89]. Regarding to the sweep frequency measurements, there were no important material changes, since the values of the storage and loss modulus were essentially steady in the whole frequency range. Additionally, the fitting of the creep measurements (Figure 7) with the SLS model addressed to elastic moduli of 38 and 50 kPa for the PVA and PVA-Cel respectively, as indicated in Table 2. The relaxation time, τ , was calculated from the values of E_2 and η given by the fitting with the model. Values of 23.3 and 15.8 seconds for the PVA and PVA-Cel, respectively were obtained as specified in Table 2. The results indicate a highly elastic performance for the two samples at the measured conditions. The bulk of the PVA and PVA-Cel samples showed ultra-soft mechanical properties in the range of the human skin at the meso and macro scales that is, within the range of few kPa, as showed in the literature [65 - 67] (Table 3).

Figure 7. Creep compliance graphs of the PVA (left) and PVA-Cel (right). The strain vs time is presented for the measured data from the DMA tests (blue line) and the calculated values based on the SLS model (red semi-transparent line).

Table 2. A collection of the results obtained from the fitting of the creep compliance data in the SLS model is presented. The values of the elastic moduli of the springs, g_1 and g_2 , the viscosity and retardation times of each material are calculated based on the SLS model.

PVA		PVA – CELLULOSE	
E_1	36 kPa	E_1	75 kPa
E_2	300 kPa	E_2	380 kPa
η	7 MPa · s	η	10 MPa · s
T_2	23.3 s	T_2	15.8 s

7.3.4 Indentation tests & friction tests of the building blocks

The value of the elastic modulus given by indentation is a required input for friction models. Hence, the elastic modulus of the PVA and PVA-Cel samples were obtained from the data analysis of indentation tests using a Poisson's ratio $\nu = 0.5$. The unloading part of the corresponding curves were fit to indentation values calculated with Equation 6. Since the studied materials have a viscoelastic performance, as observed from the DMA results, the applied force was kept constant for 5 seconds. The viscoelastic losses during indentations, which is defined by the ratio between the areas below the loading and unloading curve as shown in Figure 8 is small and viscous corrections (Equation 4) have no significant influence on the calculated elasticity. Figure 8 presents the loading and unloading curves of PVA (grey line) and PVA-Cel (green line) as a function of the depth. The double dash lines correspond to the fitting of the unloading curve according to Hertz for the two studied materials.

Figure 8. Indentation tests of the PVA (red line) and PVA-Cel (black line) samples. The green and blue lines show the fitting of the unloading curves to obtain the elastic modulus of the PVA-Cel and PVA, respectively.

Table 3. Elastic modulus of the PVA and PVA-Cel samples obtained from indentation tests.

Elastic modulus (kPa)	
PVA	PVA - CELLULOSE
45.5 – 54.8	55.7 – 81.5

Friction tests were performed in a normal force range between 1 and 80 mN to evaluate the tribological performance of the PVA and PVA-Cel samples. The COF was evaluated on non-textured surfaces and the results presented in Figure 9 display a power law function of force for the two samples. At forces below 10 mN the friction varied according to a power law function with exponents between 0.42 and 0.25 for the PVA and PVA-Cel, respectively. The PVA samples showed a friction coefficient of around 0.6 at forces lower than 10 mN whereas the PVA-Cel samples indicated a value of 0.2 at the same range of forces. The differences in the COF for PVA and PVA-Cel between 1 and 10 mN can be explained based by the differences in elasticity and surface properties of the samples, such as roughness and shear strength. As the DMA and the indentation results revealed, the elastic modulus of the PVA was lower than the PVA-Cel samples which correspond to higher areas of contact and hence, higher friction for the PVA samples. The friction coefficient at higher forces decreased noticeably. At forces up to 10 mN, the pressures generated at the contact were enough to press the water out of the porous bulk of the samples and squeeze it to the surface [90]. Thus a squeezing effect of the water in the sample occurred at higher pressures with a consequential water lubrication response. Instead, higher friction coefficients are required to approach the frictional performance of human skin which suggested modifications on the interfacial shear strength to increase the COF.

Figure 9. Friction coefficient as a function of the normal load of the PVA (black markers) and PVA-Cel (red markers).

Friction coefficients for the finger pad sliding versus a hard surface between 0.4 and 0.8 are reported [91] for applied normal forces between 1 and 5 N, respectively. The latter work shows a pressure dependence of the friction coefficient with n exponents between 0.2 and 0.5. Since adhesion is the main friction mechanism in the case of the skin – object sliding interactions [51, 56, 92], the contribution of the n exponent to friction is a consequence, either of the contacting areas or of the

interfacial shear strength, τ . Because the area of contact is defined as a function of E_{ff} according to Eq. (2) and the obtained elastic modulus for the measured blocks is in the range of the human skin modulus, the differences in the friction results can only be explained in terms of differences in the interfacial shear strength. Hence, a modification of the surface properties, in particular the interfacial shear strength of the samples, would be required to properly mimic the friction performance of the skin in the same range of forces.

7.3.5 Assembling PVA building-blocks to create a 2-Layered Skin Equivalent

Based on the previous characterization of the PVA and PVA-Cel blocks, the design and construction of a 2-Layered Skin Equivalent (2LSM) was accomplished. The production of the 2LSM started with the fabrication of a PVA top film via freezing/thawing cycles due to its higher friction. Afterwards, this layer was coupled to a viscoelastic bottom layer of PVA-Cel with a similar elastic moduli as dermis [85, 86] to complete the design.

The specific route for manufacturing the overall structure is given by:

1. In a first step, the top layer of PVA was prepared by pouring 6 gr of the 20% w/v PVA solution in deionized water inside the mould formed by parts (a) to (d) (Figure 2). Subsequently, the mould was sealed with paraffin film to avoid irregularities on the sample due to dehydration. Then, the mould was put into a programmed climate chamber to perform freezing/thawing cycles and physically cross link the polymer chains. The samples were exposed to 11 cycles of freezing for 12 h at -20 °C and thawing during 4 h at room temperature.
2. Once the top layer was ready, part (a) of the mould was removed (Figure 2) and 10 gr of the solution “20% w/v PVA-Cel” were poured in inside. Later the mould was sealed with paraffin and the same program described in step 1 was set to crosslink the bottom layer. When the cycles were finished the mould was disassembled and the model extracted.

Figure 10. Schematic image of the 2LSM on the left with indication of the layer's composition. On the right side is presented a confocal image of a cross section of the 2LSM at a magnification of x10.

Figure 10 presents a schematic view of the 2LSM and, next to it, a cross section image obtained after scanning the profile of a sample with a Confocal Microscope VK 9700 from Keyence at a magnification of 10x. The top layer of the 2LSM was moulded with 4 different textures and the resulting samples were equilibrated at different environments to evaluate the humidity gradient of the whole structure. The surface characterization and the humidity gradient are showed in the following sections.

7.3.6 Surface properties of the 2LSM

Confocal microscope images of the 4 different textures stamped on the PVA top layer of the 2LSM were obtained and they are shown in Figure 11. In addition, Figure 12 contains the arithmetic (R_a), root mean square (R_q) and peak-to-valley (R_{pv}) roughness of each layer of the model compared to human skin results from *in vivo* measurements [90] and the texturized samples. The obtained roughness values indicated a good agreement of the PVA surface layer stamped with texture number 3 (Figure 12). The non-texturized PVA and PVA-Cel samples, the ESE and the other prints showed values of roughness different to those expected for the human skin [71-73].

Figure 11. Confocal images of the PVA top layer moulded with four different textures at a magnification of x10.

Figure 12. Roughness of the material components of each layer of the 2LSM (PVA and PVA-Cel) compared to human skin results [97] and the implemented textures. The arithmetic roughness, root mean square and peak-to-valley roughness of each sample is presented.

Based on the output of the confocal measurements, the contact angle of 2LSM samples stamped with the texture number 3 was measured. This result was compared to the contact angle of the PVA, ESE and PVA-Cell layers, and the commercial skin equivalents such as Silicone Skin [39], Lorica [40, 93] or the ESE [42]. All the results were then compared to Stratum Corneum values from the literature [76]. The measured contact angles are collected in Figure 13 which indicates a good agreement between the result of the PVA texturized sample 3 and the Stratum Corneum.

Figure 13. Contact angles of the materials implemented in the 2LSM (PVA and PVA-Cel) compared to two commercial artificial human skin, Lorica and Silicone Skin L7350. A contact angle for the Stratum Corneum is also presented and compared to the value of the texture number 3 implemented on the PVA top layer of the 2LSM.

Hydration measurements were carried out after equilibration of the samples at different relative humidity for 3 days. The top PVA layer was studied at 30% and 50% relative humidity whereas the bottom layer was studied under 80% and 100% relative humidity. Hydration properties of the layers were analysed in conditions comparable to the environments of the various skin layers. Since the normal humidity at room temperature is in between 30% and 50%, the top layer of PVA, representing the Stratum Corneum properties, was measured at similar conditions that the SC is subjected to. Furthermore, the bottom layer, representing the elasticity of dermis, was measured under higher humidity conditions because it is protected against water losses by the Epidermis, and by definition it has a considerably higher amount of water compared to Epidermis. Thus, Figure 14 represents the variation of the hydration observed from the top to the bottom layer at the 4 different relative humidities (2 for each sample) and correlated to the depth of the 2LSM as indicated in the image at the right side. Thus, there is a gradient of hydration which goes from 35 ± 10 A.U. to 110 ± 5 A.U. which can be correlated to the hydration variation of the human skin through its length [94-96].

Figure 14. Hydration variation on the 2LSM as a function of the depth at 2 different relative humidity: in transparent red for the top layer (30% and 50% RH) and, in transparent blue for the bottom layer.

7.4 Conclusions

This paper presents the tribo-mechanical performance of building-blocks based on PVA hydrogel and it evaluates their suitability as a part of a 2-Layered structure to mimic the tribo-mechanical skin performance. The mechanical properties of the individual synthesized layers, analysed via DMA and indentation tests, showed an elastic modulus in the range of the ultra-soft materials which properly described the human skin elasticity. The storage and loss modulus of the PVA and PVA-Cel samples remained constant in the studied range of frequencies. The SLS model was also applied to creep measurements in compression mode and the results address to retardation times of 23.3 and 15.8 s for the PVA and PVA-Cel, respectively. The roughness values and contact angles of one of the studied textures, texture 3, showed similar values as those of the human skin found in literature. Moreover, the water presence in the PVA top layer representing the top layer of the skin, was demonstrated via TGA measurements. SEM images of the lyophilized samples also showed the existence of water cavities (or pores) where the water molecules were clustered. Due to the presence of bound and free water, the PVA-blocks absorbed/desorbed water while adapting to the temperature and humidity changes. Thus, the blocks were equilibrated at different humidity conditions to resemble the environment at which the different skin layers are subjected in normal conditions. When the blocks were implemented in the overall structure, 2LSM, a gradient of hydration from the top to the bottom was correlated to the hydration of the skin according to the given references. The frictional performance of the PVA blocks showed a COF of 0.55 at 1 mN decreasing to 0.13 at 10 mN. In this

range of low forces, the PVA blocks exhibited similar values to those of the human skin in sliding contact against a ball bearing. However, a decrease of the interfacial shear strength likely due to a squeezing effect of the water content in the sample, reduced the friction to values of 0.1 at forces higher than 10 mN. The results for the PVA-Cel blocks indicated similar performance. Lower friction values were obtained at forces between 1 and 10 mN with a COF of 0.23 decreasing to 0.07. At higher forces the existing water film decreased the friction value at around 0.07.

Based on the previous results, the mechanical properties of the PVA-based building blocks, such as the elastic moduli and viscoelastic performance were in good agreement with those expected for the human skin. The tribological performance of the samples was evaluated and the obtained values were similar to the skin at low normal loads. At higher forces the friction decreased probably due to water lubrication. The design and manufacture of a 2-Layered skin equivalent based on this blocks was accomplished. The structure showed comparable hydration capabilities and the surface properties were similar to the human skin when implemented with a specific texture. Further analysis of the tribo-mechanical performance of the overall 2LSM with evaluation of the influence of the layer's thicknesses is desirable at both, dry and hydrated conditions. Besides, a new chemically modified implementation of the top PVA layer of the 2LSM is needed to obtain interfacial shear strength values closer to the human skin.

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Chapter 8

Paper V: *Mimicking the surface properties of human skin: tribo-mechanical and adhesive properties of a new Synthetic Stratum Corneum*

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Abstract

A Synthetic Stratum Corneum (SSC) was produced from a mixture of PVA hydrogel and SDS surfactant, later mixed with rapeseed oil and, finally crosslinked with glutaraldehyde. The thermal properties of the material were studied via TGA and DSC measurements and the composition was analysed by FTIR. The hydration degree of the SSC measured at 30% of relative humidity and 25 °C was 23% whilst at wet conditions it grew up to the 80%. Moreover, the surface adhesion of the SSC, evaluated under dry, normal and wet conditions indicated substantial variations depending on the skin conditions with values for the work of adhesion of -16, -122.4 and -233.4 mN/m, respectively. The viscoelastic properties of the material were also evaluated by tensile tests and stress relaxation measurements. The elastic modulus of the samples was found between 8.8 -1.9 and 3.3 - 1.2 for dry and wet conditions, respectively. Further, stress relaxation times of 64.4 sec and 83.2 sec were calculated from the SLS model at dry and wet conditions, respectively. Lastly, the SSC presented a frictional performance in close agreement to that of human skin under normal (36 % relative humidity and 25 °C) and wet conditions. The friction coefficient, measured between 1 and 50 mN, was found between 0.8 to 0.45 at dry conditions whereas, at wet conditions, it ranged between 2 and 0.45. At higher applied forces between 0.5-3.5 N, the friction coefficient was nearly steady with values of 0.4 and 0.55 for dry and wet conditions, respectively.

8.1 Introduction

The top layer of human skin, the stratum corneum (SC), plays an essential role in the hydration and the adhesive properties of the skin and, subsequently in its tribological behaviour [1, 2]. SC comprises a thin bi-phasic layer of 10-20 cells with an average thickness between 25 – 200 μm depending on the body site. It is commonly described in the literature as a “brick and mortar” structure because is comprised of “brick” regions, the corneocytes, surrounded by a “mortar” phase of fatty acids, ceramides, cholesterol and water [4, 5]. The surface of the SC is coated with a film, the Skin Surface Lipid Film (SSLF), a mixture of sebum from the sebaceous glands and lipids secreted by keranocytes. The SSLF is composed of triglycerides, squalene, wax esters and ceramides [5-7]. The proportions of these components are known yet, there is inter-individual variability and also variations depending on the body site [8, 9]. Epidermal lipids are mainly composed of fatty acids in forms of mono-, di- or triglycerides [10, 11] such as, Palmitic, Palmitoleic, Stearic, Oleic and Linoleic acids [11, 12]. The characteristic structure of the SC and the composition of the SSLF provides rigidity to the membrane and prevents the skin dehydration [6, 13, 14]. An alteration in the chemical composition or amount of the saturated and unsaturated lipids from SSLF results in an increase of the Trans Epidermal Water Loss (TEWL) with further modifications of the interfacial and mechanical properties of the SC [15-18]. In the literature, changes in the physic-chemical properties of the SSLF have shown a reduction of the SC's barrier functions after tape stripping experiments [19-21]. Therefore, the composition of the SSLF seems to have a role in the adhesive and mechanical properties of the SC and, consequently, in its frictional performance [22-27].

Attempts to synthesize an artificial SSLF to mimic the adhesive properties of the skin have been performed previously [28-30]. A pseudo-sebum based on a mixture of nine saturated and unsaturated lipids was coated on top of Lorica to analyse the surface properties of the model in relation with its frictional performance [28]. In vitro models of SC from plastic surgery have been used to study the mechanical performance of the SC and determine its contribution to the overall skin response [31]. However, the availability of *ex vivo* samples from cadaveric or surgical source is limited and the ethical and sanitary arrangements required to manipulate biological tissue imply a limitation at industrial scale. Besides, although isolated human SC could serve as a model, the isolation process alter the natural state and properties of the SC due to the use of chemical agents. Furthermore, *in vivo* studies present inter-individual variability and testing-device limitations.

Different polymers and, particularly hydrogels have been research in the last decades for biomedical uses thanks to its inherent biocompatibility and hydration properties [32, 33]. Hydrogels have been used to manufacture contact lenses [34] or wound dressings [35], in cartilage replacement

applications [36], drug delivery systems [37] or artificial organs [38]. Within the existent hydrogels, poly vinyl alcohol (PVA) represents a relatively economical choice with ease manufacturing. PVA has been used to develop wound dressings [39, 40] due to its ability to maintain hydration and prevent dryness, its elastic and non-toxic properties and the wide number of crosslinking methods already known [41]. In our previous research [42], it was suggested a modification of the shearing properties of PVA blocks to approach the frictional performance of human skin by simply modifying the crosslinking approach. Additionally, the properties and uses of rapeseed oil (Ro) are broadly researched currently for bio diesel applications, in the biotechnology field (antibiotics, antioxidants, vitamins) and as a source of vegetable oil with positive health properties [43]. Rapeseed oil is relatively cheap and facile to acquire. It is a rich source of natural components with antioxidant properties such as vitamin E, phenolic compounds, phytosterols or phospholipids. Moreover, some of these compounds protect the skin against lipid peroxidation due to UV exposure and ionizing radiation [44]. Rapeseed oil is rich in phospholipids which are fundamental constituents of the cells membranes with an essential role their physicochemical properties such as, the homeostasis or the signal transduction. Further, the main fatty acids in the rapeseed oil are saturated fatty acids in an amount of 7.46 wt. %, 64.06 wt. % of mono un-saturated fatty acid and 28.48 wt. % of total poly-unsaturated fatty acid. These fatty acids are mainly Palmitic, Palmitoleic, Stearic, Oleic, Linoleic and α - Linoleic acids some of which are widely present in the SSLF [11, 12, 45].

Therefore, in this paper the development of an alternative Synthetic Stratum Corneum (SSC) is described and the evaluation of its tribo-mechanical behaviour is presented. The SSC was produced from a mixture of PVA hydrogel and SDS surfactant, later mixed with rapeseed oil and, finally crosslinked with glutaraldehyde. The thermal properties of the films were determined via DSC and TGA measurements. FTIR measurements were performed to confirm the presence of the rapeseed oil and evaluate the changes in the functional groups after wetting the samples. The SSC was texturized with the features of the thigh of an individual and the roughness parameters were determined. Further, the contact angle and hydration degree at different relative humidities were obtained. The mechanical properties of the films were evaluated by tensile tests and stress relaxation measurements from which the viscoelastic parameters were calculated based on the SLS model. Further, the adhesive properties were determined based on pull-off measurements and the frictional behaviour of the SSC was evaluated in two different range of forces between 1-50 mN and 0.5-3.5 N.

8.1.1 Tribo - mechanical properties of the human stratum corneum

8.1.1.1 Elastic modulus

The elastic modulus of the stratum corneum is a key parameter to describe the frictional performance of human skin. In brief, the SC has been assessed by indentation tests at different conditions which results showed an elastic modulus scattered in a wide range of values (MPa – GPa) [46-50]. Results from nano-indentation on porcine isolated stratum corneum were analysed by Yuan and Verma [51] who found an elastic modulus of 120 and 26 MPa for the stratum corneum at dry and wet conditions respectively. Isolated stratum corneum measured by indentation indicated an elastic modulus of 2.6 ± 0.6 MPa according to Geerlings [47]. Further, Pailler-mattei et al. [46] showed large differences between the results from *in vivo* skin and isolated stratum corneum. The latest indicated a mean elastic modulus around 1 GPa which decreased with the indentation depth. Moreover, Crichton et al. [52] analysed the mechanical properties of the individual layers of mouse skin. Their results indicated a larger elastic modulus for dermis (7.33 MPa) than for the stratum corneum (at 0.75 - 1.62 MPa) under similar conditions. Van Kuilenburg et al. [53] presented a collection of the elastic modulus of the human skin from indentation as a function of the length scale. These results indicate a decrease of the elastic modulus of several orders of magnitude with the increase of indentation due to the layered structure of the skin. Thus, at indentation depths no larger than 10 μm , the elastic properties of the skin can be described only by the stratum corneum which elastic modulus vary between GPa and MPa depending on the indentation and the skin conditions (dry or wet) [48-50].

8.1.1.2 Viscoelastic properties: stress relaxation measurements

The viscoelastic properties of the human skin are mostly due to the nature of dermis yet, the stratum corneum also contributes especially, at wet conditions [51, 52]. According to the literature the SC exhibits a short period of viscoelasticity which is mainly associated to the gradient of hydration from the top to the bottom of this thin layer [52]. In this paper, the viscoelastic properties of the SSC were analysed based on the Standard Linear Solid model (SLS):

$$E(t) = \frac{E_1}{E_1 + E_2} (E_2 + E_1 e^{-t/\tau}) \quad (1)$$

The elastic moduli, E_1 and E_2 , and the viscosity, η , were calculated by fitting the stress relaxation results in Eq.19. Later, the *relaxation time*, τ , was calculated as:

$$\tau = \frac{\eta}{E_1 + E_2} \quad (2)$$

8.1.1.3 Frictional performance

The frictional behaviour of the skin is significantly affected by the properties of the stratum corneum, especially at low forces [54-56]. When a smooth hard material contacts the skin, already at very low forces the true area of contact reaches the nominal one and the effect of the surface properties of the skin are mainly commanded by the Skin Surface Lipid Film (SSLF) on top of the stratum corneum. Thus, the coefficient of friction is mainly determined by means of the adhesive properties of the skin with little effects of its roughness. Previous work of several authors gives a wide overview of the most relevant factors influencing skin friction [54-60]. Derler et al. [59] analysed the contact situation of *in vivo* skin against rough and smooth surfaces at dry and wet conditions. Their results suggested the adhesive component of friction as the main friction mechanism whilst the contribution of the hysteresis due to the viscoelastic properties of the skin was minor [57, 61]. Additionally, hydration increases the coefficient of friction of skin as it has been extensively probed in other research [57, 60-64]. The effect of water, creams and moisturizers affects mainly the elastic properties of the stratum corneum with a subsequent effect in its adhesive properties and frictional performance [12]. Thus, under dry conditions the skin shows a coefficient of friction typically between 0.25 and 0.5 depending on the body location, the contacting material, age of the individual or indenter size among other. Conversely, in the hydrated case, the coefficient of friction increases as consequence of the softening effect of the stratum corneum and the increase of the adhesion forces at the contact. A range of coefficient of friction from 0.7 to 2.6 have been found for the coefficient of friction at wet conditions for a range of applied forces between 0.7 and 6.4 N [58]. The mechanism behind the differences between dry and wet conditions is not clearly known yet. Persson [63, 64] found that the effect of capillarity depends on the elastic modulus of the soft material, so that a decrease on the elasticity addresses to an increase of the capillary force that, likely enlarges the friction force.

8.2 Material and methods

8.2.1 Synthesis

The Synthetic Stratum Corneum (SSC) was created from a mixture of poly vinyl alcohol (PVA) (Mw 31,000-50,000) purchased from Sigma Aldrich (USA) and rapeseed oil (Ro) provided by Grease Factory of Lanzhou (China) with no further treatment. Besides, a 0.5 % wt. of Sodium Dodecyl

Sulphate (SDS) 99% from Sigma Aldrich (US) was combined with the PVA to enhance the solubility of the rapeseed oil in the PVA aqueous solution. Glutaraldehyde (GA) 50% solution reagent grade from AMRESCO (USA) was used as a cross linker agent. To start with the crosslinking reaction, sulphuric acid (H_2SO_4) 96% provided by Acros Organics (USA), was used as initiator. Further, a flat square Teflon mould 150x80 mm was used to create the films. To resemble the roughness of the human skin on the surface of the SSC films, a copy of the thigh surface of an individual between 25-30 years was obtained after applying the Panasil Initial Contact from Kettenbach (DE). The rubber copy was scanned by confocal microscopy and from this image an acrylate mould was obtained by laser engraving with by a Trotec Speedy 300, manufactured by TrotecLaser (USA).

The specific procedure to synthesize the films is explained as follow: first, a solution of 20 wt. % (w/v) of PVA and 0.5 wt. % of SDS in deionized water (DW) was prepared by introducing the components in a round bottom flask with a reflux and stirring over night at 90 °C. Then, 2 wt. % of Ro, respect to the PVA amount, was added to the solution and it was vigorously stirred during 3 minutes. Second, certain amount of PVA-oil solution was taken and 5 wt. % of H_2SO_4 (aq) (pH=1), with respect to the taken amount, was added as a catalyst for the formation of the acetyl groups between PVA and GA. The mixture was stirred for one minute and afterwards, GA cross linker was added in a ratio 10:1 of PVA to GA, respectively. The mixture was stirred for one minute and it was subjected to vacuum to avoid the presence of bubbles in the formed films. Finally, the acrylate mould of the thigh roughness was placed on the bottom of the Teflon mould with the texture looking up. Then, the solution labelled as PVA-oil-GA, was poured in the mould and introduced in the oven at 40 °C for 16 hours.

8.2.2 Physico – chemical characterization

8.2.2.1 *Thermal & chemical properties: TGA, DSC, FTIR*

The thermal behaviour of the SSC was analysed by TGA and DSC measurements. The weight loss, water content and degradation of the samples was evaluated from TGA measurements on a TGA 7 from Perkin Elmer (US). The samples were subjected to a heat program from room temperature to 350 °C at 10 °C/min under a N_2 flow of 25 ml/min. Moreover, thermal transitions were determined on a DSC from Mettler Toledo with temperature range from room temperature to 300 °C at 2 °C/min under a N_2 flow of 25 ml/min. The glass transition and melting points of the samples were evaluated. Additionally, a Fourier Transform Infrared (FTIR) spectroscope with a resolution of 0.5 cm^{-1} from Perkin Elmer (US) was used to collect the infrared spectra of the SSC samples. The spectroscopy of SSC samples was measured within the range of 7800 – 370 cm^{-1} at normal (36% relative humidity and 25 °C) and wet (immersed in deionized water for 2 mins) conditions.

8.3.1 Surface & hydration properties

The surface properties of the SSC, such as roughness and contact angle were determined in the texturized samples. A laser confocal microscope VK 9700 from Keyence with z-axis resolution of 1 nm was used to determine the arithmetic mean (R_a), root mean square (R_q) and peak-to-valley (R_{pv}) of the samples at a magnification of 10x. Additionally, the contact angle formed by water drops on top of the SSC was measured by a contact angle device from Dataphysics (Germany) model OCA 20. Further, the hydration degree of the SSC was determined with a corneometer MPA5 from Courage+Khazaka Electronic (Germany) at 25 °C and 30%, 70% relative humidity and also in fully hydrated samples. The latest were immersed in water 10 seconds and then, placed on a tissue paper. Later the measurements were performed.

8.2.4 Mechanical properties

8.2.4.1 *Tensile & stress relaxation tests*

The mechanical properties of the SSC were determined with an extensometer Zwick Roell model Z1.0 (Germany). Tensile tests were performed in 4 SSC samples at both, normal (25 °C and 36% relative humidity) and wet conditions. The dimensions of the tested samples are specified in the ISO 37, ASTM D412 Tensile Testing of Rubber and Elastomers. The samples, with a grip to grip separation of 45 mm, were subjected to a preload of 0.05 N and the tests were performed at a velocity of 10 mm/min. From the tensile tests, the elastic moduli of the samples were obtained as the slope of the stress – strain curve at strains of 0-0.1, 0.1-0.2 and 0.2-0.3 for the normal conditions. In the case of the wet samples, the elastic modulus was also calculated as previously at strains of 0-0.05, 0.05-0.1 and 0.1-0.15.

Additionally, stress relaxation tests at both, normal and wet conditions were carried out at strains of 2% and 10% during 150 and 300 seconds, respectively. The wet conditions were achieved after immersing the SSC samples in deionized water during one minute. The results of these tests were correlated to the Standard Linear Solid (SLS) model and the characteristic parameters of each model were calculated by fitting the experimental results.

8.2.4.2 *Adhesive properties from pull-off measurements*

Series of pull-off measurements were performed by using a Vacuum Adhesive and Friction Tester (VAFT) designed at the University of Twente [65] with a spherical indenter of 6 mm diameter made of Chrome Steel AISI-52100. The snap-off forces obtained from these measurements were used to

calculate the work of adhesion, W_{12} , between the 2 materials based on the JKR model [66]. An unloading speed of 50 $\mu\text{m/s}$ was used during the pulling-off. The work of adhesion, W_{12} , was extracted from Equation (21):

$$F_{a_{JKR}} = 1.5\pi RW_{12} \quad (21)$$

With $F_{a_{JKR}}$ the snap-off force, R the radius of the indenter and W_{12} the work of adhesion when $z = z_0$.

8.2.5 Frictional performance at two different range of forces

Friction tests were performed in a range of forces between 1 to 50 mN with the VAFT mentioned in the previous section. The measurements were carried out at a velocity 10 $\mu\text{m/s}$ during a sliding distance of 400 to 2000 μm . The SSC samples were glued to a bottom layer of PVA hydrogel obtained by freezing/thawing cycles as it was described in our previous research [42]. Additionally, friction test at loads between 0.5 and 3.5 N were conducted on a HC 4057 pin-on-disk machine (CSM, Switzerland). In this case, the friction measurements were conducted with cylindrical pins made of Chrome Steel AISI-52100 of 10 mm length and 30 mm radius. The measurements were performed at normal conditions of 36% relative humidity and 25 $^{\circ}\text{C}$ and wet conditions. It has been shown in earlier research that, the coefficient of friction presents its maximum reaction to the water effect during the first 2 min of exposure [27, 54]. Thus, several drops of deionized water were dropped on the SSC during 2 min and later the excess of water was removed with a tissue. Then, the friction measurements at wet conditions were performed.

8.3 Results and discussion

8.3.1 Thermal properties & Fourier Transform Infrared (FTIR) spectroscopy

8.3.1.1 Thermogravimetric Analysis (TGA)

The normalized loss weight as a function of the temperature is presented in Figure 1 for the SSC, PVA crosslinked with GA (PVA-GA) and PVA physically crosslinked via freezing-thawing cycles (PVA f/t). Moreover, an example of the onset of the degradation, calculated as the intersection of the two tangent lines of a drop, is also illustrated in Figure 1.

Figure 1. TGA curve for SSC sample (red line), PVA crosslinked with GA (green line) and PVA crosslinked via freezing/thawing cycles (black line).

The results extracted from the TGA curves above, such as water percentage, the onset of the degradation temperature and the residue for each sample are collected in Table 1.

Table 1. Percentage of water (between room temperature and 120 °C), degradation temperature and residue (between 400 and 500 °C) for the PVA-GA, PVA f/t cycles and SSC samples.

	WATER (%)	DEGRADATION T^a (°C)	RESIDUE (%)
PVA – GA	15	340	3.5
PVA f/t cycles	90	230	1.5
SSC	25	288	5

Although the boiling point of water is 100 °C, some water molecules remained attached to the polymer chains at higher temperatures as indicate the DSC results presented in the following section, particularly in the case of PVA-GA and SSC. Thus, the water content of the samples was calculated as the loss weight between room temperature and 120 °C which corresponded mainly to free water clustered in the hydrogel whereas another small percentage remained until higher temperatures linked to the polymer chains. Based on these results, SSC samples contained a 25% of water as indicated in Table 1, which is considerably lower than the 90% of the PVA crosslinked via freezing/thawing and similar to the 15% obtained for the PVA – GA samples. The onset of the degradation temperature for the SSC corresponded to 288 °C, lower than the 340 °C of the PVA – GA likely due to the presence of oil with smoke point around 200 °C. The degradation of the PVA crosslinked via freezing – thawing cycles started around 230 °C. Furthermore, PVA – GA and SSC samples show a less evident change of the curve around 380 °C which seems to be related to the

decomposition of main chain of PVA while, the previous step corresponded to the elimination of side-groups between 200 and 330 °C approximately. The residue of each sample is also given in Table 1. The maximum value was obtained for the SSC and it is of 5% which might corresponds to not decomposed traces of the main chain on PVA [67, 68].

8.3.2 Differential Scanning Calorimetry (DSC)

The results from the DSC measurements are shown in Figure 2. Images a), b) and c) from Figure 2 correspond to the individual tests for each analysed sample: PVA-GA, PVA f/t cycles and SSC, respectively; image d) collects the information of the three samples. In Table 2 the temperatures correlated to each transition, which were calculated as the intersection of the two tangent lines of an specific step, are presented (see image a) from Figure 2.

Figure 2. DSC curves of the (a) PVA-GA, (b) PVA f/t cycles and (c) SSC. Image d) collects the DSC results for the 3 analysed samples: SSC (red line), PVA - GA (2-point-dash line) and PVA f/t cycles (point-line).

Table 2. DSC temperature transitions for the PVA – GA, PVA f/t cycles and SSC samples.

	PVA – GA	PVA f/t cycles	SSC
1 ST (°C)	110	86	74
2 ND (°C)	338	223.5	168
3 RD (°C)	-	-	218

The first transition in a DSC curve used to be correlated to the glass transition temperature of the polymer. However, in these cases the large peak representing the first transition shows mainly the evaporation of water, overlapped with the glass transition. Thus, it was not possible to determine the exactly glass transition temperature. It is being indicated a glass transitions temperature for the PVA polymer around 80 °C [69-71] yet, for crosslinked samples the glass transition shifts. Moreover, the increase of the water content in the hydrogel causes also a change of the glass transition towards lower temperatures [72]. The SSC here presented was composed of PVA chemically crosslinked with GA. In its structure were also contained around 20% wt. of water and rapeseed oil. Thus, the effects of water and oil likely shifted the glass transition yet, the exactly temperature could not be determined probably hidden by the large peak of water. Furthermore, the second relaxation in the DSC graphs of PVA-GA and PVA f/t cycles was attributed to the melting point of the polymer. The peaks in these cases appeared at 338 and 223.5 °C, respectively. In the SSC samples a second peak emerged at 168 °C which can be attributed to the crystalline relaxations of the PVA [72] while the last peak corresponded to the melting point of the PVA at around 218 °C as indicates Table 2.

8.3.3 Fourier Transformed Infrared (FTIR) spectroscopy

The fatty acids presented in the rapeseed oil were determined by comparing SSC samples at normal and hydrated conditions. The results of the spectroscopy are shown in Figure 3 and the main peaks related to the PVA, GA and fatty acids of the rapeseed oil are indicated. Strong resonance absorption appeared around 3300 cm⁻¹ due to stretching of intermolecular and intramolecular hydrogen bonds in the PVA polymer chains and the water molecules [73, 74]. As it can be seen from Figure 3, the intensity of the peak at hydrated conditions was higher due to the increase of –OH groups in the sample linked to the water. The small peak appearing around 2950 cm⁻¹ was related to the CH₂ stretching of the in the acetal bound between PVA and GA [73, 74]. Between 2850 and 2930 cm⁻¹ two sharp peaks appeared which correspond also to CH₂ stretching bands related to fatty acids chain of the rapeseed oil [75]. Another peak related to the presence of rapeseed oil appeared at 1746 cm⁻¹ and it is most likely related to the C=O (ester/acid) stretching of the glycerides present in the oil [75]. This peak appears only in the SSC samples at normal conditions which indicates that after immersion in

water an unknown amount of oil is removed that is, in wet conditions this peak is not visible. On the contrary, the peak appearing around 1690 cm^{-1} , related to hydroxyl groups (-OH), is larger in the case of wet samples due to the higher amount of hydroxyl groups introduced by the water molecules. Further, -CO stretching bands around 1100 cm^{-1} can be attributed to the acetal ring and also to other -CO stretching groups in the fatty acids of the rapeseed oil [75].

Figure 3. FTIR curves for SSC at normal conditions of $25\text{ }^{\circ}\text{C}$ and 30% relative humidity (grey line), SSC at wet conditions compared to PVA crosslinked via freezing/thawing cycles (blue line).

8.3.4 Surface & hydration properties

The SSC samples were stamped with an acrylate mould previously texturized with the features of the human skin of the thigh of an individual. The roughness parameters R_a , R_q , R_{pv} and R_{sk} corresponding to the average of 4 SSC samples are presented in Table 3.

Table 3. Surface properties of the samples: R_a , R_q , R_{pv} , R_{sk} values of the roughness are presented as well as the contact angle of the samples.

R_a (μm)	R_q (μm)	R_{pv} (μm)	R_{sk} (μm)	CA ($^{\circ}$)
21.4 ± 0.1	26.8 ± 0.2	42.8 ± 0.1	0.19 ± 0.2	74.3 ± 3

These values have been correlated to human skin roughness from the literature which indicates values of 19.6 ± 4 for R_a [76] and 30 ± 7 for R_q , the latest obtained as an average of different body places [77].

Other researches showed similar values as well in the range of the roughness measured on the SSC [78, 79].

Additionally, the average and standard deviation of the contact angle of the samples is indicated. The contact angle of the SSC was 74.3° which is within the range of values given by Ginn et al. [80] in previous research between $58 - 104^\circ$ for different skin conditions. This results is also in agreement with other previous research [81, 82]. Furthermore, a 3D image and a 2D grey scale image of a SSC sample is shown in Figure 4.

Figure 4. Confocal microscope images of the SSC after stamping with the features of the thigh. On the left side, a 3D colour image is presented with a height scale on its left; on the right side, a grey scale image of the stamped texture is given.

The hydration of the SSC samples was evaluated after equilibration at 30% and 70% relative humidity. Further, it has been indicated that the coefficient of friction of human skin at wet conditions presents its maximum during the first 2 min after applying water [27, 55]. Thus, other 4 SSC samples were immersed for 2 minutes in water to wet them and the hydration was measured afterwards. These results are displayed in Figure 5.

Figure 5. Hydration level of the SSC samples after equilibration at 30% and 50% relative humidity and after immersion in water for 1 minute.

The average hydration measured at 30% was 23.4 A.U. and 49.7 A.U. at 70% relative humidity whilst the fully hydrated sample presented a hydration value of 80.3 A.U. Hydration of the skin changes considerably from person to person and depends also on the temperature and humidity of the environment. However, the SSC samples exhibited a hydration level in the range of the values obtained at different body sites for the human skin [83, 84].

8.3.5 Tensile & stress relaxation tests

The mechanical properties of the SSC were analysed by tensile tests and stress relaxation measurements in 4 SSC samples under both, normal and wet conditions. A discussion of the obtained results is discussed in the following sections.

8.3.5.1 Tensile tests

The elastic modulus of the SSC was evaluated as the slope of the curve ‘stress vs strain’. The initial elastic modulus, E_0 , calculated at $t=0$ s, was considerably higher than the elastic moduli, E_n , calculated between 0-0.1, 0.1-0.2 and 0.2-0.3 strains at normal conditions. At wet conditions the samples were breaking at lower elongations so that, the E_n was calculated between 0-0.05, 0.05-0.1, 0.1-0.15 strains. The average values for each case are presented in Table 4.

Table 4. Elastic modulus of the SSC at dry (left table) and wet (right table) conditions.

	Dry conditions (MPa)				Wet conditions (MPa)			
	E_0	E_1	E_2	E_3	E_0	E_1	E_2	E_3
average	8.8	2.7	2.1	1.9	3.3	1.2	1.2	1.2
SD	0.6	0.3	0.2	0.2	0.4	0.2	0.3	0.3

The initial elastic modulus, E_0 , was about 2.5 times higher at normal conditions compared to the hydrated case. Further, a considerable decrease of the modulus was observed at different strains for instance, in the normal case, where E_3 presented a decrease of 4.5 times the initial value E_0 . Figure 6 presents the evolution of the elastic modulus at normal and wet conditions as a function of the strain. There is a clear decrease of the modulus at wet conditions respect to normal with a faster stabilization of the modulus at larger strains in the wet case. Moreover, the fracture of the wet samples occurred at lower elongations, specifically at 15% strain whereas the normal samples were stretched until 30% of their elongation as indicates in Figure 6. From the previous figure it can also be observed that the elongation at fracture of the SSC depends also on the water content of the samples.

Figure 6. Evolution of the elastic modulus at dry (red line) and wet (green line) conditions at different strains.

8.3.5.2 Stress relaxation tests

Stress relaxation measurements were performed at a strain of 10%. The results were fitted in the Standard Linear Solid (SLS) model and the elastic moduli, E_1 and E_2 , and the viscosity, η , of the model were calculated. Further, the relaxation time, τ , as the quotient of the viscosity of the material and the sum of the two elasticities E_1 and E_2 , was also obtained. Table 7 collects the data of the 4 studied samples and the average values for the normal and wet conditions. As it is expected because of the plasticizer effect of water in hydrogels [85], the elastic moduli of the two springs, E_1 and E_2 presented lower values in the hydrated case. Especially notable were the differences in viscosity between normal and wet conditions. In normal conditions, the viscosity was considerably higher than the value found at wet conditions yet, the relaxation time is higher in the wet case than the normal. This can be explained due to a poorer resistance of the material to be deformed in wet conditions as a consequence of the presence of water.

Table 7. Summary of the parameters from the SLS model obtained after fitting. E_1 , E_2 , η and τ are presented for both, dry and wet conditions.

	Dry conditions (25 °C, 30% RH)				Wet conditions (2 mins wet)			
	E_1 (MPa)	E_2 (MPa)	η (MPa·s)	τ (s)	E_1 (MPa)	E_2 (MPa)	η (MPa·s)	τ (s)
average	11.6	15.0	1712.5	64.4	3.2	3.3	540	83.2
SD	1	1	74	2	0	1	68	4

Water modifies the thermodynamics of the material and it leads to a higher mobility of the PVA polymer chains within the hydrogel. Hence, the material becomes more easily deformable with a consequent decrease of the resistance to be deformed under stress so that, the viscosity, η , decreases. However, the softening effect of water in the hydrogel causes a decrease of the E_1 and E_2 and a subsequent, lower relaxation time than under normal conditions.

8.3.6 Adhesive properties from pull-off measurements

The adhesive properties of the SSC obtained from pull-off measurements at forces between 1 and 50 mN are presented in Figure 9 for very dry (20% relative humidity; 25 °C), normal (36 % relative humidity; 25 °C) and wet conditions. The wet conditions were considered after 2 mins of soaking the sample in accordance with frictional results on human skin which show a maximum variation of the COF during the first 60 sec after soaking [27, 55]. Then, the excess of water was removed with a paper tissue. The results presented in Figure 9 indicate a clear increase of the snap-off force with the humidity. Moreover, the adhesive forces presented a slight increase with the normal force which can be explain due to the increase of the area of contact at higher loads. From 10 mN on the adhesive force presented more steady values that vary depending on the environmental condition.

Figure 9. Snap-off forces of the SSC at 3 different conditions: dry SSC (red), normal SSC (grey) and humid SSC (black).

Figure 10 presents the average adhesive forces of 4 samples at 50 mN for each condition. Although the scattering is considerable, the differences between conditions are clear, especially when comparing the case of 20 % relative humidity with 36 % or wet conditions. At wet conditions, snap-off force presents the higher value due likely to the modification of the surface properties of the skin under the presence of water so that, changes in capillarity and adhesion appeared due to Van der Waals interactions.

Figure 10. Adhesive forces of the SSC at different humidity conditions and a force of 80 mN.

Additionally, the work of adhesion for the skin samples was calculated from Equation (21) at different conditions and the results are presented in Table 8.

Table 8. Work of adhesion SSC at different conditions.

20 % RH	36 % RH	Wet conditions
$-16 \pm 3 \text{ mN/m}$	$-122.4 \pm 18 \text{ mN/m}$	$-233.4 \pm 7 \text{ mN/m}$

According to these results, at very dry conditions of 20% relative humidity, the work of adhesion was considerably low at 80 mN. As the humidity increased, the work of adhesion increased almost ten-fold as it can be seen for 36% relative humidity. At wet conditions, the adhesive force indicated a highest value of $-233.4 \pm 7 \text{ mN/m}$ which evidence the influence of water in the adhesive properties of the skin which can aid to explain the differences on its frictional performance. Moreover, the results obtained for the work of adhesion at the studied conditions are in the range of values obtained in previous research [56, 86]. The differences in the adhesive force previously presented cannot be explained in terms of JKR contact area so that, they might be a result of the role of capillary forces in the adhesion of human skin as it has been previously suggested.

8.3.7 Frictional performance of the SSC at two different range of forces

The frictional behaviour of the SSC samples was evaluated between 1 and 50 mN, so-called the meso scale, and from 0.5 to 3.5 N, the macro scale, for both, dry (36 % relative humidity and 25 °C) and wet conditions (after 2 mins of wetting the samples). These results are displayed in Figure 11 as a function of the logarithm of the normal force. At dry conditions, the COF at the meso scale showed a power

law decreasing from 0.8 to 0.4 as the normal force increased. The influence of water in the adhesive properties of the skin increased the COF to values around 2 under wet conditions. As the force increased, the COF decreased to values around 0.5. At the macro scale, the coefficient of friction showed a more steady behaviour with values between 0.4 and 0.3 at dry conditions. The effect of water at the macro scale was not that relevant as for the meso scale and with a slight variation with respect to the dry case with values between 0.5 and 0.45. The results obtained for the SSC at both scales were in good agreement with the frictional results of *ex vivo* skin obtained in our previous research [87]. Additionally, these values were compared to *in vivo* skin results from the literature which indicate a COF for dry skin between 0.26 – 0.85; for wet skin the literature presents values ranging from 2.62 – 0.92 depending on the force and other boundary conditions [23, 28, 59, 62].

Figure 11. Coefficient of friction of the SSC samples presented as a function of the normal force according to a logarithmic scale.

8.4 Conclusions

The present work describes the development of a Synthetic Stratum Corneum (SSC) and its tribo-mechanical performance. To better understand the material's performance and determine its water content and melting temperature, TGA and DSC measurements at dry and wet conditions were accomplished. The surface features of the thigh of an individual copied in the SSC were analysed under confocal microscope and the roughness parameters determined. Moreover, the hydration of the SSC samples was evaluated at 20% and 36% relative humidity and for wet samples with a good correlation with results of *in vivo* skin at different body places from the literature. The mechanical

properties of the SSC were analysed under tensile and stress relaxation tests. The elastic modulus presented similar values to *in vivo* skin from the literature which supports the suitability of the SSC in terms of mechanical properties. The adhesive response of the SSC under different conditions showed the important influence of water on the surface properties of the material. Further, these values SSC were in accordance with human skin results from previous research. Lastly, the frictional performance of the SSC evaluated at the meso and the macro scale indicated a proper correlation ship with the tribological behaviour of the skin in the same range of forces. The coefficient of friction at the meso scale showed was described as a power law function of force, with values of COF at 1 mN around 2 and 5 for normal and wet conditions, respectively. At the macro scale, the results were steadier as a function of the normal force with only slight differences from dry to wet conditions and a COF around 0.4 at normal conditions and 0.5 at wet conditions.

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Chapter 9

Paper VI: *Bi-Stratified Skin model to account for the length scale dependence of the elastic modulus of human skin*

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Abstract

The length scale dependence of the elastic modulus of the skin is approached in this work by a Bi-Stratified Skin model (BSSM) composed of a bulk PVA hydrogel physically crosslinked with a top of a previously developed Synthetic Stratum Corneum. Due to the different thickness and mechanical properties of the BSSM the elastic modulus showed a length scale dependence with values between 0.5 MPa and 0.2 MPa at 20 % relative humidity and 125 to 60 kPa at 36 % relative humidity. The elastic modulus decreased as the force increased due to the layered structure and the softer bottom layer of the BSSM. Besides, the evaluation of the elastic modulus at different conditions showed a decrease of the elastic modulus from 0.5 MPa at 20% relative humidity to 95 kPa at wet conditions. The results of the material were compared to those of a theoretical model for the elastic modulus of the skin based on a skin's structure composed of stratum corneum, viable epidermis, dermis and hypodermis as underlying tissue. For the theoretical analysis both, the Hertzian, the JKR contact model and a modified version of the Hertz model with capillary force, were used with a better agreement of the Hertzian results at dry conditions. At wet conditions the role of capillarity appeared to be required to fit the model with the experimental results from the BSSM.

9.1 Introduction

The design of products in contact with the skin in the everyday life requires experimental testing to ensure the functionality and comfortability during use. Personal care and consumer products, sportswear, tools or medical devices are some of the many products which interact with the skin. Thus, an analysis of the mechanical and surface properties of the contacting materials as well as, the environmental and operational conditions need to be addressed for a more profound understanding of the interaction “skin-materials” during motion. The elastic properties of the surface of skin are one of the main aspects of research as it controls partially the area of contact, see for example work in skin tribology [1-4] or more recently, in field mechanics [5].

Human skin is a structure composed of three main layers: Epidermis, Dermis and Hypodermis, each one with different properties and hence, different contribution to the overall response of the skin under stress. Epidermis is structured in several sub-layers which outermost level, the so-called Stratum Corneum (SC), has a crucial role in the tribo-mechanical performance of the skin. It is a 20-50 μm thick layer with a relative high elastic modulus, in comparison with other skin's layers, which is also greatly influenced by hydration [6-9]. Further, on top of the SC there is a film composed of sebum, secreted by the exocrine glands, and other lipids which conform the Skin Surface Lipid Film (SSLF) [10-12]. This natural lubricant layer is fundamental to understand the role of the adhesive forces during contact and it is responsible for the hydration control of the skin [13-20]. Moreover, Dermis is a matrix of elastic fibres and cells surrounded by a ground substance and water [21] which presents a thickness between 1 – 4 mm and it provides strength and resilience to the skin. Lastly, Hypodermis, which presents a different thickness depending on the anatomical region, has no influence in the elastic response of the skin surface during interaction with products [22].

If the skin surface is considered to be fully elastic and subjected to small deformations the theory of Hertz can be applied [23-25]. Thus, the indentation depth of a half-space loaded over a small circular region on the skin, follows from the elastic moduli of the contacting materials, the ratio of the indenter and the normal force. Further, a modification of Hertz's theory was introduced by Johnson, Kendall and Roberts to account for the adhesive forces which might be of importance in the case of the human skin [26]. In that case, due to the presence of adhesive forces, the area of contact between the skin and the indenter would increase respect to that given by Hertz. According to the contact models, a key parameter in the evaluation of the contact situation and frictional behaviour of the skin is the input value of its elastic modulus. The elastic modulus of the skin has been addressed by *in vivo* human specimens via indentation tests at different loads, speeds and indenters geometry yet, the results vary between several orders of magnitude due to intra and inter-individual variability [27-31].

Moreover, isolated stratum corneum samples have shown a great influence of hydration in the elasticity properties of this layer [5-8]. In addition, Van Kuilenburg et al. [32] displayed the elastic modulus as a function of the indentation depth by showing a decrease of several orders of magnitude due to the layered structure of the skin. Based on these results, they proposed a multi-layered model for the elastic modulus which accounts for the structure and composition of the skin. Thus, to properly determine the contact parameters when the skin is under stress, an analysis of the contribution of each layer to the overall elastic modulus of the skin is required.

Otherwise, in addition to the great variability of the results when testing *in vivo* skin, the ethical and practical issues related to the manipulation of living tissue could well bring additional difficulties. The availability of *ex vivo* samples is limited and the studies *in vivo* cannot always be performed due to technical and instrumental limitations. Consequently, from an industrial perspective, it could be much more interesting if the analysis of tribo-mechanical properties of the skin was based on a reproducible alternative to provide standardized results. In this direction, different skin mechanical models have been analysed in combination with *in vivo* and/or *ex vivo* skin yet, the results indicate only a relative agreement with the skin in dry conditions. A validated model for wet or moist conditions is not reported in literature. The availability of skin mechanical models in general is very limited since, most of the options focus on the use of living tissue for healing the skin's damage, such as autographs, allografts or xenografts [33-34]. Commonly the living tissue is combined with a supportive matrix of natural and/or synthetic constituents such as, cotton, silk, natural collagen, silicone or nylon which must ensure biocompatibility, non-toxicity and preferably, promote the cellular grown and proliferation. Examples of these materials are, for instance, Alloderm™ (Lifecell Corporation, US), Integra™ (Integra LifeScience, US), Dermagraft™ (Advanced Tissue Sciences, US), Aplicraf™ (Organogenesis, US) or Biobrane™ (Dow Hickham/Bertek, US) among other similar skin substitutes typically used for wound healing [35]. Other synthetic mechanical models which have been employed for tribological testing are Lorica® (Lorica, Italy), used by Derler et al. [36] to analyse the interaction against textiles and non-woven fabrics or Silicone skin, utilized by FIFA to evaluate the abrasion and friction of artificial turf pitches [37]. However, the suitability of these material's to simulate the tribo-mechanical performance of the skin hydrated or moist conditions have been questioned. Thus, new mechanical models have been recently developed in an attempt to mimic the elasticity of the skin and its coefficient of friction in a wider range of conditions. For instance, Nachman et al. [38], synthesized a new hydrophilic silicone which shows a good agreement in dry and moist conditions with human skin, yet the material still needs to be examined in a broader range of conditions. In previous research, we also presented a new water absorbent epidermal equivalent (ESE) [39]. Our results indicate a good agreement of the hydration degree, and surface and mechanical properties of the material with respect to human skin albeit the length scale dependence of the skin not achieved.

Therefore, in this work, a bi-stratified skin model (BSSM) designed to approach the scale dependence of the mechanical properties of human skin is presented. The BSSM was composed by a thin top layer of a previously developed Synthetic Stratum Corneum (SSC) [40] glued on a poly vinyl alcohol (PVA) hydrogel-block obtained via freezing/thawing cycles [41]. The mechanical performance of the BSSM was evaluated by indentation tests in a range of forces between 1 to 80 mN to analyse the changes in the elastic modulus with the depth. Additionally, the elastic modulus was evaluated at different relative humidity and fully wet conditions. These results were compared to the findings of a theoretical model developed by van Kuilenburg et al. [32] adapted for a skin structure composed of stratum corneum, viable epidermis and dermis with hypodermis as underlying tissue, for both, dry and wet conditions. Additionally, the viscoelastic loss fraction of the BSSM was evaluated by means of indentation tests in a range of forces between 1 and 100 mN at dry conditions.

9.1.1 Theoretical background

9.1.1.1 Length scale dependence of the elastic modulus of human skin

Although the elastic modulus of the skin is an essential input parameter of the contact models, the literature shows a wide range of values for the skin depending on the skin conditions and applied force [24, 29-31, 43-45]. At the length scale of the skin's surface roughness, the elastic modulus is given by the stratum corneum whereas the influence of the below layers increases with the indentation depth [30]. Thus, in this work, the analytical model suggested previously by Van Kuilenburg et al. [32] for the elastic modulus of the skin was adapted based on the properties of the stratum corneum, epidermis and dermis whilst the hypodermis, was consider as the underlying tissue. According to the given reference, the global elastic modulus of the skin was calculated as a function of the contact radius as indicated in equation (1):

$$E_{eff}(a) = (E_{eff})^{-1} = 2 \cdot \sum_{i=1}^{n-1} \frac{t_i}{f_i(a)\pi a E_i} + \frac{1}{f_n(a)E_n} \quad (1)$$

t_i , E_i , the thickness and the elastic modulus of each individual layer and f_n , E_n referring to the properties of the underlying tissue. In this model the total number of layers, n , was 4 with $n = 1, 2, 3$ referring to the stratum corneum, viable epidermis and dermis whilst $n = 4$ concerned the properties of hypodermis. The polynomial functions f_i and f_n ensure the correct boundary conditions and, for the current case the corresponding equations were:

$$f_1(a) = 1 + \frac{2 \cdot t_{strat.corneum}}{\pi a} \quad (2)$$

$$f_2(a) = \left(1 + \frac{2 \cdot t_{\text{strat.corneum}}}{\pi a}\right) \cdot \left(1 + \frac{2 \cdot (t_{\text{strat.corneum}} + t_{\text{viable epidermis}})}{\pi a}\right) \quad (3)$$

$$f_3(a) = \left(1 + \frac{2 \cdot t_{\text{viable epidermis}}}{\pi a}\right) \cdot \left(1 + \frac{2 \cdot (t_{\text{viable epidermis}} + t_{\text{dermis}})}{\pi a}\right) \quad (4)$$

$$f_4(a) = \left(1 + \frac{2 \cdot (t_{\text{stratum corneum}} + t_{\text{viable epidermis}} + t_{\text{dermis}})}{\pi a}\right) \quad (5)$$

The analysis of the length scale dependence of $E_{\text{eff}}(a)$ given by equation 1 requires the evaluation of the contact area in a certain range of forces. The contact situation of a spherical probe against the skin can be described by Hertz's theory [24, 25, 43]. According to Hertz, the contact parameters, such as the contact radius, a , are functions of the applied force, F , the radius of curvature of the indenter, R , and the effective elastic modulus, E_{eff} , as a combination of the elastic modulus of the contacting materials [46-48]. Moreover, the effect of the adhesive properties of the top layer of the skin, the stratum corneum, are considerably affected by the influence of hydration or other substances, such as creams or cosmetics [13,49]. Hertz's theory does not account for the adhesive forces on the contact area [50, 51] due to the presence of attractive Van der Waal forces between the two solids [50], so that, the actual contact area exceeds that calculated from the theory. To correct for these forces, Johnson-Kendall-Roberts modified Hertz's theory by including the effect of the adhesive forces, F_{adh} , confined inside contact area due to the molecular interactions of the contacting materials:

$$F_{\text{adh}} = 1.5\pi W_{12}R \quad (6)$$

With W_{12} , the work of adhesion of the contacting materials which can be obtained from indentation tests. Depending on the conditions, the work of adhesion in equation (8) can increase considerably with a subsequent increase of the contact area. Thus, to correct for the effect of adhesion, the adhesive forces given by equation (6) were taken from the literature [13, 52] for dry and wet skin as 0.043 and 0.072 mN/m, respectively. Then, in the JKR model, the normal force is substituted by an apparent force which includes the adhesive forces as indicates equation (9):

$$F_{\text{app}} = F + 2F_{\text{adh}} + 2\sqrt{F_{\text{adh}}(F + F_{\text{adh}})} \quad (7)$$

Consequently, the contact radii, a , given by the JKR model imply modifications in the elastic modulus of the skin with respect to the Hertz model due to its dependence on the contact radius according to equations (1) to (5).

Further, to account for the possible effect of the capillary forces arising on the contact surface at wet conditions, a modified Hertzian approach was also implemented to evaluate the elastic modulus at

wet conditions. The capillary force, derived by the capillary pressure given by the Young-Laplace [51] can be obtained as:

$$F_{cap} = -2\pi R\gamma_L(\cos \theta_1 + \cos \theta_2) \quad (8)$$

R , radius of the sphere, γ_L , the surface tension of water and θ_1, θ_2 , the contact angles of the surfaces.

The effect of the capillary force at wet conditions was analysed by means of the Hertzian model according to equation (9):

$$F_H^{cap} = F + F_{cap} \quad (9)$$

Thus, in this work, the Hertzian, the JKR and a modified Hertzian model which included the effect of capillary forces, were applied to evaluate the evolution of the E_{eff} as a function of the normal force under dry and wet conditions. As showed in our previous research [53], the effect of the capillary forces is only relevant at wet conditions in a small range of forces at the micro level where the elastic modulus is described solely by the properties of the stratum corneum with no influence of other layers. Thus, the increase of the area of contact due to capillarity was investigated in parallel to the evaluation of the elastic modulus of the skin at wet conditions. Lastly, the theoretical results arising from both analysis at dry and wet conditions, were compared to the experimental results of a Bi-Stratified Skin Model (BSSM) to validate the physical model.

9.2 Material and methods

9.2.1 Design of the Bi-Stratified Skin Model (BSSM)

A new 2-layered model of the skin was obtained by the superposition of two previously developed materials: a poly vinyl alcohol (PVA) block obtained by freezing thawing cycles [40] and a Synthetic Stratum Corneum (SSC) made of PVA in combination with rapeseed oil and Glutaraldehyde [41]. The goal of such a structure was to mimic the length scale dependence of the human skin due to its multi-layered composition. In addition, the adhesive properties were also resembled by the use of a SSC which adhesive properties are in good agreement with those of the human skin as it described in our previous research [41]. Thus, a 1.5 mm thick block of PVA created by 11 freezing/thawing cycles of a 20% wt. PVA (aq) solution was glued to a top layer between 150-200 μm of a previously developed Synthetic Stratum Corneum. To ensure the connection between the two layers, a thin coating of waterproof acrylate glue Loctite 401 from Loctite (Germany) was extended with a brush on top of the PVA block and then, the SSC layer was positioned. Although the acrylate glue once

hardened has a considerably higher elastic modulus than the tested layers, the analysis of the BSSM was not affected due to the small thickness in comparison with the other layers. An evaluation of the length scale dependence of the elastic modulus of the BSSM was performed. In addition, the viscoelastic loss fraction was evaluated based on the differences on the loading-unloading curves due to hysteresis.

9.2.2 Testing conditions and apparatus

9.2.2.1 Elastic properties from indentation tests

The elastic modulus of the BSSM was calculated from indentation tests according to Oliver and Pharr [46]. Thus, if the stiffness of the material, S , represents the initial slope of the unloading part of the load–displacement curve as described in Figure 1, the effective elastic modulus, E^* , can be calculated as:

$$S = \frac{dP}{dh} = \frac{2}{\sqrt{\pi}} E^* \sqrt{A} \quad (10)$$

Figure 1. Schematic representation of an indentation test with indication of the loading and unloading curves. The loading area (magenta line) and the unloading area (yellow zone) were used to calculate the viscoelastic loss fraction as indicated in the figure.

To validate the length scale dependence of the BSSM with respect to the model described by [32] adapted for a skin's structure as such described in Table 1, the elastic modulus was evaluated in a range of forces between 1 and 50 mN with an indenter of 3 mm radio.

Table 1. Summary of the individual thicknesses and elastic moduli of the layers implemented in the model to evaluate the length scale dependence of the skin.

i	Skin layer	Elastic modulus, E_i (MPa)		Thickness, t_i (mm)
1	Stratum Corneum	dry	100	0.02
		wet	10	
2	Viable Epidermis		1	0.04
3	Dermis		0.05	1.1
Underlying tissue	Hypodermis		$2 \cdot 10^{-3}$	-

To account for the modifications of the mechanical properties of the skin with hydration, an evaluation of the elastic modulus of the BSSM at different humidity was performed. Thus, indentation tests were performed on three different samples at the relative humidity of 36, 80 and 100%. The surface of a fourth sample was wet and after 1 minute dried so that, the elastic modulus was also obtained at wet conditions. The measurements were performed at a velocity of 0.05 mm/s and a holding time of 5 s was applied at the maximum load.

9.2.2.2 Viscoelastic loss fraction from pull-off measurements

The viscoelastic loss fraction, β , is a frequently used parameter in assessing hysteresis losses [23]. Since the “perfect” elastic material does not exist, β represents the amount of energy dissipated through a complete load cycle during an indentation test. This is an internal energy related to the mechanical properties of the material and it can be calculated as the difference between the loading-unloading areas divided by the loading area in one indentation cycle as indicates Figure 1. The relative magnitude of the viscoelastic loss fraction gives an idea of the viscoelasticity of the material. In this case, the viscoelastic loss fraction of the BSSM was determined from indentation tests with a spherical probe of 3 mm radio at 0.05 mm/s in a range of forces between 1 to 100 mN. The measurements were performed at environmental conditions of 25 °C and 30- 36 % relative humidity and the results of β were compared to values for the human skin obtained from the literature.

9.2.2.3 Modelling the multi-layered behaviour of the elastic modulus of human skin and comparison with experimental results of the BSSM

The analysis of the elastic modulus of the skin as a function based on equations (1) to (5) was validated with experimental data obtained from indentation tests on a Bi-Stratified Skin Model in a range of forces between 1 and 30 mN. As E_{eff} is a function of the contact radii, a , and vice versa, it was necessary to implement an algorithm to evaluate both parameters in a range of forces between 1 to 30 mN. The radio of the indenter was consider 3 mm in concurrence with the experimental

method. In addition, an initial value for the elastic modulus of the skin was implemented to calculate an initial contact radii, a_0 , in that range of forces. A value of 500 kPa was used to start the iteration whereas to break it, the sum of the square differences between $E_{eff,m}$ and $E_{eff,m-1}$ was imposed to be less than 10^{-6} . The elastic modulus of the skin surface was evaluated at dry and wet conditions considering the changes between the Hertz and the JKR models due to the effect of the adhesive forces, especially at wet conditions. The thickness and elastic moduli of the skin layers and the underlying tissue are collected in Table 1.

9.3 Results and discussion

9.3.1 Elastic modulus of the BSSM

The elastic modulus of the BSSM is presented in Figure 2 at different conditions for a range of forces between 1 and 80 mN. A decrease of the elastic modulus with force was observed as was expected due to the higher elastic modulus of the top layer, the SSC, in comparison with the ultra-soft properties of the PVA hydrogel block bellow.

Figure 2. Elastic modulus of the BSSM as a function of the normal force at different relative humidity of 20 %, 36%, 80%, 100% and wet conditions.

Moreover, the influence of humidity and water in the material's properties evidenced a considerable decrease of the elastic modulus at high humidity. At 1 mN of applied force, the BSSM presented an elastic modulus around 550 kPa at 36% relative humidity whilst at 80 % relative humidity had already

decreased to 300 kPa. The differences between the elastic modulus at 100% of humidity and wet conditions were minor with an elastic modulus no larger than 100 kPa and almost steady over the applied range of forces. The results obtained for the BSSM are within the range of magnitude of the experimental values of *in vivo* skin from literature. For instance, Nachman et al. [38] carried out *in vivo* indentation measurements at the human volar forearm of a healthy woman with a spherical indenter of 2 mm radius at forces of 10, 20 and 30 mN, respectively. The results of the elastic modulus did not show pronounced differences with force. Yet, there was a slight decrease of the elastic modulus when the force increased. At 10 mN the elastic modulus was 9.7 kPa whilst at 20 and 30 mN it decreased to 9.5 and 8.9 kPa, respectively.

Indentation tests performed by Jachowicz et al. [24] with a spherical indenter of 3.94 mm radius, showed an elastic modulus for *in vivo* skin of 33 kPa. Similar results were obtained by Zahouani et al. [45] on *in vivo* skin with a value of 8.3 kPa when using a steel probe of 2.25 mm radius. The elastic modulus was also evaluated by [54] with a spherical tip of 0.5 mm at loads of 0.2 mN and 1 mN for the stratum corneum and viable epidermis, respectively. The results indicated values of 2.6 ± 0.6 MPa for isolated stratum corneum and 1.1 ± 0.2 MPa for viable epidermis. Both, isolated stratum corneum and *in vivo* skin samples were also studied by Pailler-Mattei et al. [25] at the nano and the macro scales. Nano-indentation measurements were performed on isolated stratum corneum in a range of depth between 10 nm to 1 μ m in which the elastic modulus showed a decrease with depth from 1 GPa to 100 MPa. In addition, *in vivo* measurements were carried out on the inner forearm of an individual with a spherical indenter of 6.35 mm with a resulting average elastic modulus between 7- 8 kPa. The high differences between the results of isolated stratum corneum and *in vivo* skin confirmed that, only at the nano and the micro scales the elastic modulus of the stratum corneum has a role in the mechanical response of the skin under stress. At the meso scale, according to the model proposed by [32], the elastic modulus would be influenced by the properties of the stratum corneum and the dermis with a relative dependence on the applied force whilst the elastic modulus would be well represented by the bulk mechanical properties of the skin at the macro scale.

9.3.2 Viscoelastic loss fraction, β , of the BSSM

The viscoelastic loss fraction of the BSSM was evaluated by means of indentation tests as a function of the force as indicated in Figure 3. According to the results, β seemed to be slightly higher at low forces with values around 0.2 whilst at higher forces appeared around 0.10. On average, β showed a value of 0.13 ± 0.05 as indicated in Figure 3.

Figure 3. Viscoelastic loss fraction of the BSSM at dry conditions as a function of the applied force. In the graph is also indicated the average value and the standard deviation.

In general, the viscoelastic loss fraction tends to increase as the elastic limit is reached which, commonly happens at large stresses. At low and intermediate stresses the values are steady for most of the materials [23]. The higher values obtained for the BSSM contrasts with the previous assumption and it can be attributed to the internal properties of the material, such as the crystallinity [55, 56]. Besides, the low value of β contrasts also with the generally known viscoelastic character of PVA hydrogels which would predict a higher value, especially at higher forces. Conversely, viscoelastic loss fraction values between 0.24 and 0.43 have been reported from indentation tests on *in vivo* human skin [27, 42, 57]. Other results from *in vivo* forearm skin varied in a widely from 0.175 to 0.75 with the latter value corresponding to an oldest individual [25]. The latest result is likely a consequence of the decrease of elasticity with aging which leads to a higher viscoelasticity of the skin. Thus, if the value of b for the oldest individual was not consider the authors obtained a range of β between 0.175 and 0.38. Additionally, the viscoelastic loss fraction was also reported in our previous work [53] on excised human skin samples with an average value of β of 0.35 ± 0.09 similar to those found in the literature. Thus, the viscoelastic loss fraction of the BSSM was lower than the loss fraction which is expected in general for human skin surfaces. Still, this can be definitely modified by decreasing the number of freezing/thawing cycles so that, the crystallinity of the blocks is reduced and consequently, its viscoelasticity of the bottom layer increased.

9.3.3 Modelling the multi-layered behaviour of the elastic modulus of human skin

9.3.3.1 Length scale dependence of the skin

Given the model described in section 2.1 and the parameters presented in Table 1, the evolution of the elastic modulus of the skin as a function of the contact radii is displayed in Figures 4 and 5 at dry and wet conditions, respectively. Figure 4 (a) presents the elastic modulus of skin at dry conditions calculated based on a Hertzian approach whereas Figure 4 (b) presents the results obtained based on the JKR contact model. The two graphs from Figure 4 display the evolution of the elastic modulus from an initial value of 500 kPa implemented in Equations (4) to (7) to begin the iteration and, which first output is given by the blue line on the graphs.

Figure 4. Evolution of the elastic modulus of a theoretical model of skin which properties are given in Table 1 at dry conditions. Graph (a) presents the elastic modulus calculated based on a Hertzian approach whereas graph (b) displays the elastic modulus calculated according to the JKR model.

A number of nineteen and seventeen iterations were needed to reach the point where the results of the m and the $m-1$ iteration were less than 10^{-6} , as indicated in Figure 4 (a) and (b), respectively. In addition, Table 2 collects the contact parameters of the Hertzian and the JKR model obtained for the last iteration, such as the indentation depth, contact radii and the elastic modulus at 1, 10 and 50 mN.

Table 2. Summary of the contact parameters given by the Hertzian and the JKR approaches at 1, 10 and 50 mN normal force. The contact radii, indentation depth and elastic modulus of the skin are presented in this table for dry conditions.

force (mN)	Hertzian contact case			JKR contact case		
	indentation (μm)	contact radii (mm)	elastic modulus (kPa)	indentation (μm)	contact radii (mm)	elastic modulus (kPa)
1	22.53	0.26	120.7	73.63	0.47	89.8
10	145.20	0.66	78.5	208.03	0.79	73.8
50	480.00	1.2	65.8	563.33	1.3	64.5

The differences in the indentation and contact radii between the two models are relevant, especially at lower forces. It is also clear the evolution of the elastic modulus with the increase of force due to differences in the elastic modulus and thicknesses of the implemented layers given in Table 1.

Furthermore, a similar analysis was performed to model the performance of the skin at wet conditions. In this case, the changes due to the modification of the adhesive forces at wet conditions, altered the contact parameters and consequently, the evolution of the elastic modulus was affected.

Figure 5. Evolution of the elastic modulus of a theoretical model of skin which properties are given in Table 1 at wet conditions. Graph (a) presents the elastic modulus calculated based on a modified Hertzian approach which included the capillary force; the graph (b) displays the elastic modulus calculated according to the JKR model.

As in dry conditions, the blue line indicates the beginning of the iteration process which was broken after eighteen and sixteen cycles for the modified Hertzian model and the JKR model, respectively. The Hertzian approach with capillary force yielded to slightly different results in comparison to the dry case despite of the consideration of a much lower elastic modulus of the SC as indicates in Table 1.

Figure 5 (a) and (b) show the evolution of the elastic modulus as a function of the contact radii for the modified Hertzian model with capillarity and the JKR contact model for the case of wet conditions. Similarly as in the dry case, Table 3 collects the contact parameters for the two models. The results of the modified Hertzian approach addressed to similar values as those obtained at dry conditions whereas the JKR yielded to higher indentation and contact radii at wet conditions with respect to dry. Subsequently, the elastic modulus for the JKR model at wet conditions presented lower values compared to the dry case.

Table 3. Summary of the contact parameters given by the modified Hertzian and the JKR approaches at 1, 10 and 50 mN. The contact radii, indentation depth and elastic modulus of the skin are presented in this table for the wet conditions.

force (mN)	Hertzian + capillary force contact case			JKR contact case		
	indentation (μm)	contact radii (mm)	Elastic modulus (kPa)	indentation (μm)	contact radii (mm)	Elastic modulus (kPa)
1	38.53	0.34	105.4	108.3	0.57	83.2
10	154.13	0.68	77.7	240.8	0.85	72.2
50	480.00	1.2	65.6	563.33	1.3	64.1

Further, the experimental values presented in Figure 2 at dry (36% relative humidity) and wet conditions were compared to the theoretical values obtained from the approaches given above at both conditions. The standard error associated to each case is given in Table 4 as well as a summary of the elastic modulus as a power law function of the normal force for each case.

Table 4. Summary of the elastic modulus given as a power law function of the normal force at dry and wet conditions for the different models used in each case. Moreover, the standard error due to the differences between the experimental data and the results from the given equations is also presented.

dry	Hertz	$\tau = 44.7 \cdot 10^3 \cdot F^{-0.12}$	SE=4.3 %
	JKR	$\tau = 44.7 \cdot 10^3 \cdot F^{-0.12}$	SE=7.7 %
wet	Hertz + capillary force	$\tau = 44.7 \cdot 10^3 \cdot F^{-0.12}$	SE=5.3 %
	JKR	$\tau = 44.7 \cdot 10^3 \cdot F^{-0.12}$	SE=12.4 %

From these results, the best fit was obtained by the Hertzian approach at dry conditions, whereas at wet conditions the modified Hertzian approach with capillary force presented the lower error in estimating the experimental elastic modulus.

Additionally, since the two contact models are only applicable for small deformations, a restriction of the contact radii was used to evaluate the range of forces in which the contact models can be used. If the strain is proportional to the ratio between the contact radii and the radio of the indenter [58-60],

the maximum strain according to the literature should be less than 6 %. Consequently, the maximum contact radii, a , should be of $1/3$ of the radius of the indenter, R , as indicated below:

$$\varepsilon = 0.2 \frac{a}{R} \quad \text{and} \quad \frac{a}{R} \leq \frac{1}{3} \quad \varepsilon_{max} = 6 \% \quad (11)$$

Thus, the contact radii at both, wet and dry conditions, was plotted against the normal force to identify the force at which the model cannot longer be applied. Figure 6 shows the respective curves for the Hertzian, JKR and modified Hertzian models which are intersected by a maximum contact radii of 1 mm considering an indenter of 3 mm radius.

Figure 6. Contact radii calculated according to the Hertzian contact model (red line) and the JKR model (green line) at dry conditions. At wet conditions, the contact radius was calculated according to the Hertzian approach including the effect of the capillary forces (dashed black line) and the JKR model (dashed blue line). The maximum contact radius (horizontal yellow line) was obtained based on Eq. 8 considering an indenter of 3 mm radius is also indicated.

As indicated also in Figure 6, for the given conditions the models can only be applied at forces lower than 30 mN which comprises a limitation to analyse the tribo-mechanical performance of the skin due to the uncertainty about the evolution of the contact area with the increase of force. Based on the previous consideration it can be considered that the forces given by the limitation above define two regimes for the tribo-mechanical performance of the skin. So that, at an initial regime, the assumptions of the Hertzian and JKR models are can be apply for the evaluation of the contact parameters of the skin. On the contrary, at higher forces than those imposed by the previous

limitation, the contact area would likely equal the projected area of the indenter on the skin. These suppositions would be of interest when modelling the tribo-mechanical performance of the skin yet, they need to be properly investigated.

9.3.4 Elastic modulus of the BSSM in comparison with a theoretical 3-layer model of the skin as a function of the force

The elastic modulus of the skin calculated according to equations (1) to (5) and based on the skin structure defined in Table 1 was plotted against the force in Figure 7.

Figure 7. Evolution of the elastic modulus of the skin as a function of the normal force for the Hertzian model (red line) and the JKR model (green line) at dry conditions. The evolution of the elastic modulus at wet conditions is given by a modified Hertzian approach which includes capillary forces (black dashed line) and the JKR approach (blue dashed line) at wet conditions. In addition, the experimental results of the BSSM are displayed for comparison with the model for dry (orange marker) and wet (blue marker) conditions.

These results are shown for the Hertzian case and the JKR model at dry conditions. The modified Hertzian approach with capillary forces and the JKR at wet conditions are also displayed in the graph. Additionally, the experimental results of the BSSM as a function of the force are also presented in the same figure. Thus, it can be observed the decrease of the elastic modulus of the BSSM with the force in agreement with the results of the model.

Power laws of the elastic modulus as a function of the force were obtained from the experimental results at dry and wet conditions. Moreover, the evolution of the E_{eff} based on the Hertzian and the JKR models was also defined as a power law function of the force at both conditions. Then, the experimental and the theoretical equations were compared as indicated in Figure 8 (a) and (b) for dry and wet conditions, respectively. According to Figure 8 (a), at dry conditions the best correlation for E_{eff} is given by the Hertzian model whereas at wet conditions, the JKR model showed a better agreement with the experimental data as it can be derived from the comparison of the power law functions of E_{eff} in each case.

Figure 8. Elastic modulus of the skin obtained from the experimental results (blue dashed line) and the theoretical models (red and black lines) at both dry and wet conditions. The theoretical approaches are expressed as a power law of the normal. At dry conditions, the Hertzian (black line) and the JKR (red line) models are displayed in image (a) whilst, the modified Hertzian (black line) and the JKR (red line) at wet conditions are presented in the image (b).

9.4 Conclusions

This paper presents a Bi-Stratified Skin Model based on a top film of a previously developed Synthetic Stratum Corneum (SSC) and a bottom block of PVA hydrogel obtained by freezing/thawing cycles. The model aimed to mimic the multi-layered mechanical performance of the skin which was theoretically described by [32] in previous research. Thus, indentation tests were performed in the BSSM at dry and wet conditions and a range of forces between 1 and 80 mN. Further, the viscoelastic loss fraction of the samples was also obtained with an average value of 0.13 lower than the values of human skin given in the literature. The experimental results of the elastic modulus given by

indentation were used to evaluate the length scale dependence of the mechanical model in comparison with the theoretical approach. To evaluate the elastic modulus as a function of the length scale, the classical Hertzian and JKR contact models and a modified Hertzian model which included the capillary force, were used to evaluate the contact parameters. Besides, a multi-layered model of the skin consisting in the stratum corneum, viable epidermis, dermis and hypodermis as underlying tissue, was implemented in the contact models through an iterative process at forces between 1 to 80 mN. The comparison of the theoretical approach based on the Hertzian model and the experimental results of the BSSM showed a good correlation at dry conditions. Moreover, at wet conditions the results given by the implementation of the Hertzian model which accounted for the capillary force showed a better correspondence to the experimental results. Consequently, both the experimental and theoretical results proved the suitability of the BSSM as a mechanical model of the skin.

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Chapter 10

In preparation

Paper VII: *Skin friction: a length scale dependence physical & theoretical model*

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Abstract

The frictional performance of a Bi-Stratified Skin Model (BSSM) is analyzed at the macro and the meso scale in comparison with *ex vivo* skin measurements. The results at the macro scale pointed out a COF between 0.3 and 0.45 at dry conditions for both, *ex vivo* skin and the BSSM whereas at wet conditions, a higher COF ranging between 0.56 – 0.78 was found for both samples. An important effect of the adhesive forces was observed at the meso scale where the COF showed a power law decreased from 2.2 to around 0.45 at dry conditions for *ex vivo* skin. The effect of adhesion was even higher at wet conditions with a COF ranging from 5.5 to around 0.8 for *ex vivo* skin. The BSSM presented a comparable results also at the meso scale for both, dry and wet conditions. In addition, a theoretical analysis of the frictional performance of the skin was done based on the results from *ex vivo* skin. A theoretical friction model which accounts for the length scale dependence of the skin was implemented in the Hertzian and JKR contact models. Besides, modified versions of these models which include the effect of the capillary forces were also considered to determine their role in the frictional performance of the skin.

10.1 Introduction

Several artificial skin equivalents have been developed to provide a source of data which can be used for a theoretical analysis of skin friction models or connected purposes [1-7]. However, the currently available physical models present limitations with respect to the tribo-mechanical performance, especially at wet conditions. An example is Silicone Skin L7530, a material used as skin equivalent in the FIFA test method which shows a frictional performance that is rather different to that of the human skin [8]. The results of an Epidermal Skin Equivalent previously developed in our group exhibited interesting mechanical and surface properties to that of human skin yet, the frictional performance was not precisely reproduced [4]. Raw and mixed polyurethanes with or without synthetic fibers have closely reproduced the frictional performance of human skin at dry conditions [2, 3]. Nachman et al. [1] recently developed a silicone modified with alpha-olefin sulfonate which showed interesting hydrophilic properties. The modified silicone indicated appropriate tribo-mechanical properties at dry and wet conditions yet, the material needs to be validated in a wider range of forces and conditions.

Additionally, most of the currently available physical models consist of a single layer of material so that, the elastic modulus remains constant under applied stresses. However, it is extensively demonstrated that this is different for human skin, given the structure divided in different strata with different composition and mechanical properties. The elastic modulus obtained by indentation measurements varies several orders of magnitude depending on the indenter's size and the applied loads. At small indentations, the response of the skin is determined by the stratum corneum only. Yet, from 100 nm the response of the tissue seems to be already influenced by the bulk [9, 10]. Van Kuilenburg et al. [11] presented a model to account for the variation of the elastic modulus as a function of the indentation depth due to the layered structure of the skin. Moreover, hydration has also a strong influence in the mechanical properties of the skin, especially on the stratum corneum [12-17]. Further, the effects of hydration on the mechanical and surface properties of the skin are known to affect skin friction [18-22]. At dry sliding conditions the coefficient of friction of *in vivo* skin has been found between 0.25 and 0.5 depending on the interacting surface but also on the

anatomical place and the indenter size [21 - 26]. When water is applied to the skin, the elasticity of the stratum corneum decreases noticeably due to the plasticizing effect of water. This causes an increase of the area of contact due to the lower elastic modulus and the changes on the skin's surface which subsequently, increase friction. Values between 0.7 and 2.5 have been found for the coefficient of friction of human skin at wet sliding conditions in combination with a variety of engineering surfaces [22 - 24, 27-29]. These results have also indicated a dependence of the coefficient of friction with the contact pressures particularly, at low pressures which indicates that the relative contribution of the mechanisms behind friction are also different depending on the applied force [30].

The main goal of this work was to validate the tribological performance of a physical and a theoretical skin model in comparison to *ex vivo* skin at dry and wet conditions. The effect of humidity in the frictional performance of the physical model was investigated as a function of the normal force at the meso scale. Moreover, the coefficient of friction of the BSSM and *ex vivo* skin was evaluated at 2 different regimes i.e.: the meso and the macro scale. At the meso scale, forces between 1 and 50 mN were applied whereas at the macro scale the applied forces ranged from 0.5 to 3.5 N. Further, the tribological behaviour of the BSSM and *ex vivo* skin was investigated at both, dry and wet conditions at each scale. Additionally, an analysis of the frictional performance of the skin at the meso and the macro scales was performed taking into account the length scale dependence of the skin's mechanical response. The model was applied at dry and wet conditions to analyse the role of adhesion and capillary forces on skin friction at both scales.

10.1.1 Theoretical background

10.1.1.1 Length scale dependence of the elastic modulus

To analyse the contact of a spherical ball in sliding contact on a flat skin sample, the applied force, F , the radius of the indenter, R , and the effective elastic modulus, E^* , are required input parameters in the contact models. The layered structure of the skin require to consider the mechanical properties and relative influence of each layer to the overall elasticity of the skin depending on the indentation depth [9, 11]. Thus, the elastic modulus of human skin has

been described in previous research [11] as a function of the length scale based on equation (1):

$$\frac{1}{E_{skin}}(a) = 2 \sum_{i=1}^{n-1} \frac{t_i}{f_i(a)\pi a E_i} + \frac{1}{f_n(a)E_n} \quad (1)$$

t_i , E_i , the thickness and the elastic modulus of each individual layer and f_n , E_n denoting to the properties of the underlying tissue; f_i and f_n are polynomial function to ensure the correct boundary conditions.

Figure 1. Illustration of the layers involved in the response of the skin to stress at the meso and the macro scale. At forces in the range of mN the frictional performance results on the joint response of the skin surface lipid film, stratum corneum, epidermis and dermis; at higher forces in the range of N, the response of the skin is mainly given by the properties of the stratum corneum, dermis and muscle.

The scale dependence of the skin's elastic modulus has been given by a power law function of the contact radius with a different behaviour at the micro, meso and macro scales [11]. Because of the important role of the elastic modulus of the skin in the contact models, the length scale dependence of the skin implies a subsequent variation of the contact parameters which must be taken into account. Therefore, the role of the elastic modulus of the skin as a function of the force was evaluated in this work based on Eq. (1) at the meso and the macro scales as indicated in Fig.1. Thus, at the macro scale, the elastic modulus was analysed by considering the properties of the stratum corneum, dermis and the muscle as underlying tissue. At the meso scale, the individual elastic modulus and thickness of the stratum corneum, viable epidermis and dermis with hypodermis as underlying tissue were considered. In this work, the evolution of the E_{eff} was described at each scale by a different skin structure which was adapted to fit with friction experimental results. The composition of the skin at each scale is presented in Table 1 as well as a collection of the input parameters required in equation (1) at the meso and the macro scales.

Table 1. Summary of the individual thicknesses and elastic moduli of the layers implemented in the model to evaluate the length scale dependence of the skin [11].

MESO SCALE				
<i>i</i>	Skin layer		Elastic modulus E_i (MPa)	Thickness t_i (mm)
1	Stratum Corneum	dry	100	0.02
		wet	10	
2	Viable Epidermis		1	0.04
3	Dermis		0.05	1.1
Underlying tissue	Hypodermis		$2 \cdot 10^{-3}$	-
MACRO SCALE				
<i>i</i>	Skin layer		Elastic modulus E_i (MPa)	Thickness t_i (mm)
1	Stratum Corneum	dry	50	0.02
		wet	5	
2	Dermis		0.06	1100
Underlying tissue	Muscle		0.25	1.1

10.1.1.2 Contact model & mechanics of friction force

To analyse the frictional performance of human skin at dry and wet conditions, different contact models were applied in this work to evaluate the effect of the adhesive forces on the contact. These models were also used to describe the evolution of the elastic modulus defined

in the previous section as a function of the force and, successively, the coefficient of friction. The first contact model applied to evaluate the frictional performance of human skin was based on Hertz's theory, i.e.: the contact radius, a_H , is a function of the applied force, F , the radius of the probe, R , and the Effective Elastic modulus, E^* , a combination of the elasticity of the contacting materials, as in Eq. (2).

$$a_H = \left(\frac{3RF}{4E^*} \right)^{1/3} \quad (2)$$

Since Hertz's theory does not take the adhesive forces into account for the contact area [31, 32], a modification of this model, proposed by Johnson-Kendall-Roberts [33, 34], was implemented to introduce the adhesive forces related to the van der Waals forces. Thus, the apparent force acting on the material's surface was calculated as:

$$F_{app} = F + 2F_{adh} + 2\sqrt{F_{adh}(F + F_{adh})} \quad (3)$$

With F_{adh} the adhesive force defined as:

$$F_{adh} = \frac{3}{2}\pi RW_{12} \quad (4)$$

Where W_{12} is the work of adhesion between the two contacting surfaces, see also [32, 35]. Moreover, the capillary force due to meniscus formation has also been suggested to have an influence in the frictional performance of the skin [36, 37]. The capillary force can be derived from the capillary pressure given by the Young-Laplace [38] as indicate equation (5):

$$F_{cap} = -2\pi R\gamma_L(\cos \theta_1 + \cos \theta_2) \quad (5)$$

R , radius of the sphere, γ_L , the surface tension of water and θ_1, θ_2 , the contact angles of the surfaces.

The introduction of the capillary force in the contact models would increase the area of contact, especially, at low forces which might be a proper approach to predict the coefficient of friction of the skin. The role of the capillary force can be analysed by means of the Hertzian model or the JKR approach which corresponding formula are given by equations (6) and (7), respectively, see also [39].

$$F_H^{cap} = F + F_{cap} \quad (6)$$

$$F_{JKR}^{cap} = (F + F_{cap}) + 2F_{adh} + 2\sqrt{F_{adh}((F + F_{cap}) + F_{adh})} \quad (7)$$

10.1.1.3 Two-term friction model

The elastic modulus as a function of the length scale and the contact parameters Eqs. (2), (3), (6) and (7) were used to evaluate the frictional performance of the skin at the meso and the macro scale based on the “Two-term friction model” [22, 23, 40]. According to this model, the friction force results from two non-interacting terms: the adhesive and the deformation component. Typically, adhesion is considered the main part of the frictional behaviour of skin, whereas the contribution of the deformation component is minor [20, 23]. The adhesion component depends on the true area of contact, A_{real} , and the interfacial shear strength, τ , between the contacting surfaces.

$$F_{\mu,adh} = \tau A_{real} \quad (8)$$

The deformation component is a function of the viscoelastic losses due to hysteresis and it is proportional to the viscoelastic loss fraction, β , the normal force, F and the relative indentation of the probe into the skin, $\frac{a}{R}$:

$$F_{\mu,def} = \frac{3}{16}\beta \frac{a}{R}F \quad (9)$$

The friction force arising from the two term friction model is commonly described by a power law of the normal force in which the exponent, m , can be related to the deformation component or to the adhesion component.

$$\mu = k \cdot F^m \quad (10)$$

The coefficient of friction as a function of the normal force obtained from this analysis were compared to experimental results of *ex vivo* skin and the BSSM by fitting the interfacial shear strength at both scales for dry and wet conditions. Table 2 summarises the parameters introduced in the friction model, such as the, indenter radius, R , the viscoelastic loss fraction, β , or the applied range of forces at each scale. Additionally, the work of adhesion, W_{12} , at dry and wet conditions is also indicated in Table 2 [41].

Table 2. Conditions applied for the evaluation of the COF at each scale: range of forces and indenter size, viscoelastic loss fraction and work of adhesion at dry and wet conditions.

	MESO SCALE	MACRO SCALE
Normal force range, F (N)	0.001 – 0.08	0.5 – 4
Indenter radio, R (mm)	3	15
Elastic modulus indenter (GPa)	210	210
Viscoelastic loss fraction, β (-)	0.35	0.35
Work of adhesion, W_{12} (mN/m)	dry	0.043
	wet	0.072

10.2 Material and methods

10.2.1 Materials

10.2.1.1 *Ex vivo* human skin samples

Ex vivo human skin samples belonging to the abdomen of one individual were used to evaluate the frictional performance of the skin and validate the physical and theoretical model. The samples were selected from the Radboud Hospital of Nijmegen, with the consent of the patient and the agreements of the Ethical Committees of the Hospital and the University of Twente. Previous to the measurements, the hypodermis was removed from the samples to flatten them and to keep a similar thickness around 1.2 mm. Later the samples were cut in square pieces of 20x20 mm and they were glued in a petri dish. The average roughness parameters: peak-to-valley roughness (R_{pv}), arithmetic roughness (R_a), root mean square (R_q) and skewness (R_{sk}) of the skin samples are presented in Table 3. Moreover, a three dimensional image of one of the skin samples is displayed in Fig. 2.

Table 3. Average roughness parameters of the analysed skin samples.

R_{pv} [μm]	R_a [μm]	R_q [μm]	R_{sk} [μm]
74.1 ± 3	46.2 ± 4	57.7 ± 5	-0.53 ± 0.05

Figure 2. Three dimensional morphology of the surface of a skin sample obtained from confocal microscopy with a scale indication of the height in μm .

10.2.1.2 Physical models

The frictional performance of Bi-Stratified Skin model (BSSM) was compared to the *ex vivo* skin results to evaluate the suitability of the material as skin substitute for frictional testing. The physical model, BSSM, was created by gluing two previously developed materials: a poly vinyl alcohol (PVA) block obtained by freezing thawing cycles [42] and a Synthetic Stratum Corneum (SSC) made of PVA in combination with rapeseed oil and glutaraldehyde [43]. A 1.5 mm thickness ultra-soft block of PVA was glued to a 150-200 μm layer of SSC with a waterproof acrylate glue Loctite 401 (Loctite, Germany).

10.2.2 Experimental conditions and apparatus

10.2.2.1 Friction measurements at the meso scale

Series of friction measurements were conducted with the Vacuum Adhesive and Friction Tester (VAFT) [44], using a pin-on-disk setup. The coefficient of friction was obtained in a normal force range of 1 to 50 mN by using a spherical pin of 6 mm diameter made of Chrome Steel AISI-52100. The measurements were carried out at a velocity 10 $\mu\text{m/s}$ and a sliding distance of 400 to 2000 μm . The setup for the VAFT measurements is shown in Fig. 3 (a).

10.2.2.2 Friction measurements at the macro scale

Tribological measurements at the macro scale were performed on a HC 4057 tribometer (CSM, Switzerland), in a pin-on-disk setup. Cylindrical pins made of Chrome Steel AISI-52100 with a spherical tip of 30 mm diameter were used to perform the measurements. Figure 3 (a) displays the set-up of the pin-on-disk tests with a skin sample placed on the holder. The coefficient of friction was determined at normal loads between 1 and 3.5 N at intervals of 0.5 N at a velocity of 50 mm/s.

Figure 3. Setups used for the frictional measurements: image (a) shows the VAFT during one measurements on excised skin; image (b) displays the macro scale tribometer also during a measurement on excised skin.

10.3 Results and discussion

10.3.1 Frictional performance of the BSSM in comparison with *ex vivo* skin

10.3.1.1 Analysis at the meso scale

The friction performance of the BSSM was compared to *ex vivo* skin results at the meso and the macro scale and for at dry and wet conditions. The measurements at the meso scale are presented in Fig. 4. As it can be seen from Fig. 4 (a), the resulting COF for *ex vivo* skin was around 2.2 at very low forces likely due to high adhesion interactions whereas, at higher normal forces it decreased to values around 0.60-0.45. The friction performance of BSSM indicated COF values between 0.50-0.45 at dry conditions. Although the initial COF of BSSM

was lower than that obtained with *ex vivo* skin, the results obtained at higher forces showed similar values to those of *ex vivo* skin.

Figure 4. Coefficient of friction of the BSSM as a function of the normal force in comparison to *ex vivo* skin at the meso scale: (a) and (b) indicate dry and wet conditions, respectively.

The tribological results obtained at wet conditions, see Fig. 4 (b), show a significantly high COF of around 5.5 at low normal forces for *ex vivo* skin likely due to the presence of meniscus bridges in the contact. The resulting COF for the BSSM was also higher at low normal forces because of a modification of the surface properties of the BSSM due to contact with water. The results showed a COF for the Chrome Steel - BSSM sliding contact of around 2.2 which approached the values of *ex vivo* skin at higher normal forces as indicated in the right hand part of Fig. 4 (b).

10.3.1.2 Analysis of the BSSM at different humidity levels

The coefficient of friction of the BSSM was also analysed at the meso scale at different relative humidity to evaluate the changes in friction related to hydration. These results are displayed in Fig. 3 at 20, 36, 80 % relative humidity and at wet conditions. The highest friction level was measured at 80 % relative humidity, COF around 3.8, decreasing to 1.5 at 50 mN. At 20 % relative humidity the same operational conditions resulted in with considerably lower friction

with COF values between 0.8 and 0.45 as a function of the applied force. At wet conditions, the COF was also high but, slightly lower than at 80% relative humidity maybe due to a water lubrication effect after applying water on top of the BSSM.

Figure 5. Coefficient of friction of the BSSM for different relative humidity and wet conditions as a function of the normal force at the meso scale.

The results presented in Figure 5 show a COF variable with the hydration level which confirmed modifications on the BSSM top layer depending on the conditions. This confirmed the ability of the model to adapt to the environmental changes and to resemble the frictional performance of the skin at different hydration levels.

10.3.1.3 Analysis at the macro scale

Further, the coefficient of frictions are also plotted at the macro scale for dry and wet conditions. These results are presented as a function of the normal force in Fig. 6 in which, the coloured areas represent a range of COF's for *in vivo* skin at dry and wet conditions given by the literature [45]. The coefficient of friction of *ex vivo* skin were within the range of the *in vivo* skin values from the literature and showed a relatively steady coefficient of friction at the macro scale at both conditions. The resulting COF for *ex vivo* skin varied between 0.45 – 0.34 and 0.65 – 0.55 for dry and wet conditions, respectively. BSSM showed a similar friction behaviour to *ex vivo* skin with a COF between 0.37 and 0.28 at dry conditions. At wet conditions, the BSSM displayed an increase of the coefficient of friction with respect to the

dry conditions with values for the COF between 0.66 and 0.76 as a function of the normal force, slightly higher compared to human skin at the macro scale.

Figure 6. Coefficient of friction of the BSSM as a function of the normal force in comparison to ex vivo skin at the macro scale: (a) and (b) indicate dry and wet conditions, respectively. Coloured areas' s indicate a range of friction values for in vivo skin based on the literature at both, dry (red area in (a) image) and wet (blue area in (b) image) conditions.

The nearly constant COF for the skin at the macro scale suggested a tribological performance independent of the area of contact at high forces which is consistent with Amonton's law. The frictional results presented above at the meso and the macro scales indicated a good agreement between the results of human skin and the frictional performance of the BSSM at both, dry and wet conditions. Moreover, this was demonstrated at the meso scale, where the coefficient of friction was described as a power law function of the normal force and at the macro scale where the coefficient of friction followed Amonton's law.

10.3.2 A length –scale dependent model to predict skin friction

10.3.2.1 Friction at the meso scale

The evolution of the elastic modulus a function of the applied force was determined at dry conditions by means of the Hertzian model. To account for the changes at the contact due to

hydration, a modified Hertzian model which includes the effect of the capillary forces was implemented based on Eq. (6) to determine the elastic modulus and the contact parameters at wet conditions. Similarly, the JKR model and a modified JKR model given by Eq. (7) were used to evaluate the contact situation also at wet conditions. The evolution of the elastic modulus obtained from the previous contact models as a function of the normal force at dry and wet conditions is presented in Table 4.

Table 4. Summary of the elastic modulus of the skin as a function of the normal force at the meso scale according to different contact models for dry and wet conditions.

MESO SCALE		
dry	Hertz	$E_H(F) = 4.2 \cdot 10^4 \cdot F^{-0.14}$
	Hertz & capillary force	$E_{H+CAP}(F) = 4.5 \cdot 10^4 \cdot F^{-0.12}$
wet	JKR	$E_{JKR}(F) = 5.1 \cdot 10^4 \cdot F^{-0.074}$
	JKR & capillary force	$E_{JKR+CAP}(F) = 5.2 \cdot 10^4 \cdot F^{-0.072}$

These equations were implemented in the “Two-term friction model” to derive the evolution of the interfacial shear strength as a function of the force by fitting the experimental results on *ex vivo* skin presented in the previous section for both, dry and wet conditions. In this work, the real area of contact was considered the 80% of the nominal area of contact. Based on these considerations the results for interfacial shear strength are presented in Table 5 for each case. The standard error (SE) is associated to the differences between the experimental COF from *ex vivo* skin and the coefficient of friction calculated from the given definitions of the elastic modulus and the interfacial shear strength for each model. Thus, according to these results the best fitting at wet conditions was given by the modified Hertzian approach with capillary force.

Table 5. Interfacial shear strength as a function of the normal force obtained from the fitting of the experimental COF of *ex vivo* skin and the theoretical models at the meso scale for dry and wet conditions. The standard error is also given for each case.

fitting	dry	Hertz	$\tau = 800 \cdot F^{-0.21}$	SE=12.05%
		Hertz & capillary force	$\tau = 990 \cdot F^{-0.15}$	SE=15.8%
	wet	JKR	$\tau = 620 \cdot F^{-0.05}$	SE=17.1%
		JKR & capillary force	$\tau = 550 \cdot F^{-0.05}$	SE=21.2%

Based on the results presented above for the elastic modulus and the interfacial shear strength, a predictive model is presented in Figure 7 for both, dry and wet conditions. The theoretical results are displayed in comparison to the experimental results on *ex vivo* skin and

the results of the BSSM. At dry conditions, a coefficient of friction around 2 underwent a power law decrease until values around 0.3 at 20 mN. Similarly, an evolution of the COF as a power law function of the applied load is presented for wet conditions in Figure 7 (b) in comparison to the experimental results of *ex vivo* skin and the BSSM. The coefficient of friction at wet conditions was obtained according to the contact situation given by a modified Hertzian model which considered the capillary forces due to meniscus formation. At wet conditions, the experimental results indicated a higher COF at low forces due to the influence of the adhesive forces with a value of 5.5 at low forces, decreasing to around 0.35 as the normal force increased.

Figure 7. Prediction for coefficient of friction at the meso scale as a function of the normal load compared to the experimental results of *ex vivo* skin and the BSSM: (a) and (b) indicate dry and wet conditions, correspondingly.

Further, the coefficient of friction is presented in Figures 7 (a) and (b) as a power law function of the normal force with an n exponent of -0.43 and -0.63 for dry and the wet conditions, respectively. The higher n at wet conditions indicates a more important role of the adhesive forces with higher COF's at low forces with respect to dry conditions. Moreover, the increase of the COF at wet conditions indicated also an increase of the interfacial shear strength in opposition to the decrease of the elastic modulus. This highlights the likely influence of the adhesive forces in the mechanical properties on the interface between materials which suggests that, at the meso scale the role of adhesion is especially important to understand the changes in the COF.

10.3.2.2 Friction at the macro scale

Similarly as at the meso scale, an evaluation of the length scale dependence of the elastic modulus of the skin was achieved at the macro scale for dry and wet conditions. According to the definition of the skin structure at the macro scale, the elastic modulus was considered to be influence by the muscle as indicated in Table 1. At dry conditions the evolution of the elastic modulus was evaluated by means of the Hertzian model whereas, at wet conditions, the JKR approach was applied. The resulting elastic modulus as a power law function of the normal force are presented in Table 6 for both, dry and wet conditions. The effect of the capillary force was not considered at the macro scale because the initially applied forces in this range were considerable larger than the contribution due to the meniscus formation.

Table 6. Summary of the elastic modulus of the skin as a function of the normal force at the macro scale according to different contact models for dry and wet conditions.

MACRO SCALE		
dry	Hertz	$E_H(F) = 4.8 \cdot 10^5 \cdot F^{-0.066}$
wet	JKR	$E_{JKR}(F) = 4.7 \cdot 10^5 \cdot F^{-0.063}$

Although the elastic modulus of the stratum corneum was higher at dry than at wet conditions, no important differences in the overall elasticity of the skin were evidenced based on the results of equation (1). The implementation of these results in the “Two-term friction model” by considering a real contact area as the 80% of the nominal contact area, addressed to the evaluation of the interfacial shear strength. These results are presented in Table 7 as a power law function of the normal force for the dry and wet cases as well as the standard error due to the differences with respect to the experimental data.

Table 7. Interfacial shear strength as a function of the normal force obtained from the fitting of the experimental COF of ex vivo skin and the theoretical models at the macro scale for dry and wet conditions. The standard error for each case is also given for each case.

fitting	dry	Hertz	$\tau = 13.5 \cdot 10^3 \cdot F^{0.086}$	SE=3.1%
	wet	Hertz	$\tau = 17 \cdot 10^3 \cdot F^{0.16}$	SE=2.9%
		JKR	$\tau = 16.8 \cdot 10^3 \cdot F^{0.13}$	SE=2.7%

Given the results presented in Table 6, at dry conditions the Hertzian approach showed a good agreement with the experimental data with an error of 3.1%. Conversely, at wet conditions, the JKR model presented a better correlation with the experimental results with an error of the 2.7% lower than that obtained with the Hertzian assumptions. The equations

given in Table 7 show an m exponent lower at dry conditions compared to the wet case which indicates a relationship between the adhesive forces and the value of the exponent. At wet conditions, the Hertzian model presents an m exponent slightly larger than that given by the JKR which reveals that, to fit the theoretical values with the experimental data it was necessary to increase the influence of the adhesive forces. As the JKR model includes this effect, the value of the exponent is lower whereas the Hertzian approach required a higher m exponent. Both approaches showed a similar error when comparing the experimental results and the theoretical data from both models. Further analysis was done in this case by using the JKR approach.

According to the previous results, a theoretical model was obtained at the macro scale at both, dry and wet conditions. Thus, the elastic modulus and the interfacial shear strength obtained from the Hertzian approach were implemented to predict the COF at dry conditions. At wet conditions, the elastic modulus and the interfacial shear strength calculated from the JKR model were implemented to evaluate the COF. Figure 8 (a) displays the evolution of the coefficient of friction as a function of the normal force at dry conditions. An equation for the COF as a power law function of the normal force is also included.

Figure 8. Prediction for the coefficient of friction at the macro scale as a function of the normal load compared to the experimental results of ex vivo skin and the BSSM: (a) and (b) indicate dry and wet conditions, correspondingly.

Similarly, the theoretical model was calculated at wet conditions based on the JKR model and the results are displayed in Figure 8 (b) in which is also presented the COF as a function of

the normal load. The analysis of the m exponent at dry and wet conditions showed the same value of -0.14 which indicates that the coefficient of friction at the macro scale is nearly constant and independent of the contact area. Then, the differences between the COF at wet and dry conditions at the macro scale might be due a consequence of the changes that water causes in the interfacial shear strength rather than modifications on the contact area.

10.3.3 A function fit for the COF of the skin as a function of the length scale

Based on the previous analysis of the COF, in this section the corresponding function fits at the meso and the macro scales for both, dry and wet conditions are displayed as a function of the normal force. In Table 8, the coefficient of friction at the meso and the macro scales is presented as a power law function of the normal force at dry and wet conditions. Due to the absence of experimental data at the micro scale, the performance of the skin in this range was not included yet, according to our considerations it might not change significantly from the results obtained at the meso scale. From the previous analysis at the meso and the macro scale, it has been demonstrated that the contribution of the interfacial shear strength to the COF is considerable higher at the macro scale a cause of the higher elastic modulus due to the consideration of the muscle's influence in the mechanical response of the skin. Moreover, the influence of the adhesive forces was not important the macro scale whereas at the meso scale presented an essential contribution to the total friction force, especially at wet conditions. Thus, at high loads the contribution of the adhesion forces in the COF becomes trivial, even in wet conditions, as indicate the values of the exponent m at the macro scale with a crucial role of the interfacial shear strength.

Table 8. Summary of the function fits obtained for the COF at the meso and macro scale at both, dry and wet conditions.

	DRY CONDITIONS	WET CONDITIONS
Meso scale	$\mu_{meso} = 0.08 \cdot F^{-0.43}$	$\mu_{meso} = 0.04 \cdot F^{-0.68}$
Macro scale	$\mu_{dry} = 0.36 \cdot F^{-0.19}$	$\mu_{macro} = 0.46 \cdot F^{-0.15}$

Figure 9 displays the coefficient of friction of the skin obtained from the previous analysis at the meso and the macro scale based on the frictional results performed on *ex vivo* skin. The

outcomes of this study indicated a crucial role of the interfacial shear strength at the meso scale with further influence of the surface properties of the skin given by the Skin Surface Lipid Film or the modifications implemented on it. Conversely, at the macro scale, the COF indicated a more steady behaviour with only differences from dry to wet conditions likely due to the changes in the interfacial shear strength from dry to wet conditions. At this scale, the influence of the elastic modulus of the skin seemed to be more important than the changes in the area of contact and consequently, in the adhesive properties of the skin. The discontinuity in Fig. 9 results of the error associated to the different equipment and because of a discontinuity of the elastic modulus between the meso and the macro scale. This issue will be analysed further in the ongoing work.

Figure 9. Coefficient of friction of the skin as a function of the normal force at different scales. The COF at the meso (red area) and the macro (violet area) scales the COF is described as a power law function of the normal load with n exponents between -0.43 and -0.63 for dry and wet conditions, respectively at the meso scale. At the macro scale, the n exponent was -0.19 at dry conditions while at wet conditions was -0.15. The full lines indicate the COF at dry conditions whereas the dashed lines represent the COF at wet conditions on each case.

10.4 Conclusions

In this work is presented a Bi-Stratified Skin model (BSSM), which accounts for the structure of the skin and the relative contribution of each layer to the tribo-mechanical response depending on the applied force. With regards to the physical model the main conclusions are appointed below:

- The frictional performance of the BSSM at the meso and the macro scale indicated a good agreement of the model with experimental results on *ex vivo* skin and other *in vivo* skin measurements from previous research.
- The friction results as a function of the relative humidity showed the ability of the model to adapt to the environmental changes and to accomplish modifications of its performance as a function of the hydration level.

Additionally, a theoretical skin model which considers the relative contribution of the skin's layers depending on the scale is presented in this work. Moreover, the contact situation was analysed by means of the classical Hertz and JKR models and modifications of these which included the effect of the capillary forces by fitting the interfacial shear strength with the experimental results on *ex vivo* skin. Thus, the main conclusions extracted from this analysis are:

- A better agreement between the predictive model based on a Hertzian approach and the experimental results was observed at dry conditions at the meso scale.
- Under wet conditions, a closest fit between the Hertzian approach with capillary forces and the experimental results was found at the meso scale.
- At the macro scale, the results of the model calculated according to the Hertzian contact model showed a good agreement with the experimental results at dry conditions.
- At wet conditions, the predictive model indicated the lowest standard error for the JKR model although the differences with the Hertzian approach were minor.

- The results of this analysis pointed out the importance of the scale and magnitude of the applied force for a proper evaluation of skin's friction to properly assess the relative influence of the adhesive forces accordingly to the scale of work.
- According to the results, at dry conditions the Hertzian model can properly described the frictional performance of the skin at both, the meso and the macro scale.
- At the meso scale the role of the capillary forces is crucial to understand the high values for the coefficient of friction obtained on *ex vivo* and *in vivo* skin under wet conditions.
- At the macro scale, the predictive model based on the JKR approach predicted the experimental results without further influence of the capillary force.
- A lower elastic modulus of the stratum corneum at both scales under wet conditions is required to correlate the theoretical and the experimental results.
- The results of the interfacial shear strength presented as a power law function of the applied force indicated a stronger influence of the adhesive forces at the meso scale whereas, at the macro scale it was strongly influenced by the elastic modulus. Thus, at the meso scale, the role of the adhesive forces showed a strong influence on the COF whereas, at the macro scale the elastic modulus had a key role.

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Chapter 11

11.1 Conclusions and Recommendations

In this thesis the development and tribo-mechanical characterization of a new synthetic skin equivalent is described and compared to the results from *in vivo* and *ex vivo* skin as well as other commercial synthetic surrogates. The model, so-called Bi-Stratified Skin model (BSSM), is based on two material layers with different mechanical properties to account for the length scale dependence of the skin's elastic modulus. The bottom layer, a soft PVA hydrogel obtained by freezing/thawing cycles, ensures the elastic response of the skin at large deformations. On its top, a mixture of PVA hydrogel and rapeseed oil with higher elastic modulus and similar adhesive properties as the stratum corneum is implemented. The elastic modulus and the frictional performance of the model was compared to *ex vivo* human skin results in two different range of forces: at the meso and the macro scale. Further, changes in the elastic response and the adhesive properties of the BSSM due to the effect of water are analysed in correlation ship with variations in its tribo-mechanical performance. Finally, a theoretical model for the frictional performance of the human skin is proposed based on the experimental results on *ex vivo* skin and the BSSM. The predictive model is defined in two regimes by a different skin layer-composition to assess the scale dependent response of the elastic modulus of the skin. The analysis of the contact situation is conducted by means of the Hertzian and JKR models. Moreover, to evaluate the frictional performance of the skin at wet conditions, the effect of the capillary force is also included in the previous models. The conclusions arising from the correlation between the BSSM and the results from *ex vivo* skin or other results from *in vivo* skin from the literature, are presented in the following sections. Further, the conclusions from the theoretical analysis in comparison to the frictional performance of the BSSM and *ex vivo* skin are also summarized below.

11.1.1 Conclusions

➤ **Analysis of the frictional performance of *ex vivo* skin**

- In a tribological system based on a hard smooth surface applying force to a flat piece of skin, the adhesive properties are essential in describing the frictional performance of the skin at the meso scale.
- If the coefficient of friction of the skin is described as a power law function of the normal force, the differences in the n exponent depend essentially on the skin condition (untreated or treated) and they are related to the contribution of the adhesive forces to the total friction force.
- The coefficient of friction of the skin is mainly governed by the value of the overall elastic modulus of the skin at the macro scale (forces in the N range) with an effect of the adhesive forces under wet conditions which is related to the softening of the stratum corneum.
- The physic-chemical properties of the contacting materials and the resulting interfacial shear strength are essential aspects which influence the interaction “skin-materials”.
- The sliding contact of a spherical ball on the skin causes surface lipid removal and other surface modifications which affect the integrity of the stratum corneum although no histological damage is observed.
- The high coefficients of friction obtained at the meso scale could be explained as a combined effect of the adhesive forces due to Van der Waals forces and capillary meniscus formation specially the latest, at wet conditions.
- The presence of debris due to stratum corneum and surface lipid removal might explain, together with an increase of the ploughing effect, the rise of the coefficient of friction with the increase of the force at the macro scale.

➤ **Mimicking the tribo-mechanical performance of the human skin**

- The combination of hydrophobic PDMS and a hydrogel of PVA shows water absorbent capabilities and it presents an elastic and the shear modulus in the same order of magnitude as the human skin according to the literature.
- The frictional performance of Silicone Skin L735 reveals a limited suitability as skin equivalent for testing applications at both, dry and wet conditions. Lorica and the ESE present a more similar frictional performance as *ex vivo* skin at both, dry and wet conditions at the macro scale.
- PVA based building-blocks obtained by freezing/thawing cycle exhibit hydration properties and a viscoelastic behaviour in the range of the ultra-soft materials similar to the human dermis.
- The tribological performance of PVA blocks differs from the human skin due to a low interfacial shear strength resulting from a squeezing effect of the water content of the blocks.
- The viscoelastic, adhesive and hydration properties of the Synthetic Stratum Corneum (SSC) indicate a good agreement with *in vivo* skin results from the literature.
- The elastic modulus of a BSSM is described as a power law function of the applied force at both, dry and wet conditions based on a previously developed length scale dependent model.
- The frictional performance of the BSSM indicates a length scale dependence with results varying from the meso to the macro scale due to the relative effect the adhesive forces and elastic modulus. The coefficient of friction of the BSSM is similar to that of *ex vivo* and *in vivo* human skin at dry conditions whereas, at wet conditions a close agreement is found.
- The frictional performance of the BSSM at the meso scale is a function of the skin's surface properties with a special influence of the adhesive forces resulting from the skin condition (wet, hydrated, coated with oil, creams, etc.) in the area of contact.

At the macro scale, the frictional performance of the skin shows a nearly constant coefficient of friction as a function of its elastic properties.

➤ **Modelling the tribo-mechanical performance of human skin**

- There is a large influence of the surface conditions, especially of the adhesive forces, in the frictional performance of the skin at low forces. This effect is reflected in an increase of the n exponent when the coefficient of friction is described as a power law function of the load, particularly under hydrated or wet conditions.
- The capillary force affects the frictional performance of the skin under hydrated or wet conditions at forces in the range of mN (the meso scale).
- The tribological analysis of the skin at the meso scale requires to consider the evolution of the elasticity in a structure composed of stratum corneum, viable epidermis and dermis.
- At the meso scale, the frictional performance of a length scale dependent predictive model is properly described by the Hertzian contact model at dry conditions. The effect of the capillary force needs to be implemented in the Hertzian approach to include the increase of the nominal contact area arising from the increase of the adhesive forces at wet conditions. Thus, the role of the capillary force is crucial at the meso scale to describe the frictional performance of the skin under wet conditions.
- The mechanical response of the muscle must be included in the evaluation of the contact parameters at loads in the range of N. Then, the overall skin's elastic modulus is nearly constant with a slight variation from dry to wet conditions.
- At the macro scale, the coefficient of friction of the skin is almost constant as function of the normal force and it can be approached by the Hertzian contact model at dry conditions. At wet conditions, a slightly higher coefficient of friction is calculated by considering the JKR contact model to account for the effect of the adhesive forces. Nevertheless, the experimental results from *ex vivo* skin presented in this thesis show that, the coefficient of friction at the macro scale does not depend

on the area of contact and it is nearly constant in a range of forces between 0.5 and 3.5 N.

- The interfacial shear strength, as the fitting parameter in the models presented in this thesis, holds a crucial influence in the evolution of the frictional performance of the skin. At the meso scale, it is greatly influenced by the adhesive forces at the contact whereas, at the macro scale it depends mostly on the overall elastic modulus of the skin.

11.1.2 Recommendations for future research

- The BSSM developed in this thesis simulates the frictional performance of the skin at equilibrated conditions either dry or wet. A fast-responsive material should be created to adapt more rapidly its physical and mechanical properties to the changes in temperature and humidity.
- The particularly high coefficient of friction of *ex vivo* skin obtained at the meso scale, especially under wet conditions, could not be fully represented by the BSSM. Thus, surface modifications of the present model should be considered to approach the results of human skin.
- An analysis of the frictional performance of the skin at the micro scale under different conditions (range of forces within few μm) should be conducted to better understand the role of the adhesive forces on skin friction.
- A broader analysis of the effect of water in the physical and mechanical modifications of the stratum corneum should be achieved to properly understand its influence in the surface properties of the skin and the interfacial shear strength.
- Alternatively to the analysis of the adhesive and capillary effects on friction, the role of the electrical properties in the interfacial shear strength might be relevant to understand the changes in the frictional performance of dry, normal and hydrated skin.

- The analysis of the frictional performance of the skin has been performed in this thesis by considering a constant viscoelastic loss fraction. However, it might be possible that a higher contribution of hysteresis occurs at higher loads with a consequent increase of the deformation component of friction. Consequently, investigations about this likely influence must also be conducted.
- The properties and performance of the developed physical model can be analysed for further purposes such as:
 - Diffusion of vaccines, creams and moisturizers or drugs through the skin.
 - As a replacement in problems related to spinal disc protrusion due to degeneration.
 - For cartilage replacement applications.
 - The model could also be adapted for using as dressing to avoid the trans epidermal water loss in burn skin.

List of publications

• Journal publications

- I. **Morales Hurtado M**, Zeng X, Gonzalez-Rodriguez P, Ten Elshof JE, Van der Heide E. A new water absorbable mechanical epidermal skin equivalent: The combination of hydrophobic PDMS and hydrophilic PVA hydrogel. *Journal of the Mechanical Behaviour of Biomedical Materials* 2015; 46: 305-3017.
- II. **Morales Hurtado M**, Peppelman M, Zeng X, Van Erp PEJ, Van der Heide E. Tribological behaviour of skin equivalents and *ex-vivo* human skin against the material components of artificial turf in sliding contact. *Tribology International* 2016; 102: 103 – 113.
- III. **Morales Hurtado M**, de Vries EG, Peppelman M, Zeng X, van Erp PEJ, van der Heide E. On the role of adhesive forces in the tribo – mechanical performance of ex vivo human skin. Under review: *Tribology International* (September, 2016).
- IV. **Morales Hurtado M**, de Vries EG, Zeng X, Van der Heide E. A tribo-mechanical analysis of PVA-based building blocks for implementation in a 2-layered skin model. *Journal of the Mechanical Behaviour of Biomedical Materials* 2016; 62: 319- 332.
- V. **Morales Hurtado M**, de Vries EG, Zeng X, Van der Heide E. Mimicking the surface properties of human skin: tribo-mechanical & adhesive properties of a Synthetic Stratum Corneum. Under review: *Materials & Design* (September, 2016).
- VI. **Morales Hurtado M**, de Vries EG, Zeng X, Van der Heide E. Bi-Stratified Skin model to account for the length scale dependence of the elastic modulus of human skin Submitted to the *International Journal of Solids and Structures* (July, 2016).
- VII. **Morales Hurtado M**, de Vries EG, Zeng X, Van der Heide E. Skin friction: a length scale dependent physical & theoretical model; manuscript in preparation.

- **Book chapters**

- a) **Morales Hurtado M**, Zeng X, van der Heide E. The human skin and hydration, Chapter 4. In: Hydrated Materials: Applications in Biomedicine and the Environment; Nakanishi Y, edr. CRC Press; 2015.

Papers not included in this thesis

- **Contributions**

- b) Zhang, S, AC Rodriguez Urribari, **Morales Hurtado M**, Zeng X, Van der Heide E. The role of the sliding direction against a grooved channel texture on tool steel: an experimental study on tactile friction. International Journal of Solids and Structures, 2015; 56-57: 53-61.

- **Conference proceedings**

- c) Zeng X, **Morales Hurtado M**, van der Heide E, Yang H, Lin L, Li J. The development of skin equivalent for tribological applications, 3rd International Conference on BioTribology, London, September 11-14, 2016.
- d) **Morales Hurtado M**, Zeng X, Peppelman M, van Erp P, van der Heide E. The Tribological Behaviour of a Newly Developed Water Absorbable Skin Equivalent Compared to Isolated Human Skin during Sliding, International Tribology Conference ITC2015, Tokyo, September 17-20, 2015.
- e) Van der Heide e, Zhang s, Rodriguez Urribarri A, **Morales Hurtado M**, Zeng X. Soft Tribology: Research and Applications, Annual meeting JSME, Sapporo, Japan, September 14-15, 2015.
- f) Zeng X, **Morales Hurtado M**, van der Heide E. Active layered tribo mechanical skin equivalent, Japan-the Netherlands symposium on Soft-Tribology, Kumamoto, Japan, September 10-13, 2015.

- g) Van der Heide E, Zhang S, Rodriguez Urribarri A, **Morales Hurtado M**, Zeng X. Soft Tribology: Research and Applications, Japan-the Netherlands symposium on Soft-Tribology, Kumamoto, Japan, September 10-13, 2015.
- h) **Morales Hurtado M**, Zeng X, Peppelman M, van Erp P, van der Heide E. Tribological performance of skin equivalents and ex vivo human skin during sliding contact with artificial grass, VIII Iberian Conference on Tribology, Cartagena, Spain, June, 2015.
- i) **Morales Hurtado M**, ZengX, van der Heide E. Artificial skin for tribological testing based on polydimethylsiloxane (PDMS) with a polyvinyl alcohol (PVA) hydrogel coating, 7th World Congress on Biomechanics, July 6-11, 2014, Boston, MA, US.

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Marina Morales Hurtado was born May 22th 1985 in San Miguel de Salinas (Alicante), Spain. She studied Mechanical Engineering at the Polytechnic University of Elche where she graduated within the 3rd first track positions of her promotion. Afterwards, she worked in a company in the field of civil engineering and later decided to continue the studies in Biomedical Engineering. Then, she joined the interdisciplinary Master of Biomedical Engineering of the University of Valencia and the Polytechnic University of Valencia, Spain.

During her master she specialized in Biomechanical Engineering and Tissue Regeneration, particularly she worked in the development and characterization of materials with biomedical applications. For her master's thesis she accomplished the synthesis and characterisation of a reinforced hydrogel for cartilage replacement and pericardial dressings which she defended on July 2012 by obtaining highest honours. On November 2012, she joined the Laboratory of Surface Technology and Tribology at the University of Twente in Enschede, The Netherlands. The aim of her research was to develop a mechanical skin substitute for industrial applications which could simulate the frictional performance of the human skin at different conditions. The results of this research are reflected in this thesis.