Full Length Article

Bioactive calcium phosphate coatings applied to flexible poly(carbonate urethane) foils

P. Farjam a, M. Luckabauer b, E.G. de Vries b, V.R. Rangel a, E.E.G. Hekman a, G.J. Verkerke a,c, J. Rouwkema a,c

Abstract

Long-term fixation of orthopaedic implants can be enhanced by tissue ingrowth techniques. As such, the deposition of a bioactive bone-like coating could be considered a promising method to facilitate the integration of implants onto bone tissue. In this study, we identified the optimized osteo-conductive Calcium Phosphate (CaP) coating parameters for deposition on PolyCarbonate-Urethane (PCU) foils. The oxygen plasma surface-activated PCU specimens were suspended in simulated body fluid (SBF) and supersaturated SBFs for 4 h, 8 h, 24 h, or 6 days at a temperature of 20 °C, 37 °C, or 50 °C. This resulted in semi-crystalline CaP coatings on a thin flexible foil via a one-step low-temperature aqueous technique. The deposited CaP coatings demonstrated high stability and remained intact upon bending deformation. According to the in vitro cell assessments, the conducted CaP coatings did not influence cell viability nor cell proliferation compared to the bare PCU substrate. In addition, the deposited CaP coatings enhanced the cell-mediated calcium deposition. All in all, this paper demonstrates a promising method to apply stable bioactive coatings to flexible PCU foils, which can be a promising strategy for the enhanced integration of PCU implants onto bone.

Keywords: PolyCarbonate-Urethane, Calcium phosphate coating, Osteo-conductive, Thin film, Mechanical integration.

1. Introduction

Polyurethanes (PUs) have been used in a vast variety of biomedical applications due to their favourable biological characteristics combined with tuneable mechanical properties. PUs represent a class of different synthetic polymers that are derived from isocyanates (R – N=C=O). The key feature of PU elastomers lies in their structures consisting of alternating soft and hard segments [1]. The segmented PUs comprise three main components; a polyol (soft segment, mainly polyethers or polyesters), an isocyanate (hard segment, mainly diisocyanate), and a chain extender (a low molecular mass molecule, e.g. 1,4- butanediol) [2]. Due to this structure comprising three tuneable moieties, PUs can be designed to exhibit different physical properties. The use of segmented PU elastomers in medical implants was first suggested for cardiovascular devices such as cannulas, heart valves, catheters, and pacemakers based on reports regarding the short-term biocompatibility with blood [3]. Depending on the chemical and physical properties of PUs, they can be used as both bio-degradable and permanent implants.

PolyCarbonate-Urethane (PCU) is a particular member of the PU elastomers which displays excellent characteristics to be biostable in long-term applications. It has been reported that PCU has great resistance to hydrolysis, environmental stress cracking, and metal ion oxidation [4]. In addition, PCU possesses promising characteristics to be used in orthopaedic implants [5]. These include biocompatibility, an elastic modulus which is similar to natural cartilage, and favourable friction and wear properties. One conventional method to prepare PCU is the polyaddition of aromatic diisocyanates and polycarbonate diol [6]. PCU can be involved in a diverse range of orthopaedic implants including, but not limited to, joint-bearing prostheses. PCU has been suggested as an appropriate prosthetic material for hemiarthroplasty of the hip and shoulder in very early stages of osteoarthrises (OA). In hemiarthroplasty, which is the replacement of half of an affected joint with an artificial bearing surface, the low rate of apposing cartilage wear is a key characteristic [7] which makes PCU an appropriate candidate. Use of PCU has been reported in total meniscus replacements as well [8–10]. Trammpolin® (made of PCU) and NUrsurface® (made of PCU) were used as both bio-degradable and permanent implants.

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reinforced with high-density polyethylene) are two synthetic meniscus prostheses that are currently under clinical trials.

Successful implantation depends on the design and material of the implant, surgical procedure, short-term stability, and long-term fixation. Insufficient long-term stability might lead to serious issues such as undesired movement and detachment of an implant which causes implant failure [11]. Generally, polymeric implants possess low mechanical stiffness compared to bone, which may cause micromotion that could lead to failure of the implant [12]. Tissue ingrowth techniques are practical methods to ensure the long-term stability of dental and orthopaedic implants. One promising technique would be deposition of a bone-like apatite coating to improve the integration of the implant into the bone tissue. Applying a bioactive, osteoconductive coating to joint implants could contribute to their long-term fixation to bone. Several bioactive materials from the calcium phosphate (CaP) family elicit enhanced bone integration and osteo-conductivity. It has been reported that hydroxyapatite (HAp) promotes osteogenesis and bone ingrowth. Apatite coatings have also appeared to prevent fibrous encapsulation of polymeric implants [13].

Various methods and strategies have been reported to deposit a bioactive coating consisting of material from the CaP family on metals and polymers. One conventional technique is plasma spraying which is usually used to deposit HAp on Titanium alloys [14]. In plasma spraying, a plasma gun creates a high-energy electric arc, through which an inert gas is passed at high velocity to create a plasma flame. Then, HAp powder is introduced into the flame. The plasma flame melts the HAp powder and deposits it on the target substrate. Even though there is an array of parameters in this technique that have an effect on the final coated layer, the thermal plasma spray coating usually requires elevated temperature (often more than a thousand degrees Celsius) [14, 15]. This makes plasma spraying unsuitable for most polymeric materials including PCU, as these materials are sensitive to severe conditions such as high temperature and concentrated etching solutions [16, 17]. According to literature [17–19], thermal degradation might start between 120 °C and 200 °C depending on the structure of the polymer (chemistry and composition of soft and hard segments) and the presence of any additives. To overcome these restrictions, a low-temperature aqueous coating technique is required for PCU.

Several alternative techniques involving immersion of the substrate in an ionic solution have been suggested. Conventional simulated body fluid (SBF) contains an ion concentration equal to that of blood plasma. It has been reported that using SBF as the soaking solution would require a soaking time of >7 days to result in a uniform coating [13]. To lower the required soaking time, researchers adapted the process by changing the immersion solution to a super saturated, concentrated SBF [20, 21]. These modified SBFs revealed to be capable of depositing both crystalline CaP such as HAp, carbonated-HAp, octacalcium phosphate(OCP), dicalcium phosphate dihydrate (DCPD) also known as brushite, dicalcium phosphate anhydrous (DCPA) that is known as monetite, and amorphous CaP [13].

Deposition of an apatite coating on flexible polymeric substrates has not been extensively investigated. Only a few studies have reported the deposition of osteoconductive CaP coatings on thin polymeric films consisting of polyether ether ketone (PEEK), high density polyethylene, ultra-high molecular weight polyethylene (UHMWPE), and poly methyl methacrylate (PMMA) with thickness range of 60–500 μm [22–24]. One crucial concern about deposition of an apatite coating on a thin flexible polymeric film is its mechanical stability due to the mechanical mismatch of the coated material and the coating. The adhesion of coating layers to thin polymeric substrates was assessed qualitatively in a few studies [22,24]. In these studies, the evaluated coatings demonstrated adequate adhesion when they were applied on surfaces which were chemically pre-treated (etched) to increase the roughness of the substrates. However, PUs are not resistant to strong and concentrated etching solutions. Thus, the destruction and delamination of an apatite coating formed on thin flexible PUs remained as a critical concern.

The goal of this research was to evaluate and optimize the formation of apatite coatings on a thin flexible PCU foil. We hypothesize that the morphology, percentage of the deposited elements, and crystal characteristics of the coated layer depend on surface pre-treatment, ion concentration of immersion solution, immersion duration, and immersion temperature. Apart from evaluating the composition and stability of the coating, this research investigates the biocompatibility and osteogenic properties of the coating using human meniscal stromal cells. An optimized bone-like apatite coating applied to PCU implants could promote bone tissue integration by enhancing the attachment, proliferation, and differentiation of cells.

2. Materials and methods

Extruded PCU foil (Carbothane AC-4085A) with a thickness of 150 μm was obtained from the Fraunhofer Institute for Manufacturing Engineering and Automation (Stuttgart, Germany). Square samples of 1 cm² were cut and used. To clean the samples before the coating procedure, the specimens were thoroughly washed with 70 % ethanol and diH₂O and then dried with N₂ gas. Samples were then placed in a plasma cleaner (CUTE, Femto science) and exposed to cold low-pressure oxygen plasma for 40 s at 0.5 Torr with an intensity of 50 W. The plasma-treated specimens were immediately suspended in immersion solutions for 4 h, 8 h, 24 h, or 6 days (with solution replenishment each 24 h) at a temperature of 20 °C, 37 °C, or 50 °C respectively. For this, each sample was placed vertically into a well of a 48-well-plate and entirely covered by 1 mL of immersion solution. The coated specimens were then thoroughly washed with diH₂O and dried with N₂ gas. For cell culture tests, the samples were placed in a commercial UV sterilization chamber (3UV™ Transilluminator) to be treated with UV-C light (245 nm) for 30 min.

2.1. Preparation of immersion solutions

SBF was prepared according to the protocol suggested by Oyane et al. [25]. Briefly, all reagents were added to diH₂O at 36.5 °C according to the sequence of Table 1 after each preceding reagent was completely dissolved. The SBF solution was then stored at 4 °C for up to 30 days. Modified SBFS with increased ion concentrations were prepared based on the work reported by Costa et al. [13] and Tas et al. [26]. Concentration of ion species were raised approximately 5 times (5-SBF) and 10 times (10-SBF) by dissolving the reagents according to the Table 2 into diH₂O at room temperature. The prepared 5-SBF and 10-SBF were stored at room temperature for up to 7 days. Prior to the coating process, approximately 0.1 g of NaHCO₃ was added to 50 mL of 5-SBF and 10-SBF while stirring at 500 rpm at 37 °C to increase the pH to 5.5–6.

2.2. Physical and chemical coating characterisation

The surface microstructure and morphology of the deposited coatings were examined using a scanning electron microscope (SEM), JEOL JSM-IT 100. Samples were first coated with gold using a sputter coater (CRESSINGTON) at 10 mA for 60s. Cross-sectional images of CaP coating layers to thin polymeric substrates was assessed qualitatively in a few studies [22,24]. In these studies, the evaluated coatings demonstrated adequate adhesion when they were applied on surfaces which were chemically pre-treated (etched) to increase the roughness of the substrates. However, PUs are not resistant to strong and concentrated etching solutions. Thus, the destruction and delamination of an apatite coating formed on thin flexible PUs remained as a critical concern.

Table 1

<table>
<thead>
<tr>
<th>Reagent (g)</th>
<th>Amount (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.036</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.352</td>
</tr>
<tr>
<td>KCl</td>
<td>0.225</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>0.230</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.311</td>
</tr>
<tr>
<td>HCl (1 M)</td>
<td>40</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.293</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>0.072</td>
</tr>
<tr>
<td>TRIS</td>
<td>6.063</td>
</tr>
<tr>
<td>HCl (1 M)</td>
<td>0.2</td>
</tr>
</tbody>
</table>
were collected from 4000 cm\(^{-1}\) by FTIR, PerkinElmer Spectrum 100. Transmittance spectra were taken by a SEM equipped with an energy dispersive X-ray detector with DIFFRAC.EVA software (Version 4.3.1.2). Chemical functional groups of coatings were identified using a Fourier Transform Infrared Spectrometer (FTIR), PerkinElmer Spectrum 100. Transmittance spectra were collected from 4000 cm\(^{-1}\) to 500 cm\(^{-1}\) wavenumbers.

2.3. Mechanical integrity of coatings

2.3.1. 180° bend test

A 180° bend test was performed to assess the stability of the coated layer on the PCU substrate. Bend tests are qualitative methods to identify the extent of debonding after inducing cracking in the coating by bending. The coated specimen was bent over a stainless steel rod with a bend radius of 500 μm and was kept in the bent position for 30 s. Subsequently, the samples were studied by SEM to detect potential cracking and delamination of the coatings.

2.3.2. Scratch test

A scratch test was performed on coated PCU samples using an UMT2 (Bruker, USA) material tester equipped with a diamond Rockwell cone with a tip radius of 10 μm and an angle of 120°. The normal load during the scratch tests was increased from 0.1 to 1.5 N over a track length of 8 mm, using a low velocity of 100 μm/s to reduce viscoelastic effects. The normal and shear forces were monitored and the resulting wear tracks were measured using a Keyence VHX 7000 digital microscope (Keyence, Japan). Before analysing the wear tracks under the microscope, the samples were tape-protected, using Scotch® tape, which was pressed against the sample after the scratch experiments and pulled off to remove all residual wear particles and the released CaP coating. The maximum contact width (MCW), as well as the track width (TW) where the coating was released, were identified. Based on the MCW and TW, the shear stress was calculated at the initial failure point and maximum failure point. Per group, 8 scratch lines were prepared on a single sample. The detailed test procedure and analysis methods are reported in the Appendix A. Supplementary data.

2.4. In vitro cell assessment

2.4.1. Cell culture

For cell viability assessment, human bone marrow mesenchymal stromal cells (hBMSCs) (Lonza) were seeded at a density of 5000 cells/cm\(^2\) on the samples which were subsequently incubated in 500 μl proliferation medium per well in 24-wells plates at 5% CO\(_2\) at 37 °C. The proliferation medium comprised α-MEM 86 % (v/v) (gibco), FBS 10 % (v/v), L-glutamine 2 mM (GlutaMAX, gibco), Ascorbic acid 0.2 mM, Pen/Strep 1 % (v/v), dexamethasone 10 \(^{-7}\) M, β-Glycerophosphate 0.01 M. For all experiments, culture medium was refreshed every 2–3 days.

2.4.2. Cell viability assessment

Cell viability was assessed 24 h, 3 days, and 5 days after seeding of the samples using a PrestoBlue® (Invitrogen, Thermo Fisher Scientific) assay. After washing the cells twice with phosphate-buffered saline (PBS) (gibco), 50 μl PrestoBlue® reagent was added to 450 μl of fresh medium for each well and incubated for 30 min. Three samples of 100 μl PrestoBlue-cultured medium supernatant were measured in microtiter plates using absorbance at 560 nm with a reference wavelength set at 590 nm using a plate reader (Victor3, PerkinElmer). Three samples were analysed per group and measurements were performed once. The statistical analysis to indicate significant difference between the different means were performed using ANOVA one-way and Turkey post hoc correction.

2.4.3. Cell-mediated calcification assessment

Alizarin Red-S (ARS) assay was performed to detect the effect of the different coatings on calcium deposition by hBMSCs differentiated towards the osteogenic lineage. Calcification assessment was performed after 21 days of culturing hBMSCs on samples in osteogenic medium. Samples that were not seeded with cells and that were incubated in osteogenic medium for 24 h served as controls. ARS solution (40 mM) was prepared by dissolving alizarin red-S dye (Sigma) in diH\(_2\)O. The pH of ARS was adjusted to 4.2 using 25 % ammonium hydroxide (Fluka) or HCl (1 M) without the formation of a precipitate. Cells were washed three times with PBS and fixed in 10 % (v/v) formaldehyde (Sigma) for 15 min at room temperature. The fixative was removed and the cells were washed three times with diH\(_2\)O. After removing the diH\(_2\)O completely, 500 μl of ARS (40 mM) was added to each well. Plates with ARS were incubated at room temperature for 20–30 min with gentle shaking. After removing the ARS dye, the cells were washed five times with diH\(_2\)O. 200 μl of 10 % acetic acid (Sulpco) was added to each well to extract the dye after storing the plates at −20 °C. Well-plates with extraction solution were incubated for 30 min with shaking at room temperature. Samples were scraped and the cells in 10 % acetic acid were transferred into 1.5 mL microcentrifuge tubes, vortexed for 30 s, then the tubes were sealed with parafilm and were heated at exactly 85 °C for 10 min. The tubes were subsequently incubated on ice for 5 min to become fully cooled. Then the tubes were centrifuged for 15 min at 20,000 g (Centrifuge 5425, Eppendorf). After centrifugation, 200 μl of the supernatant was transferred to a new tube and 75 μl of 10 % ammonium hydroxide was added to neutralize the acid. 150 μl of each sample were aliquoted in a 96-well plate (opaque-walled, transparent-bottomed plates) to measure the absorbance at 405 nm with a plate reader (MULTISCAN GO, Thermo Scientific). The bound ARS concentration of the samples was calculated by using an ARS standard curve based on a calibration series that was taken along the assessment of the samples. Three samples were analysed per group and measurements were performed twice per sample. The statistical analysis to indicate significant difference between the different means were performed using ANOVA one-way and Turkey post hoc correction.

3. Results

3.1. Topological characterization of coatings on PCU

PCU samples were coated with different immersion solutions (SBF, 5SBF, or 10SBF), at different temperatures (20 °C, 37 °C, or 50 °C), for different durations (4 h, 8 h, 24 h, or 6 days). Subsequently, the coatings were visually inspected using SEM (Fig. 1). Nucleated particles can be seen for all coating durations, where the shape, size and quantity of the...
particles vary both with the incubation temperature and incubation time. The suspension of oxygen plasma treated PCU in SBF resulted in the nucleation of calcium phosphates on the PCU surface, but the coatings deposited by immersion in SBF did not form a homogenous layer across the PCU surface for all investigated temperatures, even after 6 days of incubation.

Increasing the ion concentration of the immersion solution from SBF to 5SBF at both 37°C and 50°C led to the formation of a continuous multilayer porous structure with cracks in a short time. Uniform coatings with a network of micro-cracks were deposited after 4 h in 5SBF at 37°C. However, upon increasing the time of immersion to 8 h, only non-homogenous rocky particles were detectable on the surface. Uniform non-continuous multilayer coating with cracks and without further distinct surface features formed after 24 h. After 6 days of immersion in 5SBF at 37°C, a noncontinuous uniform coating with a porous surface structure was detected. Immersing the oxygen-plasma-treated PCU foils in 5SBF at 50°C resulted in the formation of a multilayer coating with microcracks after 4 h. The coating deposited at this temperature demonstrated a porous surface covered with homogenous porous globules after 8 h of immersion, no further distinct surface features after 24 h of immersion, and a multilayer deposited coating covered with a dense layer of integrated/fused porous globules after 6 days of incubation.

By increasing the ion concentration of the immersion solution to 10SBF, all the coatings that were deposited at 37°C or 50°C showed a uniform homogenous multi-layer coating for all immersion times. The general structure of the coating consisted of a dense layer with a porous surface, including micro-cracks that were covered with porous globules. By increasing the immersion time, more layers of the globules formed on top of each other. The dense base layer of the coating deposited by immersion in 10SBF at 50°C after 6 days was not exposed since it has been covered totally with layers of globules (around 5 μm in diameter) deposited on each other.

### 3.2. Thickness and mechanical integrity of coatings on PCU

Further evaluations were performed on selected coatings to investigate the effects of the coating parameters (immersion temperature, immersion solution, and immersion duration) on the physical and chemical characteristics of the deposited coatings. The thickness and the elemental atomic ratio of the deposited coatings in 5SBF and 10SBF at 37°C were measured. Additionally, to evaluate the effects of incubation temperature, the coatings deposited in 5SBF and 10SBF at 20°C or 50°C after 24 h of suspension were also included in these analyses.

The cross-sectional images of the deposited coatings in 5SBF at 37°C (Fig. 2A) revealed the process of nucleation and growth of the coating over the incubation time. After 4 h of immersion, only a thin, discontinuous layer was detected. Subsequently, a non-homogenous coating including rocky particles (similar to Monetite crystals [35]) formed after 8 h, a dense layer containing cracks was deposited after 24 h, and a dense layer with the porous structure on the surface was formed after 6 days of immersion. The multilayer structure of the coatings consisted of a dense base layer covered by porous globules on top of each other,
which was noticeable in the cross-sectional images of coatings treated in 10SBF at 37°C (Fig. 2A) or 50°C (Fig. 2B). The thickness of the coatings was measured by SEM-EDX which is reported in Fig. 2C. This analysis showed that the thickness of the coatings was mainly affected by the ion concentration of the soaking solution. Increasing the ion concentration of the soaking solution from 5SBF to 10SBF resulted in an increased thickness of 5–10 folds.

To evaluate the mechanical stability of the deposited coatings to the PCU substrate, the 180° bend test and the scratch test as described in Section 2.3 were performed. As a result of the bending test, none of the examined coatings showed delamination of the deposited layer. By analysing the SEM images before and after the bend test, only propagation of the already existing cracks in the coating was detected (Fig. 3). The mechanical stability assessment of all evaluated coatings are reported in Appendix A. Supplementary data.

Six coated samples, 5SBF-37-24, 5SBF-37-6d, 5SBF-50-24, 10SBF-37-24, 10SBF-37-6d, and 10SBF-50-24 were selected for further evaluations. The selection was based on the SEM-EDX results to assess the coating’s parameters which showed feasibility to deposit a homogeneous layer. In the scratch test, the first point of coating failure with respect to
the increasing normal load was defined as the initial failure (Fa-init), as determined from the individual wear track photos, Fig. 4A. At this point, the maximum contact width (MCW), as well as the track width (TW) where the coating was released were determined. The initial failure, the appearance of the wear track, happened at beginning of the scratches mainly at low normal forces of 0.1–0.5 N. The contact pressures and shear stresses causing the Fa-init were high for all tested samples, in the range of 23–32 (MPa) and 13–29 (MPa) respectively. From the results, Fig. 4A, it can further be observed that after a certain load, the MCW and TW remain constant due to the limited thickness of the PCU sample which limits further indentation depths. The transition to constant TW was found at a track distance of 3 mm for all samples. Therefore, force measurements were taken at this distance as the maximum failure (Fa-max) of the coating, and used to calculate the pressure which causes the maximum track width. The resulting stresses forces are summarized in Fig. 4B. The Fa-max happened shortly after the Fa-init at normal force of approximately 0.6 N.

SSBF-37-24 showed a partial delamination at the sides of the wear track, as a result of the tape peeling. For this sample, an average TW was used for the calculations. The coatings of the other samples were able to resist the tape peeling before taking the microscopic images, indicating that the coatings were still adhered to the PCU base material. Compared to the other samples, the marked scratches on SSBF-37-6d, and 10SBF-37-6d revealed different failure profiles, as the induced wear tracks

Fig. 3. SEM micrographs of polycarbonate urethane (PCU) discs with different apatite coating (coating thicknesses = t) B) before, and C) after a 180° bend test (A). Scale bars = 20 μm.
were not regularly propagated over the remaining length of the scratch path. Due to this, TW was difficult to define for the initial failure and therefore a constant value of 4 μm was assumed for these samples as the minimum wear track radius for all replicate measurement.

### 3.2.1. Chemical characterisation

To further investigate the composition of the coatings, the atomic percentage of Ca, P, C and O in the coatings were determined by EDX analysis (Fig. 5A). For all the evaluated samples with the exception of

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**Fig. 4.** Scratch test performed on polycarbonate urethane (PCU) discs with different apatite coatings A) track width (TW), and maximum contact width (MCW) were identified at two sites: initial failure (Fa-init), and maximum failure (Fa-max) where TW became constant (parallel red dashed line). Scale bars = 500 μm B) Applied normal force on the indenter ($F_N$ (SD)), contact pressure (Normal stress (SD)), and Shear stress(SD) at Fa-init and Fa-max.

<table>
<thead>
<tr>
<th>Coating</th>
<th>$F_N$ (N)</th>
<th>TW (μm)</th>
<th>MCW(μm)</th>
<th>Normal stress(MPa)</th>
<th>Shear stress(MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Failure (Fa-init)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5SBF</td>
<td>37-24</td>
<td>0.1(0.02)</td>
<td>5.1 (1.4)</td>
<td>88.2 (22)</td>
<td>26.2 (3.5)</td>
</tr>
<tr>
<td></td>
<td>37-6d</td>
<td>0.2(0.05)</td>
<td>4.0 (-)</td>
<td>232.9(26.1)</td>
<td>29.0(2.8)</td>
</tr>
<tr>
<td></td>
<td>50-24</td>
<td>0.2(0.09)</td>
<td>11.5 (6.7)</td>
<td>153.8(46.5)</td>
<td>23.1(4.4)</td>
</tr>
<tr>
<td>10SBF</td>
<td>37-24</td>
<td>0.2(0.03)</td>
<td>3.8 (1.7)</td>
<td>150.8(18.3)</td>
<td>29.2(2.7)</td>
</tr>
<tr>
<td></td>
<td>37-6d</td>
<td>0.5(0.14)</td>
<td>4.0(-)</td>
<td>280.8(55.5)</td>
<td>32.4(2.0)</td>
</tr>
<tr>
<td></td>
<td>50-24</td>
<td>0.3(0.04)</td>
<td>5.4(5.3)</td>
<td>170.1(14.6)</td>
<td>28.2(2.9)</td>
</tr>
<tr>
<td><strong>Maximum Failure (Fa-max)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5SBF</td>
<td>37-24</td>
<td>0.6(0.01)</td>
<td>31.6(6.3)</td>
<td>249.7(33.9)</td>
<td>20.5(5.3)</td>
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<tr>
<td></td>
<td>37-6d</td>
<td>0.6(0.00)</td>
<td>52.1(19.1)</td>
<td>461.5(30.5)</td>
<td>18.5(5.9)</td>
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<td></td>
<td>50-24</td>
<td>0.6(0.01)</td>
<td>59.6(24.0)</td>
<td>249.0(26.5)</td>
<td>16.5(6.4)</td>
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<td>10SBF</td>
<td>37-24</td>
<td>0.6(0.01)</td>
<td>26.8(6.8)</td>
<td>262.1(19.7)</td>
<td>21.6(4.9)</td>
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<td></td>
<td>37-6d</td>
<td>0.6(0.00)</td>
<td>44.8(14.3)</td>
<td>401.0(42.1)</td>
<td>18.9(5.7)</td>
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<td>50-24</td>
<td>0.6(0.01)</td>
<td>46.0(4.0)</td>
<td>275.5(10.2)</td>
<td>18.1(6.0)</td>
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5SBF-37-6d and 10SBF-37-6d, the percentage of carbon was significantly higher than the percentage of deposited calcium and phosphorus in the coatings. By increasing the immersion time from 24 h to 6 days, the percentage of carbon decreased considerably. The Ca/P elemental ratio of the coatings was in the range of 1.37–1.68 which corresponds to a wide range of calcium phosphate materials including octacalcium phosphate (Ca/P = 1.33), α-tricalcium phosphate (Ca/P = 1.5), β-tricalcium phosphate (CaP = 1.5), amorphous calcium phosphates (Ca/P = 1.2–2.2), calcium-deficient hydroxyapatite (Ca/P = 1.5–1.67), or Hydroxyapatite (Ca/P = 1.67) [36].

![Table of elemental composition](image)

<table>
<thead>
<tr>
<th>Coating</th>
<th>Elemental Composition (At%)</th>
<th>Ca/P</th>
</tr>
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<tr>
<td></td>
<td>Ca</td>
<td>P</td>
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<tr>
<td>20-24</td>
<td>NA</td>
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<tr>
<td>37-4</td>
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<td>37-8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>37-24</td>
<td>1.0 (0.104)</td>
<td>0.7 (0.090)</td>
</tr>
<tr>
<td>37-6d</td>
<td>16.3 (0.748)</td>
<td>11.7 (0.446)</td>
</tr>
<tr>
<td>50-24</td>
<td>7.2 (0.283)</td>
<td>4.6 (0.047)</td>
</tr>
</tbody>
</table>

B: XRD pattern of the bare PCU film and the coated samples (% Crystallinity Index).

C: FTIR spectra of the surfaces of coated samples.

Fig. 5. Polycarbonate urethane (PCU) discs with different apatite coating were tested A) Elemental composition percentage (SD) of the coatings as determined using EDX analysis, NA: The coating was too thin, the elemental composition was not possible to be detected by EDX. B) XRD pattern of the bare PCU film and the coated samples (% Crystallinity Index), and C) FTIR spectra of the surfaces of coated samples.
The crystal structure of the selected coatings was determined by X-ray diffraction (XRD) as shown in Fig. 5B. None of the evaluated coated samples revealed the peaks of the bare PCU specimen. An amorphous halo was detected for all of the coatings. Although the calculated crystallinity index (CrI) of 5SBF-37-24, 5SBF-50-24, 10SBF-37-24 and 10SBF-50-24 indicated the deposition of a semicrystalline structure, the XRD patterns of 5SBF-37-24, 5SBF-50-24, and 10SBF-50-24 showed an amorphous halo with absence of discrete peaks. The calculated crystallinity value may stem from the non-smooth appearance of the patterns, indicating the onset of intermediate range ordering in the structure. In comparison, well-developed discrete peaks were visible for 5SBF-37-6d, 10SBF-37-24, and 10SBF-37-6d. Increasing the suspension time from 24 h to 6 days while the pre-treated PCU sample was immersed in SSBF at 37 °C, changed the mainly amorphous structure of the deposited calcium phosphate layer to a semi-crystalline structure. The intense sharp peaks at $2\theta = 26^\circ, 2\theta = 31.7^\circ$, and $2\theta = 32.14^\circ$ and

![Graph A](image1)

![Graph B](image2)

Fig. 6. Polycarbonate urethane (PCU) discs with different apatite coating were tested in vitro. A) After one (d = 1), three (d = 3), and five (d = 5) days of culture in proliferation medium, cell viability was determined using a PrestoBlue assay, B) After 21 days (d = 21) of culture and differentiation in osteogenic medium, calcification was calorimetrically quantified using an alizarin red S assay. Significant differences (p < 0.001) were determined using ANOVA one-way and Turkey post hoc correction.
other small peaks at 2θ = 13.8°, 2θ = 29.4°, 2θ = 39.5°, 2θ = 44°, 2θ = 46.7°, 2θ = 49.5°, 2θ = 53.4° could be characteristic of HA (data retrieved from form COD, cif: 2106757, and cif: 2300273). The XRD analysis of the 10SBF-37-24 sample exhibited intense sharp peaks at 2θ = 12.1° and 2θ = 23.9°, and small peaks at 2θ = 29.8°, 2θ = 32.11°, 2θ = 45.8°, and 2θ = 48.4°. The coated layer in 10SBF at 37 °C after 6 days also displayed a semi-crystalline structure with intense sharp peaks at 2θ = 11.5° and 2θ = 25.8°, and small peaks at 2θ = 28°, 2θ = 32°, 2θ = 33.8°, 2θ = 49.4°, and 2θ = 53.3° which reveals similarities with HA and Brushite (data retrieved form COD, cif:1533075) crystal structures.

Chemical functional groups of the coatings were further determined using Fourier transform infrared vibrational spectroscopy. Absorbance spectra from 4000 cm\(^{-1}\) to 500 cm\(^{-1}\) wavenumbers were plotted in Fig. 5C. The moderate peaks representing PO\(_4\)\(^3-\) group were detectable at 560 (cm\(^{-1}\)) and at 603 (cm\(^{-1}\)) [37] in 10SBF-37-6d, 10SBF-37-24, and 5SBF-37-6d. While the peak at 1020 (cm\(^{-1}\)) was an intense peak of the PO\(_4\)\(^3-\) group [37] for 10SBF-37-6d, 10SBF-37-24, and 5SBF-37-6d samples, it only appeared as a moderate peak for 10SBF-50-24, 5SBF-50-24, and 5SBF-34-24 samples. The strong peak at 1225 (cm\(^{-1}\)) and 1730 (cm\(^{-1}\)) could be referred to the presence of C–O, and C=O bonds of the CO\(_2\)\(^2-\) respectively [38], which were detected in the spectra of all the evaluated coatings except the 5SBF-37-6d, and 10SBF-37-6d samples (appeared as a moderate peak). A broad weak peak representing absorbed H\(_2\)O [37] was identified for all the coatings at 2880–3000 (cm\(^{-1}\)).

3.3. In vitro cell viability and calcification assessment

In order to evaluate the biocompatibility of the different coatings, both cell viability and calcification of hBMSCs cultured on the coated samples was evaluated (Fig. 6). Cell viability tests were performed after 1, 3, and 5 days to evaluate the effect of the selected coatings on the viability and proliferation of hBMSCs. According to the in vitro cell viability results (Fig. 6A), none of the examined coatings showed a significant (p < 0.001) increase or decrease in the number of viable cells present compared to the bare PCU foil at any of the time points. When compared with cells cultured on cell culture plastic, all the coated groups and the bare PCU foil demonstrated a significantly lower amount of viable cells after 3 and 5 days.

The amount of deposited calcium by hBMSCs differentiated towards the osteogenic lineage was quantified after 21 days using an Alizarin Red-S assay. To assess only the cell-mediated calcification after 21 days and eliminate the deposited Ca of the coatings, the bound amount of ARS of the control group has been deducted. Calcium deposition was not detected for the cells cultured and differentiated on cell culture plastic. According to the results (Fig. 6B), the cells that were cultured on PCU-10SBF-37-24, 5SBF-50-24, and 10SBF-50-24 revealed enhanced deposition of calcium, significantly (p < 0.001) higher than all the other groups after 21 days and compared to the bare PCU foil.

4. Discussion

This study demonstrates that PCU foils were successfully coated with a layer of calcium phosphate material using low-temperature aqueous solutions. Surface activation of PCU by employing low-pressure, and low-frequency oxygen plasma showed to be an effective pre-treatment that allowed for the formation of the coating. This is comparable to the finding of Kikanî et al. that reported the low-frequency plasma treatment as the superior choice compared to high-frequency plasma treatment and dielectric barrier discharge for surface activation of polyethylene. Plasma treatment changes the methyl groups into hydroxyl groups on the surface of the substrate which results in a higher surface energy and consequently an activated surface to create new bonds [39].

We evaluated the effect of the ion concentration of the immersion solution, immersion temperature, and immersion duration as the coating parameters on the physical and chemical characteristics of the coating layer. Ion concentration of the immersion solution was shown to affect the surface morphology of the coating, coating thickness, and elemental ratio of deposited components. Where immersion of PCU samples in conventional SBF solution did not result in the deposition of a homogenous coating regardless of other coating parameters, the use of SSBF or 10SBF resulted in a homogenous coating after 4 h at 37 °C or 50 °C. Increasing the ion concentration increased the coating thickness and ratio of Ca/P. Immersion at a low temperature of 20 °C did also not result in the deposition of a homogenous coating even after 6d of soaking and even by immersion in the concentrated 10SBF solution. The nucleation on the PCU substrate occurred, but the process did not further develop to the growth phase of coating deposition. Increasing the temperature from 37 °C to 50 °C did not have a distinct effect on the mechanical and chemical characteristics of the deposited CaP coatings. By immersing the PCU in SSBF and increasing the temperature from 37 °C to 50 °C, the thickness of coating and percentage of deposited Ca and P increased while the crystallinity index decreased. By immersing the PCU in 10SBF and increasing the temperature from 37 °C to 50 °C, the thickness of coating and percentage of deposited Ca and P kept almost at the same level, and the crystallinity index decreased slightly. By increasing the incubation time, some of the nucleated crystals dissolved while some of them increased in size, which reveals the dynamic process of nucleation and growth of the deposited coatings. This was especially evident at earlier time points in less concentrated immersion solutions.

The adhesion of the coating layer onto the substrate, particularly when depositing a brittle CaP coating on a thin flexible foil is a critical aspect. Previous research has pointed out that performing a scratch test on thin compliant samples is challenging, as the created scratch is not easily detected on SEM images due to the high number of cracks on the surface of the CaP coatings, and the high elastic recovery of the underlying flexible substrate. The elastic nature of the substrate also means that performing a lap shear test with a standard epoxy adhesive is not a suitable method [40,41]. The aim of the mechanical integrity tests in this study was to qualitatively and quantitatively check and compare the stability of the different coatings on the PCU substrate. The performed 180° bend test in this study confirmed the adhesion strength of the coatings in a qualitative manner. Even though the performed scratch test was informative to assess the adhesion of the coatings quantitatively, the indication of the exact onset for coating failure was subjective, as indicated previously [17,41]. Regardless, the scratch test shows lower normal and shear strength for the coatings with longer immersion duration (5SBF-37-6d, and 10SBF-37-6d) due to higher affected surface areas induced by the wear track, resulting in lower contact stresses. Observation under a microscope after tape-stripping revealed that for all samples the coatings were still largely adherent, which implies higher adhesion strength between the coatings and the PCU than the coatings and Scotch® tape. The shear stresses that will be exerted on a bone implant prepared using the PCU foil are hard to predict, as these will depend on the application. An approximation can be made by regarding values found in literature for contact pressure [42–46], coefficient of friction (COF) [47–49] and density of trabecular bone [50–52] as the total contact area (CA) between the polymeric prosthetic and the cancellous bone is dependent on the bone surface density. By applying maximum pressure, maximum COF, and minimum contact area in $\sigma = \frac{P \cdot COF}{CA}$ where $\sigma$ is the shear stress in the contact, a maximum shear stress of 3.5 MPa can be found, which is significantly lower than the lowest limiting shear strength of 5 MPa for 5SBF-37-6d in this study. However, further analyses are needed to determine the actual coating stability under the exact conditions that may arise for instance during the insertion of a coated PCU implant.

The physical and chemical characteristics of biological and synthetic nanocrystalline apatite coatings reported in literature reveal remarkable
distinctions compared to stoichiometric HAp. These differences include stoichiometry, crystallinity features, the morphology of the surface, and hydration state of the apatites [53]. By immersing the PCU samples in 5SBF or 10SBF at 50 °C, mainly amorphous calcium phosphate (ACP) formed after 24 h according to SEM-EDX, XRD, and FTIR analysis. The XRD results (Fig. 5B) of these two coatings clearly show no peaks with only amorphous halos. Regarding the Ca/P ratios for the 5SBF-50-24 and 10SBF-50-24, 1.57 and 1.56 were measured respectively which could be a sign of ACP (CaH2(PO4)x.nH2O, Ca/P: 1.2–2.2) [37]. The samples coated at 37 °C in 5SBF for 24 h also exhibited the same characteristics of an ACP coating. By increasing the immersion duration to 6 days for samples coated in 5SBF at 37 °C, the coating exhibited a semi-crystalline structure with high crystallinity index according to the analytical analysis. The XRD graph shows the amorphous halo but also peaks representing (002), and (211,112) planes similar to HAp. The FTIR analysis demonstrates the presence of PO2− groups similar to HAp. The combination of 10SBF, 37 °C, and 24 h of immersion led to the formation of a semi-crystalline structure with low percentage of crystallinity as determined using XRD analysis with an amorphous halo and peaks which indicate (020), and (021) planes with some similarities to brushite. Another semi-crystalline coating was formed by immersing the PCU in 10SBF at 37 °C for 6 days. The XRD analysis of the 10SBF-37-6d coating featured an amorphous halo and peaks at (020,002), and (211,112) with similarities to both brushite and HAP coatings. The peaks at 1730 cm−1 in the FTIR graphs could represent the C=O bond in CO2− which is not found in the FTIR analysis of conventional nano-crystalline coatings [37]. One potential reason for formation of the amorphous CaP coatings could be high amount of CO2− as indicated by the high fraction of carbon in the EDX analysis, which is an inhibitor of crystal growth. Increasing the time of immersion from 24 h to 6 days exhibited a great loss in concentration of the CO2− and consequently promoted the formation of the crystalline CaP coatings as shown for 5SBF-37-6d, and 10SBF-37-6d.

As the goal of applying a CaP coating to the PCU foil was to enhance bone integration of PCU implants, the effects of the CaP coatings on cell viability, cell proliferation, and cell-mediated calcification was investigated. By using a PrestoBlue™ assay with which the metabolic activity of viable cells was measured, none of the evaluated CaP coatings adversely or favourably affected either the viability or the proliferation of hBMSCs compared to the bare PCU substrate. Both mainly amorphous CaP coatings (5SBF-50-24 and 10SBF-50-24) and semi-crystalline CaP coatings (10SBF-37-24) significantly enhanced the calcium deposition compared to the bare PCU samples. It should be noted that the coated samples were cultured for 21 days at 37 °C, which could alter the characteristics of the coatings due to the bioactivity of the deposited CaP coatings. Amorphous calcium phosphate as a mineral precursor for bone formation has been reported by several authors [54–56]. The Ca/P ratio of physiological bone apatite has been reported to be 1.51 [56]. According to Fig. 5A, 10SBF-37-4 and 10SBF-37-24 express the closest Ca/P value to bone. Moreover, the maximum peak position of FTIR analysis of fresh bone was reported at 565 cm−1, 604 cm−1 (ν3(P-O) vibrational mode), 1035 cm−1 (ν2(P-O) vibrational mode), and 1415 cm−1 (ν3(C-O) vibrational mode) [57]. Even though the coatings in this study replicate some characteristics of the mineral fraction of bone and the coatings do show an osteoinductive effect on hBMSCs in vitro, performing an in-vivo assessment is needed to clarify if and to what extent the CaP coatings reported here can positively influence cell-mediated calcification and bone bonding in-vivo.

5. Conclusion

This research shows the feasibility of depositing amorphous and semi-crystalline CaP coatings on a thin compliant PCU foil with adequate adhesion strength by a low-temperature aqueous immersion technique. Pre-treating the PCU substrate by employing low-pressure, low-frequency oxygen plasma is a suitable method to activate the PCU surface and potentially increase the adhesion of a CaP coating to the PCU substrate. The ion concentration of the immersion solution, the immersion temperature, and the immersion duration all affect the morphology, thickness, and elemental composition of the deposited coatings. In vitro cell assessments revealed that the deposited CaP coatings do not decrease cell viability compared to the non-coated PCU specimens. Moreover, CaP coatings deposited on the PCU substrate exhibited the potential to enhance calcium deposition mediated by hBMSCs differentiated towards the osteogenic lineage.

CRediT authorship contribution statement


Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References
