Artificial substitutes of the human corneal stroma were developed using fibrin and fibrin-agarose with different agarose concentrations (0.025%, 0.05%, 0.1%, 0.2%, 0.3%) with human keratocytes immersed within. Samples of the different stromal substitutes were studied weekly until 8 weeks of development in culture. After each week in culture, samples were subjected to a novel nanostructuring technique and measurements of the elastic (G') and viscous (G") moduli were performed for using a controlled shear-stress rheometer (Bohlin CS-10, UK). The highest values of G' and G" were found for the corneal substitutes with higher agarose concentration (0.1%, 0.2% and 0.3%) (Figure 1).

Strikingly, the fibrin substitute with 0.1% agarose, in the first weeks of culture, showed G' values equal to a half or one third of the values of the substitutes with higher concentrations of agarose, whereas from the fourth week on, these values are comparable.

The statistical analysis determined a high correlation between the agarose concentration and the elastic and viscous moduli with statistical significance (p<0.05) and also, an inverse correlation between the time in culture and the viscous modulus. In conclusion, these preliminary results suggest that the nanostructured artificial corneal stroma substitutes show good viscoelastic stability during time of development in culture.


Keywords. nanostructured cornea; elastic modulus; viscous modulus.

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(33.O4) NON-INVASIVE QUALITY CONTROL FOR ISLET TRANSPLANTATION USING RAMAN SPECTROSCOPY

Hilderink J (1), de Koning EJP (2), Engelse MA (2), Otto C (3), van Blitterswijk CA (1), van Apeldoorn AA (1), Karperien M (1)

1. Department of Tissue Regeneration, University of Twente; 2. Department of Nephrology, University Medical Center Leiden; 3. Department of Medical Cell Biophysics, University of Twente

Abstract. Type 1 diabetes patients with poorly controllable glucose levels, can be treated by intrahepatic transplantation of donor islets of Langerhans. During isolation, islets are exposed to mechanical stress and their cell-matrix relationship is disrupted, which may induce apoptosis. Before islets are transplanted into the patient, their quality needs to be assessed. Current quality control requires fixation and labeling and does not allow time-lapse studies on the same tissue. In this study we explore the feasibility of using Raman spectroscopy to perform functional studies on pancreatic islets and to monitor their quality over time.

We first used Raman spectroscopy to measure purified insulin and glucagon, the two main hormones produced by pancreatic islets. Raman bands at 520 and 640 cm⁻¹ can be assigned to cysteine and tyrosine, amino acids that are present in insulin. Tryptophan, one of the building blocks of glucagon, causes specific bands at 759 and 1552 cm⁻¹ (fig.1). These bands can be used as markers for the identification of beta and alpha cells in islet preparations.

We subsequently measured human islets and compared their spectral characteristics to those of insulin and glucagon. Tryptophan-specific Raman bands were observed in the islets spectrum, suggestive for the presence of glucagon-producing alpha cells. Bands suggestive for the presence of insulin were not observed in the average islet spectrum, possibly because insulin is a weaker Raman scatterer (fig 1). High resolution local measurements on individual islet cells are currently performed to identify the presence of insulin-vesicles inside these cells.

Currently, we are extending these studies by investigating the effects of different substrates and extracellular matrix components on islet function using Raman spectroscopy. Our data provides the first steps towards a non-invasive and label-free method to study the quality of pancreatic islets, before transplantation in patients with type 1 diabetes.

Keywords. Raman spectroscopy, Quality control, Islet transplantation, Diabetes

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(33.O5) TAILORABLE, HIGHLY-ALIGNED POLY-DL-GLUTAMIC ACID FOR LIGAMENT TISSUE ENGINEERING

May JR (1), Gentilini C (1), Clarke DE (1), Stevens MM (1)

1. Departments of Materials and Bioengineering, Imperial College London

Introduction. A tissue-engineered ligament requires appropriate mechanical properties, resistance to bulk degradation, and biocompatibility. Poly-DL-γ-Glutamic Acid (γ-PGA) is a water-soluble, non-toxic polypeptide produced by many Bacillus species, and undergoes enzymatic degradation rather than hydrolysis. We have modified γ-PGA by esterification[1] and utilized tensile deformation to produce highly-aligned molecular structures with ultra-high tensile strength[2]. This has enabled us to tailor γ-PGA’s mechanical properties over a broad range, targeting that of native ligaments.

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