

IN VITRO EVALUATION & SURFACE CHARACTERISATION OF BIORESORBABLE POLYLACTIDE FOAMS SURFACE COATED WITH FIBRONECTIN & COLLAGEN

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INTRODUCTION: Synthetic biodegradable polymers are ideal for their application in tissue engineering owing to their mechanical strength, easy manipulation into desired shapes, and optimal degradation kinetics, creating the needed (micro) stress environment for the growing tissue [1]. However, their hydrophobic nature, lack of cell-recognition signals, and poor surface structure and chemistry for cell attachment, proliferation, and differentiation limits their application as supportive three-dimensional matrices for cells [2].

Coating surfaces with extracellular matrix (ECM) proteins such as fibronectin, vitronectin, and collagen, provides an 'adhesive interface' between the scaffold material and the cells that resembles the native cellular milieu, whose organization and production modulates and enhances cell adhesion through transmembrane integrin receptors [3]. The reason for selecting complete ECM proteins as opposed to peptide sequences is that fibronectin and collagen not only provide the cell binding sequence for cell adhesion but also provide secondary interactions with other ECM proteins and interactions with growth factors that stabilise the binding of the cells thus strengthening cell adhesion, which results in enhanced cell growth and maturation. Furthermore ECM proteins do not cause any harmful side effects, as they are the natural ligands found *in vivo*.

In the present work highly porous poly(D,L-lactide) (PDLLA) scaffolds were prepared by thermally induced phase separation (TIPS) process of polymer solutions and subsequent solvent sublimation. The scaffolds were characterised in terms of their surface properties, before and after protein surface modification.

METHODS: Purasorb® PDLLA with inherent viscosity of 1.62 dl/g was purchased from Purac biochem (Goerinchem, The Netherlands). Dimethylcarbonate (DMC, 99% in purity), used for the fabrication of PDLLA foams was purchased from Sigma Aldrich. The polymers and solvent were used without further purification.

The polymer was dissolved in dimethylcarbonate to give a polymer weight to solvent volume ratio of 5 %. The mixture was stirred overnight to obtain a homogeneous polymer solution. The resulting solution was transferred to a lyophilisation flask, which was immersed in liquid nitrogen and maintained at -196°C for 2 h. The frozen mixture was then transferred into an ethylene glycol bath at -10°C and connected to a vacuum pump (10⁻² mbar). The solvent was sublimed at -10°C for 48 h and then at 0°C for 48 h. The sample was completely dried at room temperature in a vacuum oven until reaching a constant weight. More details about the preparation of the foam scaffolds have been presented elsewhere [4].

Surface characterisation of the porous PDLLA scaffolds was carried out pre- and post-modification of the surfaces with human plasma fibronectin purchased from Chemicon Europe (Hampshire, UK) and bovine collagen type I purchased from Sigma Aldrich (Dorset, UK). To determine the biocompatibility of the scaffold surface, murine fibroblast cultures were established to evaluate cell adhesion and proliferation.

The porous morphology of the samples was examined with scanning electron microscopy (SEM) working at 20 kV. Specimens were cut with a razor blade to enable examination of longitudinal and transverse sections.

Time and pH-dependent Zeta potential measurements in 1 mM KCl supporting electrolyte solution were performed to characterise the material

swelling in water and the changing surface chemistry. Wettability measurements using the capillary-rise method and scanning electron microscopy were used to characterise the samples as a function of surface modification and time in culture. Zeta potential, as well as wettability measurements allow the changes of the polymer surface character to be followed in a non-destructive manner.

RESULTS: The foams possess two distinct pore sizes, as shown in Figure 1 i.e., macropores with diameters $> 100 \mu\text{m}$ interconnected by micropores of $10\text{-}50 \mu\text{m}$ diameter. The pores are preferentially orientated in the cooling direction, which is typical of the TIPS process [4].

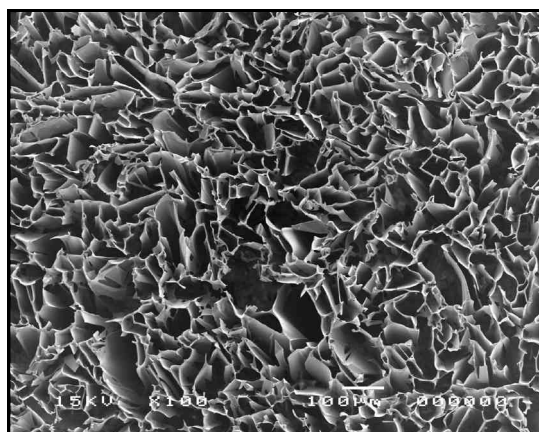


Fig 1: SEM micrograph perpendicular to the tubular pore direction in a PDLLA scaffold.

As determined by previous studies [4], the apparent density of the PDLLA foams is 0.06 g/cm^3 ; porosity of the scaffolds is $> 93\%$, with a total porous volume of $9.5\text{-}11 \text{ cm}^3/\text{g}$.

The measured Zeta potential of the pure polymer scaffold is negative over the whole pH range and the isoelectric point (i.e., where Zeta potential is zero) is in the remit of pH 2.2. The zeta potential plateau value is -25 mV . The results of the Zeta potential measurements clearly indicate that the polymer contains acidic functional groups. The polymer is quite hydrophobic prior to protein adsorption as shown by the wettability measurements.

DISCUSSION & CONCLUSIONS: The objective of the current work was to characterise the PDLLA scaffold in terms of porosity, pore size, wettability, and electrokinetic behaviour by zeta potential measurements.

The porous morphology of the PDLLA scaffolds as examined with SCM supports their application in tissue engineering. The high porosity, void fraction, interconnected pores and continuous channels provide sufficient space for cell adhesion, distribution, and ECM regeneration. However, the application of such polymers for cell adhesion, proliferation, and differentiation may be compromised owing to their hydrophobic nature.

Surface characteristics of polymers for biomedical application are highly important. Further work entails optimisation of these parameters through surface chemistry modification and protein surface coating, resulting in higher seeding density of murine fibroblast cells in comparison to the unmodified scaffolds and generating an environment that promotes normal physiological cellular behaviour.

REFERENCES: ¹C.T. Laurencin, A.M.A. Ambrosio, M.D. Borden, et al (1999) *Ann. Rev. Biomed. Eng.* **1**: 19-46. ²M. Kantlehner, P. Schaffner, D. Finsinger, et al (2000) *CHEMBIOCHEM* **1**: 107-114. ³K.E. Healey (1999) *Current opinion in Solid State and Materials Science* **4**: 381-387. ⁴AR Boccaccini, JA Roether, LL Hench, et al. (2002) *Ceram. Eng. Sci. Proc.* **23**: 805-816.

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