

Microfabrication of biodegradable polymeric structures for guided tissue engineering

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ABSTRACT

In this study, we report a method for the fabrication of poly-(ϵ -caprolactone) (PCL) scaffolds, which utilizes the heating energy of a laser beam to sinter polymer microparticles according to computer-guided geometries, thus providing a powerful means to study cell response to an environment similar to that found in vivo.

INTRODUCTION

Tissue engineering and guided tissue repair are rapidly developing new areas of science, which generally require the use of special biodegradable and biocompatible scaffolds, as three-dimensional (3D) supports for initial cell attachment and subsequent tissue organization and formation. Advanced manufacturing technologies, known as rapid prototyping (RP) technologies, are now developing to fabricate scaffolds with controlled architecture [1-11]. RP methods combine computer-assisted design (CAD) with computer-assisted manufacturing (CAM): 3D computer models are sliced into two-dimensional (2D) layers to fabricate complex 3D structures layer-by-layer [12]. In this study, we present a new developing RP method and apply it to the fabrication of poly(caprolactone) (PCL) scaffolds.

EXPERIMENTAL DETAILS

Polymer used was a commercial poly-(ϵ -caprolactone) (PCL) supplied by Polysciences, Inc. (M_w = 45,000). PCL microspheres for SLS were prepared by a solvent evaporation procedure based on a single oil-in-water (O/W) emulsion [13]. PCL films were obtained from a 4% w/v solution in chloroform by casting. The SLS machine of this study is a prototype designed and built at the Department of Mechanical, Nuclear and Production Engineering of the University of Pisa. The machine consists of a work chamber equipped with a platform able to shift along the z-axis and a laser block, including the laser beam and a system of galvanometric mirrors, which are positioned to precisely address the beam. The CO₂ laser used (SYNRAD model J48-5S) has a nominal power of 50 W. The machine is PC-driven by means of a software for laser guidance (CADMARK, Quantasystem s.r.l). The materials to be processed may be either in powder or slurry form. Slurries are obtainable by dispersing the polymer in a small amount of a volatile, non-inflammable, non-toxic non-solvent. Two-dimensional scaffolds (grids and films) were prepared for cell culture according to the procedure present in [5]. NIH-3T3 mouse fibroblasts

were cultured in Dulbecco's modified Eagle's medium (DMEM; Cambrex, Italy) with high glucose, 10% fetal calf serum (Cambrex, Italy), 1% glutamine (Cambrex, Italy), penicillin (200 U/ml) (Cambrex, Italy) and streptomycin (200 $\mu\text{g/ml}$) (Cambrex, Italy). Culture was maintained in an incubator equilibrated with 5% CO_2 at 37 $^\circ\text{C}$. The polymer structures and films were seeded with a 100,000 cells/ml suspension. After a culture time of 2, 4 and 24 h respectively, they were fixed and stained. Samples were analysed under an optical microscopy (Olympus AX 70) and the ratio between the number of cells on the polymeric structures and the total area of the polymer substrate was calculated as an index of cell density [5]. Both cells of a reference sample (a petri dish coated with gelatin) and cells seeded on scaffolds with different geometries (cast films and square-meshed grids) were examined for comparison to evaluate the effect of scaffold topography on cell growth.

DISCUSSION

PCL laser sintered structures were produced under a fill laser power of 2 W and a beam speed of 20 mm/s. The energy density (ED), regarded as the applied laser energy per unit area, is directly proportional to the ratio between the fill laser power and the beam speed. Its value is a fundamental parameter as it affects properties such as surface roughness, density, tensile strength, dimensional accuracy, occurrence of curling and cracking [9]. Photos of two and three dimensional scaffolds fabricated by SLS are reported in Figs 1 (a) and (b), respectively.

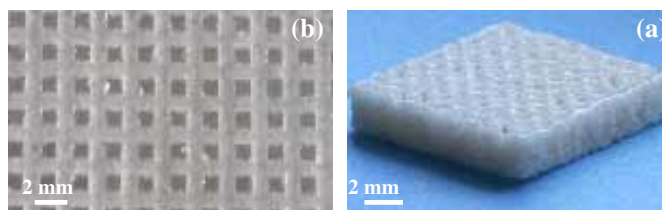


Figure 1. Pictures of laser-sintered two dimensional (a) and three dimensional (b) PCL structures

Fig. 2 shows cell density on scaffolds (cast films and grids) and on a gelatin layer, as a function of culture time. Cell density on laser sintered grids is the lowest at low culture times (2-4 h), whereas, after 24 h, it increases greatly and becomes higher than that on films. This finding suggests that cell adhesion is affected by scaffold topography (geometry and surface roughness). Flexible rapid prototyping techniques such as SLS can help in identifying the optimum architecture for a particular tissue.

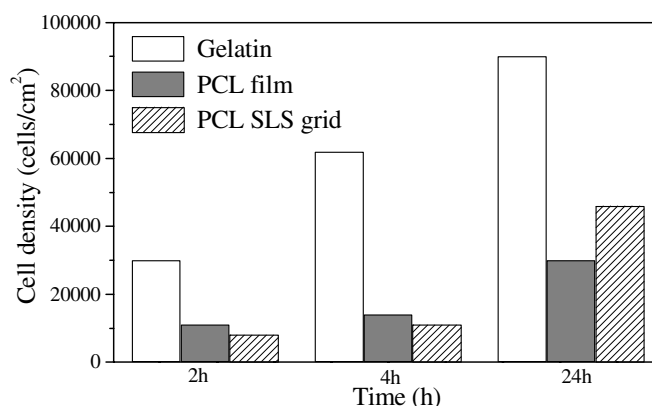


Figure 2. Cell density data for copolymer and PCL scaffolds (films and grids) as compared to control layer (gelatin) at different culture times (2, 4, 24 h).

CONCLUSIONS

In this paper a rapid prototyping technology, SLS was investigated and successfully applied to produce simple 2-D and 3-D PCL scaffolds, by a new custom made prototype machine. The purpose was that to evaluate the actual performance of the machine in the scaffold production field and to eventually improve it through technical modifications. SLS structures have a resolution of about 300 μm (height) \times 700 μm (width). General advantages and disadvantages of the method as a rapid prototyping technology for tissue engineering were investigated. Cell attachment to scaffolds was higher than to membrane samples after 24h culture time, indicating that surface topography and roughness have a positive influence on adhesion.

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