Scaffolds for muscle tissue engineering

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Abstract

Two different approaches can be followed in muscle tissue engineering: implantation of in vitro cultured polymer scaffolds, specifically designed to promote cellular orientation (e.g. micropatterned surfaces), or in vivo delivering of cells to the site of action by use of biodegradable carriers (e.g. microcapsules). Both strategies are currently developed at BioMatLab.

Key Words: human muscle, permanent denervation of the lower extremity, Scaffolds for muscle tissue engineering

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Two different approaches can be followed in muscle tissue engineering: A) implantation of *in vitro* cultured polymer scaffolds, specifically designed to promote cellular orientation (e.g. micropatterned surfaces) [1-3], B) *in vivo* delivering of cells to the site of action by use of biodegradable carriers (e.g. microcapsules).

A) Microgrooved substrata were obtained by a soft lithography technique (Replica Molding) out of a biodegradable PLLA-TMC (L lactide - trimethylene carbonate) copolymer. PLLA-TMC was chosen, among different biocompatible and biodegradable polymers, as the one showing the most appropriate mechanical properties. Two different masters were employed: an alumina master, obtained by laser fabrication (groove width 60 μm, groove depth 20 μm); and a modeling paste sheet micro-imprinted with a home-made array of fibers (Ø 50 μm). From each master, a silicone mold was prepared and a solution of the PLLA/TMC polymer was cast in the mold, as shown in figure 1.

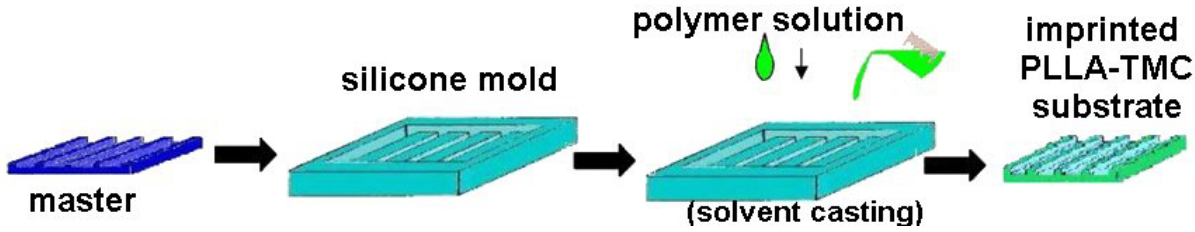

Figure 1. *Schematic procedure for micro-imprinting PLLA-TMC substrata.*

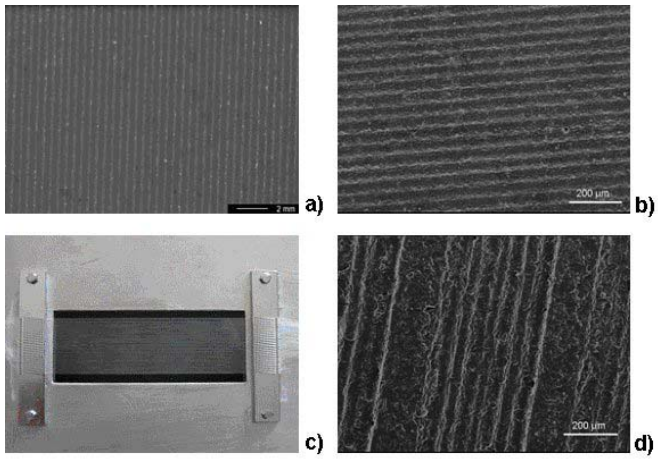

Figure 2. *(a) Alumina master and (b) PLLA/TMC film; (c) plate with an array of fibers and (d) PLLA/TMC film obtained using a modeling paste sheet micro-imprinted by the array of fibers on the plate.*

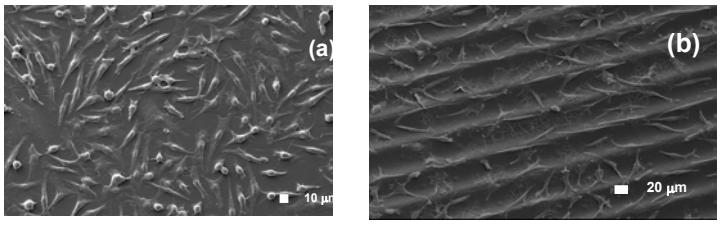

Figure 3. *L929 cells onto PLLA/TMC substrates from (a) smooth film and (b) alumina master, r 3 days after seeding.*

Results indicated that all masters patterns were successfully reproduced. As shown by scanning electron microscopy (SEM), PLLA-TMC replica from alumina master appeared homogeneous with a regular alternation of grooves and ridges (Fig. 2b), whereas from the modeling paste master imprinted with the array of fibers, an irregular alternation of grooves and a micro-rough surface was obtained (Fig.2d). The adhesion of L929 to smooth and microgrooved surfaces was evaluated by SEM analysis and MTT test after 1, 2 and 3 days from seeding. Cells cultured from 24 to 72 hours well adhered on the surfaces of each polymer film (Fig.3). At 48 and 72 hours, a high number of cells well spread on the surface was observed. In particular, for PLLA-TMC film from alumina, a certain degree of alignment on the top of the ridges was observed, whereas the cells tended to overcome the grooves by extending their philopodia with a random orientation (Fig.3b). On films obtained from the modeling paste/fiber array master a good adhesion and proliferation of cells was detected even though no cells alignment was observed. Cell culture experiments under dynamic conditions (i.e. under flow and cyclic mechanical testing) are now in progress with the C2C12 cell line.

B) For cell delivering, a microencapsulation approach was followed. A microencapsulation system based on a syringe pump and a coaxial air flow was developed to obtain microspheres with diameter smaller than 500 m. Alginate-based materials were employed for cell encapsulation. A thermogelling copolymer (Lutrol F127) and chitosan were used to adjust degradation profiles. The influence of processing parameters on microcapsule morphology was investigated and the prepared microspheres were characterized with respect to degradation. Preliminary experiments to assess cell release were performed with murine myoblasts C2C12. By changing the composition of the gel forming material, complete degradation of the microspheres can be adjusted between few hours and more than 1 month. Good cell viability after encapsulation was observed (see figure 4). Released myoblasts were observed on the culture wells after few days for the fastest degrading compositions (calcium alginate) and after 1 or 2 weeks for the most slowly degrading microcapsules (Ca-alginate/chitosan). Future developments are considering the *in vivo* implantation in the rat animal model.

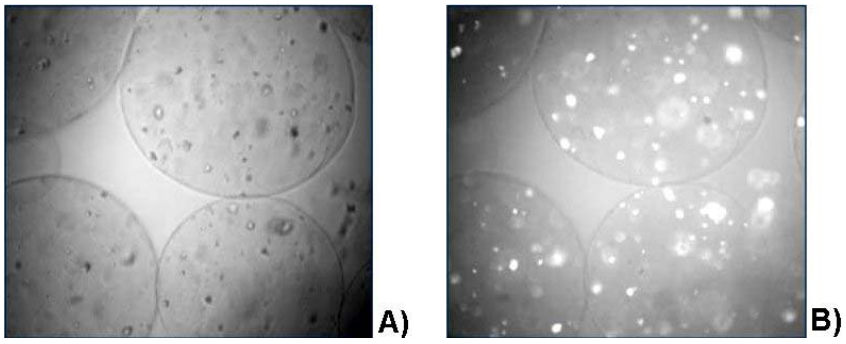

Figure 4. *A, optical image of cells encapsulated into alginate microcapsules; B, fluorescein staining of viable cells.*

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