A functionally flexible, biomimetic scaffold for tissue repair

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I. Introduction. A great array of biodegradable materials, both natural and synthetic, is available for the creation of scaffolds for the repair of damaged or lost tissue. Collagen and poly (α-hydroxy esters) are among the most popular choices. Poly(L-lactic acid) (PLLA) is widely used due to its well-characterized degradation mechanisms and good mechanical properties. Nonetheless, PLLA possesses an inert nature that affects cell-matrix interactions. Its modification using bioactive molecules can lead to the creation of a construct that mimics the physiological biochemistry. Cell attachment, proliferation, migration, and specific cellular responses can be achieved through the use of these biomimetic scaffolds. We have prepared a biomimetic 3D scaffold by entrapping poly (L-lysine) (polyK) in the surface of poly (L-lactic acid) (PLLA) foams in a controllable fashion. Further functionalization of the surface makes this construct suitable for a wide range of tissue engineering applications. We have linked RGD peptides to the entrapped polyK to enhance cell adhesion and demonstrate the functional flexibility of the surface.

II. Materials and Methods

Preparation of the foams. Porous foams were made out of PLLA (Birmingham Polymers, MW: 100,000) salt leaching, with a 90 % porosity and a pore size of 300 mm and dimensions of 8mm in diameter by 3mm in thickness.

Entrapment of poly(L-lysine). Scaffolds were soaked in an acetone-water mixture (7:3) for 1 h. Then, they were placed in 600µl of a solution of PolyK in DMSO for 12 h and rinsed. All steps were carried out under vacuum.

Detection of entrapped PolyK. PolyK-modified foams were incubated in 600µl of a solution of 1mM N-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP) for 30 min. The SPDP was activated in 600µl of 1mM dithiothreitol (DTT). Scaffolds were then incubated in 0.1mM fluorescein-5-maleimide for 2 hrs. Fluorescence spectroscopy was performed on transversal sections of the foams. All steps were carried out under vacuum. The amount of entrapped polyK was determined by linking glutaraldehyde HRP and applying an AmplexRed assay (Invitrogen), which consists of H_2O_2 and a fluorescent dye.

Improvement of cell adhesion: PLLA surfaces were incubated in equal parts of 0.1mg/ml N-hydroxysuccinimide (NHS) and 10mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiamide HCl (EDC) for 2 hours, under vacuum. After rinsing, they were soaked in 100µM RGDC for an hour and rinsed thoroughly. $5x10^5$ rat mesenchymal stem cells were seeded statically on RGDC-modified scaffolds. Cell attachment was assessed by performing PicoGreen® DNA quantification assay (Invitrogen).

III. Results. Figure 1 shows fluorescent micrographs of transversal sections of 3D foams treated in different ways. The scaffold treated with polyK, SPDP and DTT gave the highest fluorescent signal. Thus, it is demonstrated that polyK only reacts with DTT and is available on the scaffold surface. A homogeneous intensity was observed throughout the sections, implying that polyK was evenly distributed throughout the porous network. To control the entrapment, different concentrations of polyK were used to incubate the scaffolds. Sections of modified scaffolds treated with fluorescein-5-maliemide are shown in Figure 2. At polyK concentrations of 0.1 and $5x10^{-4}$ mg/ml there is no appreciable difference in the intensity of the fluorescence, implying that, at those concentrations, the surface is saturated with polyK. A lower concentration of $5x10^{-6}$ mg/ml however, yields a weaker signal, and a further decrease ($5x10^{-7}$ mg/ml) seems to resemble the plain scaffold. Nonetheless, quantification of the polyK in the surface reveals that, at 10^{-8} mg/ml, the amount entrapped is still significant when compared with the plain scaffolds (Figure 3a). Cell adhesion is greatly improved by the presence of RGDC peptides linked to the polyK, demonstrating the viability of further functionalization of the functionally flexible scaffold (Figure 3b).

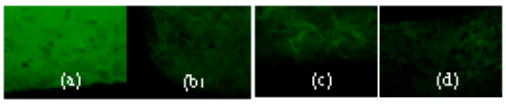

Figure 1. Transversal sections of 3-D scaffolds modified with (a) polyK-SPDP (b) no polyK-no SPDP (c) no polyK-SPDP (d) plain. All discs were treated with DTT and fluorescein-5-maleimide

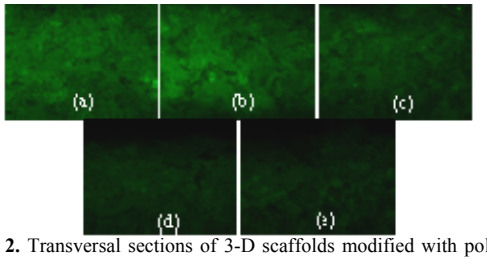

Figure 2. Transversal sections of 3-D scaffolds modified with polyK at (a) 0.1 mg/ml (b) 0.5 µg/ml (c) 5 ng/ml (d) 0.5 ng/ml (e) unmodified. All discs were treated with SPDP, DTT and fluorescein-5-maleimide

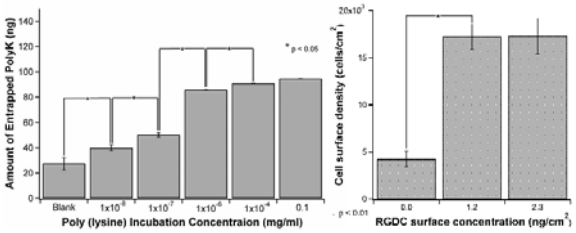

Figure 3. (a) Controlled entrapment of polyK in the PLLA scaffold surface (b) Cell adhesion test on polyK-modified scaffolds

IV. Conclusions. We have produced a functionally flexible, biomimetic scaffold for tissue engineering. The entrapment of polyK in the material surface has been done in a controllable fashion, and further biomimesis has been demonstrated. The utilized modification technique allows for the evaluation of the optimal surface concentration of bioactive molecules for specific applications.