Controlling MSC behavior on 3D PLLA scaffolds using physical entrapment of functional biomolecules

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I. Introduction. Poly(L-lactic acid) (PLLA) is widely used in tissue engineering applications due to its degradation characteristics and mechanical properties but possesses an inert nature, affecting cell-matrix interactions. Its modification using bioactive molecules can lead to the creation of a construct that mimics the physiological extracellular matrix. Cell attachment, proliferation, migration, and specific cellular responses can be achieved through the use of these biomimetic scaffolds. We have prepared biomimetic 2D discs and 3D scaffolds by entrapping poly (L-lysine) (polyK) in the surface of poly (L-lactic acid) (PLLA) 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 mesenchymal stem cell (MSC) adhesion. Other biomolecules can also be incorporated easily via amine coupling.

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 µm and dimensions of 8mm in diameter by 3mm in thickness.

Entrapment of poly(L-lysine). Discs or 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. Incubation polyK concentrations were 0.1, $1x10^{-4}$, $1x10^{-6}$, $1x10^{-7}$ or $1x10^{-8}$ mg/ml. 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: PolyK-modified surfaces were incubated in 600µl of a solution of 1mM N-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP) for 30 min. After rinsing, they were soaked in 100µM RGDC for an hour and rinsed thoroughly. Rat MSCs were seeded statically on 2D and 3D RGDC-modified surfaces by adding the cell suspension in a drop-wise manner. Cell spreading on 2D discs was determined by labeling the cells with BODIPY® FL phalloidin (Invitrogen), a high affinity probe for F-actin. Cell attachment on 3D scaffolds was assessed by performing PicoGreen® DNA quantification assay (Invitrogen).

III. Results. At polyK concentrations of 0.1 and $1x10^{-4}$ mg/ml there is no appreciable difference in the amount of entrapped polyK, implying that, at those concentrations, the surface is saturated with polyK. At lower incubation concentrations, the amount of entrapped polyK decreases with the incubation concentration. At 10^{-8} mg/ml, the amount entrapped is still significant when compared with the plain scaffolds (Figure 1). Figure 2 shows fluorescence micrographs of fluorescently tagged MSC that were seeded on plain and modified discs. In polyK/SPDP/RGDC-modified discs, at polyK incubation concentrations of 0.1 and $1x10^{-4}$ mg/ml, cells greatly stretched once attached to the surface after two hours, with elongated extensions of the cell membrane and a uniform distribution throughout the disc surface, while at a polyK incubation concentration of $1x10^{-6}$ some cells started to display a rounded appearance, a phenomenon that was more pronounced on unmodified discs. Cell surface areas at different extents of surface modification are shown in Figure 3. Greater cell spreading area was obtained at polyK incubation concentrations of 0.1 and $1x10^{-2}$ mg/ml, without any significant difference between the two. Significant declines were observed at polyK incubation concentrations of $1x10^{-4}$ and $1x10^{-6}$ mg/ml, but there was not a significant decrement when the incubation concentration was reduced to $1x10^{-7}$ mg/ml. Furthermore, polyK/SPDP/RGDC-modified surfaces provided the greater cell stretching than polyK-modified or polyK/RGDC-modified discs. Cell surface area was the lowest when cells were seeded on plain discs and plain discs incubated in an RGDC solution. In the three-dimensional polymeric foams, the incorporation of small amounts of RGDC onto the surface (polyK incubation concentration of $1x10^{-7}$ mg/ml) increased cell attachment ($2.7\pm0.4x10^5$ cells), which was about three times higher than that obtained in the unmodified surface, ($0.8\pm0.2x10^5$ cells). However, no significant difference compared to the lower polyK incubation concentrations in cell attachment was observed when scaffolds were modified with RGDC at a polyK incubation concentration of 0.1 mg/ml.

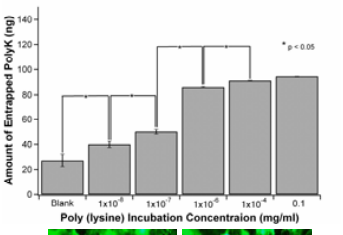

Figure 1. Controlled entrapment of polyK in the PLLA scaffold surface

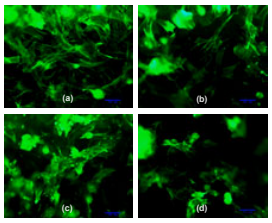

Figure 2. Cell adhesion test on RGDC-modified PLLA discs. The RGDC was linked to polyK through the creation of a disulfide bond using SPDP. Mesenchymal stem cells were seeded on the surface of disks modified with polyK at: (a) 0.1 mg/ml, (b) 10^{-4} mg/ml, (c) 10^{-6} mg/ml, or (d) plain

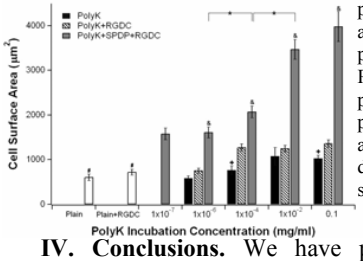

Figure 3. Effect of the extent of polyK entrapment on cell surface area after linkage of RGDC peptides to polyK entrapped in PLLA discs. Controls included polyK-modified and polyK/RGDC-modified surfaces, as well as plain discs and plain discs incubated in an RGDC solution.

IV. Conclusions. We have produced a functionally flexible, biomimetic surface for tissue engineering. The entrapment of polyK has been done in a controllable fashion. Cell stretching was greatly affected by the RGDC surface concentration.