Soft Scaffolds for Adipose Tissue Engineering Based on Poly Ethylene Glycol -Gelatin Systems

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Statement of purpose: Strategies aiming either *in situ* or *de novo* adipose tissue regeneration require a scaffold capable of promoting stem cell or preadipocytes proliferation and differentiation, while providing the adequate structural support. Poly ethylene glycol (PEG) is a biocompatible, *non-fouling* polymer whose acrylate derivatives have already been investigated as possible *in situ* UV photo-polymerizable hydrogels, showing to support preadipocytes viability if adequately modified with peptidic sequences [1].
Aim of this work is the preparation of soft scaffolds for adipogenesis based on PEG diacrylate (PEGdA) and gelatin. UV-cured PEGdA of different MWs were compared to novel gelatin-PEGdA hydrogels covalently crosslinked via a catalyst-/organic solvent-free Michael-type addition between amino groups of the protein and unsaturated acrylic bonds of PEGdA.
Materials and Methods: *PEG hydrogels*: PEGdA of different MWs (258, 575, 700 Da, Sigma) and different concentrations in water (10, 15 and 20% v/v) were photo-crosslinked into a Teflon mold by exposure to a UV-A lamp (OSRAM Ultravitalux, 300W), using 2,2-dimethoxy-2-phenyl acetophenone (DMPA, 0.4% w/v_{PEGdA}) as photo-iniziator. *PEGdA-Gelatin hydrogels*: Gelatin A (bloom ~300, from bovine skin, Sigma) was cross-linked by Michael-type addition with PEGdA (M_W = 575 Da). The reaction was carried out at 37°C in a water/buffer solution (pH= 8.7) until complete gelation. *Chemico-physical characterization*: all hydrogels were dried in oven for 48h prior to subsequent characterization. The effectiveness of the reactions was evaluated with FT-IR spectroscopy (*Nicolet 6700, Thermo Electron Co.*), by checking the disappearance of acrylate double bonds bands at 1615 and 1407 cm^{-1} in PEGdA spectra after the photocrosslinking reaction, and the appearance of PEGdA-related ester carbonyl at 1727 cm^{-1}, and ether C-O-C stretching at 1098 cm^{-1} in gelatin spectra after the Michael-type addition. Swelling and weight loss behavior of the prepared samples was studied in distilled water at 37°C, at increasing time points. Mechanical properties of the swollen samples were assessed in an unconfined cyclic compression tests (n=5 cycles) in water at 37°C (*DMA 2980 TA*). *Cytocompatibility* of UV-cured PEGdA 575 and cross-linked PEG-gelatin hydrogels was evaluated *in vitro* by a preliminary indirect contact test. L929 fibroblasts were put in contact with the eluates obtained by immersing the materials in DMEM for 24 and 48h. Cells viability was assessed by optical microscopy and MTT assay 24h after seeding.

Results and Discussion: FTIR analysis confirmed the complete UV-curing for PEGdA 575 and 700 MW. PEG hydrogels were stable in distilled water up to 2 months, while PEGdA-crosslinked gelatin degraded within 7 days. Tangent moduli of the different samples at first and last compression cycle are reported in Fig.1. By increasing PEG MW, stiffness, brittleness and deformation recovery of the hydrogel increased too. PEGdA-gelatin hydrogels appeared weaker, with a low hysteresis area.

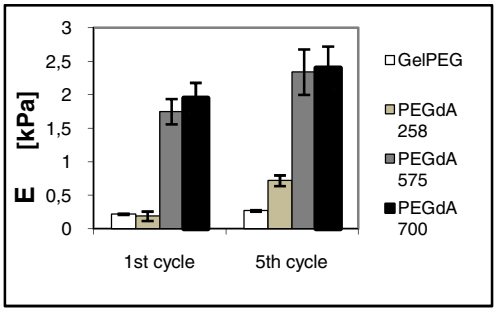

Figure 1: Tangent compressive moduli of PEG hydrogels and PEGdA-gelatin (GelPEG)

The DMEM eluates of UV-cured PEGdA resulted no cytotoxic (Fig.2), whereas PEGdA-gelatin dissolved after 1 day in DMEM, making impossible cytotoxicity evaluation.

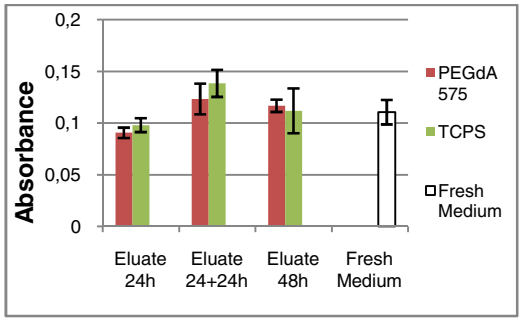

Figure 2: MTT test results on the eluates from UV-cured PEGdA.

Conclusions: PEGdA 575 hydrogel showed the most adequate mechanical properties and was chosen as a platform for subsequent modifications. In this work, we demonstrated the possibility to covalently bind PEGdA to gelatin through a Michael type-addition, even though this system did not show adequate stability in cell culture medium. An optimized approach would be that of combining PEGdA photo-polymerization with Michel-type addition of gelatin, to adjust the degradation kinetic, modulate mechanical properties and provide a chemistry favorable to adipose cells adhesion. Preliminary results have already shown prolonged degradation times in water for these new PEG-gelatin systems, overcoming 2 weeks.

References: [1] Patel BN, Tissue Eng. 2005; 11(9/10):1498-1505.