EVENT ABSTRACT

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Hybrid polyurethane scaffolds interpenetrated with newly cross-linked gelatin for adipose tissue regeneration

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Introduction: Adipose tissue (AT) is a connective tissue that can be affected by severe pathologies (breast cancer, lipoatrophy). Strategies aiming to AT regeneration represent a valid alternative approach to clinical strategies (lipofilling, autologous tissue grafting), preventing loss of volume after implantation[1]. We are here proposing the use of an innovative hybrid polyurethane (PU) scaffold interpenetrated with cross-linked gelatin to promote adipose-derived stem cells (ADSCs) differentiation, while providing the adequate structural support.

Materials and Methods: PU foams (PUf) were synthesized by gas foaming in an one-step bulk reaction. The reagents used were: MDI prepolymer (Bayer), an ad hoc polyether polyol mixture, FeAcetylAcetonate as catalyst, and water (2% w/wpolyol) as expanding agent. Gelatin type A (6% w/v) was crosslinked by a Michael-type addition with N,N'-methylene-bysacrylamide[2]. Hybrid scaffolds (PU/gel) were produced by interpenetrating PUf samples with gelatin during the crosslinking reaction, by use of a vacuum chamber, to promote the coating of the internal pores of the PUf. Scaffolds were observed at SEM; compression and frequency sweep tests were performed by DMA. ADSCs were isolated from mice adipose tissue by enzymatic digestion and expanded in DMEM/Ham's F12 medium. Cells were seeded onto PU-gelatin scaffolds (TCPS wells as control) and cultured in adipogenic medium (0.5 mM isobutylmethylxanthine, 50 lM indomethacin and 0.5 lM dexamethasone). After 3, 6 and 12 days of culture, the number of differentiated cells was counted by Oil Red staining (OR) and cell morphology assessed by SEM.

Results and Discussion: SEM images (Fig.1) showed the presence of the gelatin coating until the inner pores of the PU foam.

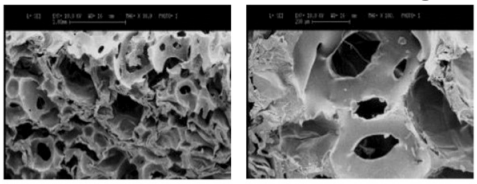

Figure 1 – SEM images of PU/gel scaffold showing the presence of gelatin inside the pore walls (scale bar 1 mm, left; 200 mm, right)

PU/gel bulk mechanical properties (Fig.2) were not statistically different from PUf ones, proving the gelatin coating not to influence the mechanical properties.

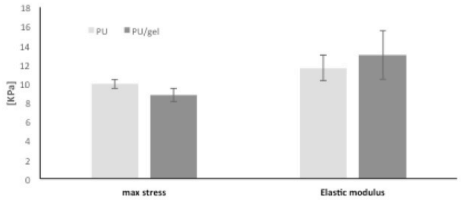

Figure 2 – Maximum stress and Elastic modulus values as obtained in the compression mechanical tests for PU foams (left light grey bars) and interpenetrated PU/gel scaffolds (right dark grey bars)

After 3 and 6 days of culture, the number of OR positive cells into PU/gel vs the total number of seeded cells was close to 50 and 80%, respectively (Fig.3A) and significantly different from the control (20% and 60%), proving the PU/gel to promote a faster adipogenesis. After 12 days, no significant differences were detected between PU/gel and the control, showing 95% of OR positive cells. The presence of mature adipocytes with the typical round morphology was detected at SEM (Fig.3B, left), confirming the ability of PU/gel to support ADSC differentiation. On the contrary, a small percentage of cells resulted positive to OR onto TCPS wells (Fig.3B, right).

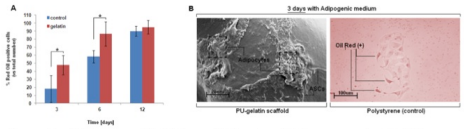

Figure 3 – A) Number of ASDCs on PU/gel scaffolds and control TCPS; B) SEM image of differentiated ADSC into PU/gel scaffolds (scale bar 20 mm) and Oli-Red-O staining of ADSC onto TCPS (scale bar 100 mm)

Conclusions: PUf were successfully interpenetrated with crosslinked gelatin, thus obtaining a more hydrophilic ECM-mimicking surface. These novel hybrid scaffolds were able to promote ADSCs adhesion and adipogenesis in a very short period (3 days), proving their suitability as valid substrate for AT regeneration.

AC was partially supported by PRIN 2010–2011 (PRIN 20102ZLNJ5_006), financed by the Ministry of Education, University and Research, Italy.

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Keywords: Regenerative Medicine, material design, 3D scaffold, Cell interaction

Conference: 10th World Biomaterials Congress, Montréal, Canada, 17 May – 22 May, 2016. **Presentation Type:** General Session Oral

Topic: Regenerative medicine: biomaterials for control of tissue induction

Citation: Fare S, Cochis A, Contessi N, Uberti F, Bertoldi S, Tanzi M and Rimondini L (2016). Hybrid polyurethane scaffolds interpenetrated with newly cross-linked gelatin for adipose tissue regeneration. *Front. Bioeng. Biotechnol. Conference Abstract: 10th World Biomaterials Congress.* doi: 10.3389/conf.FBIOE.2016.01.02265

Received: 27 Mar 2016; **Published Online:** 30 Mar 2016.