Surface modified PLLA as drug delivery scaffold for bone regeneration

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Statement of Purpose: In orthopedics and dentals issues about bone regeneration have shown a high potential in favoring bone repair and regeneration. The ability of substrates to support osteoblast-like cells and to induce crystal formation corresponds to the ideal characteristics of a material for orthopedic or dental applications. To favor tissue regeneration, a scaffold which provides a suitable ECM-like environment with signals for cell survival and functions has been developed and tested in this study. We have modified the biodegradable PLLA scaffold to enhance the ability of human osteoblast-like cells to attach, grow, and differentiate in view of a bone tissue engineering application. PLLA is known for its poor cell–material interactions (Hu Y, J Biomed Mater Res A. 2003;64:583-590) so to increase osteoblasts adhesion and tissue integration into the implant we have functionalized its surface following two approaches: one is the PLLA coating with bioactive cell adhesion proteins found in extracellular matrix molecules (fibronectin and laminin); as second approach we have grafted PLLA surface with active peptides derived from ECM proteins (RGD and SIKVAV). Moreover, to improve osteoblasts differentiation we have incorporated a bioactive molecule into the biodegradable material that shows better cellular inetaction. For this purpose we have used 1,25-$(OH)_2D3$ (VitD3) shown to be a potent transcriptional activator of the genes encoding osteocalcin (Noda M, PNAS 1990;87(24):9995-99) and to increase human osteoblast-like cells ALP and mineralization in vitro (Bosetti M, Biomol Eng. 2007;24:613-618).

Methods: PLLA films have been obtained by casting technique, solubilizing 5% solid PLLA in chloroform, and pouring this solution in a mold. Materials with VitD3 have been obtained adding 1% VitD3 to chloroform PLLA solution. Films obtained were hydrolyzed for 30' in a solution of 0,5 M NaOH in 50% ethanol/water solution. Grafting procedure has been performed pouring over the PLLA film a solution of 14mM EDC, 5,5mM NHS and 50mM MES, for 1 h. This solution has been then replaced with 0,5 mg/ml RGD and SIKVAV, for 24 h at 4° C. Grafted surfaces have been then characterized by water contact angle, to investigate wettability of the surface, and XPS spectroscopy, to verify the chemical composition of PLLA surfaces and the accomplishment of the reaction. The release of VitD3 from the materials has been analyzed with GC-MS.

To verify cell adhesion and proliferation crystal violet assay has been used with human primary osteoblast-like cells (h-OB). To study cell differentiation, alkaline phosphatase activity (ALP) of h-OB has been quantified while cell phenotype has been evaluated with RT-PCR analisys of differentiation markers such as Osteopontin, osteocalcin and Rankl on h-OB and on human mesenchymal stem cells (h-MSC). Substrate induction of osteoblast-like cell mineralization has been evaluated by fluorescence microscopy (calceine test) and results evaluated also considering acellular mineralization test performed by soaking samples in SBF for 2 months and SEM/EDX analysis.

Results: All the studied treatments resulted to increase cell adhesion respect to untreated PLLA but even if h-OB cultured on fibronectin and laminin coated PLLA showed better adhesion at 3 h with respect to control PLLA, they showed low proliferation rates in the course of time both at 3 days and 6 days of cell culture. Differently the two grafting treatments showed to induce cell proliferation without significant upregulation of osteogenic markers and no effect on ALP activity. When RGD grafted PLLA was enriched with VitD3, like shown in Figure, a statistically significative increase of bone differentiating markers has been shown together with an increased ALP activity of both h-OB and h-MSC.

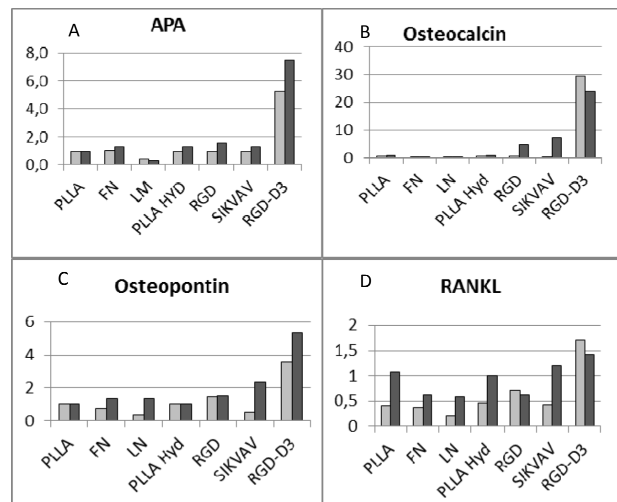

Figure 1. A) Alkaline Phosphatase assay on osteoblast (light grey) and MSC (dark grey) and RT-PCR for B) osteocalcin, C) osteopontin and D) RANKL on osteoblast (light grey) and MSC (dark grey). All the results are normalized with respect to the control (ctr = 1)

Conclusions: Our data showed that in view of bone integration and bone regeneration, PLLA grafting with RGD can be considered a good substrate to induce h-OB adhesion and proliferation but having no significant effect on the osteogenic induction, the scaffold has to be reinforced with osteoinductive molecules. In this work VitD3 reinforced RGD-PLLA keep increased cell proliferation supported by an up-regulation of the studied osteogenic markers. These results can lead to a future application of RGD-D3 PLLA as an osteogenic material for bone replacement.

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