

**OP94**  
**Hierarchical structuring of 3D prototyped scaffolds with human platelet's lysates and marine-origin polysaccharides induce and improve biomineralization of human adipose derived stem cells**

S Oliveira, RL Reis and JF Mano

*3B's Research Group, University of Minho, Guimarães, Portugal*

*Introduction:* Bone tissue engineering demands hybrid 3D structures able to provide both mechanical stability and an adequate nano and micro-environment. Herein, we investigated a methodology to perform the structuring of 3D prototyped scaffolds, with nanocoatings and fibrillar structures where autologous growth factors (GFs) are stabilized and

able to conduct and induce the osteogenic differentiation of human adipose derived stem cells (hASCs).

**Materials and methods:** Poly( $\epsilon$ -caprolactone)-PCL,  $\iota$ -carrageenan and chitosan were obtained from Sigma-Aldrich. Human platelet's lysate was obtained as explained elsewhere<sup>1</sup>. The assembling of the polyelectrolytes (PEs) with PL was studied by QCM-D. PCL scaffolds were prepared by Bioplotter<sup>TM</sup> and modified by dipping LbL similar to what we described before<sup>2</sup>. hASCs were culture in osteogenic (+dexamethasone,+Dex) and osteoconductive media (-Dex). After 28 days in culture, samples were harvested and characterized by several techniques for calcium, osteocalcin (OC) and fat deposition; and for chondro-, adipo-, angio-, and osteogenic-specific gene expression.

**Results:** PL was incorporated in the LbL assembled structures in the form of nanocoatings and fibrillar structures (Fig. 1).

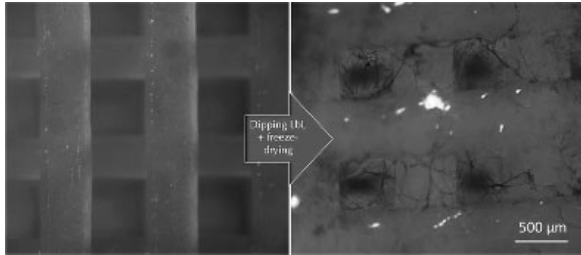


Figure 1 PCL scaffold before and after the modification using LbL.

The new structures supported hASCs osteogenesis both in the presence and absence of PL. These structures conducted the deposition of CaP not only onto the surfaces (as in the case of PCL) but also in the void spaces of the pores. In the absence of Dex, PL and the hierarchical structures both induced the deposition of mineralized matrix and osteocalcin (Fig. 2). An inhibitory effect was observed when the number of bilayers was triplicated (results not shown).

**Discussion and conclusions:** The structuring method has shown to be effective for the introduction and stabilization of osteo-inductive factors derived from PL. The GF's showed its bioactivity by inducing the deposition of OC and CaP, by hASCs, in absence of Dex. Gene expression was influenced by both structuring and PL; which tended to increase angio- and osteogenic-related expression. With this method, 3D scaffolds can be structured with hybrid, multi-functional, inductive and hierarchical micro/nanostructures. By playing with the structuring parameters the density of the GFs which are presented to cells, the profile and even the spatial-temporal release can be controlled.

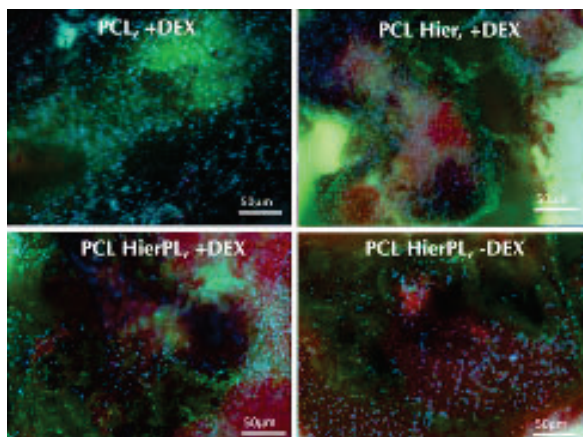


Figure 2 Immunodetection of osteocalcin (green) and nucleus (blue); ARS staining (red).

**Acknowledgments:** FCT is gratefully acknowledged for fellowships of S.M.O. (SFRH/BD/ 70107/2010).

**Disclosures:** The authors have nothing to disclose.

**References**

1. Santo EV, et al. Journal of Controlled Release. 162, 19-27, 2012.
2. Oliveira SM, et al. Adv Health Mat, 2, 3, 2013.