Development of multifunctional platelet lysate membranes for tissue engineering applications

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Statement of Purpose: The development of scaffolds for cells/tissue ingrowth that also enable the entrapment and controlled release of growth factors (GF) targeting regeneration of damage tissues has been a major focus in tissue engineering research. Nevertheless, issues concerning the safety, cost, and effectiveness of these systems have overshadowed their attractiveness.

Platelet-based products (Platelet rich plasma- PRP; Platelet Lysate – PL) hold an enormous potential for regenerative therapy as an alternative source of GFs as they can be obtained using simple and cost-effective procedures and used in autologous approaches. The GFs derived from platelets are involved in essential stages of wound healing and regenerative processes such as chemotaxis, cell proliferation and differentiation. Moreover, platelets release numerous cell adhesion molecules (fibrin, fibronectin and vitronectin) that are important for the formation of ECM and for the adhesion and migration of cells. The autologous application of endogenous GFs would largely reduce the risks of disease transmission and simultaneously allows the induction of the "wound healing" cascade in a physiological manner.

In this work we propose strategies for the development of PL membranes obtained either by self-crosslinkage of PL proteins using the natural-origin crosslinker genipin or by incorporating PLs in photopolymerizable hyaluronic acid (HA) hydrogels, targeting applications in periodontal ligament regeneration.

The sustained release of proteins from the PL-based membranes crosslinked with genipin is expected to stimulate the migration and proliferation of endogenous cells upon implantation, facilitating the tissue regeneration.

Methods: Platelet concentrates with a cell count of $10^6.mL^{-1}$ were processed with repeated freezing and thaw cycles to generate PL. Increasing concentrations of genipin (0.10, 0.18 and 0.25 % w/v), a natural crosslinker, were used to crosslink PL proteins and, after a solvent casting process, PL-based membranes were formed. The crosslinking reaction was confirmed by FTIR. The mechanical properties of the produced membranes were assessed in wet stage by Dynamic Mechanical Analysis (DMA). The release of specific GFs was quantified by ELISA. Also *in vitro* assays were performed both using human adipose derived stem cells (hASCs) and periodontal ligament fibroblasts (hPDLFs).

Photocrosslinkable membranes were produced by dissolving methacrylated HA in solutions of increasing concentration of PL (0, 50 and 100% v/v), and using the photoinitiator Igacure 2959.

Results: The morphological and mechanical features of PL-genipin membranes are dependent on the crosslinking degree. Regarding the mechanical properties, these membranes presented viscoelastic behavior, and the elastic storage modulus was dependent on the amount of crosslinker used. The membranes presented a sustained release of FGF, a GF with mitogenic and angiogenic properties, up to 14 days.

hASCs showed reduction of metabolic activity proportional with the genipin amount. In the other hand, the metabolic activity of hPDLFs seeded in the membranes was not affected, suggesting the potential of these membranes for periodontal ligament regeneration approaches.

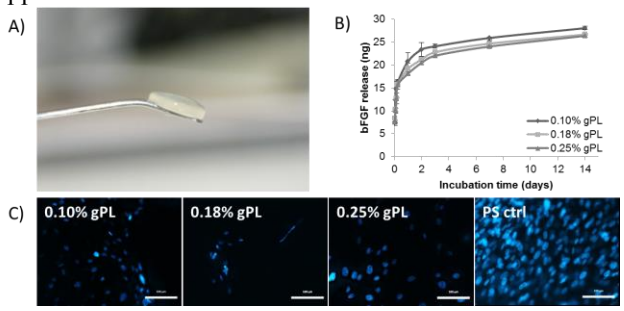

Figure 1: A) Photocrosslinked HA hydrogel incorporating PL. PL membranes crosslinked with genipin: B) release of specific GFs; C) DAPI stained hASCs seeded on the PL membranes after 7days in culture

The incorporation of PL in photocrosslinkable membranes produced stable hydrogels that offers a wider versatility for processing technologies enabling to obtain, for example, membranes with defined topography, or the crosslinking *in situ* for the directed delivery of GFs.

Conclusions: The PL-based membranes developed in this work, present high stiffness and elasticity and, consequently, a great potential in the regeneration of elastic and mechanically active tissues. Moreover these membranes have demonstrated to act as a valuable substrate for hPDLFs attachment and growth while enabling the sustained release of GFs relevant for tissue healing. These results suggest that it is possible to produce stable PL-based membranes with great potential for the regeneration of periodontal ligament, but also for tissue engineering approaches targeting various other tissues.

References:

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