In vivo potential of functionally graded platelet lysates scaffolds for osteochondral repair

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Statement of Purpose: Platelet lysates (PLs) are an enriched pool of growth factors (GFs) obtained from activation of platelets that can promote the recruitment, proliferation and differentiation of stem cells. PLs can be used as either a GFs source or as a three-dimensional (3D) hydrogel for simultaneous GFs and cell delivery. However, most of current PLs-based hydrogels lack stability, exhibiting constant and significant shrinking behavior. In this work we report the *in vivo* assessment of 3D PLs-based scaffolds, crosslinked with genipin and incorporating a gradient of Bioglass® microparticles obtained by a supercritical fluid based methodology. Previous studies[1,2] have shown that this system presents adequate morphology with *in-situ* pore forming ability and a controlled delivery profile of several GFs involved in bone and cartilage repair. Tuning the properties of the scaffolds allow to create regions with distinct mechanical properties. Furthermore, they showed the ability to provide appropriate mechanical support and biological cues to promote *in vitro* osteogenic differentiation of human adipose derived stem cells (hASCs) in response to the gradient of ceramic particles. On the other hand, when the hydrogels were developed in the absence of Bioglass®, chondrogenic differentiation of hASCs was also achieved. In this study, the constructs were evaluated *in vivo* for their ability to act as autologous templates for osteochondral regeneration.

Methods: The PLs scaffolds reinforced with mineral microparticles were prepared as described elsewhere.[2] Briefly, 1.5 ml of PLs suspension (pooled from three different donors) was mixed with genipin (0.18% w/v) and Bioglass® microparticles (2.5% w/v) and placed inside the high-pressure vessel until undergoing phase inversion. Two different experimental set-ups were then designed: 1) subcutaneous implantation of the materials in rats for 1, 4 and 12 weeks: acellular or pre-seeded with hASCs, for assessment of *in vivo* stability and degradation of the scaffolds, local inflammatory response and ectopic osteochondral extracellular matrix (ECM) deposition; 2) orthotopic implantation of the materials in rat critical size ulna defects for 4 and 8 weeks and for evaluation of new bone formation. The harvested constructs from both experimental set-ups were characterized through histological and immunohistochemistry analysis. The explants from the ulna defects were also assessed by Micro-Computed Tomography (Micro-CT).

Results: The subcutaneous implantation of PLs hydrogels shows that the materials remained stable for at least 4 weeks (as easily distinguished in dark blue in figure 1C and 1D). The hydrogels were well integrated in the subcutaneous space, already showing signs of strong vascularization around the implant. The hydrogel explants did not show any signs of inflammatory response or infection, even the ones seeded with hASCs, further supporting the immunomodulatory behavior of these cells that has been previously reported. The constructs were also able to induce the ectopic deposition of osteogenic and chondrogenic matrix proteins in a gradient manner, with the Bioglass-enriched region promoting an enhanced osteogenic differentiation of host stem cells which migrated into the constructs.

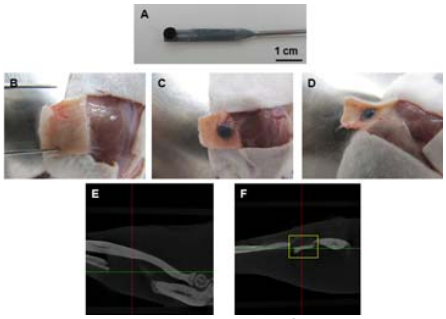

Figure 1. A. Macroscopic visualization of the PLs hydrogels; **B-D:** Macroscopic images of the subcutaneous region 4 weeks post-implantation of the empty, acellular PLs and PLs-hASCs scaffolds, respectively. **E-F:** Micro-CT 2D imaging of bone regeneration in the ulna defect 4 weeks post-implantation in the empty (E) and PLs scaffold-filled defects (F).

Regarding the orthotopic implantation, PLs-Bioglass scaffolds were able to induce strong deposition of mineralized ECM in the critical size ulna defect, indicating new bone formation and almost complete bone bridging of the defect region 4 and 8 weeks post-implantation (highlighted in fig. 1F). This was confirmed by histology through Hematoxylin & Eosin and Masson Trichrome stainings and by immunohistochemistry through the strong detection of collagen type I and osteocalcin.

Conclusions:

The present study successfully proposed a new methodology to develop stable and biofunctional PLs hydrogels that can act simultaneously as a template, with a well-defined gradient of a mineral component, and as a multiple GFs release system. It represents a feasible strategy for an autologous-oriented osteochondral engineering approach.

References: 1. Santo VE et al. J Tiss Eng Reg Med 2012, (6 Suppl 1, 197); 2. Santo VE et al. J Tiss Eng Reg Med 2012; (6 Suppl 2, 32).