Pre-vascularized Gellan Gum-Hyaluronic Acid Spongy-like Hydrogels improve Skin wound healing

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Statement of Purpose: Extensive-full thickness adult skin wounds, heal by both undesirable wound contraction and scar formation leading to non-functional skin. Thus, strategies capable of limiting these two events, by promoting skin tissue regeneration, have been intensely pursued by tissue engineers. It is believed that insufficient re-vascularization after grafting is one of the events that most contribute to fibrosis (1, 2). Pre-vascularization of skin substitutes, despite the limitation still encountered with the source of endothelial cells, turned out to be a promising approach for a more efficient inosculation consequently improving wound healing.

Under this context, we proposed a tissue engineered (TE) strategy that combines an off-the-shelf scaffold with two types of cells, human adipose stem cells (hASCs) and microvascular endothelial cells (hAMECs) to promote skin tissue regeneration by modulating neovascularization and the intrincate cascade of events that drive wound healing. The innovative character of the proposed approach relies on taking advantage of a powerful cell-machinery obtained from a single cell source yet holding a potential immunomodulatory capacity within a gellan gum-hyaluronic acid spongy-like hydrogel (GG-HA), which, unlike traditional hydrogels, depict cell adhesive properties and improved mechanical stability upon hydration.

Methods: The GG-HA hydrogels were prepared by dissolving hyaluronate in deionized H_2O, followed by the addition of gelzan with further dissolution, with temperature. The solution was crosslinked with divalent cations and then freeze-dryed to achieve dried polymeric networks. The structures were analysed in terms of microarchitecture (uCT and SEM) and of water uptake ability. hASCs and hAMECs were isolated from the digestion of human lipoaspirate with collagenase and characterized by flow cytometry considering the mesenchymal and endothelial phenotypic markers, respectively.

Spongy-like hydrogels were formed by hydration, at the time of cell seeding, either by dropwise addition of the different cell suspensions, GG-HA Spongy-like hydrogel with hASCs (homotypic spongy-like gels), GG-HA Spongy-like hydrogel with hASCs and hAMECs (heterotypic spongy-like gels), or the respective culture medium, GG-HA Spongy-like hydrogel. Constructs were implanted in nude mice skin full-thickness excisional wounds and analyzed at day 3, 7, 14 and 21 post surgery regarding wound closure, repithelialization and vascularization. Empty wounds were set as control group.

Results: Dried GG-HA polymeric networks were able to uptake about 2000% of water within 15 minutes when hydrated with culture medium, forming the spongy-like hydrogels and enabling the natural entrapment/encapsulation and consequent attachment of the cells within its porous microarchitecture (Fig. 1).

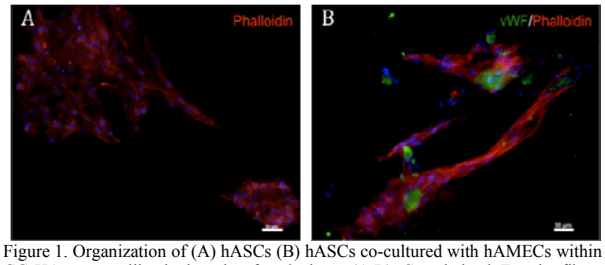

Figure 1. Organization of (A) hASCs (B) hASCs co-cultured with hAMECs within GG-HA spongy-like hydrogels after 2 days. (A,B) Cytoskeletal F-actin fibers stained with rhodamine-labeled phalloidin (red) confirmed hASCs spreading and (B) the expression of wWF (green) showed the interaction of the hAMECs with the hASCs. Nuclei were stained with DAPI. Scale-bars correspond to 50 μm.

Upon transplantation GG-HA spongy-like hydrogels showed to facilitate the early inflammatory cell infiltration, translated by a dense granulation tissue formation. Consequently, spongy-like hydrogel rapid degradation, matrix remodeling, wound closure and complete reepithelization, were observed. More importantly, GG-HA spongy-like hydrogels promoted a superior neovascularization (Fig. 2A), which was enhanced when enclosing hAMECs that directly incorporated the neovessels formed (Fig. 2 B).

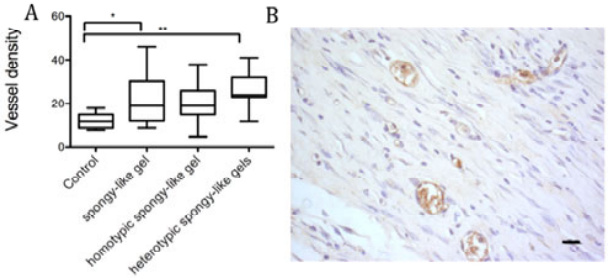

Figure 2. Vascularization potential of the proposed spongy-like hydrogel-based constructs. (A) Vessels density in the wounded area after 21 days of implantation. Quantification was performed on the vessels positive for α-smooth muscle actin. *$p < 0.05$ and ***$p < 0.001$. (B) Immunolocalization of anti-human CD31 in histological section of heterotypic spongy-like gel group at day 21 post-transplantation. Scale-bar corresponds to 20 μm.

Conclusions: This work proposes an approach that takes advantage of a powerful cell-machinery combined within an improved GG-HA spongy-like hydrogel, to promote skin regeneration by promoting neo-vascularization. Through the assemblage of an off-the-shelf polymeric network with cellular elements, obtained in a relatively short timeframe from the same source, it was demonstrated the possibility of creating a clinically relevant skin tissue substitute that acts in promoting neotissue vascularization.

References: 1. Boyce ST (2001) *Burns* 27(5):523-533, 2. Metcalfe AD & Ferguson MW (2007) *Biomaterials* 28(34):5100-5113.