Biomimetic approaches for tissue healing and restoration

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Statement of Purpose: Tissue engineering (TE) has continued to evolve as an exciting and multidisciplinary field aiming to restore, replace or regenerate defective tissues in the past twenty years. Classical TE approaches have been mainly focused on the development of biomaterials able to tune stem cells behavior [1]. Recently, the host immune response has been suggested as a crucial aspect to consider in the development of implantable biomimetic devices for TE. The ability to guide the fate of various classes of infiltrating cells will help reducing side effects (such as acute and chronic inflammation and/or implant rejection) and improving the therapeutic outcome (healing, tissue restoration). In particular, the successful implantation of a biomaterial depends on the biological processes activated by macrophages as they constantly monitor the presence of foreign material in the body [2]. With this in mind our laboratory is actively developing different strategies to trigger the immune reaction toward a functional regeneration. Our approaches include a direct functionalization of and the release of bioactive factors from naturally inspired biomaterials. Here we propose one of the platforms we developed, which consists on chondroitin sufate (CS)-functionalized scaffolds. CS was chosen, as it is one of the most represented components of cartilage extracellular matrix and due to its proven immune-modulating potential [3]. Despite its role in supporting cartilage formation and suppress inflammation, a recapitulated analysis regarding its tissue engineering potential is still lacking, and the cascade of biological events that follow its implant in vivo have yet to be determined.

Methods: Porous Collagen scaffold (CL) and the Chondroitin Sulfate functionalized (CSCL) one were fabricated by freeze dried method. CSCL were also subsequently cross-linked for 4 h at 37 C using 50mM 2-(N-morpholino)ethanesulfonic acid, 5mM 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 5 mM N-Hydroxysuccinimide (NHS). The scaffolds have been characterized by SEM, FTIR and TGA for their physical-chemical features. The efficacy of such functionalization was assessed in vivo in rat subcutaneous implants. The biological events produced by the presence of CS were characterized at a molecular and proteomic level at early (24h and 72h) and long (7 and 21 days) time points and compared to those obtained implanting bare collagen scaffolds (CL).

Results: At 24 hours, macrophages represented the 90% of the total cells recruited by CSCL against the 40% found in CL. Interestingly, the 90% of these cells was positive for anti-inflammatory associated markers, such as CD206 and IL-10. The same results were achieved with CL only at 7 days. And these data have been confirmed by flow cytometry and immunofluorescence of tissue sections. Consistent with this, PCR arrays demonstrated that at early time points cells recruited by CSCL expressed higher levels of chemokines and cytokines involved in the positive regulation of the immune system processes and chemotaxis. All these data have been confirmed by histological and immunofluorescence analysis performed on the explanted scaffold (Figure 1).

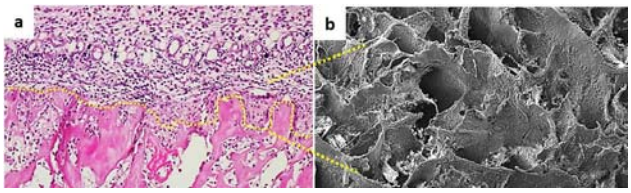

Figure 1. Cells colonization of CSCL after 24hs. a) H/E staining of the interface tissue/scaffold (dotted yellow line) b) SEM images of CSCL

After 21 days, a marked reduction in the expression of markers associated to chronic inflammation (IL-6) and fibrosis (F13a1, Fga, Plat, and Plaur) was found in CSCL compared to CL. Although histology and immunofluorescence revealed new forming vessels in both CL and CSCL, the expression of angiogenesis stimulators (such as FGF-2, TGF-a/b, PGE_2) was down-regulated in CSCL, this suggested that a quicker vascularization occurred. We performed also histological analysis of the 21 days samples and we observed a higher level of vascularization of the CSCL in comparison with CL and a homogenous distribution of vessels within the scaffold.

Conclusions: All together, these data suggest the platform we propose is able to accelerate the biological processes started by implantation toward a faster restoration of the tissue and healing. We believe this study will lay the foundations to establish a new biological paradigm for the development of biomaterial for tissue engineering.

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