homogenous tissue similar to native cartilage in composition and organization in contrast to the more elastic hydrogel formulations. When compared with the standard approach using single cells, encapsulation of organoids led to a higher proliferation, matrix deposition and superior tissue organization. The resulting neocartilage was mechanically superior and stable after 24 days of culture. This study describes a multimodal approach that involves chondrocyte expansion, organoid formation and their in vitro assembly into neocartilage which proved to be superior to the current standard approaches used in cartilage tissue engineering.

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SILK-BASED MATERIALS TO CREATE HIGH RESOLUTION THREE-DIMENSIONAL STRUCTURES USING ELECTROHYDRODYNAMIC PRINTING

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Mimicking the complex hierarchical structure of the extracellular matrix (ECM) has always been a major goal in tissue engineering (TE) approaches [1] [2]. Despite the great advances in biomaterial processing technologies, the main limitation concerns the resolution of the fibers, which hampers the reproduction of ECM.

Here, we combine Silk Fibroin (SF) [5], a highly potent biomaterial that intrinsically has the characteristics of making fibrous structures, with Electrohydrodynamic printing, an innovative 3D printing technique that allows patterning at micro and sub micro scale.

To fabricate these complex structures, Electrohydrodynamic printing applies a voltage between the needle and the collector screen to charge the polymer solution, with a consequent thinning of the fibers, making it possible to reach optimal resolutions for recreating the hierarchical and fibrillar structure of ECM [3] [4].

We have studied SF in its chemical structure to allow a better understanding of the structural and mechanical behaviour of the material before and after printing. We have demonstrated the printability of SF with Electrohydrodynamic printing and, just by tuning the rheological properties, it is possible to obtain straight fibers with a resolution of 10-20 mm. We have also demonstrated that these fibers can be physically crosslinked inducing the formation of b-sheets structure in the protein chain; after crosslinking the fibers are stable and don't dissolve in water.

SF is therefore proving to be an optimal material for this application and is gaining strong interest in soft tissue engineering.

Keywords: Electrohydrodynamic printing ; Silk Fibroin

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NICHE SCAFFOLDS FOR OSTEOCHONDRAL REGENERATION

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Aging worldwide population demands new solutions to permanently restore damaged tissues, thus reducing healthcare costs. Stem cells are a promising alternative due to their differentiation potential into multiple lineages. Yet, better control over cell-material interactions is necessary to maintain tissue engineered constructs in time. In particular, it is crucial to control stem cell quiescence, proliferation and differentiation in 3D scaffolds while maintaining cells viable in situ. Here, we developed a biomimetic scaffold inspired by the mesenchymal stromal cell niche. The multi-compartment scaffold is composed of a hydrogel, aimed at maintaining cell quiescence, encapsulated on a proliferative electrospun cup that is surrounded by a 3D printed scaffold, aimed at differentiation via peptide sequences.

In vitro, cells encapsulated in alginate remained quiescent for a low concentration of RGD peptides bound to the hydrogel matrix. Cells seeded on electrospun meshes were able to proliferate and migrate through the entire mesh thickness, depending on the pore size and fiber diameter of the scaffolds. Cells seeded on the 3D printed scaffolds functionalized with osteogenic and chondrogenic peptides on a gradient manner were able to support cell differentiation, as measured by classical osteogenic and chondrogenic gene markers expression and protein secretion.

In vivo, the scaffolds showed to maintain biocompatibility. The gel compartment served as a pool of quiescent cells that were able to migrate and proliferate on the electrospun mesh. The 3D printed compartment appeared fully infiltrated by neo-tissue and served as a support for cell differentiation with large amounts of collagen and glycosaminoglycan deposition.

Keywords: Stem cell niche; 3D printing; hydrogel

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BIOFABRICATION OF VASCULARIZED MYOGENIC SPHEROIDS FOR MUSCLE ENGINEERING

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Engineering muscle tissue is challenging due to the limited degree of diffusion. To overcome this problem, the incorporation of a vascularized network is important. Therefore, a possible strategy is generating vascularized tissue-specific spheroids. The use of these microtissues is advantageous compared to single cells because of their high cell density and accelerated ECM production. When spheroids are combined with a hydrogel, a bioink can be obtained and used for 3D bioprinting.

Vascularized spheroids were generated by seeding 7,5x10^5 endothelial cells, fibroblasts and mesenchymal stem cells (MSC) in a 200 μm-microwell system. Myogenic spheroids were generated by seeding 1,0x10^6 myoblasts, whether or not together with MSC and endothelial cells, in a 400 μm-microwell system. After cultivation in expansion medium, spheroids were exposed to differentiation medium. Subsequently, vascularized myogenic spheroids were generated by combining myoblasts, MSC and endothelial cells. To assess the effect of different hydrogel compositions, encapsulation experiments were performed in gelatin-methacryloyl and gelatin-norbornene. Morphology, ECM production and spheroid viability was evaluated.