Use of Advanced 3D Bioprinting Technologies for the Engineering of Tendon-Biomimetic Magnetic Nanocomposites

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Besides acting as structural support and anchorage for resident cells, the physical properties of fibrous native tissue extracellular matrices (ECMs) also exquisitely dictate several morphogenetic processes. The complex anisotropic topography and hierarchical structure of native ECMs not only provide tissues with highly anisotropic mechanical properties, but also impact on cells response by regulating their spreading, migration and morphology, specific features that are highly tissue-dependent [1]. Among the wide range of different materials proposed to engineer biomimetic constructs analogous to native tissues, polymeric hydrogels are particularly appealing options due to their water-rich composition, high biocompatibility, and easy tunability of their physical and chemical properties [2]. However, unlike most human tissues, which are characterized by anisotropic cell patterns and ECMs organized into hierarchical fibrillar structures, bulk polymeric hydrogels have inherent isotropic architectures that limit their potential to recreate these rich biophysical cues of living tissues. In this point, the modification of polymeric hydrogels with magnetic nanomaterials has emerged as a promising approach not only for creating anisotropic ordered patterns within their structural networks, but also for providing the nanocomposites with additional remote response functionalities [3]. Thus, besides microstructural patterning, the application of external magnetic fields on magnetic hydrogels can further be used for the programmed contactless magneto-mechanical stimulation of the encapsulated cells, during their *in vitro* maturation and after construct transplantation *in vivo*.

On the other hand, the combination of 3D (bio)printing technologies with magnetic hydrogel (bio)inks allows an impressive control over the resolution of the designed anisotropic constructs and a significantly improved versatility and reproducibility in comparison with other fabrication methods. However, although traditional 3D bioprinting strategies can be exploited to fabricate biomimetic scaffolding systems, they fail to provide control over the distribution of magnetic materials incorporated in the bioinks while preserving the fidelity of the designed composites.

To overcome this dichotomy, here we combine the concepts of magnetically- and matrix-assisted 3D bioprinting (Figure 1A) [4]. For such purpose, bioinks composed of methacrylated gelatin, short magnetically responsive electrospun microfibers (sMRFs) and human adipose-derived stem cells (hASCs) were extruded into fibrillar support baths under the application of external magnetostatic fields that align the sMRFs, thus obtaining high-resolution constructs with controlled anisotropic architectures [5]. The developed support baths, based on cellulose nanocrystals partially self-assembled through the controlled addition of calcium chloride, showed the adequate rheological properties to: i) maintain the low viscosity bioinks uncrosslinked after printing to enable the magnetically induced arrangement of the incorporated sMRFs, thus creating anisotropic patterns within the designed constructs; and ii) preserve the resolution of the 3D bioprinted structures before inducing their solidification.

The sMRFs incorporated within the scaffolds have been prepared by modifying poly(caprolactone) microfibers obtained through electrospinning techniques with pre-synthesized magnetic nanoparticles (MNPs). Here, we have addressed an usually overlooked but not less important variable for magnetic tissue engineering strategies, such as the fine design of MNPs capable of delivering high magnetic responses. For that, different synthetic strategies were explored, being determined that the preparation of highly crystalline zinc-doped iron oxide MNPs doped with zinc through a thermal decomposition route is an effective approach obtain nanostructures with large magnetization values [6]. The design of these highly responsive MNPs enabled to minimize the concentration of magnetic material required to provide the 3D bioprinted hydrogels with the desired magnetic behavior, as well as the intensities of the magnetic fields necessary to manipulate them, two critical safety/toxicity factors in view of the potential clinical translation of the obtained biomaterials.

To test the potential of the proposed concept on a specific tissue engineering application, sMRFs composed not only of poly(caprolactone) and zinc-doped iron oxide MNPs, but also containing tendon decellularized ECM, were produced and magnetically aligned within 3D bioprinted constructs. The biophysical cues stemming from the created tendon-like anisotropic fibrous microstructures revealed effective to induce the elongated growth and alignment of the encapsulated hASCs, thus replicating the morphology and organization of resident cell populations in native tendon tissues (Figure 1B). Moreover, the synergy between these anisotropic architectures and the biochemical cues derived from the incorporation of tendon decellularized ECM, in combination with the

application of remote magneto-mechanical stimulation during *in vitro* maturation, promoted the commitment of encapsulated hASCs toward the tenogenic phenotype, as demonstrated by the up-regulation of representative tendon-related markers such as scleraxis or tenomodulin (Figure 2).

Overall, we foresee that the combination of highly responsive MNPs with magnetically- and matrix-assisted 3D bioprinting, while further leveraging on the inherent magneto-mechanical actuation functionality of produced constructs for cell stimulation, represents an attractive biofabrication strategy to engineer not only tendons, but also the wide range of native tissues with anisotropic structures and mechanosensitive properties.

References

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Figures

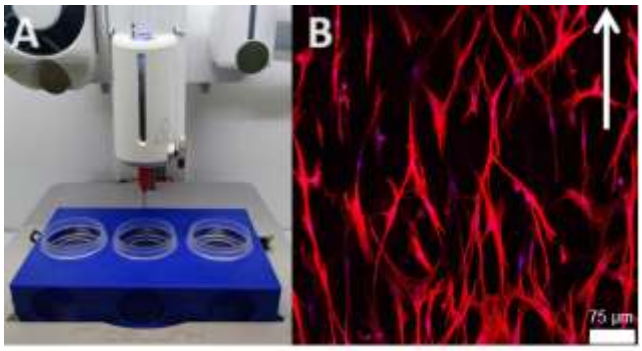

Figure 1. A) Image of the experimental setup used for magnetically- and matrix-assisted 3D bioprinting procedures. B) Confocal microscopy image of elongated hASCs aligned in the direction dictated by the sMRFs oriented within anisotropic polymeric hydrogels.

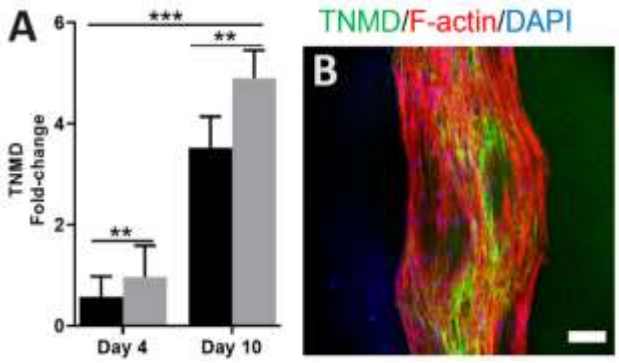

Figure 2. A) Expression levels of tenomodulin (TNMD) after 4 and 10 days of *in vitro* maturation of cell-laden anisotropic hydrogels cultured in static conditions (black columns) and under magneto-mechanic stimulation (grey columns). B) Confocal microscopy image of cell-laden hydrogels after 10 days incubation with stained cell cytoskeletons (red), cell nuclei (blue) and TNMD (green).