Growth factors-controlled delivery systems and 3D biomimetic cultures: a study of tenogenic and chondrogenic events on human mesenchymal stem cells

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Chemical stimulation using appropriate concentrations of growth factors (GFs) is required to promote in vitro differentiation of human stem cells toward specific phenotypes. However, GFs' limited half-life and slow tissue diffusion render conventional administration methods ineffective1. Therefore, GFs' encapsulation within different polymeric carriers has been proposed to allow their controlled delivery and enhance their pharmacokinetic and pharmacodynamic properties2. Additionally, advancements in manufacturing techniques have enabled the direct integration of these carriers into 3D scaffolds, bioengineered with stem cells, thereby creating artificial biomimetic extracellular matrices (ECMs) that are highly effective for tissue engineering applications3,4. In this work, PLGA carriers, obtained by Supercritical Emulsion Extraction (SEE) technology5, were fabricated to encapsulate human Transforming Growth Factor-β1 (hTGF-β1) and Growth Differentiation Factor-5 (hGDF-5), well-known to trigger chondrogenic and tenogenic events, respectively, in mesenchymal stem cells. Two different types of 3D biomimetic cultures were established, using a collagen hydrogel (in the case of chondrogenic differentiation) and a fibrin hydrogel (for tenogenic differentiation), both bioengineered with mesenchymal stem cells, functionalized with TFG-β1/GDF-5 loaded PLGA carriers and cultured in a dynamic microenvironment. Compared to static conditions, significant results in terms of gene expression were obtained when the mechanical stimulus was applied, as indicated by the transcriptional upregulation of both cartilage (type II Collagen) and tendon (Scleraxis-A, type I Collagen, Decorin, Tenascin-C) related markers. Histological and quantitative immunofluorescence (qIF) analysis confirmed cell activity by remodelling the synthetic ECM when cultured in dynamic conditions. The results suggested that PLGA carriers can be effectively incorporated into a 3D structure, serving as microenvironmental regulators. Indeed, the presented 3D scaffolds facilitated the controlled delivery of the GFs along the culture, and it was effective in inducing cell differentiation. The adaptability of this system offers the potential for future designs that enable the sustained release of various GFs and/or bioactive signals, opening new possibilities for the development of different 3D in vitro models.