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Indirect co-cultures of stem cells with chondrocytes for cartilage tissue engineering using PCL electrospun nanofiber meshes

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Mesenchymal Stem Cells (MSCs) have been recognized for their ability to differentiate into cells of different tissues such as bone, cartilage or adipose tissue, and therefore might be of interest for potential therapeutic strategies. These cells are induced to differentiate by growth factors supplementation in culture medium that will trigger differentiation in the desired cell type. Chondrocytes are responsible for maintaining the extracellular matrix (ECM) integrity of articular cartilage. Chondrocytes have been shown to release growth factors that can ultimately induce chondrogenic differentiation of undifferentiated cells, for example MSCs. It is well known that chondrocytes tend to de-differentiate when in 2D culture, losing their ability to produce a rich ECM. In this process occurs a shift from collagen type II production to collagen type I, among other factors, giving rise to a fibrocartilage tissue. In order to overcome this problem, several tissue engineering strategies have been proposed, involving different combinations of cells, including the use of co-cultures. The present work presents a co-culture strategy using human articular chondrocytes and stem cells (Wharton's jelly stem cells) for cartilage-like tissue production. We aimed at assessing the paracrine effect that chondrocytes may have on stem cells by co-culturing directly both cells on the two faces of NFM. The aim is to allow communication of the two cells communities by soluble factors released, but not having direct contact between them. Polycaprolactone (PCL) nanofiber meshes (NFM) were produced by electrospinning. The NFM were further placed into inserts (two in each insert) in order to allow seeding each type of cells in opposite faces of the NFMs. Cells were isolated from human samples collected at the local hospital, under donors' informed consent. After cells expansion, chondrocytes were seeded on the top of the NFMs, whereas stem cells were seeded on the bottom of the NFMs. Controls were performed by seeding chondrocytes or stem cells in NFM. For evaluation of cell viability, proliferation and distribution within the scaffolds, DNA, Alamar Blue and SEM methods were used. Chondrogenic differentiation was evaluated using histological staining, glycosaminoglycan quantification, qRT-PCR and immunolocalization. Cells kept viable along the experiment. Stem cells were able to over express cartilage related genes such as aggrecan, sox9 and collagen type II when compared to the undifferentiated controls. Articular chondrocytes induced the chondrogenic differentiation of stem cells and ECM formation. The obtained results showed that this new strategy enables the development of new therapies for cartilage repair.

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Magnetic-responsive hydrogels for cartilage tissue engineering

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The use of magnetic nanoparticles (MNPs) has been explored as an alternative approach to overcome current limitations of regenerative medicine strategies. Cell engineering approaches where MNPs are incorporated within three-dimensional constructs, such as scaffolds or hydrogels may constitute a novel and attractive approach towards the development of a magnetically-responsive system. These systems would enable remote controlled actions over tissue engineered constructs *in vitro* and *in vivo*. Moreover, growing evidence suggests that the application of a magnetic field may enhance biological performance over commonly used static culture conditions providing stimulation for cell proliferation, migration and differentiation. In this work we analyze the role of magnetic stimulation on the behavior of human adipose derived stem cells (hASCs) laden in k-carrageenan hydrogels aiming at cartilage tissue engineering approaches. Thermo-responsive natural-based κ-carrageenan hydrogels were used as 3D templates since previous studies[(1)] report the adequate environment provided by these materials to support the viability and chondrogenic differentiation of several types of cells. K-carrageenan (k-carr) was mixed with MNPs in different ratios, namely 2.5, 5 and 10%. Human ASCs previously isolated from surplus tissues from elective plastic surgery procedures, were encapsulated in these k-carr-MNP hydrogels and cultured *in vitro* for up to 21 days in chondrogenic culture medium either in the presence or absence of magnetic stimulation generated by a bioreactor device. The hASCs-laden constructs were assessed for cell viability, cell proliferation as well as deposition of a cartilaginous-like extracellular matrix. Human ASCs appear to preferentially adhere to MNPs as they could be found in higher concentrations in regions enriched with the magnetic component. The presence of MNPs within the κ-carrageenan hydrogels did not significantly influence the viability or proliferation of encapsulated hASCs, whose values were similar to hydrogel MNP-free controls. Results also indicate that the formation of vacuoles typically observed in chondrocytic cells, was noticed in cell laden k-carr-MNP hydrogels supplemented with chondrogenic medium. Stem cell performance on k-carr-MNP hydrogels can be modulated by the presence of MNPs stimulated by a magnetic field. Magnetic responsive hydrogels can stimulate hASCs towards chondrogenic differentiation, without affecting cell viability or cell proliferation rates. Therefore, magnetic-based systems may provide new opportunities in regenerative medicine applications towards cartilage engineered tissues.

Reference:
1. Popa EG, Gomes ME, Reis RL, (2011) *Biomacromolecules*, 12 (11), 3952-61.