Abstract

Articular cartilage lesions cannot spontaneously heal because of the lack of vascularization and low metabolic activity of resident chondrocytes making clinical management difficult. Indeed, current therapies are restricted only to counteract inflammatory symptoms without promoting cartilage healing, and tissue engineering could represent a valid treatment option in patients with these disorders. However, to date, cell therapies are still not curative and have several technical issues, especially for cell harvesting and *in vitro* expansion before transplantation to the injury site. For example, a proper scaffold choice for in vitro cultures is essential for effective cell proliferation and differentiation, and collagen-based biomaterials are the best supports for hosting chondrocytes or stem cells, because of their similarities to natural extracellular matrix (ECM). Therefore, collagen-based scaffolds are being investigated as a valid alternative for *in vitro* chondrogenic commitment of human stem cells for further clinical use. In this thesis work, we aimed to develop a novel bioengineered three-dimensional (3D) scaffold for induction and maintenance of chondrogenic phenotype by seeding human bone marrow-derived mesenchymal stem cells (hBM-MSCs) in a type I collagen hydrogel functionalized along with biopolymeric microcarriers for providing a controlled release of transforming growth factor β1 (TGF-β1), a well-established chondrogenic growth factor. First, the appropriate TGF-β1 concentration in culture medium for successful chondrogenic induction of hBM-MSCs was investigated using standard monolayer culture approaches. Cells cultured using a medium supplemented with 10 ng/mL of TGF-β1 showed higher chondrogenesis-related genes (*SOX9*, 4-fold; *COL2A1*, *2*-fold) and protein expression (type II collagen, 2.74-fold) after 16 days, and cells showed a prevalent expression of pro-inflammatory cytokines (*IL-12A*, 6-fold). Once optimized culture conditions, a 3D high-density approach was employed for chondrogenic commitment of hBM-MSCs, because standard 2D cultures promote cell de-differentiation and a switch toward a fibroblastic phenotype. 3D high-density spheroids were assembled using hBM-derived or Wharton's Jelly (WJ)-derived MSCs, an alternative source of stem cells. This culture

condition promoted a more marked upregulation of all chondrogenic genes and proteins compared to the 2D approach, probably because of synergic effects between TGF-β1 delivery and high-density culture promoting cell-to-cell contacts. Moreover, we showed that WJ-MSCs might represent a good alternative for 3D models in cartilage tissue engineering. Next, hBM-MSCs were cultured in a collagen hydrogel functionalized with poly-lactic-co-glycolic acid microcarriers (PLGA-MCs) loaded with TFG-β1 to provide a 3D biomimetic environment for chondrogenic commitment of stem cells. Cells were cultured under static and dynamic conditions using a perfusion bioreactor, and chondrogenic commitment was studied showing that chondrogenesis was induced only under perfusion conditions (*COL2A1,* 5-fold). Finally, to improve mechanical properties and enzymatic resistance of collagen hydrogel, we synthesized a novel composite scaffold using an equine type I collagen and hyaluronic acid under a reaction in heterogeneous phase.

In conclusion, our proposed data open new perspectives for clinical uses of collagen-based scaffolds and PLGA carriers as regenerative tools for cartilage lesion healing.