Abstract

Skeletal muscle injuries can spontaneously heal, because of an intrinsic regenerative potential of a resident stem cell pool, known as satellite cells. After injury, quiescent satellite cells start proliferating and differentiating toward mature muscle cells. Immune cells also play a central role in skeletal muscle regeneration process, by switching from a pro-inflammatory to an anti-inflammatory microenvironment, as healing process proceeds, and injured muscle is structurally and functionally recovered. However, skeletal muscle regenerative potential is limited, and severe injuries frequently result in incomplete recovery and chronic inflammation. Current therapies are restricted to conservative management, including RICE protocol (rest, ice, compression, and elevation) and nonsteroidal anti-inflammatory drugs or intramuscular corticosteroids for inflammatory symptom relief. These approaches do not provide a complete restoration to preinjury status, thus cell-based approach may represent a valid treatment option, even if these therapies show several technical issues, especially regarding cell collecting, harvesting and *in-vitro* expansion before transplantation. Therefore, peripheral blood mononuclear cell (PBMC) infusion can be an alternative and promising approach even though *in-vitro* and clinical studies are still few. Based on this evidence, the aim of this thesis work is to develop innovative *in vitro* models to study myogenic commitment of human bone marrow-derived mesenchymal stem cells (*h*BM-MSCs) by adopting two different approaches:

(i) two-dimensional (2D) co-culture of human stem cells and PBMCs under dynamic conditions, such as perfusion;
(ii) bioengineered three-dimensional (3D) co-culture of human stem cells and PBMCs.

First, appropriate culture conditions, including culture medium composition and cell seeding ratio between *h*BM-MSCs and human primary skeletal myoblasts (*h*SkMs), were investigated using standard monolayer culture approaches. Cells cultured using medium supplemented with 10 ng/mL of b-fibroblast growth factor (b-FGF) and co-cultured with *h*SkMs showed higher myogenesis-related gene expression (*Desmin,* 141-fold; *Myosin Heavy Chain II (MYH2*)*,* 32-fold) after 21 days, and a prevalent expression of anti-inflammatory cytokines (*IL10,* 15-fold; and *IL4*, 11-fold) rather than pro-inflammatory ones (*IL1*, 1.4-fold). Subsequently, PBMCs collected using a commercial filtration system commonly used in clinical practice were added in an upper transwell chamber to investigate their potential paracrine effects on myogenic events. Myogenic commitment of *h*BM-MSCs in the presence and absence of PBMCs was investigated by gene expression profiling by qRT-PCR and protein expression using immunofluorescence (IF) assay and flow cytometry. In the presence of PBMCs, *h*BM-MSCs significantly upregulated myogenic genes, such as *Desmin* and *MYH2* starting

at day 7 of culture, as confirmed by both qRT-PCR and IF, while pro-inflammatory cytokines (*IL12A* at day 14) were downregulated.

Subsequently, myogenic commitment of *h*BM-MSCs was further implemented using a 3D approach employing fibrin-based scaffolds embedded with *h*BM-MSCs plus *h*SkMs with and without PBMCs, cultured in dynamic conditions using a perfusion bioreactor. This culture system appeared as a valid biomimetic environment for myogenic commitment of stem cells. Indeed, all myogenic-related genes tended to be upregulated in the presence of PBMCs, and Desmin and Myosin Heavy Chain were also detected at protein level. Moreover, the presence of PBMCs in 3D culture induced a significant downregulation of pro-inflammatory cytokine genes, such as *IL6*.

This blood filtration system was structurally investigated by optical and electron microscopy, Fourier-transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and Thermogravimetric analysis (TGA). We investigated filter membrane structure and their polymer compositions showing a characteristic morphological structure composed by polyhydroxy butyrate (PHB) polymer. Filtered PBMCs were characterized by flow cytometry immunophenotyping to confirm the efficacy of this filtration system in concentrating PBMCs in smaller volumes by simultaneously preserving their composition.

In conclusion, our works confirm that *h*BM-MSCs represent a valid source of stem cells for skeletal muscle tissue engineering, as *h*BM-MSCs have a versatile myogenic potential, enhanced and modulated by blood filtered PBMC fraction co-culture. Moreover, our 3D biomimetic *in vitro* model better resembles physiological tissue architecture, improving stem cell survival and proliferation, and promoting complex biological *in vivo* cross-talks.