Summary

Tendon injuries affect millions of people worldwide annually and still remain a major challenge for clinicians. Tendon tissue is a connective tissue mainly composed by collagen fibrils organized with a hierarchical structure. Considering the scarce functionality of healed tendons and the unsuccessful clinical outcome of current conventional treatments, the development of tissue engineering and regenerative medicine (TERM) approaches have been proposed. To this end, a multidisciplinary strategy that resemble the specific 3D architecture, biological (e.g. progenitor cells) biophysical (e.g. stiffness and mechanical-sensitivity) and biochemical (growth factors, matrix proteins) characteristics of the microenvironment of tendon is strictly required. Three-dimensional (3D) bioprinting represents a promising tool in this contest, since it may allow production of highly precise 3D-structures by controlled placement of cells and biomaterials mimicking morphology and functionality of the native target tissue. However, the most advanced studies for its application in musculoskeletal system are mainly focused on bone and cartilage reconstruction. Among mesenchymal stem cell (MSC) sources, adipose derived stem cells (ASCs) have been already successfully employed in vivo for a wide range of pathological conditions including the treatment of tendon injuries. The precious hallmarks of ASCs for their use in TERM applications include, among others, multipotency, anti-inflammatory and immune-modulation properties as well as trophic and angiogenic effects. Some of the growth factors (GFs) known to be involved in the tendon healing process, have been proven to trigger MSC tenogenesis in vitro. However, the literature lacks a consensus about the exact medium culture composition that efficiently drives MSC tenogenesis in vitro. Moreover, optimization of standards and culture protocols, cell density and the use of clinical grade reagents are urgently needed to achieve their clinical application.

In order to meet these needs and move toward clinical scale-up of the use of ASCs in regenerative cell-based therapies for tendon treatment, in this Ph.D. thesis a bottom-up approach has been adopted encompassing three main steps: i) the development of xenogenic-free protocol for adipose-derived stem cell culture and

differentiation toward a tenocyte-like phenotype in 2D in vitro condition; ii) the design of a 3D bioprinted construct fabricated with a precise positioning of ASCs within a natural-based bioink for tendon TERM; and iii) the thorough analysis of regulatory aspects, availability of standards and guidelines within the current legislation framework about the possible clinical use of 3D bioprinting.

First, ASC cultured with the novel chemically defined serum-free and xenogenic-free (SF) medium and human platelet lysate (hPL) medium maintained MSC features, including the expression of stem-cell markers. Both SF and hPL tenogenic media (TENO) consisting in the supplementation of AA (ascorbic acid), CTGF (connective tissue GF), TGFβ-3 (transforming GF beta-3) and BMP-12 (bone morphogenic protein-12), efficiently triggered the differentiation of ASCs in 2D in vitro condition as demonstrated by the statistically significant up-regulation of specific tendon-related genes and proteins. Finally, for the first time in literature, ASCs were embedded in a nanofibrillar cellulose/alginate (NFC/A) hydrogel and 3D bioprinted into grid square structures suitable for tendon TERM application. 3D printed cells showed good cell viability suggesting the safety of the printing protocol and high tendon-related proteins synthesis when TENO induced. The absence of ASC inflammatory response to the 3D NFC/A scaffold ensured the safety of the xeno-free FDA approved hydrogel constituents and of the xeno-free GMP-compliant tenogenic differentiation protocol. In addition, the careful examination of the existing regulation in the European Union indicated that a 3D bioprinted product could be classified as a tissue engineered combined medicinal product that would fall under the scope of the ATMP Regulation, although, as an emerging field, there is still a lack of bioprinting-related standards. In conclusion, the first attempt on the suitability of ASC-laden hydrogel for tendon TERM showed successful preliminary results on the applicability of 3D bioprinting of ASCs, suggesting, also, novel insight about tendon development. The evaluation of MSCs in a tissue-like construct is another key aspect to better understand their behavior in 3D-environments. Finally, there is an urgent need for an adequate standard to ensure that a bioprinted product can be reproducible had a high quality and is effective and safe.