Engineering the bone marrow microenvironment

Abstract

In the past few decades, biomaterials have become highly relevant for tissue engineering (TE). Currently, instead of producing inert biomaterials, their design is based on attempts to harness and improve the natural regenerative ability of the human tissues. By mimicking their complex architectures and intrinsic properties, researchers are able to create new artificial microenvironment. However, there is still a lack of fundamental molecular and cellular biology knowledge that is crucial for the success of tissue engineering medical products (TEMPs). In fact, most of the research in adult stem cells (ASCs) has been conducted in two-dimensional (2D) microenvironments instead of three-dimensional (3D) ones, which had led initial TEMPs to be ineffective. Hence, this thesis has been focused on understanding the interaction between biomaterials and human mesenchymal stem cells (hMSCs). More particularly, on how to replicate the stem cell niche, a source of ASCs necessary for tissue regeneration. In fact, this microenvironment has a set of unique properties that are fundamental for tissue homeostasis. Through a number of self-regulatory processes, the stem cell niche keeps a pool of quiescence stem cells, i.e. dormant, non-replicative cells, which are recruited and expanded upon the tissue injury. These processes and the existing 3D cell culture systems recreating the bone marrow stem cell niche microenvironment have been reviewed in depth in **Chapter 2**. In this chapter, we identified four major applications of 3D artificial stem cell niches (to promote stem cell expansion, to maintain stem cell quiescence/stemness, to drive cells towards differentiation, and to induce stem cell cytokine production and secretion) and focused our literature review on the latest research on maintaining quiescence of stem cells in 3D biomaterials.

The literature review has been pivotal for the design of 3D culture systems in **Chapter 3** and **4**. In **Chapter 3**, a direct comparison between hMSCs culture as scaffold-free self-assembled aggregates of low and high cell number or encapsulate into alginate hydrogels with and without arginine-glycine-aspartic acid (RGD) peptides was conducted. Both culture systems have successfully kept hMSCs alive throughout 14 days of cell culture, with alginate hydrogels showing more living cells than their scaffold-free self-assembled aggregates. Additionally, in a weeklong culture, both systems were able to induce cells to become non-proliferative. The findings of this study improve our understanding of how aggregate cultures differ with or without a hydrogel carrier, and whether aggregation itself is important when it comes to the 3D culture of hMSCs. In the follow-up study, we sought to unravel the molecular pathway leading to the low replicative potential of hMSCs when encapsulated in alginate hydrogels modified with the RGD peptide motifs (**Chapter 4**). Yet again, encapsulated hMSCs became non-proliferative and halted in the G_1 phase of the cell

cycle. Their quiescent state was further characterized by an upregulation of quiescence-related proteins when compared to hMSCs grown in two-dimensional (2D) tissue culture polystyrene (TCPS). Additionally, the results found for RGD-alginate were similarly replicated in other 3D hydrogels that presented higher number or different adhesion molecules, thus revealing that 3D hydrogel cultured hMSCs are induced to become quiescent. In contrast, such observations were not found in cells grown in 2D, even when quiescence was achieved by fetal bovine serum (FBS) deprivation, leading to conclude that 3D cultures have a unique molecular pathway to induce quiescence. This different molecular pathway has been further characterized through the expression of the mammalian target of rapamycin complex 1 (mTORC1), which was found to be downregulated in hMSCs cultured in 3D hydrogels. Such activation might be the key regulator in keeping "deep" quiescent cells alive both *ex vivo* and *in vivo*.

In **Chapter 5**, we sought to increase the complexity of our alginate hydrogel culture system by modifying the alginate backbone with all the possible combinations of five different proteins found in the bone marrow niche. Hence, hMSCs were encapsulated and cultured in hydrogels modified with combinations of peptide motifs of collagen type I, collagen type II and fibronectin (bone marrow's ECM proteins), as well as angiopoeitin-1 and N-cadherin (bone marrow's neighboring cells). By optimizing a high-throughput screening (HTS) platform, we were able to characterize a correlation between cell shape and proliferation when cells were exposed to the different peptide combinations. Both the HTS platform and the automated data analysis optimized in this chapter are less time- and material-consuming alternatives towards designing new artificial stem cell niches that can better mimic the native microenvironments.

Finally, in **Chapter 6** and **7**, we sought to unravel how 3D culture can affect cell's secretion. In **Chapter 6**, we showed how stanniocalcin-1 (STC-1) is secreted by hMSCs through a mechanosensitive pathway involving zyxin, actin-myosin and Rho-associated protein kinase (ROCK). In conditions where these proteins were downregulated, including in two distinct cell 3D culture systems, hMSCs increased their STC-1 secretion. Such response from the cells illustrate how secretion can be strongly affected by biophysical changes in the artificial matrix. In a rather similar way, in **Chapter 7**, a newly optimized hybrid material made of a hollow electrospun scaffold filled with an alginate hydrogel designed to fix implanted hydrogels in place, the *hydrocup*, showed that hMSCs change their secretome profile when 3D encapsulated. In fact, when cultured inside the *hydrocup*, hMSCs secretome profile seemed to be more associated with a less inflammatory profile, when comparing to alginate hydrogels. Moreover, the *hydrocup* was successful on maintaining in place alginate hydrogels in a 6-week *in vivo* implantation model, thus showing its relevance in a clinical setting design for hMSCs secretome delivery.

All the findings described on this thesis were thoroughly discussed in **Chapter 8** and their impact on society at large was address in **Chapter 9**.

Overall, this thesis adds fundamental knowledge to our understanding on microenvironments design through a deeper comprehension on molecular pathways involved between biomaterials and cells, while providing new technological tools to speed-up the translational research process of TEMPs.