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The optimization of a scaffold for cartilage regeneration

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Natural polymers offer various advantages in cartilage tissue engineering applications, thanks to their intrinsic bioactivity and adaptability, which can be exploited for the optimization of scaffold properties. In particular, silk fibroin has multifunctional features driven by the self-assembly of molecular subunits in appropriate environmental conditions. For these reasons, it was used in combination with hyaluronic acid to produce porous sponges for cartilage regeneration. The added amount of hyaluronic acid and the cross-linking with genipin modulated scaffold properties in a synergistic way, showing a strong inter-correlation among macroscopic and microscopic characteristics. Interestingly, hyaluronic acid affected silk fibroin conformation and induced a physical separation between the two material components in absence of genipin. Instead, this was prevented by the cross-linking reaction, resulting in a more interspersed network of protein and polysaccharide molecules partially resembling the structure of cartilage extracellular matrix. In addition, the systematic evaluation of sponge properties and how they can be modulated will represent a significant starting point for the interpretation of the complex outcomes driven by the scaffold in vitro and in vivo.

Keywords: cartilage tissue engineering, silk fibroin, hyaluronic acid, scaffold

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Successful Tissue Engineering therapies require scaffold materials with physical properties consistent with the application and ability to promote cells adhesion, proliferation and ECM production/assembling. The last requirement is mandatory, since the ultimate goal of tissue engineering is to rebuild the architecture of the tissues to restore, possibly quickly and with the full recovery of its structural and functional properties.[1] Even if synthetic materials can offer multiple choices in terms of adaptability to specific physical properties requirements, they do not generally possess bio molecular recognition features that are needed for the induction of the regenerative pathway. Natural materials can offer for many application favorable solutions, thanks to their intrinsic bioactive properties, provided that these are properly selected and addressed.[2,3]

Scaffold materials and architecture, cells and biochemical and/or mechanical signals, must be established for each application and, eventually, for each pathologic condition. On this basis, energies have been devoted to the exploration and the management of the micro environmental niche experienced by cells in the TE system, such as oxygen concentration, pH, electric potential, cytokine gradients and mechanical forces.[4]

These are the general considerations that supported our recent study.[5] The aim was to produce a scaffold tailored for cartilage regeneration.

Silk fibroin (SF) and hyaluronic acid (HA) were selected to fabricate porosity-controlled sponges by means of the salt leaching technique. SF isolated from *Bombyx mori* cocoons filament is a multifunctional responsive polymer whose adaptability is driven by the self-assembly of nanostructures (hydrophobic and hydrophilic blocks) into molecular conformations which depend on the environmental conditions.[6-8] Many studies have already demonstrated the adaptability of this material for the fabrication of tissue engineering scaffolds. Proposed applications range from bone[9-15] to tendons[16-18] or skin.[19,20] Scaffolds with tunable characteristics can be fabricated with several methods, such as gelation, salt leaching, casting, spinning and so on.[21]

However, the peculiar aspect of silk fibroin molecule is in the presence of two different active sequences, VITTDSDGNE and NINDFDED, recognized by integrin promoting cell growth, that were identified in the N-terminal region of the heavy chain,[22] lending the silk protein with multifunctional bio-recognition capabilities.

Hyaluronic acid is ubiquitous in mammalian tissues and particularly in cartilage. Cartilage is one of the most interesting examples of design from Nature. An engineered combination of collagen fibers with different orientation along the thickness impart cartilage the required mechanical properties, while the matrix materials, i.e., glycosaminoglycans comprising hyaluronic acid, and its swelling-deswelling assure the nutrients diffusion to cells while reducing to physiologically acceptable values the friction among bones of the joint.[2,23] As an attempt to mimic these functions, we modulated both hyaluronic acid concentration and distribution in the scaffold matrix with respect to silk fibroin.

Results showed a strong inter-correlation of scaffold properties, which were affected by the amount of hyaluronic acid and cross-linking in a complex and synergistic way. The addition of HA in silk fibroin sponges determined a modification of SF conformation and arrangement with respect to the pure material: hyaluronic acid not only enhanced the formation of crystalline stable structure in the protein, but also induced the segregation of silk fibroin due to its higher hydrophilicity. Interestingly, HA acted as the hydrophilic sericin during the extrusion of the silk bave from the silkworms glands.[24] The cross-linking reaction, instead, competed against the physical separation of the two material components, forming a more interspersed network of protein and polysaccharide molecules and partially resembling the structure of cartilage extracellular matrix. As expected, all scaffold properties were affected by the different molecular arrangements, even if it is worth to mention that the role of porosity was crucial in the determination of scaffold water content and mechanical properties. Nevertheless, the systematic characterization allowed a deep and critical understanding of the strong relationships among sponge properties. In addition, two different perspectives were considered in this study, focusing on the macroscopic characteristics of the scaffolds (morphology, porosity, mechanical properties), the microscopic features (molecular arrangement, cross-linking degree, silk fibroin crystallinity) and their connection.

However, beyond the premises and approach adopted in this study, a significant question is still left to answer: will this systematic picture be effective in helping clarify and guide cell behavior, once chondrocytes or even stem cells are cultured on the produced sponges or the best scaffolds are implanted in vivo? Or, on the contrary, is the big picture too much complex? Most likely, the answer is yes, to both. In fact, complexity arises not only from dealing with biological systems, but also from the mutual interactions between the material and the cells and tissues involved. This means that, even if all elements of the TE system are well known, they may be changed by their being in communication, adding a further level of complication. Nevertheless, a deep understanding of the playing forces remains as a fundamental starting point for tissue engineering, as it can effectively help reduce the complexity of interpreting the outcomes of a TE system, even if it cannot remove it.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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