

Cyclodextrins as Multipurpose Materials for Bone Regeneration [†]

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Abstract: The increase in life expectancy is favoring the prevalence of musculoskeletal diseases that compromise the quality of life of millions of people all over the world. Moreover, some life styles promote the early apparition of degenerative diseases. The unmet clinical demand of tissue regeneration prompts the searching of novel strategies. Cyclodextrins (CDs) have an outstanding track record as versatile tools for drug formulation. Although still less explored, CDs are being found useful for a variety of purposes in the design of advanced scaffolds for regenerative medicine. CDs can simultaneously host therapeutic agents and structural components of the scaffolds serving as reversible tie-junctions. Soft 3D supramolecular structures can be prepared as syringeable materials to be administered using minimally invasive techniques, with the additional advantage of ensuring a full filling of the gap in the damage tissue. Additionally, CDs have recently been demonstrated suitable to prepare patient-personalized scaffolds by means of 3D printing, opening novel approaches for scaffold design using additive manufacture techniques. This talk aims to provide an overview of the state-of-the-art of the use of CDs in bone regeneration. Although most information still refers to in vitro and preclinical studies, recent progresses in the field point out CDs as key components of improved scaffolds.

Keywords: cyclodextrin; electrospinning; supramolecular; 3D printing; regenerative medicine

1. Introduction

According to several World Ageing Reports, we are living through a period of population ageing that is without parallel in the history of humanity. The percentage of people above 65 years old was 8.5% in 2015 but it is expected to reach 16.7% in 2050. Japan and Spain are the countries with the highest average age of the world [1]. Ageing means that degenerative processes are becoming prevalent diseases, mainly musculoskeletal diseases, diabetes and cancer. For example, bone and cartilage diseases affect mobility, independence and quality of life of more than 100 million Europeans [2]. The needs to repair or regenerate tissues can also manifest at younger ages. In the practice of sports, tissues and joints are subjected to efforts that, as is well known, can also cause injuries even when well-planned training programs are followed. In athletes of such high level as those participating in the Olympic Games the problem is also of great importance, and about 10% suffer serious injuries.

The regenerative medicine field is paying huge efforts in finding the way to regenerate the tissues that became injured and thus to increase the quality of life of both old and young persons. In this context, cyclodextrins are being revealed as useful multipurpose tools. The peculiar cyclic structure and size makes cyclodextrins (CDs) to be one of the most versatile substances produced by

nature. They can form inclusion complexes with a variety of molecules, and they can be likely considered as the first drug nanocarriers ever used. It is in water and in the physiological aqueous environment where the multifaceted potential of CDs can be completely unveiled [3].

In the next sections, some examples of CD-based scaffolds are analyzed: syringeable supramolecular hydrogels, 3D printed scaffolds, and composites, that have already demonstrated to be useful for the repair or regeneration of a variety of tissues, particularly bone and cartilage.

2. Syringeable Supramolecular Hydrogels

In situ forming scaffolds that can be administered as a fluid material through minimally invasive maneuvers are advantageous to get access to profound tissues, to completely fill the gap in the lesions as the fluid perfectly adapts to the shape of the lacking tissue, and to minimize the risk of collateral events such as infections. Syringeable cyclodextrin supramolecular gels are mainly prepared via self-assembly of poly(pseudo)rotaxanes and via zipper-like coupling of CD-functionalized and guest-functionalized macromolecules in water.

Most poly(pseudo)rotaxane systems rely on the threading of poly(ethylene glycol), PEO, copolymers mainly triblock copolymers of poloxamer (Pluronic) or poloxamine (Tetronic), through α -cyclodextrin (α -CD) units. The interactions among the polypseudorotaxanes are reversible and the supramolecular gels behave as a thixotropic fluid [4]. Under mild stress (e.g., pressure of the plunger of a syringe), the assemblies are broken and the syringe delivers a low viscosity solution, which recovers the gel state in the static conditions of the site of injection.

Several syringeable poly(pseudo)rotaxane-based hydrogels have been evaluated in critical bone defects, which are bone defects resulting from nonunion fractures or tumor resections. In contrast to poloxamers that trigger differentiation of MSCs to adipocytes, some poloxamines have been shown able to induce differentiation to osteoblasts both in vitro and in vivo [5]. Studies in calvarial critical defect have evidenced the capability of poloxamines to act synergically with BMP-2 or simvastatin in bone recovery [6]. Addition of α -CD, decreased the concentration of poloxamine required for the sol-to-gel transition at the body temperature and reinforced the viscoelastic properties [4]. The poly(pseudo)rotaxane gels evidenced good ability to sustain in vitro release of simvastatin hydroxy acid form (SV, osteoinductive agent) diffusion. Overall, α -CD favored an earlier and prolonged differentiation of the mesenchymal stem cells (MSCs) to osteoblasts, and also favored bone repair in vivo in critical size defects in rat calvaria [7].

Syringeable supramolecular scaffolds can be also suitable for cartilage regeneration. Recent research in the field is devoted to reverse the osteoarthritis (OA) phenotype by direct transfection of genes encoding for anabolic growth and/or transcription factors. However, there are some barriers that have to be overcome for an efficient use of viral vectors for cartilage repair: (i) patients medicated with anticoagulants, such as heparin, show inhibited transduction because heparin binds to the viral particles inhibiting the interaction with the cell membrane receptor, a heparin sulfate proteoglycan (HSPG); (ii) there may exist in the patient a humoral response against the viral capsid as a consequence of a previous natural infection by the wild type virus; and (iii) a long-term recovery requires a platform from which the virus can be sustainedly delivered to the cells, delaying the clearance. Polypseudorotaxane gels of Pluronic® F68 (PF68) or Tetronic® 908 (T908) mixed and hyaluronic acid (HA) or chondroitin sulfate (CS) were shown able to provide sustained release of the rAAV vectors. The gels were able to promote the transfection when they were placed in direct contact with MSCs in a monolayer culture. The gels prepared with either chondroitin sulphate or HA notably enhanced the capability of the viral vectors to transfer the gene to the cells, and they exhibited sustained expression of the genes upto 21 days [8,9].

3. 3D Printing

3D printing is causing a revolution in the way of addressing health problems, and especially tissue regeneration. 3D printing allows to prepare scaffolds adapted to the specific needs of each patient, that is, personalized, with sophisticated geometries that adjust to the irregularities of each

defect and, in addition, mimic the complexity of the tissues by means of a precise distribution of organic materials and inorganics, cytokines and growth factors and, in some cases, also cells.

Polypseudorotaxanes and zipper-like assemblies possess two characteristics that make them particularly suitable for extrusion 3D bioprinting: (i) shear-sensitive behavior, exhibiting high viscosity at rest but easy flowability under moderate pressure, which facilitates the printing and also the maintenance of shape fidelity; and (ii) feasibility of secondary stabilization by means of post-processing techniques already implemented in additive manufacturing, such as photocross-linking of the polymer chains involved in the supramolecular assemblies [10]. For the 3D printing, two approaches have been investigated: the injection of the zipper-like gel into a preformed soft hydrogel (support hydrogel) that can easily accommodate the injected strands; or the modification of HA with methacrylate groups for UV cross-linking, which can be exploited for the direct 3D printing of subsequent layers of the zipper-like gel [11].

Only recently, wet masses of CDs and cellulose ethers have been shown useful for 3D micro-extrusion printing. Regulating the components ratio, the mass can be endowed with self-healing behavior, which is very useful for a correct 3D printing, and a variety of drug release profiles could be obtained [12].

4. Composites

CDs themselves can trigger cell differentiation to distinct lineages depending on the substituent groups and also promote salt nucleation. α -CDs by themselves facilitate differentiation to chondrocytes, while α -CDs substituted with methyl or phosphate groups stimulate adipogenic and osteoinductive differentiation [13]. Scaffolds that combine organic and inorganic components or that promote mineralization are particularly suitable for bone regeneration purposes. A bioinspired strategy consists in the use of polymers bearing ionic moieties that can bind calcium or phosphate ions *in vivo* and, in turn, regulate the growth of the crystals as the neotissue is being formed. Also, the alcohol functionalities present in the CD structure accelerate the nucleation of hydroxyapatite through electrostatic attraction of calcium ions. Hydrogel networks of β -CD functionalized with succinic anhydride were shown to trigger hydroxyapatite formation while also regulate the release of indomethacin during the biomineralization process [14]. Also, 3D printed scaffolds of polyesters, such as poly(ϵ -caprolactone) or poly(L-lactic acid), were surface modified with adamantane groups in order to immobilize β -CD-grafted hydroxyapatite on the surface. Scaffolds loaded with simvastatin evidenced in *in vivo* studies increased bone repair [15].

5. Conclusions

Although still incipiently explored, CDs are being pointed out as versatile multi-task agents in the preparation of scaffolds for tissue regeneration. CDs enable the preparation of a wide variety of soft and hard 3D scaffolds acting as structural agents. Simultaneously, CDs maintain their capability to host a variety of active substances that can be precisely delivered to the growing tissue, facilitating cell growth and differentiation. Further steps in the field will unveil the multipurpose roles that CD may play in regenerative medicine.

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