Development and characterization of an injectable dextrin-based hydrogel for bone regeneration

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Abstract

Bone is a dynamic, highly vascularized tissue that remodels itself continuously over an individual´s lifetime. It plays several important roles in maintaining homeostasis of the body systems [1, 2]. However, this regenerative capacity is limited and, as in the case of large bone defects, where the template for an orchestrated regeneration is absent, surgical procedures are needed [2]. In this respect, bone tissue engineering is a very challenging and promising field given the need to mimic bone mechanical and biological functions and also due to the failure of current orthopedic implants. The general concept consists in the development of three-dimensional scaffolds, from biocompatible materials (natural or synthetic), which confer temporary support for the regeneration of bone tissue, while the scaffold itself will be resorbed and replaced by newly formed tissue [2, 3].

Hydrogels are cross-linked networks made of natural or synthetic polymers, which are able to support high water contents [4]. These materials are usually biocompatible, have the ability to mimic physiological conditions, promote an environment that can protect cells or unstable drugs, their physical characteristics can be controlled to some extent and some can be injected *in vivo*. These features make them attractive materials in the biomedical field for cell encapsulation, drug or gene delivery or to act as an interface between tissue and materials [4-7]. Natural polymers are advantageous for this kind of applications since they are cheap raw materials, bear a great biocompatibility and are usually biodegradable [8]. Dextrin is low molecular weight carbohydrate, generally regarded as safe (GRAS), obtained from partial hydrolysis of starch or glycogen [9]. It is a glucose polymer linked by α-1,4 glycosidic linkages with some degree of branching due to the presence of α-1,6 bonds [10]. It is biocompatible and non-immunogenic, degradable by α-amylases and can undergo renal clearance avoiding tissue accumulation [11, 12].

This work describes the preparation and characterization of an injectable dextrin-based hydrogel (oDex) able to incorporate nanoparticles, cells, biomolecules or Bonelike® granules [13]. Bonelike® is a Biosckin-molecular and cell therapies S.A. proprietary synthetic bone graft, and the outcome of the project will result in a novel injectable presentation of this product. The hydrogel was produced by dextrin oxidation with sodium periodate followed by cross-linking with a dihydrazide [14]. In vitro characterization of oDex hydrogel has shown acceptable mechanical properties, overall good biocompatibility and the ability to be combined with other materials such as a nanogel and urinary bladder matrix, without affecting its structure. The cytotoxicity of the free dihydrazide was evaluated and only a mild inhibitory effect on cell proliferation was observed for the concentration used in the hydrogel crosslinking. The biocompatibility of oDex hydrogels was confirmed through the encapsulation of cells, which were able to endure the gelation process. Subcutaneous implants were performed in Sasco Sprague Dawley rats in order to evaluate the inflammatory response and systemic effects of oDex hydrogels and their combination with Bonelike® and human mesenchymal stem cells isolated from umbilical cord's Wharton jelly. After 3 and 15 days post-implantation, a quantitative evaluation of both responses was performed according to ISO 10993 by a scoring system leading to a classification of the implanted material as slight irritant even when associated to Bonelike® or to the cellular system. The

performance of oDex hydrogel combined with Bonelike granules and/or UBM in bone defects was investigated in New Zealand rabbits. Bone defects in several anatomical locations (tibiae and cranium) of non-critical and critical size were filled with those materials. Histological analysis showed that oDex does not constitute a barrier for cellular colonization and proliferation since the defects that were filled with these materials presented a higher degree of regeneration and a higher amount of collagen fibers with higher organization degrees, when compared with the empty defects. Even though oDex hydrogels purpose is to act as an injectable carrier for osteoconductive materials, like Bonelike® granules, the hydrogel itself seems to assists the regenerative process when compared with the empty defects. This is due to the 3D support conferred by hydrogels that facilitates cell migration to the defect site. Moreover, the presence of UBM strongly stimulates the bone regeneration, for levels comparable with the Bonelike® conditions, since an increase in cellular colonization and organization in the defect site can be denoted. A sterilization protocol for oDex hydrogels by gamma and beta radiation was investigated through irradiation of oxidized dextrin solutions. Despite both kinds of radiation induced slight differences in the storage modulus of the hydrogels, indicating the occurrence of chain scission/cross-linking effects on the dextrin chain, all materials were gelable after the irradiation treatments. These effects seem to not be dose or temperature dependent and the irradiation process in liquid or solid state also does not induce major differences in the rheology of the final hydrogels. Due to its known advantages, gamma radiation seems to be a suitable sterilization method for oxidized dextrin solutions. The stability of gamma irradiated dextrin solutions was evaluated up to 8 months. Despite the increase of storage modulus of the hydrogels over the time, this effect does not constitute a disadvantage since it improves elastic behavior of the hydrogels. oDex hydrogels provides a system that can carry and stabilize cells, nanogels, Bonelike® granules and other biomolecules. It is a promising biomaterial due to its biocompatibility, and potential to promote an adequate environment for bone regeneration. Its injectability allows a minimal invasive surgical procedure with decreased patient morbidity, lower risk of infection and reduced scar formation.

This work has been developed in the scope of an European project that allowed collaborations with research groups, which have complementary expertise. The tight collaboration between University of Minho and Bioskin S.A. company, envisioning technology transfer and product valorization, has resulted in a published international patent of the product (WO2011070529A2) [15]. Currently, a new set of pre-clinical trials in sheep models are being planned as well as the submission of a request for the authorization for the clinical trials.

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