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Research Paper

Composite scaffolds for controlled drug release: Role of the polyurethane nanoparticles on the physical properties and cell behaviour

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ABSTRACT

Localised delivery of appropriate biomolecule/drug(s) can be suitable to prevent post-operative infections and inflammation after scaffold implantation *in vivo*. In this study composite shell scaffolds, based on an internally produced bioactive glass and a commercial hydroxyapatite, were surface coated with a uniform polymeric layer, embedded with thermo-stable polyesterurethane (PU)-based nanoparticles (NPs), containing an anti-inflammatory drug (indomethacin; IDCM). The obtained functionalised scaffolds were subjected to physico-mechanical and biological characterisations. The results indicated that NPs incorporation into the gelatin coating of the composite scaffolds: 1) not changed significantly the micro-architecture of the scaffolds in terms of mean pore diameter and pore size distribution; 2) increased the compressive modulus; and 3) allowed to a sustained IDMC release (65–70% of the loaded-drug) within the first week of incubation in physiological solution. On the other hand, the NPs incorporation did not affect the biocompatibility of composite scaffolds, as evidenced by viability and alkaline phosphatase (ALP) activity of MG63 human osteoblast-like cells.

1. Introduction

Tissue engineering aims to restore and maintain the function of damaged or diseased human bone tissues by combining biodegradable scaffolds with isolated functional cells and signalling molecules, such as growth factors (Langer and Vacanti, 1993). An ideal scaffold to be employed in bone tissue regeneration and repair should be biocompatible,

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highly porous with a morphology resembling the microstructure of trabecular bone, osteoconductive and biodegradable, and should have adequate mechanical strength, sufficient to ensure mechanical stability prior to synthesis of new extracellular matrix by seeded cells (Rezwan et al., 2006). Both calcium phosphates, in particular hydroxyapatite (HA), and bioactive glasses have been extensively used in the last years as scaffolding materials, tissue surgery and dentistry. HA is chemically and structurally similar to the mineral component of human bone and it is able to create a bond with the surrounding bone tissue *in vivo* (Orlovskii et al., 2002). On the other hand, HA degradation rate after implantation in the body is remarkably low, and this is an undesirable feature for the realisation of scaffolds, which are expected to be resorbed *in vivo* at rates appropriate to new tissue regeneration (Ducheyne et al., 1993; Oonishi et al., 2000). Moreover, the production of HA based scaffolds requires high temperature thermal treatments, which may lead to HA decomposition with the formation of CaO, α- or β-tricalcium phosphate, and their presence produces decohesion of the whole material, hinders sintering and modifies the biodegradability of the final product (Cihlar et al., 1999; Suchanek et al., 1997). Bioactive glasses offer important advantages as an alternative to HA, mainly due to their higher bioactivity index. Specific compositions, such as the so-called 45S5 Bioglass®, are able to bond to hard tissues as well as to soft ones, to stimulate angiogenesis and osteoblast turnover (Day, 2005; Xynos et al., 2000). A fundamental research topic is the design and development of new composite systems, which combine a bioglass and a calcium-phosphate phase, in order to overcome the intrinsic limits of the single constituents. In this sense, several works have been devoted to investigate the sintering and mechanical behaviour of HA with phosphate or silicate-based bioglass addictions (Bellucci et al., 2011b, 2013b; Goller et al., 2003; Ravarian et al., 2010). Recently a novel binary composite ("HA_BGCaM") has been obtained by sintering a CaO-rich bioglass (named BG_Ca/Mix), characterised by a low tendency to crystallise, with the addition of HA as the second phase (Bellucci et al., 2013a). Thanks to the peculiarities of the glass (Bellucci et al., 2010, 2012b), it was possible to treat the composites at a relatively low temperature (818 °C), thus minimising the interaction between the constituent phases and avoiding HA decomposition. Moreover, the obtained composite has been successfully employed to realise scaffolds for bone tissue engineering by means of a protocol recently developed to go beyond the limits of the conventional replica technique. The new samples, called "shell scaffolds", were characterised by an internal high porosity combined with an external resistant and permeable surface, which ensures an adequate mechanical strength (Bellucci et al., 2012a).

As a further step, composites based on bioceramics and natural polymers can be developed in order to reproduce at best the natural bone structure, which is composed by a mineral phase (biological HA) and collagen. These new generation scaffolds are able to preserve the structural and biological functions of bone tissue in a biomimetic way. Among them, gelatin is a non-expensive and commercially available biomaterial that has gained interest in biomedical engineering, mainly because of its biodegradability. Gelatin is obtained by thermal denaturation or physical and chemical degradation of collagen, the most widespread protein in the body, occurring in most connective tissues as skin, tendon and bone. With respect to collagen, gelatin does not express antigenicity in physiological conditions, it is completely resorbable *in vivo* and its physicochemical properties can be suitably modulated. The main limitation of gelatin for the production of tissue substitutes arises from its rapid dissolution in aqueous environments (Huang et al., 2005). Chemical and physical crosslinking methods have been used to increase gelatin stability in aqueous media (Ciardelli et al., 2010; Kuijpers et al., 2000); Genipin, the aglycone of geniposide (an iridoid glucoside isolated from the fruits of *Genipa americana* and *Gardenia jasminoides Ellis*) is a naturally occurring compound that can be used as a coupling agent for amino containing materials (Tonda-Turo et al., 2011). Genipin has been widely used as a crosslinking agent for natural polymers in tissue engineering and biomedicine. For example, genipin was used to crosslink (1) gelatin microcapsules for drug delivery (Chen et al., 2010; Huang et al., 2009), (2) gelatin conduits for peripheral nerve regeneration (Chen et al., 2005) and (3) composite films based on gelatin and hydroxyapatite/bioactive glass for bone tissue engineering (Gentile et al., 2010).

Recently a feasibility study has been devoted to discuss 45S5 Bioglass®-derived shell scaffolds coated by bio-resorbable gelatin (Bellucci et al., 2012c). Unfortunately, the uncoated scaffolds were completely crystallised, since high temperatures are required in order to consolidate 45S5 (Boccaccini et al., 2007; Chen et al., 2006a). On the other hand, the main objective was to investigate the effect of the biomimetic coating either on scaffolds' porosity and on their bioactivity *in vitro*. In this sense, *in vitro* tests in a Simulated Body Fluid solution confirmed the bioactivity of the gelatin-coated samples, whose porosity remained open and interconnected.

Here, for the first time, HA_BGCaM binary composite powders have been employed to produce gelatin-coated shell scaffolds, according to the protocol developed in (Bellucci et al., 2012c). In order to investigate potential applications of a gelatin coating as drug-delivery, thermo-stable polyesterurethane (PU)-based nanoparticles containing an anti-inflammatory drug, indomethacin (IDMC), were incorporated during the fabrication of the polymeric coating to fix the location of nanoparticles within the porous composite scaffolds, with the aim to prevent postoperative infections and inflammation after scaffold implantation *in vivo*.

IDMC is a non-steroidal anti-inflammatory drug with a high relevance in the treatment of disorders like several kind of arthritis, pericarditis, bursitis, tendinitis or spondylitis. Approved by FDA in 1965, it is widely used due to its antipyretic and analgesic properties, more potent than aspirin (Manjoo et al., 2010; Temussi et al., 2011). Its chemical structure is presented in Fig. 1.

Furthermore, the effect of PU nanoparticles incorporation on the physical and biological properties of the coated composite scaffold(s) was investigated by analysing the scaffold micro-architecture, porosity, chemical composition, cell attachment, cell viability and ALP activity using MG63 human osteoblast-like cells. Then, the drug release was analysed to evaluate the therapeutic potential of the functionalised coated scaffold(s).