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Engineered porous scaffolds for periprosthetic infection prevention

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ABSTRACT

Periprosthetic infection is a consequence of implant insertion procedures and strategies for its prevention involve either an increase in the rate of new bone formation or the release of antibiotics such as vancomycin. In this work we combined both strategies and developed a novel, multifunctional three-dimensional porous scaffold that was produced using hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), coupled with a pectin (PEC)-chitosan (CHIT) polyelectrolyte (PEI), and loaded with vancomycin (VCA). By this approach, a controlled vancomycin release was achieved and serial bacterial dilution test demonstrated that, after 1 week, the engineered construct still inhibits the bacterial growth. Degradation tests show an excellent behavior in a physiological and acidic environment (<10% of mass loss). Furthermore, the PEI coating shows an anti-inflammatory response, and good cell proliferation and migration were demonstrated *in vitro* using osteoblast SAOS-2 cell line. This new engineered construct exhibits excellent properties both as an antibacterial material and as a stimulator of bone formation, which makes it a good candidate to contrast periprosthetic infection.

1. Introduction

Coupling bone graft and titanium implant is still one of the best available solutions to replace bone and dental tissue loss. In particular, in large defects, where the residual bone is not enough to ensure stability for the implant, it is necessary the insertion of the so-called bone filler material. This kind of surgical approach promotes bone formation and gives stability, guiding bone regeneration around the implant. Many of these procedures are successful, with an implant survival rate > 90% at 10–15 years of follow-up [1–4]. Despite this success rate, the implant fails in 1–5% of procedures and must be removed [5,6]. The reason for this could be explained by the biomechanical issue on one side, due to an overloading at the bone implant surface [7–9], and by the biological failure on the other side, associated with microbial plaque accumulation and bacterial contamination [10–12].

Current strategies for the prevention of periprosthetic infection (PPI) involve either an increase in the rate of new bone formation by implanting a graft material or the systemic administration of antibiotics. In the last decades, the development of grafting materials has aroused great interest [13–15]. In particular, synthetic ceramic materials such as tricalcium phosphate (βTCP) and hydroxyapatite (HA) have been widely used due to their good reproducibility, biocompatibility, and non-immunogenicity, but especially because of their similarity to the components of the native bone mineral phase [16–18]. In large bone defects, filling completely the void and giving a sufficient mechanical resistance to the implant are the principal issues as they are a key factor for the induction of cell migration and proliferation inside the scaffold, in order to achieve a satisfactory osteointegration. The HA/βTCP mix is one of the most used biphasic materials due to its great biocompatibility, good degradation rate and mechanical support [19]. Morra et al., in a recent work [20], developed a new bone filler in particles, named Synergoss®, made with HA/βTCP in percentage of 25 and 75 wt.% respectively. *In vivo* studies demonstrated that this filler allows new bone formation, coupled with a compatible degradation rate [20]. The balance between HA and βTCP is a key point to obtain both mechanical strength and degradation, and to stimulate excellent osteointegration [21,22].

However, dentistry practice for large bone defects is expected to use three-dimensional (3D) porous scaffold in order to fill large voids, thus stimulating cell infiltration through interconnected pores and increasing mechanical stability for the immediate implant loading. Scaffold architecture and pore characteristics can be properly tailored by making use of different processing methods, including foam replication [23], polymeric filler burning-out [24] and 3D printing [25]. However the scaffold properties, alone, are not enough to treat the injury successfully, as large bone defects are often the result of the removal of necrotic tissue or of a zone with an acute infection. Furthermore, considering

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the natural function of the oral cavity – which is at the interface with a lot of bacteria, there is a high possibility of bacterial growth and re-infection of native bone [26–28].

The traditional antibiotic therapy is not effective in the control of PPI, due to a specific adhesion of bacteria on the biomaterials and the very low penetration of the antibiotic into the osseous defect [10,29,30]. Furthermore, systemic delivery of a high dose of drug can cause systemic toxicity with associated renal and liver complications, resulting in the need for hospitalization for monitoring [3,31–33]. Development of a complex release system in order to reduce infection and, at the same time, promote bone formation, could be a valuable solution.

Antibiotics used in drug release complexes should be effective against bacteria and biocompatible, *i.e.* they should not damage the surrounding native tissues. Vancomycin is a widely used antibiotic that provides bactericidal activity against the most relevant germs and shows efficacy and biocompatibility in clinical use [34–36]. A smart tissue-engineering approach involves the combination of ceramic material with a polymeric coating, which allows encapsulation of drugs and increases the implant biocompatibility generating biomimetic surface [13,37]. In particular, collagenous materials were used, since collagen is a key component of bone, is biocompatible and its degradation products are nontoxic [20]. Collagen is a very expensive component and, in order to obtain a stable surface functionalization without a faster degradation *in vivo*, a chemical crosslinking is needed, which implies the use of chemical crosslinkers such as glutaraldehyde that may cause cytotoxic effect on the surrounding tissues [38].

Pectin and chitosan are nontoxic natural polysaccharides that have aroused great interest in the last years in tissue engineering, due to their capability to crosslink by means of intermolecular interactions and to form stable and biocompatible complexes which may simulate the extracellular matrix and interact with cells from surrounding tissues [39–44]. Pectin is a natural anionic polysaccharide, a major component of citrus cell walls or apple peel by-product, consisting in a poly(D-galacturonic) chain with carboxyl groups, which could be ionically crosslinked by calcium ions (Ca^{2+}) thus forming the so called “egg box” structure. Furthermore, ionic interactions occur with polycationic polysaccharides, in order to form a well-known polyelectrolyte structure (PEI) [39]. Pectin is already widely used in the food industry and, in the last decades, it has found application in bone tissue engineering, in particular as a drug carrier [45–48]. The limit of pectin is its great water-solubility, which causes fast dissolution, and when it is used as a drug carrier a burst release of the therapeutic molecules occurs. To overcome this problem, many research groups try to combine pectin with other materials [49–54]. In particular, chitosan is a natural polycationic material that could form a stable polyelectrolyte composite in acidic environment when mixed with pectin. Chitosan derives from chitin, which can be extracted from crustaceous exoskeleton and is composed by β-(1,4)-glucosamine and *N*-acetyl-D-glucosamine. Owing to its biocompatibility, its intrinsic antibacterial nature, and its ability to induce no foreign body reaction and to promote cell adhesion, proliferation and differentiation, chitosan has aroused great interest in tissue engineering and pharmaceuticals, finding many application especially in bone tissue engineering [42,43,55–57].

In this work, we developed and produced an engineered scaffold coupling inorganic and organic phases loaded with vancomycin, as a novel system to prevent and control periprosthetic infection in dental large defects. As inorganic phase we used a highly porous 3D biphasic ceramic (25 wt.% of HA and 75 wt.% of βTCP). We hypothesized and demonstrated that the functionalization of the ceramic scaffold with a pectin-chitosan PEI coating allows the control of vancomycin release, inhibits bacterial proliferation and biofilm formation, stabilizes the degradation rate in physiological and acidic environment and promotes osteoblast proliferation without compromising the mechanical properties; moreover, gene expression results demonstrated that PEI treatment elicits anti-inflammatory responses. We therefore successfully manufactured a 3D construct that could prevent the generation of periprosthetic infection and promote new bone formation in large dental bone defects.

2. Materials and methods

2.1. Materials

In this work we designed, developed and characterized a macroporous ceramic scaffold, functionalized with a polyelectrolyte coating loaded with vancomycin. βTCP, chitosan with medium molecular weight (Mw = 400 kDa) from crab shell, pectin from citrus peel, vancomycin and all other chemicals were purchased from Sigma-Aldrich. HA (5 μm particles) was purchased from Fluidinova (Portugal) and the dispersing agent Dolapix CE 64 was purchased from Zschimmer&Schwarz (Germany).

2.2. Three-dimensional porous scaffold preparation

HA was used for enhancing the mechanical strength of scaffold while β-TCP for its degradability; they were mixed in a percentage of 25 and 75 wt.%, respectively, to reach an optimum compromise between the two properties (this compositional ratio was selected on the basis of previous results reported elsewhere [20]). Briefly, the preparation of the ceramic scaffolds involved the mixing of the HA and β-TCP powders (45 wt.%) with a binding agent (poly(vinyl alcohol), 8 wt.%), and ultrapure water (47 wt.%) to obtain a ceramic slurry. Dolapix CE 64 was added as a dispersing agent (0.5 wt.% of the solid load). Polyurethane (PU) sponge impregnation method was used to obtain a macroporous ceramic scaffold [58,59]. A commercial PU sponge cube (45 ppi) of $11 \times 11 \times 11$ mm^3 was soaked into the ceramic slurry for 90 s, followed by compression along the three spatial directions (40 kPa) until 30% of height and left at room temperature for 5 min before repeating the cycle. Impregnation/compression cycles were repeated for 3 times. The ceramic-coated PU sponge was left to dry overnight at 37 °C and sintered in a furnace at 1150 °C for 12 h in air (heating rate: 5 °C/min), in order to obtain a porous HA/βTCP scaffold of $10 \times 10 \times 10$ mm^3 (a volumetric retention of 24% was calculated).

2.3. Engineered coating preparation

Pectin powder was dissolved in a concentration of 1% in acetate buffer (pH 5.5); then, in the same solution, 1% of vancomycin was dissolved. Separately, chitosan 0.5% powder was dissolved in acetate buffer (pH 5.5). The coating process involves two steps of immersion. In the first step, the sintered ceramic scaffold was immersed in the pectin-vancomycin (PEC-VCA) solution for a defined time (60 s). In this step, pectin polyanion polysaccharides were crosslinked on the surface of ceramic scaffold due to the Ca^{2+} ions released from the scaffold; then, the clad material was freeze-dried overnight and a HA/βTCP_PEC/VCA scaffold was obtained. The first step allows obtaining a pectin-vancomycin coating with a mass of 7.24 ± 1.77 mg, of which 5.04 ± 0.31 mg of vancomycin (calculated by release studies, see the Section 2.6). In the second step the HA/βTCP_PEC/VCA scaffold was immersed for 30 min in a chitosan solution: in this step a polyelectrolyte was generated from polycationic chitosan and polyanionic pectin. The construct was then freeze-dried overnight, and a final material called HA/βTCP_PEC/VCA_CHIT was obtained. The coating process was evaluated on 20 samples and results repeatable with a final mass value of 11.07 ± 1.70 mg.

2.4. Chemical and microstructural analysis

The microstructure of the scaffold was studied in a nondestructive manner by micro-computed tomography (μ-CT), with a desktop μ-CT scanner (SkyScan 1174, Aartselaar, Belgium). The scanner was set at a voltage of 50 kV and a current of 800 μA, and the sample was scanned at 9.23 μm pixel resolution. The exposure time per projection was