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Research paper

Development and characterization of clay-polymer nanocomposite membranes containing sodium alendronate with osteogenic activity

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ABSTRACT

The preparation of new biocompatible polymer systems could accelerate processes underlying guided bone tissue regeneration (GBR) by acting as adjuvants. Here, different reaction conditions were investigated to engineer nanocomposites based on chitosan and sodium montimorilonite (Na-Mt) that can be used to generate biocompatible membranes. The goal was a balanced combination of flexibility and tensile strength for use in orthopedic and periodontal surgical procedures for improved GBR. The execution of different experimental protocols aimed to control the polymer intercalation process inside the Na-Mt lamellae, and to avoid their exfoliation, by varying its concentration while applying, at same time, ultrasonic energy as a pretreatment. To increase the therapeutic efficacy of the membranes formed, sodium alendronate was included in the compositions as well as plasticizers to optimize their mechanical and physical-chemical properties. All membranes were analyzed by powder x-ray diffraction, nuclear magnetic resonance, differential scanning calorimetry and thermogravimetric analysis, as well as high performance chromatography to characterize the sodium alendronate content. Surface roughness, water absorption (swelling) and tensile strength were evaluated by scanning electron microscope and profilometry. As a model for human osteoblasts, the proliferation of Saos-2 cells was measured by colorimetric assays and microscopy to assess the potential performance of the membranes in GBR. Membranes with improved mechanical and physical-chemical properties along with excellent biocompatibility could be generated from 33% sodium montmorillonite (w/w) intercalated with chitosan and containing sodium alendronate, glycerol (20% [w/w]) and Labrasol® (3% [w/w]) that also supported the induction of Saos-2 cell proliferation and differentiation, exhibiting potential to develop a novel biomaterial device for GBR

1. Introduction

A major benefit of approaches based on nanotechnology is the potential to introduce new properties into bioengineered materials that can control their interactions with biological material and modulate cellular responses (Klippstein and Pozo, 2010; Yang et al., 2015). The combination of biomaterials and nanotechnology represents an important tool to allow significant progress in the field of bone tissue engineering. Bone is a complex structure composed of an organic matrix, approximately 90% Type I collagen (Moura et al., 2013), and an inorganic matrix (60–70%) that contains mainly hydroxyapatite (Krzesińska and Majewska, 2015). Recent advances have enabled the development of novel biomaterials for bone tissue engineering applications that include bone substitutes, healing of bone defects, stabilization of fractured bones, and periodontal tissue regeneration (Bottino et al., 2012; Farraro et al., 2014). Current nanotherapeutic approaches include nanoparticles for targeted delivery of drugs or immunotherapies, nanofibers that serve as tissue scaffolds and nanomembranes or nanodevices for guided tissue regeneration (GTR) (Basile et al., 2015; Farhatnia et al., 2013; Saiz et al., 2013; Su et al., 2012).

Among the materials used today for GTR applications, the most commonly utilized, and marketed, are non-degradable metals such as stainless steel and titanium alloys, as well as non-reabsorbable membranes due to their excellent biocompatibility, mechanical strength and

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corrosion resistance (Farraro et al., 2014). However, their permanence requires a second surgical procedure for their removal, which increases the risk for patient infections and can offset their advantages (Ribeiro et al., 2015; Villar and Cochran, 2010). For this reason, both academic and industrial research has focused extensive studies on the development of resorbable membranes based on biodegradable polymers that can deliver the required structural performance profiles.

Bioresorbable membranes have also attracted great interest in the field of functional tissue engineering to delivery bioactive molecules that can enhance tissue healing and can ultimately be replaced by patient tissue (Basile et al., 2015; Nunes-Pereira et al., 2015). Biomaterial scaffolds provide adequate porosity, adherence and incorporation into adjacent tissue for cellular attachment, proliferation, migration and differentiation (Karuppuswamy et al., 2014; Kavya et al., 2013; Yang et al., 2015). Several natural biodegradable polymers including the polysaccharides chitosan (CS) and cellulose, as well as proteins, such as collagen (CG), have been considered as important substrates for bone grafts (Serra et al., 2015).

Chitosan is a linear cationic amino-polysaccharide obtained by the partial deacetylation of chitin and is comprised of copolymers of glucosamine and *N*-acetylglucosamine (Sabaa et al., 2015). Chitosan has been widely used on account of its biodegradability, biocompatibility and low cost, in addition to its excellent antimicrobial property against a wide range of microorganisms (Kavya et al., 2013; Kumar-Krishnan et al., 2015). Similar to chitosan, the polysaccharide hydroxyethylcellulose (HEC) is also both biodegradable and bioadhesive (Stoyneva et al., 2014). It is a non-ionic cellulose derivative presenting high water solubility, swelling, chemical stability and compatibility with tissue and blood (Villegas-Pañeda et al., 2014). The major organic component of bone, collagen, has been commonly used for bone tissue engineering owing to its good bioactivity, biodegradability and negligible immunogenicity (Chen et al., 2014).

Despite the excellent adhesion of tissue cells to natural polymers, promoted by biological recognition, their weak mechanical properties and fast degradability are often inappropriate for the biomedical applications (Chen et al., 2014; Hsu et al., 2012; Nunes-Pereira et al., 2015). An effective approach to overcome this limitation is to form nanocomposites through the combination of biodegradable polymers with clay minerals, which improves the physical properties of the resulting membranes (Sainitya et al., 2015). In comparison to natural polymer membranes, clay-polymer nanocomposites often display improved characteristics such as durability, mechanical resistance, thermal stability, flame resistance, gas-barrier properties, surface properties, biodegradability and biocompatibility (Chang and Lee, 2015; Suin et al., 2013; Taheri and Sadeghi, 2015).

Sodium montmorillonite (Na-Mt) is a hydrophilic clay mineral of the smectite group with a 2:1 aluminosilicate layered structure that has a negatively charged surface (Ali and Bandyopadhyay, 2015). The negative charges, resulting from the isomorphous substitutions in the layers, are balanced by exchangeable cations located in the interlayer space, most commonly Ca^{2+} or Na^{+} (Petra et al., 2015). Na-Mt has been widely studied as a naturally nano-sized layered material with a large surface area that provides the ability to insert different molecules into the interstitial space due to their high cation exchange capacity (Sun et al., 2015). It can be obtained in either an intercalated or exfoliated form and membranes formed with exfoliated Na-Mt nanocomposites demonstrate superior properties in comparison to those from intercalated products (Barick and Tripathy, 2011; Alboofetileh et al., 2013).

The association of Na-Mt nanocomposite membranes with sodium alendronate (ALN), a potent nitrogen-containing bisphosphonate widely used for the treatment and prevention of osteoporosis, can further increase the efficacy of this type of membrane in guided bone regeneration (GBR) due to the specific inhibition of osteoclast-mediated bone resorption (Ke et al., 2016). Bisphosphonates are synthetic analogs of the pyrophosphate that bind to the hydroxyapatite present in bones. Despite the numerous publications about the development of ALN-polymeric systems, no investigations have been described on the production and evaluation of ALN-Na-Mt. scaffolds or membranes associated with biodegradable polymers. This study focused on the development, characterization and evaluation of ALN-Na-Mt-polysaccharide membranes for biomedical applications such as GBR.

2. Materials and methods

2.1. Materials

Low molecular weight chitosan (75–85% deacetylated, 20–300 cps), commercial bovine tendon collagen type I and hydroxyethylcellulose (viscosity ~145 mPa·s, 1% in H_2O - 20 °C) were purchased from Sigma-Aldrich (Saint Louis, United States). Sodium bentonite (Bentocol®) used for Na-Mt preparation was acquired from Laviosa Minerals (Livorno, Italy). Bentocol® consists predominantly of montmorillonite (Mt) (96%), carbonates and accessory minerals, such as quartz, feldspar and calcite (maximum concentration 4%). The average chemical formula of Mt., as described by the manufacturer, is expressed as $Na_{0.67}[Si_8Al_{0.33}][Al_{3.04}Fe_{0.44}Mg_{0.67}]O_{20}(OH)_4$ and the cation exchange capacity (CEC) is 87 meq/100 g, determined after saturation with ammonium cations using 1 M ammonium acetate solution. Mt obtained from Bentocol® was purified and posteriorly saturated with NaCl solution according to a procedure adapted from Neaman et al. (2003), in order to reduce the accessory minerals concentration. The clay material was washed with distilled water, recovered by centrifugation (10,000 rpm), and kept in an oven at 60 °C to remove all water. Sodium alendronate trihydrate was supplied by Apotex (Toronto, Canada). Glycerol, glacial acetic acid, polyethylene glycol (PEG 6000) and all other analytical reagent grade chemicals were obtained from Vetec (Rio de Janeiro, Brazil). Labrasol® and Transcutol® were purchased from Gattefossé (Saint-Priest, France). MTT (3-[4, 5 dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) was acquired from Tokyo Chemical Industry (Tokyo, Japan). Distilled water was used to prepare all solutions.

2.2. Preparation of membranes from Na-Mt nanocomposites containing HEC, CG CS

Na-Mt:HEC, Na-Mt:CG and Na-Mt:CS nanocomposite membranes were prepared by casting solutions onto a glass plate followed by solvent evaporation at 50 °C. Two approaches were employed to create nanocomposites solutions. In the first, Na-Mt (5% or 7%, w/w final clay mineral/polymer ratio) was pre-treated in acetic acid (2%) or water by high intensity ultrasonic energy in a Hielscher immersion probe UP100H (Teltow, Germany), under 100% amplitude with magnetic stirring for 30 or 60 min at room temperature. Next, HEC or CG was added to Na-Mt pre-treated in water, while CS was added to Na-Mt pre-treated in acetic acid at two different concentrations, 95% or 93% (w/w polymer/clay mineral ratio) under mechanic stirring (Nova Ética M110, São Paulo, Brazil) for 3 h. In the second, nanocomposites were formed under vigorous mechanical stirring (430 rpm) of polymer solutions with the addition of the respective pretreated Na-Mt dispersions at room temperature for two reaction times; 24 and 48 h. The stirring method utilized the same solvents and concentrations as described for the first approach. Larger quantities of Na-Mt were also tested for nanocomposites preparation using mass ratios of 2:1, 1:1 and 1:2 (Na-Mt:polymer [w/w]) maintaining the other parameters described.

2.3. Incorporation of plasticizers and sodium alendronate into nanocomposite membranes

Different plasticizers were analyzed to reduce the brittleness and improve mechanical properties of Na-Mt:polysaccharide nanocomposite membranes (Lee et al., 2014). The hydrophilic plasticizers polyethylene glycol (PEG) and glycerol were incorporated at ratios of 20%,