Materials Science and Engineering C 75 (2017) 418–424

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Improvement of mechanical and biological properties of Polycaprolactone loaded with Hydroxyapatite and Halloysite nanotubes

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ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form 10 February 2017
Accepted 16 February 2017
Available online 20 February 2017

Keywords:
Polycaprolactone
Hydroxyapatite
Halloysite
Curcumin
Tissue engineering

ABSTRACT

Hydroxyapatite (HA) and Halloysite nanotubes (HNTs) percentages have been optimized in Polycaprolactone (PCL) polymeric matrices to improve mechanical, thermal and biological properties of the composites, thus, to be applied in bone tissue engineering or as fixation plates. Addition of HA guarantees a proper compatibility with human bone due to its osteoconductive and osteoinductive properties, facilitating bone regeneration in tissue engineering applications. Addition of HNTs ensures the presence of tubular structures for subsequent drug loading in their lumen, of molecules such as curcumin, acting as controlled drug delivery systems. The addition of 20% of HA and different amounts of HNTs leads to a substantial improvement in mechanical properties with values of flexural strength up to 40% over raw PCL, with an increase in degradation temperature. DMA analyses showed stability in mechanical and thermal properties, having as a result a potential composite to be used as tissue engineering scaffold or resorbable fixation plate.

1. Introduction

Skull injuries are the most frequent traumas found in urban trauma centers [1,2], increasing the risk of stroke, which is one of the leading causes of death and disability worldwide [3]. Elderly people are the group most affected by craniofacial injuries, due to normal aging and decreased estrogen production, which can lead to bone loss and osteoporosis, the latter being especially prominent in women after menopause [4]. According to the National Institute on Aging (US Department of Health and Human Services), an estimated 8.1% percent of the world's population was over 61 years old in 2014. By 2050, this number is expected to triple. Accordingly, large efforts are being focused on the research of novel bioabsorbable materials capable of fulfilling the requirements for bone fracture remodeling as a promising alternative to current metal prostheses.

Commonly used metal prostheses are biocompatible and highly resistant to breakage. However, metal prostheses are heavy, expensive, do not promote bone regeneration, nor are degraded over time. Consequently, a second surgery is usually required in order to remove the metal prosthesis once the fractured bone is healed, entailing an extended recuperation process and eventual infections. One of the most significant disadvantages present in metal prostheses is the stress shielding effect caused by a larger support of the load by the plate instead of the bone, yielding a decrease in bone mass and an increase in bone porosity (bone atrophy) [5]. For the purpose of preventing the stress shielding effect, several studies have focused their efforts on the development of low-modulus biodegradable polymers in order to get prosthesis stiffness values similar to that of the host tissue [6–9], resulting in a desirable tissue remodeling.

Osteogenic biomaterials are an alternative to traditional bone repair techniques, leading to faster healing of the involved joint. Materials used for this purpose must be able to overcome important medical issues such as implant rejection, chronic inflammatory reaction, infections, corrosion and metal toxicity (commonly present in typical metallic prostheses) [8,10]. Natural and synthetic polymers have been broadly studied as fixed systems, bioabsorbable synthetic polymers being the most commonly used, e.g., Polycaprolactone (PCL), Polylactic acid (PLA), and Polyglycolic acid (PGA) [6,7,11,12].

Among them stands out PCL, for its better inflammatory response, slower resorption rate (required to maintain structural properties when used as bone fixation plates) and due to its hydrophobic character [13]. Nonetheless, the mechanical properties are lower compared to other biopolymers such as PLA (PCL flexural modulus is around 0.48–0.58 GPa, while PLA flexural modulus is around 3.1–3.6 GPa). For this reason, the use of PCL as a tissue engineering scaffold, sometimes, need to study possible modifications by adding fillers or blends [14–18]. Few studies have tested PCL composites as internal fixation systems, such as Lowry et al. [13] who prepared fixations of PCL reinforced with glass fiber for rabbit humerus. The results revealed a higher strength for PCL/bone complex compared with bony humerus healed with a stainless steel implant. As such, craniofacial repair fixations

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http://dx.doi.org/10.1016/j.msec.2017.02.087
0928-4931/

appear to be a plausible application for PCL, as it has also been studied by Rudd and co-workers for the last decade [19–23]. In other strategies, PCL scaffolds have been reinforced with Hydroxyapatite (HA) as one of the most common and inexpensive fillers broadly used [24,25] because it provides osteoconductive properties, causes no inflammatory response, and has very low toxicity in humans [26,27]. Reinforcement of PCL with different amounts of HA was studied by Biqiong Chen and Kang Sun [28], who reported an enhancement of mechanical properties, reaching the greatest strength value when the concentration of HA was 20%. Nevertheless, when the content of HA increased over 20%, the strength decreased significantly. Halloysite Nanotubes (HNTs) have also been used in PCL nanocomposites due to their biocompatibility, high mechanical strength, thermal stability and abundance in nature. PCL with polymeric matrices were reinforced with HNTs by Liu et al. [29] and Prashantha et al. [30], who tested the decrease of flexural properties when exceeding 7.5 wt% of HNTs, correlating the overloading of HNTs with the generation of agglomerates acting as weak points and failure initiation sites. Kang-Suk Lee et al. [31] also studied mechanical properties of PCL/HNTs nanocomposites. The results showed an improvement of tensile strength and modulus of the PCL matrices by the inclusion of HNTs, attributed to the good compatibility between HNTs and PCL. This is due to the formation of strong hydrogen bonding between the hydroxyl groups (—OH) present on the HNT surfaces and carbonyls (C═O) in the ester linkages of the PCL molecular chains [29]. All these features make HNTs ideal as polymer filler highlighting its nanotubular structure which allows the loading, storage, and controlled release of drugs to be used as a delivery system [32,33].

The introduction of curcumin in HNTs lumen appears promising, being it a commonly used food colorant which possesses excellent pharmacological features such as anti-oxidative [34], anti-inflammatory [35], anti-tumorigenic [36], in addition to numerous desirable biological benefits [37]. Curcumin is a yellow lipid-soluble natural pigment extracted from the powdered rhizome of the plant *Curcuma longa*, with the chemical formula [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepadiene-3,5-dione] [34,38]. Considerable attention has been paid to curcumin, being one of the most natural compounds studied so far with almost 3000 research articles published regarding its properties [39]. Curcumin has demonstrated to have very low activity in physiological environment owing to its poor solubility in water (<1 μg/mL); besides, it exhibits rapid degradation in alkali conditions (pH 7.4) at physiological temperature [40], which makes curcumin hard to absorb from the gastrointestinal tract after oral administration. Consequently, studies are continuously being developed to achieve appropriate drug delivery systems to enhance in-vivo curcumin stability. Some of these efforts have based on the use of polymer nanospheres of poly(DL-lactide [41,42] and (NIDAM-NVP-(PEG-A)) copolymers [43] as carriers, but also the encapsulation in different bio-microspheres using bovine serum albumin and chitosan [44] or lipid-based nanoparticles [45].

The purpose of this paper is two-fold. Firstly, an optimization of the amount of HA and HNTs loading has been carried out in different blends based on PCL. Previous studies on the reinforcing effect of PCL have been done using HA and HNTs separately, showing both a significant improvement of mechanical properties, but not combined simultaneously to take advantage of their individual suitability. The main objective is to achieve the adequate PCL strength increase and provide osteoconductive properties to be applied in craniofacial fixed systems. In the second part of the work, the functionalization of HNTs has been implemented through the addition of curcumin aiming to confer additional anti-inflammatory properties to the nanohybrids.

2. Materials and methods

2.1. Reagents and materials

Polycaprolactone (PCL) named commercially as CAPA 6500 was kindly provided by Solvay Interox UK. CAPA 6500 is a high molecular weight thermoplastic linear polyester derived from caprolactone monomer. Its melting point is between 58 and 60 °C and its molecular weight is 50,000 Da. Hydroxyapatite ($HCa_5O_{13}P_3$; HA) was supplied by Sigma Aldrich, Madrid, Spain. Halloysite nanotubes (HNTs), with molecular formula $Al_2Si_2O_5$ $(OH)_4$ 2 H_2O, were supplied by Sigma Aldrich, Madrid, Spain. Curcumin was supplied by Sigma Aldrich, Madrid, Spain.

2.2. Preparation of the PCL-based hybrids

In the first part of the study PCL-based hybrid samples were prepared, varying the mass fraction of HA and HNTs, and characterized to select the inorganic load that optimizes the mechanical properties of the hybrids. The HA and HNTs mass fractions were chosen based, on the one hand, on the work by Biqiong Chen and Kang Sun's [28], who reported an enhancement of mechanical properties when PCL was reinforced with different amounts of HA, the greatest strength value being reached when the concentration of HA was 20%, and noticing a significant strength decrease when the content of HA increased beyond 20%, and, on the other hand, on the works of Liu et al. [29] and Prashantha et al. [30], who observed an improvement in flexural properties of the polymer matrix loaded with HNTs not exceeding 7.5 wt%. In the latter, the authors correlating the overloading of HNTs with the generation of aggregates acting as weak points and failure initiation sites.

In this first stage, seven different types of hybrids were herein extruded and injection molded, with the mass percentage of HA and HNTs specified in Table 1. Pure PCL samples were used as control. Of these seven hybrids samples, the optimal was chosen in terms of its mechanical and thermal properties, with the aim of studying the influence of HNTs loaded with curcumin on the final properties of the PCL-based hybrids, being reflected in the second stage of the study. Different samples related with both stage of the study are specified in Table 1.

Firstly, the components of each blend formulation were dried separately in a vacuum oven (PCL at 45 °C for 24 h; HA and HNTs at 200 °C for 48 h). 600 g of each manufacturing mixture were next prepared following the percentages specified in Table 1, manually mixed in a zip bag and mechanically mixed in a twin screw corotating extruder at 40 rpm, mass dosing of 2.5 kg/h and a mass flow rate of 30 kg/h. The temperature profile for the four extruder zone of the barrels was 65 °C/75 °C/85 °C/90 °C. After this process a room temperature cooling during 5 min was needed before the pelletization of the mixture in a jaw mill at room temperature. The mill used was a jaw crusher BB 300 supplied by Retsch GmbH (Retsch Spain). Samples were injection molded in a Meteor 270/75 injection machine (Mateu and Solé, Barcelona, Spain). The temperature profile of the extruder was 80 °C/80 °C/85 °C/85 °C/90 °C, with a mold temperature of 50 °C and a cooling time of 6 s. The pressure value of the injection molding process was 800 pSi and the mold used was a steel one with mirror finishing with the dimensions recommended by the corresponding standards: strength samples type 1A had dimensions of 170 × 10 × 4 mm^3 (UNE-EN ISO 527), and flexural samples were 90 × 10 × 4 mm^3 (ISO 178).

Table 1
PCL/HA/HNTs mass percent composition for sample injection.

		Mass fraction (%)		
	Sample ID	PCL	HA	HNTs
1	PCL HA 20	80	20	0
2	PCL HA 20 HNTs 2,5	77.5	20	2.5
3	PCL HA 20 HNTs 5,0	75	20	5.0
4	PCL HA 20 HNTs 7,5	72.5	20	7.5
5	PCL HNTs 2,5	97.5	0	2.5
6	PCL HNTs 5,0	95	0	5.0
7	PCL HNTs 7,5	92.5	0	7.5
8	PCL	100	0	0
9	PCL HA20 HNTs7.5 + curcumin	72.5	20	7.5 loaded