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PCL/EGGSHELL SCAFFOLDS FOR BONE REGENERATION

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ABSTRACT

Eggshell (ES) is one of the most common biomaterials in nature. For instance, the ES represents 11% of the total weight of a hen's egg and it is composed of calcium carbonate, magnesium carbonate, tricalcium phosphate and organic matter. Hen ES are also a major waste product of the food industry worldwide. Recently, ES have been used for many applications such as coating pigments for inkjet printing paper, catalyst for biodiesel synthesis, bio-fillers for polymer composites and matrix lipase immobilization. It is also considered a natural biomaterial with high potential for the synthesis of calcium enriched implants that may be applied in tissue engineering applications, such as bone regeneration. The aim of this research regards the production of poly(ε-caprolactone) (PCL) scaffolds enriched with hen ES powder for bone regeneration applications, using an extrusion-based process called Dual-Bioextruder. The main objective is to investigate the influence of the addition of ES powder on the PCL matrix. For this purpose the structures were characterised regarding morphological and chemical properties. Morphological images of the PCL scaffolds enriched with hen ES, demonstrated the interconnectivity of the pores within the scaffold and revealed that the addition of the ES powder combined with the screw rotation velocity has a large influence on the resulting filament diameter and consequently on the porosity of the scaffolds.

1. INTRODUCTION

The evolution of science and knowledge, coupled with increased of average life expectancy and demand for better quality of life is leading to an increased demand for implants for bone dysfunction caused by diseases such as osteoporosis and cancer [1, 2]. Over the last few years, a range of biomaterials have been investigated to be applied in bone substitutions [3, 4]. From synthetic polymers such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) [5] to natural biopolymers, such as collagen [6] and hyaluronic acid [7]; and ceramics such as hydroxyapatite [8] and calcium phosphate [9, 10, 11] have been used in bone regeneration. In spite of the high number of many different possible biomaterials, composites made of different materials represent the optimal solution in meeting the restrictive requirements of bone regeneration by taking advantage of their specific properties [5, 12, 13, 14].

Nowadays, thermoplastic polymers are produced and consumed in massive quantities and play a major role in many aspects of our everyday lives [15]. PCL is one of the most interesting thermoplastic materials that has been reported as an excellent material to be used in bone regeneration due to its excellent mechanical properties, nontoxicity, biodegradability and biocompatibility [5, 16, 17, 18]. However, its properties can be improved with the combination of other material(s) [19]. Ding et al. [20] successfully produced PCL/HA composites for regeneration of goat femoral head and observed that the addition of different materials was an added value for the success of bone regeneration. In turn, Annabi et al. [21] developed PCL/elastin composites and demonstrated that the fabrication of synthetic/natural hybrid composites allows to reach a suitable balance of biological and mechanical properties.

Bone is the hardest tissue of the human body and is a hierarchically structured natural composite material. It mainly consists of a biopolymer (type I collagen), a mineral phase (carbonated hydroxyapatite) and water [14, 22, 23]. Recently, due to its characteristics, the eggshell (ES) has been considered a promising bone substitute material. ES contains many traces of elements such as Na, Mg and Sr, resembling to mineralized bone matrix [24, 25, 26]. The natural composition of eggshells consists in hydroxyapatite with a crystalline structure and composition similar to human bone, with considerable benefits to the overall physiological function posteriorly to implantation [27, 28]. This is one of the most common biomaterial in nature and is the main waste product of food industry [29, 30]. The ES represents 11% of the total weight of the egg and it is mainly composed by calcium carbonate (94%), magnesium carbonate (1%), tricalcium phosphate (1%), and organic matter (4%) [28, 31]. In recent years, ES has been used for many applications such as catalyst for biodiesel synthesis [32, 33], coating pigment for inkjet printing paper [29, 34, 35], bio-filler for polymer composites [36], matrix lipase immobilization [38] and bone substitutes [29].

To restore and/or regenerate damaged bones, transplantation (autograft and allograft) is a usual therapy. However, this activity involves a range of risks. Bone tissue engineering has been introduced as the alternative strategy to overcome such limitations [12, 14, 39].

Materials engineering and molecular biology principles have been applied in tissue engineering to create artificial constructs that enables the formation of biological substitutes to repair or replace organs or tissues failure. In a common approach, cells isolated from the patient are cultured in a biocompatible scaffold supplemented with growth factors to provide a temporary artificial cell development for regeneration of new tissue [40, 41]. The creation of more complex organs and tissues requires three dimensional scaffolds that offer high porosity to allow both cell penetration and mass transport [42, 43]. The scaffold provides structural and chemical cues that strongly influences cell activity and tissue growth [44, 45, 46]. Far from being a passive element, there is an acute need to improve scaffold compositions and geometries that modulate the temporal and spatial conditions for neotissue formation [40, 47]. Therefore, the major challenge in tissue engineering lies on the development of reproducible manufacturing methods and products for translation to the clinic [47]. Several processing techniques have been developed to fabricate biodegradable 3D scaffolds based on additive manufacturing methods including stereolithography, selective laser sintering, fused deposition modeling and inkjet printing, and these are considered as effective routes to fabricate custom-made scaffolds with a given anatomical contour for hard tissue regeneration [48, 49, 50]. In particular, extrusion-based processes have been widely used to fabricate 3D constructs with a high level of control over the architecture. In this technique, thin filaments are extruded from molten material that passes through a nozzle guided by a robotic device controlled by a computer, and deposited in a platform forming layers of strands to obtain the 3D object [51, 52, 53, 54].

In this study, PCL and PCL/ES scaffolds with different parameters were produced. The effects of varying processing conditions and material composition of extruded scaffolds on morphological and chemical features were evaluated and compared.

2. MATERIALS AND METHODS

2.1. Materials

The PCL pellets used in this research work (CAPA 6500) have a molecular weight of 50.000 and was obtained from Perstorp Caprolactones (Cheshire, United Kingdom).

All the eggs used in this research work were obtained at a local market.

A Glutaraldehyde solution grade II 25% was used to treat the ES powder and was purchased from Sigma (USA).

2.2. ES extraction and treatment

The extraction and treatment of the ES was performed in several stages. First the eggs were cleaned and immersed in boiling water during 30 minutes to remove impurities. Then the eggshells were broken and the albumen and yolk were discarded. The shell membranes were carefully removed and the ES were treated with glutaraldehyde. For this purpose the ES were washed with distilled water and immersed in 2.5% glutaraldehyde solution. After 20h, the ES were removed from the glutaraldehyde solution, washed with copious amounts of distilled water and dried for 1h at 50^{o}C. Then the ES was grinded into powders with a particle size below 63 µm.

2.3. Scaffolds production

PCL and PCL/ES scaffolds were produced using the Dual-Bioextruder system (Fig. 1), which was developed by the Centre for Rapid and Sustainable Product Development of the Polytechnic Institute of Leiria (Portugal). This system enables the fabrication of mono and multi-material scaffolds through a layer-by-layer manufacturing process.

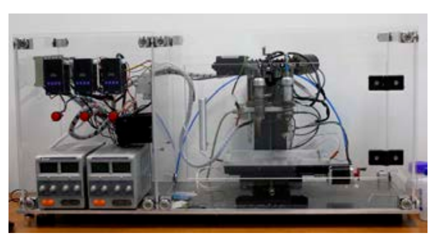


Fig. 1 Dual-Bioextruder System.

PCL/ES blends were prepared through the melt of PCL pellets at 100°C and addition of 20 wt% of ES powder.

All the scaffolds were produced by deposition of fibbers with a diameter (RW) of 300 µm of previously molten mixtures, using a 0º/90º pore configuration, filament distance (FD) of 650 µm, filament gap (FG) of 350 µm and slice thickness (ST) of 280 µm (Fig. 2). PCL and PCL/ES scaffolds with dimensions of 15 x 15 x 2.24 mm^3 were processed at 90ºC, using a deposition velocity (DV) of 400 mm/min and with three different screw rotation velocities (SRV) (22, 26 and 30 rpm).

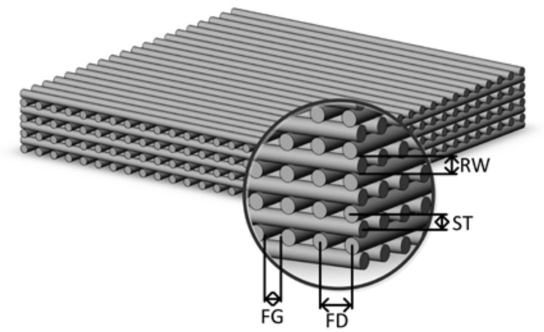


Fig. 2 Scaffold design parameters.

2.4. Morphological analysis

PCL and PCL/ES scaffolds were morphologically analysed to observe and determine the dimension of the filaments and pores of the produced scaffolds. These analyses was performed using a Stereomicroscope (from The Imaging Source, camera David Cam-1-F, Germany).

2.5. FTIR-ATR analysis

FTIR-ATR of ES powder, PCL pellet, PCL/ES blend and PCL and PCL/ES scaffolds were performed with an ATR Fourier transform infrared spectrometer (Alpha FT-IR spectrometer, Bruker, Belgium). The spectra was averaged over 64 scans at a resolution of 4 cm-1. All tests were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Scaffolds production

PCL and PCL/ES scaffolds were successfully produced and presented both good geometric accuracy and pore interconnectivity. The scaffolds production revealed that the SRV parameter has direct influence on the material's flow behaviour. Morphological analysis (Fig. 3) shows that an increase of SRV produces a higher RW and, consequently, a FG reduction in both materials. In Table 1 are summarized the obtained results for SRV, RW and FG values. The results demonstrated that PCL and PCL/ES presented an increment in the RW value from 276 ± 5 µm to 356 ± 10 µm and 317 ± 9 µm to 403 ± 7 µm, respectively, and consequently a decrease of FG value from 370 ± 10 µm to 288 ± 6 µm and 328 ± 7 µm to 280 ± 13 µm, respectively. This observed effect is a consequence of the increase of the amount of extruded material.

Results also demonstrated that PCL and PCL/ES materials had different flow behaviours, because for the same parameters, they presented different results (Fig. 4).

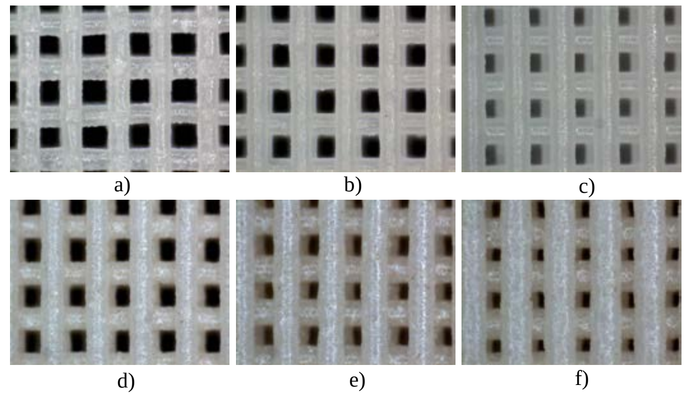


Fig. 3 Stereoscopic images of scaffolds with different SRV values (magnification: 100x): a) PCL 22 rpm, b) PCL 26 rpm, c) PCL 30 rpm, d) PCL/ES 22 rpm, e) PCL/ES 26 rpm and f) PCL/ES 30 rpm.

Table 1 Scaffolds morphological values

Scaffold material	SRV (rpm)	RW (µm)	FG (µm)
PCL	22	276 ± 5	370 ± 10
	26	301 ± 7	341 ± 7
	30	356 ± 10	288 ± 6
PCL/ES	22	317 ± 9	328 ± 7
	26	363 ± 5	303 ± 9
	30	403 ± 7	280 ± 13

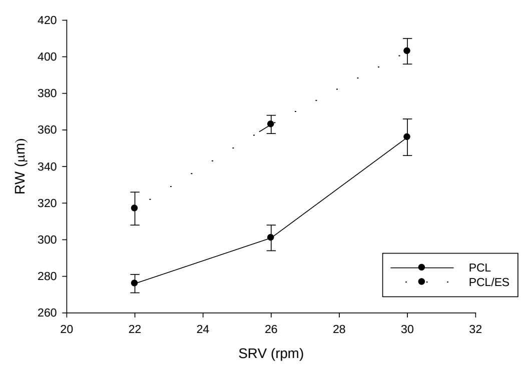


Fig. 4 PCL and PCL/ES RW behaviour with SRV variation.

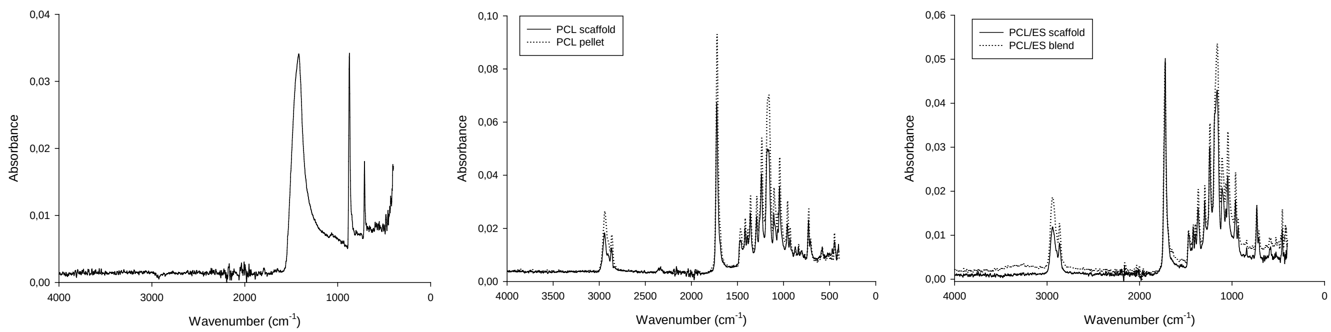
3.2. FTIR-ATR analysis

In order to evaluate the interaction between PCL and ES and to identify any changes in the chemical structure that could have occurred during the materials processing, FTIR-ATR assays were performed. For this purpose were executed analysis for ES powder (Fig. 5 a), PCL pellet and PCL scaffold (Fig. 5 b) and PCL/ES blend and PCL/ES scaffold (Fig. 5 c). The spectrum of ES powder reveals the characteristic peaks of calcium carbonate at 1417 cm-1, 875 cm-1 and 712 cm-1 [28, 38 and 58]. The peak at 1417 cm-1 is strongly associated with the presence of carbonate minerals within the eggshell matrix. The other peaks (875 cm-1 and 712 cm-1) are associated with the out-plane and in-plane deformation modes, respectively, in the presence of calcium carbonate [25, 58].

The PCL pellet spectrum (Fig. 5 b) reveals important peaks at 2943 cm-1 and 2867 cm-1 that can be attributed to the asymmetric and symmetric CH2 stretching, respectively [55, 56 and 57]. The strong band at 1725 cm-1 was assigned to the carbonyl (C=O) stretching mode and the peak at 1293 cm-1 corresponds to the stretching of C–O and C–C in the crystalline phase. The spectrum also showed one peak at 1240 cm-1 that corresponds to the asymmetric COC stretching vibrations and other peak at 1163 cm-1 that can be attributed to symmetric COC stretching [16, 55, 56, 57].

The PCL/ES blend spectrum (Fig. 5 c) is similar to the pristine material FTIR spectrum, displaying typical adsorption bands of PCL. Due to the high PCL ratio and to the coincident characteristic peaks of the calcium carbonate, the PCL peaks remain overlapped with the ES peaks.

The PCL and PCL/ES scaffolds spectra indicates that the extrusion process has no effect on the functional groups present in the resulting structure.



a) b) c)

Fig. 5 FTIR-ATR spectra of: a) ES powder, b) PCL pellet and PCL scaffold and c) PCL/ES blend and PCL/ES scaffold.

4. CONCLUSIONS

This research work focused on the fabrication of poly(ε-caprolactone) scaffolds incorporating ES powder. Materials such as ES are considered good candidates for use as a filler for preparing polymer composites because it is a promising biomaterial to use in bone tissue regeneration and has high abundance and low cost.

In this paper, PCL and PCL/ES scaffolds with interconnected pores and good geometric precision were successfully produced using the Dual-Bioextruder system. The obtained results showed that the screw rotation velocity has a large influence in terms of filament diameter and consequently in terms of scaffold porosity. We also concluded that the PCL and PCL/ES materials have different flow behaviours and that the fabrication process does not chemically modify the extruded materials.

ACKNOWLEDGMENTS

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