TOUGHENING 3D-PRINTED Sr–HT–GAHNITE SCAFFOLD THROUGH NATURAL AND SYNTHETIC POLYMER COATING

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ABSTRACT

Bone scaffold for aiding bone regeneration in large bone defects should have following ideal characteristics; biocompatibility, biodegradability, bio-activity, high porous and interconnected-pore architecture, as well as, mechanical characteristics similar to the cortical bone for supporting loads. 3D printed Sr–HT (Sr–$Ca_2ZnSi_2O_7$)–gahnite scaffold with hexagonal pore structure is an interesting bone scaffold meeting most of these ideal features. To explain, biocompatible, osteoinductive, and osteoconductive properties as well as unique high compressive strength are obtained from Sr–HT–gahnite, glass-ceramic, material. With hexagonal pore structure, the scaffold has compressive strength comparable to cortical bone balancing with high porosity and large pore size. Nonetheless, the scaffold had a limited feature on the flexural strength. Therefore, in this study the printed glass-ceramic scaffold will be coated with polycaprolactone (PCL) and chitosan with the purpose of improving its toughness. The study reported coating the prepared ceramic scaffold with PCL and chitosan enhanced its toughness seen from an increase in its flexural strength from 12 ± 3 to 19 ± 2 and to 32 ± 5, respectively.

Keywords: bone scaffold, glass-ceramic scaffold, 3D printing, polymer coating.

1. INTRODUCTION

Scaffolds utilized to aid and direct bone regeneration by design are not intended to be permanent implants but to ideally facilitate host cells to deposit extracellular matrix (ECM) and to replace the scaffold structure over time. To allow this perfect healing process, scaffolds should has following ideal characteristics; biocompatibility, biodegradability, bioactivity, high porous and interconnected-pore architecture, as well as, mechanical characteristic similar to the host bone [1]. Particularly, scaffolds had better have mechanical features comparable to corti-cal bone for supporting loads balancing with porosity of 60-90% and average pore size of >300 μm for enhancing bone formation [1, 2]. To control certain porosity and pore size of scaffolds, 3D printing techniques are suitable as scaffold fabrication method [1].

3D printed Sr–HT (Sr–$Ca_2ZnSi_2O_7$)–gahnite scaffold with hexagonal pore structure is an interesting scaffold with ideal features. To explain, Sr–HT gahnite, glass-ceramic, used as scaffold material provides the scaffold to have biocompatible, osteoinductive, and osteoconductive properties as well as unique high compressive strength [3]. Designed to have hexagonal pore structure, the scaffold was reported to have compressive strength comparable to cortical bone balancing with porosity of 60% and average pore size of 900 μm [4]. Nonetheless, the scaf-fold still had reported a limited feature on the flexural strength which was approximately more three times less than the compressive strength limiting their use for applications in load-bearing sites [2, 4].

Coating ceramic scaffolds with biocompatible polymer, particularly poly-caprolactone (PCL) as well as chitosan, is a simple and effective approach to improve their toughness [5] through crack bridging behaviour—after crack initiation occurs, the polymer fibres will expand as bridging the crack which impedes further crack propagation [6].

In this study, Sr–HT–Gahnite scaffold with hexagonal pore geometry fabricated by 3D-bioprinting technique was dip-coated with PCL and chitosan to enhance its toughness making it preferable in regenerating large bone defects under loads.

2. MATERIALS AND METHODS

2.1 Material and Ink Preparation

Sr–$Ca_2ZnSi_2O_7$ or Sr–HT powder was prepared by sol-gel method as previously described [3, 4]. Tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$), calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) and strontium nitrate ($Sr(NO_3)_2$) were used as raw materials. TEOS material was purchased from TCI, Japan while others was purchased from Himedia, India. The TEOS was mixed with water and 2 M HNO_3 (mol ratio: TEOS/H_2O/HNO_3 = 1:8:0.16) and hydrolyzed for 30 min under stirring. Then, the $Zn(NO_3)_2.6H_2O$, $Ca(NO_3)_2.4H_2O$ and $Sr(NO_3)_2$ (5 wt%) solution was added into the mixture (mol ratio: TEOS/$Zn(NO_3)_2.6H_2O$/$Ca(NO_3)_2.4H_2O$ = 2:1:2), and reactants were stirred for 5 h at room temperature. After

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the reaction, the solution was maintained at 60 °C for 1 day and dried at 120 °C for 2 days to obtain the dry gel.

The prepared Sr–HT was combined with 15 wt% of aluminium oxide (Al_2O_3, Himedia) which was then dry-grinded and wet-grinded with a high energy ball mill machine (Emax, Germany). For dry-grinding, 15 g sample with 8 zirconia balls (diameter: 12 mm) was added into zirconia jars and grinded for 15 min at 800 rpm. For wet-grinding, the dry-grinded sample with 110 g zirconia balls (diameter: 1 mm) and 15 mL ethanol was grinded at 800 rpm. Wet grinding time is varied for 15, 30, and 60 min in order to obtain particles in the 0.5–2 μm size range. The size of obtained particles after grinding was calculated by a laser diffraction particle size analyzer (Mastersizer 3000, MALVERN, UK).

Sr–HT 3D-ink was composed of 45 vol. % of powder in aqueous solution, Then, 1 wt. % of dispersant (sodium polyacrylate, MW~ 5000, Sigma Aldrich, USA), 1 wt. % of viscosifying agent (5 wt. % hydroxypropyl methylcellulose solution, 2,600-5,600 cP, Acros Organics, USA), and 0.2 g flocculant (10 wt% polyethyleminine solution, branched PEI, MW~25,000, Sigma-Aldrich) were dissolved into the powder solution, respectively. Sonication was performed for 3 min after each addition. The viscosity of water-based organic ink was tuned by adjusting its pH with 5M HNO_3 or 5M NH_4OH as needed before passed through a test sieve with the pore size 53-μm of in order to prevent clogging of the ceramic ink during printing.

2.2 Direct ink writing of scaffolds

The scaffolds were fabricated using 3D-Bioplotter device (ENVISIONTEC, USA). The prepared ink was dispensed through 410-μm conical nozzle on an oil coated film sheet. Detailed setting parameters for printing process and the scaffold pattern can be seen in Appendix A. The fabricated scaffolds were left at room temperature overnight making the scaffold detach from the sheet. Then, the dried samples were heated at the rate of 2 °C/min to 450 °C which was hold for 1 h to remove organic components from the sample. After that, the samples were heated at the rate of 2 °C/min to 1250 °C which was hold for 3 h to sinter the struts.

2.3 Optimization of Polymer Concentration for Dip Coating

Dip coating of the prepared scaffold in PCL and chitosan solutions was adapted from the previous study [5, 7]. For preparation of PCL solutions, PCL beads (average Mn 45,000, Sigma, USA) were dissolved in toluene at different concentrations: 20% and 25% (w/v). For preparation of chitosan solutions, chitosan (TCI, Japan) was added into an aqueous solution with a 2 vol% acetic acid at different concentrations: 20% and 25% (w/v). Both solutions were stirred at room temperature for 24 h.

The dip coating of the printed scaffolds were performed by immersing the scaffold into the synthetic and natural polymer solutions with different concentrations at 60 °C for 30 min. Then, the coated scaffolds were incubated at 37 °C for 2 days in order to evaporate the solvent.

2.4 Scaffold Characterization

2.4.1 Physicochemical properties of the scaffolds

A tabletop scanning electron microscope (TM-1000, Hitachi, Japan) was used to observe the microstructure and to determine pore size of the prepared scaffold. The pore size of the Sr–HT–gahnite scaffold is determined from mean pore diameter of prepared scaffolds.

The porosity of the prepared scaffolds was determined using micro-computed tomography (Skyscan 1173, Bruker, Belgium). The reported porosity and are average values derived from this method.

Chemical composition of the glass-ceramic scaffolds and the coating polymers was analyzed by X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR), respectively.

2.4.2 Physicochemical properties of the scaffolds

The compressive strength of the scaffolds was measured according to ASTM C165 with using a universal testing machine at a constant crosshead speed. Three scaffolds (n=3) with a dimension of 7 mm × 8.4 mm × 6.4 mm are used for analysis.

The flexural strength of the scaffolds was performed according to ASTM D790 with using a universal testing machine at a constant crosshead speed. Three scaffolds (n=3) with dimension of 21 mm × 5 mm × 3 mm are used for analysis.

3. RESULTS AND DISCUSSION

3.1 Effects of ball milling time on the particle size

The final particle size required for this study is <10 μm, thus wet grinding with small grinding balls of 0.1 - 3 mm had to be performed. To explain, dry grinding allow small particles to attract to each other by their electrostatic charges due to their significantly enlarged surface in relation to their volume. Therefore, adding dispersants such as alcohol in grinding, called wet/colloid grinding, is needed to neutralize the charges on the particle surfaces. The wet grinding is varied herein for 15, 30, and 60 minutes in order to obtain ceramic particle size as possibly same as the previous research [4] whose ink formulation was followed in order to avoid any effect on the colloid stabilization and the optimum solid loading for 3D-printing [8]. As can be seen in Figure 1, the final particle sizes of the obtained particles after grinding for 30 and 60 min had smaller than for 15 min and were nearer to the desirable one. Thus, 30 min and 60 min ball milling time are preferable. D_{10}, D_{50} and D_{90} values of the obtained particles after grinding for 30 min and 60 min

show not considerable difference, therefore, 30 min grinding time is chosen for this experiment.

Grinding Time (minute)	D_{10} (µm)	D_{50} (µm)	D_{90} (µm)
15	0.66	2.5	9.5
30	0.61	2	7.1
60	0.59	1.8	6.9
Desired Size	0.52	1.0	2.0

Figure 1. Particle final sizes after wet-grinding

3.2 Morphology and microstructure of the ceramic scaffolds coated with different polymer concentration

By dip-coating method 25% w/v solution of PCL caused the polymer to partially fill the scaffold pre-designed macropores. On the other hand, 20% w/v solution of PCL can preserve the macroporosity of the scaffold. For chitosan coating, 5% (w/v) solution of chitosan make the scaffold macropores partly clogged. Meanwhile, 3.75% (w/v) solution of chitosan still preserve the internal pore structure (Figure 2). Partially filling the scaffold macropores decreases the scaffold interconnectivity—an important feature contributing to bone ingrowth process by allowing inwards diffusion of oxygen and nutrients and outwards diffusion of waste products from the scaffold [1, 9]. Therefore, 20% w/v solution of PCL and 3.75% (w/v) solution of chitosan were optimal for this study.

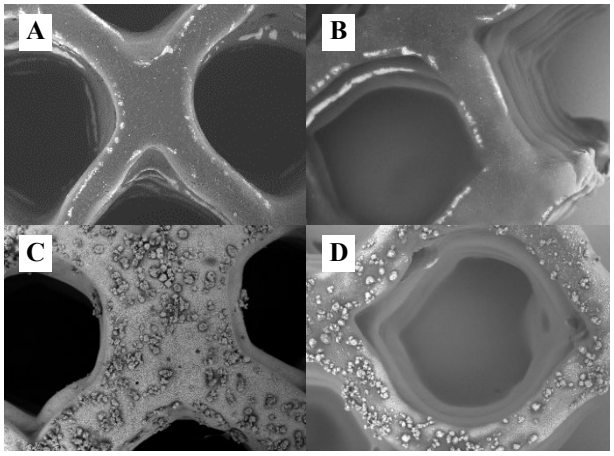

Figure 2. SEM image of the PCL-coated scaffolds prepared with 20% (w/v) (A) and 25% (w/v) (B) solutions of PCL and of the chitosan-coated scaffolds prepared with 3.75% (w/v) (C) and 5% (w/v) (D) solutions of chitosan.

3.3 Morphology and microstructure of the scaffolds

Figure 3 shows the microporous network and the strut microstructure of Sr–HT–gahnite scaffolds before [(A) to (B)] and after coating with PCL [(C) to (D)] and chitosan [(E) to (F)] at magnification of 50X and 5000X, respectively. The Sr–HT–gahnite scaffolds (A) showed interconnected hexagonal pores with average pore size of about 1420 µm. The pore size is suitable to be used in bone tissue engineering requiring a pore size between 20 and 1500 µm. Moreover, the pore size was >300 µm which may assist in vascularization and enhance bone formation leading to significant bone growth [8]. The average porosity of the prepared scaffolds de-termined from their pore and solid volumes obtained from micro-CT was 28.30 ± 0.24 % which is still too low for desirable bone scaffold requiring porosity should between 60% and 90% [1]. However, the porosity could increase by using smaller tip size to reduce the solid volume of the scaffold.

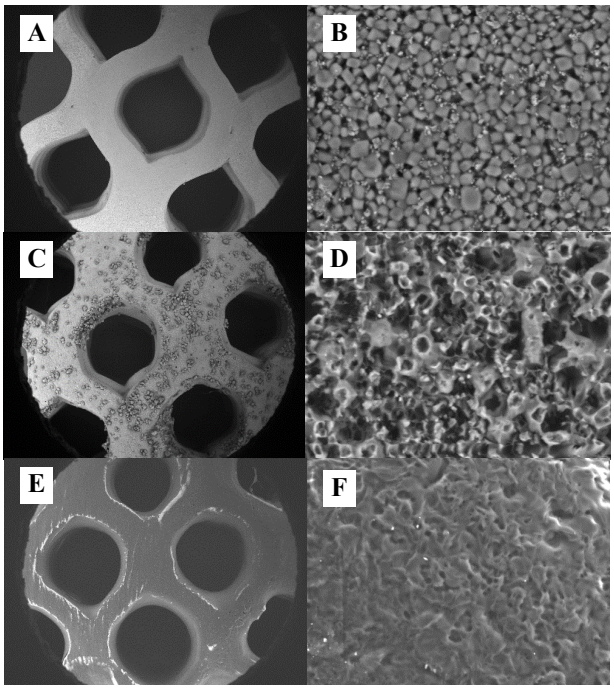

Figure 3. SEM image of Sr–HT–gahnite, PCL coated, and chitosan coated scaffolds.

3.4 Physicochemical properties of the scaffolds

The bare Sr–HT–gahnite ceramic or uncoated scaffold is composed of two crystalline phases: Sr–$Ca_2ZnSi_2O_7$ (Sr–HT) and gahnite ($ZnAl_2O_4$). These phase which are confirmed by X-ray diffraction (XRD) pattern as shown in Figure 4. The reflections of Sr–HT were detected at 21.09° (101), 24.06° (111), 25.70° (210), 29.13° (201), 31.38° (211), 32.70° (220), 35.72° (002), 36.69° (310), 37.62° (102), 39.20° (112), 44.49° (212), 50.40° (401), 52.04° (312) 61.32° (213), and 63.87° (110) [3]. The gahnite ($ZnAl_2O_4$) phase shows the peaks at 31.38° (220), 36.95° (311), 44.78° (400), 49.14° (331), 59.44° (511), and 65.24° (440) [10].

Figure 5 demonstrates the FT-IR spectral analysis of Sr–HT–gahnite, PCL-coated, and chitosan-coated scaffold. The characteristic absorption bands of PCL coated on the Sr–HT–gahnite scaffold include at 2864 cm^{-1} and 2942 cm^{-1} related to symmetric and asymmetric

C–H stretching of the CH_2 group of PCL. The band at 1720 cm^{-1} ascribed stretching vibration to carbonyl (C=O) groups. The C-O-C symmetric and asymmetric stretching belong to the bands at 1239 cm^{-1} and 1162 cm^{-1}, respectively. Moreover, the band at 1193 cm^{-1} are ascribed to O-C-O stretching. Lastly, C-O and C-C stretching in crystalline phase of PCL belongs to the band at 1293 cm^{-1} [11]. The typical spectral of chitosan coated on the ceramic scaffold show board peaks at 3290 cm^{-1} and 3356 cm^{-1} related to O-H and N-H stretching, respectively. N-H bending of amide II relates to the peak at 1563 cm^{-1} whereas C-O stretching of amide I belongs to the peak at 1643 cm^{-1}. The deformation vibrations of CH_3 and CH_2 groups relate to the peaks at 1377 cm^{-1} and 1410 cm^{-1}, respectively. The peak at 1151 cm^{-1} corresponds to C-O-C symmetric stretching meanwhile the band at 1026 cm^{-1} related to C-O stretching. The characteristic peak of the ring of monoscaccharides also appear at 895 cm^{-1} belonging to C-H out-of-plane vibration [12].

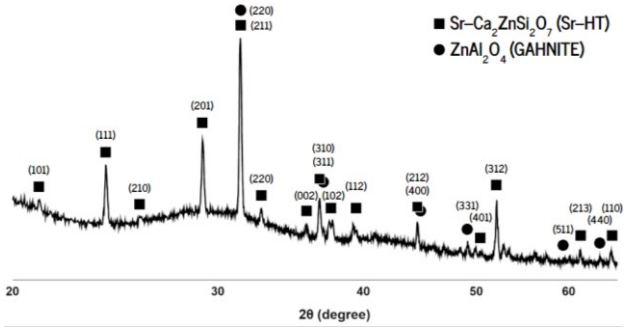

Figure 4. X-ray diffraction patterns and FTIR spectra of Sr–HT–gahnite scaffold.

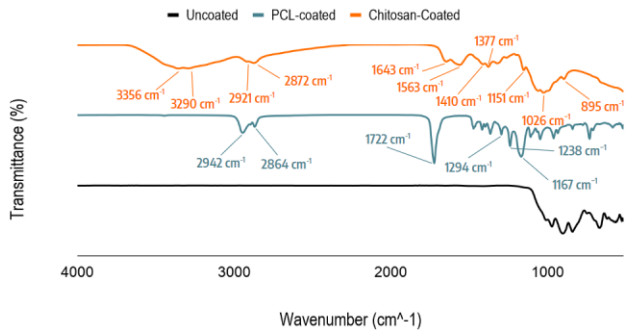

Figure 5. FTIR spectra of Sr–HT–gahnite, PCL coated, and chitosan coated scaffolds.

3.5 Mechanical properties of the scaffolds

The compressive strength of the Sr–HT–gahnite with 28.3 % porosity is 335.3 MPa showing the unique high compressive strength of the prepared Sr–HT–gahnite like one of the previous research [3]. The compressive strength of the scaffold had greater than one of the cortical bone which is 100-230 MPa. By the way, the compressive strength could be reduced by increasing the porosity of the scaffold.

The flexural strength of the Sr–HT–gahnite or uncoated scaffold is 12 ± 3 MPa. The flexural strength of the Sr–HT–gahnite scaffold was increased from 12 ± 3 to 19 ± 2 and to 32 ± 5 after coating with PCL and chitosan, respectively. The result showed coating the ceramic scaffold with PCL and chitosan enhanced its toughness.

4. CONCLUSION

Wet grinding with fixed condition was performed for 30 min to obtain the most desirable size of the prepared ceramic scaffold. For polymer dip-coating process, 20% w/v solution of PCL and 3.75% (w/v) solution of chitosan were preferred due to preserving the internal pore structure of the prepared scaffold. With 28.3% porosity and hexagonal pore size of 1420 µm, the Sr–HT–gahnite or uncoated scaffold had the compressive strength of 335.3. Coating the ceramic scaffold with biopolymers was reported to enhance toughness of the prepared scaffold by increasing the flexural strength of the Sr-HT granite scaffold from 12 ± 3 to 19 ± 2 and to 32 ± 5 after coating with PCL and chitosan, respectively.

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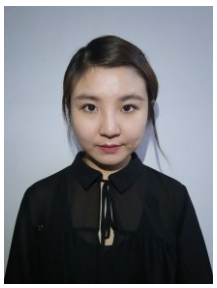

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