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MATERIALS ENGINEERING

Bioceramic scaffolds by additive manufacturing for controlled delivery of the antibiotic vancomycin

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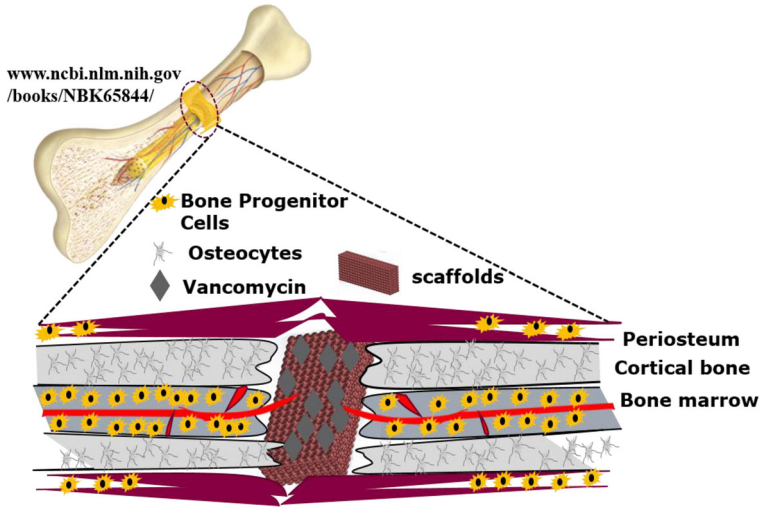
Abstract. Silicon–calcium silicate scaffolds were fabricated by selective laser melting (SLM). Rectangular composite scaffolds with a pore size of 400 µm were designed with dimensions of 10 × 20 × 5 mm^3. For imparting controlled drug release capability, scaffolds were covered with polycaprolactone (PCL) coatings for the sustained delivery of vancomycin. The drug release profile of the coated scaffolds was studied by UV–visible spectroscopy. The encapsulated drug within the PCL coated scaffold exhibited a controlled release of vancomycin. Nearly 50% of the vancomycin release from the scaffolds was observed during the first 40 h followed by the sustained release of vancomycin of nearly 20% of the actual loaded drug for the next six days. These findings suggest that SLM synthesized scaffolds with PCL coating can expand their applicability to be used as a target for *Staphylococci aureus* bacteria, which often cause chronic infections such as chronic osteomyelitis in bone.

Key words: selective laser melting, 3D printing, bioceramic scaffolds, drug delivery.

Graphical abstract



Si-$CaSiO_3$ scaffolds for drug delivery and bone repair

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1. INTRODUCTION

Vancomycin is a peptide drug needed for the treatment of serious, life-threatening infections by Gram-positive bacteria, particularly for the treatment of chronic osteomyelitis [1]. 'Small colony variants', a subpopulation of *Staphylococci aureus*, have been described as emerging pathogens and as another mechanism by which *S. aureus* can evade the immune response and antimicrobial therapy [2]. These bacteria often cause antibiotic-refractory recurrent and chronic infections such as chronic osteomyelitis [3].

Various studies have suggested that the local application of antimicrobials clearly provides higher local antibiotic concentrations than those achieved with intravenous application [4,5]. The most frequently studied material is polymethylmethacrylate (PMMA) serving as a carrier for the local delivery of antibiotics [6]. However, PMMA beads are associated with some drawbacks including an antibiotic delivery, as the carrier material is not degradable, and induction of foreign body reaction of the immune system [7]. The emerging 3D printing technologies, 3D scaffolds, are nowadays used as carriers for a better and controllable local delivery of antibiotics.

The composite scaffolds manufactured by 3D printing are used for drug loading to study the sustained release properties for bone tissue engineering [8]. Du et al. [9] recently printed macro/meso-porous composite scaffolds, which are loaded with high dosages of isoniazid/rifampin against anti-osteoarticular tuberculosis. These scaffolds show a greatly prolonged drug release time as compared to the commercial calcium phosphate scaffolds both in vitro and in vivo, with the combined merits of osseous regeneration and local multi-drug therapy. In the study by Zhang et al. [10] magnetic Fe_3O_4 nanoparticles incorporated onto mesoporous bioactive glass/polycaprolactone (PCL) composite scaffolds were fabricated by 3D printing. Incorporation of magnetic Fe_3O_4 nanoparticles into bioactive glass/PCL scaffolds not only influenced the apatite mineralization (bioactivity) ability, but also resulted in an excellent magnetic heating ability and significantly stimulated cell proliferation and differentiation. Moreover, when doxorubicin was used as a model anticancer drug, Fe_3O_4/bioactive glass/PCL scaffolds exhibited a sustained drug release for use in local drug delivery therapy. Zhou et al. [11] tested and designed a novel composite scaffold with antibacterial efficacy for treating bone infections using a 3D printed poly(ε-caprolactone) (PCL) scaffold coated with polydopamine (PDA) for the adsorption of polylactic acid–glycolic acid (PLGA) microspheres loaded with vancomycin.

The innovative concept of surgical implantation of high osteogenic scaffolds with tailored hierarchical meso-macroporosity followed by local drug delivery could provide a new synergistic strategy for treating bone repair and infection. Herein, we synthesized novel Si–$CaSiO_3$ composite scaffolds with the single-step technology and without addition of binders by selective laser melting (SLM) for local delivery of vancomycin. The high surface area, large pore volume, and mesoporous structure of Si–$CaSiO_3$ scaffolds printed by SLM is beneficial for the enhancement of local delivery of vancomycin. At the same time, the 3D printing technique allows controlling the level of porosity of the as-synthesized scaffolds. The aim of the study was to use bioceramic scaffolds synthesized by 3D printing and to investigate the local sustained delivery of vancomycin for treating chronic osteomyelitis infection.

2. EXPERIMENTAL

2.1. Synthesis of the bioceramic scaffolds

The starting powder materials were >99.9% purity wollastonite ($CaSiO_3$) (Aldrich) with a particle size of 1–5 μm and >99.5% purity silicon (Silgrain-Elkem) with a mean particle size ranging from 10 to 44 μm. The powders were mixed in 50/50 wt% in ethanol with 50 rpm for 3 h, and later the powder mixture was kept for drying in a furnace.

The melting of the mixed powder was performed by the SLM technique using a Metal 3D printer (ReaLizer GmbH SLM-50, Germany) equipped with a YAG: Nd^{3+} laser with a maximum output power of 120 W, laser spot size of 15–80 μm, and computer-controlled laser beam scan velocity of up to 1000 mm/s. The process was performed in a chamber sealed with high-purity argon (99.999 vol%) to avoid oxidation and degradation of the powder.

2.2. Procedures of loading vancomycin onto PCL-coated bioceramic scaffolds

The coating of the as-fabricated bioceramic scaffolds (Si–$CaSiO_3$) was prepared by dissolving 7.5 wt% of polycaprolactone (PCL) solution in dichloromethane (DMC), both from Sigma Aldrich, for 10 min at 37 °C using a magnetic stirrer. Vancomycin hydrochloride (Fig. 1) with a concentration of 19.2 mg/mL was added to the coating solution and stirred additionally for 5 min. Then, the as-fabricated scaffolds with a weight of 1 gm were dipped into the coating solution for 10 min in vacuum. Under vacuum the coating solution containing

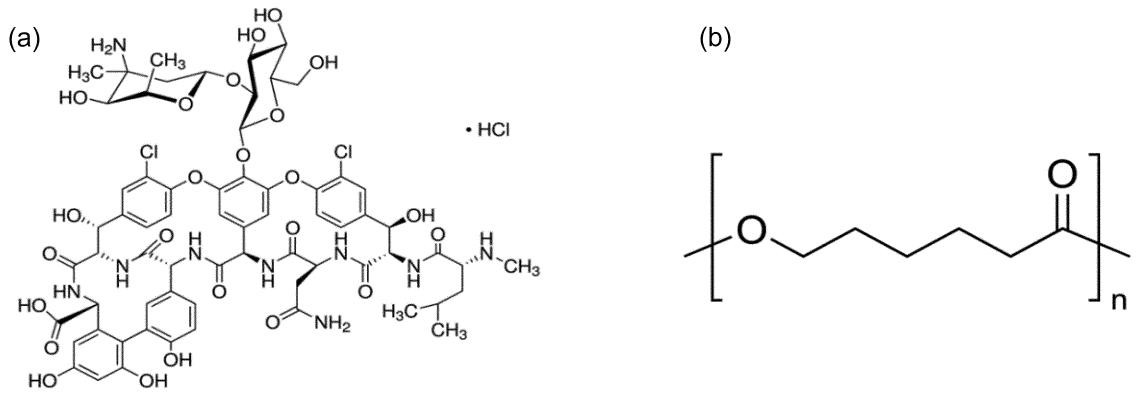

Fig. 1. Vancomycin hydrochloride molecule (a) and PCL molecule (b).

vancomycin promotes the penetration of the vancomycin inside the porous network of the bioceramic scaffolds. In fact, under normal pressure, the surface tension of the liquid prevents the infiltration of vancomysin into the pores of the scaffold. Allowing displacement of the air within the pores of the scaffolds with vancomycin solution by lowering the pressure can enhance the amount of vancomycin adsorbed and/or the rate of its penetration.

The scaffolds were placed in 60 mL vials containing simulating body fluid (SBF) into a shaking incubator at a constant speed of 240 rpm at 37 °C for some days. The vancomycin release measurements were carried out by using UV/vis spectroscopy at a wavelength of 280 nm with the correlation coefficient of $R^2 = 0.9999$ for the calibration curve between the absorbance and vancomycin concentration.

3. RESULTS AND DISCUSSION

3.1. Synthesis of the bioceramic scaffolds by selective laser melting

Figure 2(a) illustrates the as-synthesized bioceramic scaffold (Si–$CaSiO_3$) printed by SLM, and Fig. 2(b) depicts the microstructure of the scaffolds. Both of the figures show that scaffolds can be fabricated by layer-by-layer melting despite the fact that the ceramics have a low absorption of laser beam energy and poor thermal shock resistance [12].

The macroporosity (pore size 400 ± 20 μm) achieved was in accordance with the design of the model. Additionally, mesoporosity (pore size 15–50 μm), indicated by a blue circle on the image presented in Fig. 2(b), is observed and can be attributed to the interconnectivity of the macropores for the infiltration of SBF.

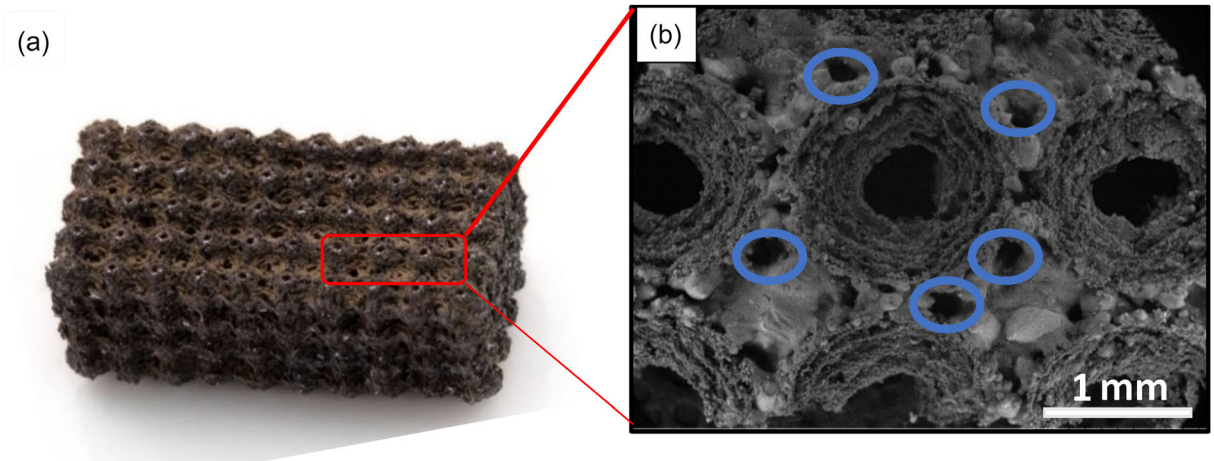

Fig. 2. As-synthesized Si–$CaSiO_3$ scaffolds printed by selective laser melting (a) and the microstructure of the scaffolds with macropores (~400 μm) and mesopores (blue circles, pore size ~15–50 μm) (b).

The capability of 3D printing techniques of producing scaffolds with structural anisotropy is well documented [13]. Macroporosity is required for large drug loading capacity, osteoid growth, which facilitates the proliferation of cells, vascular growth, and internal mineralized bone formation (osseous regeneration) [14]. The mesoporosity obtained can serve for cell attachment and for the infiltration of the SBF [15]. Hence, the fabricated bioceramic scaffold with hierarchical porosity (designed macroporosity with mesoporosity around the pores) can mimic the structure of the native tissues with concurrent drug delivering ability and simultaneously mimic the biological requirements.

3.2. PCL coatings onto bioceramic scaffolds

The SEM images of the bioceramic scaffolds with PCL coating are represented in Fig. 3. The porosity of the

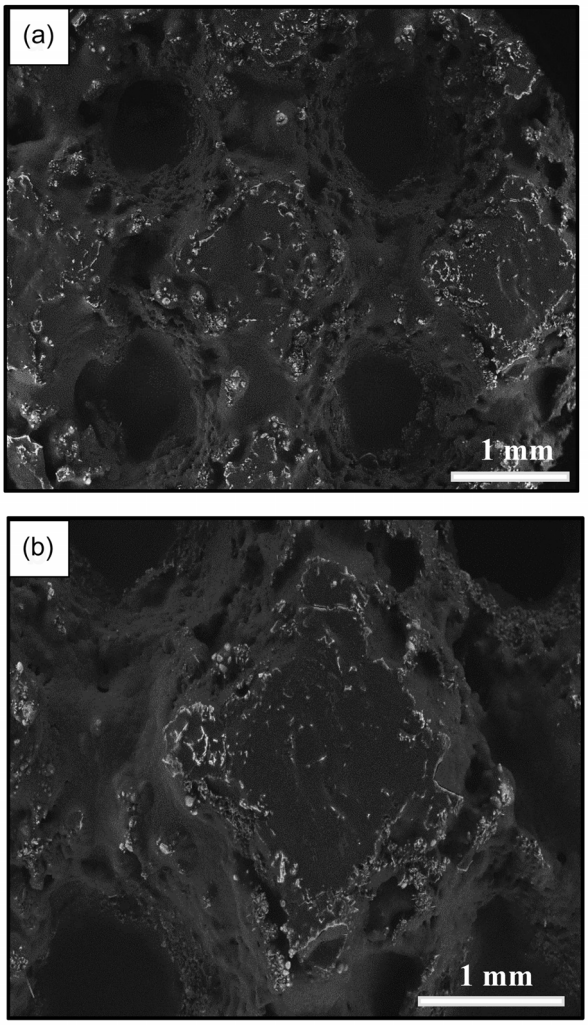

Fig. 3. PCL-coated bioceramic scaffold: (a) the whole scaffold after PCL addition and (b) the interconnective stem.

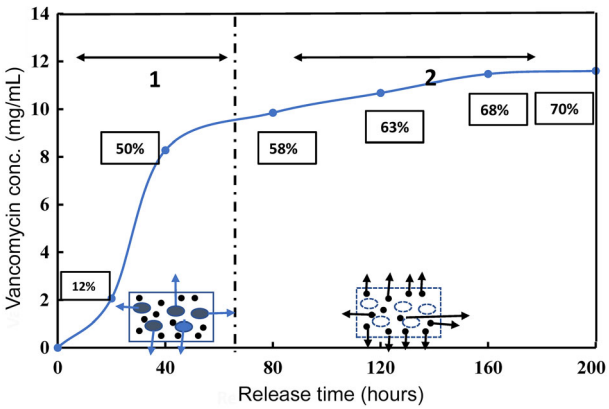

Fig. 4. Vancomycin release profiles from bioceramic scaffolds as a function of release time of vancomycin immersed in simulating body fluids SBF indicating typical drug release kinetics: 1 – fast/burst release, 2 – slow release of vancomycin.

Si–$CaSiO_3$ scaffolds measured by mercury intrusion porosimetry was approximately 35%. When PCL was coated onto the scaffolds, the morphology of the scaffolds changed slightly. The interconnective stem connecting the pores became thicker (Fig. 3b) and some mesopores were partially closed (Fig. 3a). As they possess good bioresorbability and biocompatibility and show a shielding behaviour for the controlled drug release applications, PCL coatings are widely used in bone tissue engineering [16]. The incorporation of PCL coatings onto bioceramic has yielded a class of biomaterials with remarkably improved mechanical properties, controllable degradation rates, and enhanced bioactivity, which makes these materials suitable for bone tissue engineering. Henceforth, scaffolds are coated with PCL to achieve controlled vancomycin delivery as illustrated in Fig. 4. The morphological features of the coating layer are similar to the reported scaffolds with exactly similar pore size [17].

3.3. Drug release profiles of scaffolds

The fabricated bioceramic scaffolds with porosity may have noteworthy applications for the release of vancomycin. The results demonstrated that the vancomycin loading efficiency ($m_{\text{loaded drug}}/m_{\text{carrier}}$, mg/g) reached up to 16.73 ± 0.82 mg/g. Figure 4 shows the release profiles of vancomycin drug from the scaffolds in SBF at 37 °C. It is clear that vancomycin loaded onto the scaffolds had an obvious two-step release behaviour with an initial fast release and a subsequent relatively slow release stage. The scaffolds exhibited sustained release behaviour throughout the whole study period. More than 50% of the cumulative loaded vancomycin was released from the scaffolds during the first 40 h, followed by a sustained release of nearly 20% of the

vancomycin during the next six days. The subsequent release rate significantly decreased with time, but the cumulative release kept slowly increasing during the next six days, reaching the maximum release of 70% of the loaded vancomycin. The fast drug release can be attributed to the macropores (open porosity) of the scaffolds [18,19]. On the contrary, a slow and sustained drug release can be ascribed to the microporosity of the scaffolds and the release of the vancomycin molecule on the external macropore walls bound by the PCL molecule. To put it in a nutshell, the macro/mesoporous structures of bioactive materials have a sustained release behaviour of vancomycin delivery.

4. CONCLUSIONS

Selective laser melting allows fabrication of 3D porous bioceramic (Si–$CaSiO_3$) scaffolds. The results showed that bioceramic scaffolds had a regular circular macropore structure with pore size and porosity of ~400 μm and 35%, respectively. Additionally, mesopores were obtained with pore size from 15 to 50 μm. The controllable porosity at both macro- and mesolevels combined with a biocompatible polymer (PCL) coating made it possible to fabricate scaffolds for bone regeneration and to sustain the release of vancomycin. Release of nearly 50% of the vancomycin from the scaffolds was observed during the first 40 h followed by sustained release of nearly 20% of the actually loaded vancomycin for the next six days.

ACKNOWLEDGEMENTS

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Biokeraamiliste nanovõrgustike aditiivne valmistamine vankomütsiini kontrollitud vabanemiseks

Nikhil Kamboj, Miguel A. Rodríguez, Ramin Rahmani, Konda Gokuldoss Prashanth ja Irina Hussainova

Selektiivset lasersulamit on käesolevas töös kasutatud 3D struktureeritud poorsete biokeraamiliste (Si–$CaSiO_3$) struktuursete nanovõrgustike valmistamiseks. Valmistati ristkülikukujulised komposiidist nanovõrgustikud mõõtmetega 10 × 20 × 5 mm^3, mille pooride suurus on 400 µm. Tulemused näitasid, et biokeraamilised struktuurid olid regulaarsed ja ümara kujuga makropoorsusega, kusjuures pooride suurus ja poorsuse väärtus olid vastavalt ~400 µm ning 35%. Lisaks sellele saadi ka mesopoorid poori suurusega 15–50 µm. Kaetud nanovõrgustike puhul uuriti ravimi vabanemise profiili UV-nähtava spektroskoopia abil. Ligikaudu 50% vankomütsiini vabanes nanovõrgustikust esimese 40 tunni jooksul, millele järgnes ühtlane vankomütsiini vabanemine järgmise kuue päeva jooksul peaaegu 20% ulatuses algsest ravimi kogusest.