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Selective laser melting porous metallic implants with immobilized silver nanoparticles kill and prevent biofilm formation by methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Implant-associated infection and limited longevity are two major challenges that orthopedic devices need to simultaneously address. Additively manufactured porous implants have recently shown tremendous promise in improving bone regeneration and osseointegration, but, as any conventional implant, are threatened by infection. In this study, we therefore used rational design and additive manufacturing in the form of selective laser melting (SLM) to fabricate porous titanium implants with interconnected pores, resulting in a 3.75 times larger surface area than corresponding solid implants. The SLM implants were biofunctionalized by embedding silver nanoparticles in an oxide surface layer grown using plasma electrolytic oxidation (PEO) in Ca/P-based electrolytes. The PEO layer of the SLM implants released silver ions for at least 28 days. X-ray diffraction analysis detected hydroxyapatite on the SLM PEO implants but not on the corresponding solid implants. *In vitro* and *ex vivo* assays showed strong antimicrobial activity of these novel SLM PEO silver-releasing implants, without any signs of cytotoxicity. The rationally designed SLM porous implants outperformed solid implants with similar dimensions undergoing the same biofunctionalization treatment. This included four times larger amount of released silver ions, two times larger zone of inhibition, and one additional order of magnitude of reduction in numbers of CFU in an *ex vivo* mouse infection model.

1. Introduction

Multifunctional orthopedic biomaterials [1] that improve bone regeneration and fixation [2–4] and at the same time offer protection against infections [5–11] are intensively researched. Unmet clinical needs, particularly in the case of large bony defects [12–14], complex bone reconstructions [15,16], and patients with compromised bone metabolism and immune systems such as those with malignant bone tumors that might receive large limb salvaging prostheses [17–20] motivate most of that research. Recent advances in additive manufacturing techniques have given rise to a new paradigm in which the novel functionalities of biomaterials do not necessarily depend on discovery of new materials with unique properties, but are rather driven by rational design of biomaterials.

The properties and, thus, functionalities of rationally designed biomaterials are direct functions of their topologies otherwise

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known as their micro-architectures. The seemingly limitless form-freedom offered by additive manufacturing techniques in fabricating arbitrarily complex topologies has been exploited in the last few years to design and manufacture porous biomaterials with unique properties. It has been, for example, shown that the type and dimensions of the repeating unit cell can be adjusted to achieve mechanical properties close to those of native bone [21], thereby preventing stress-shielding and improving implant longevity. The other geometrical parameters of the porous biomaterials such as pore size [22], pore shape [23], porosity [22], and curvature [24,25] that have been shown to influence bone tissue regeneration [26] can be also rationally designed to achieve improved tissue regeneration performance and implant fixation. Finally, rationally designed and additively manufactured fully porous biomaterials can achieve surface areas that are up to several orders of magnitude larger than those of the corresponding solid biomaterials. Such a huge surface area, as we have shown before [27], can then be used for biofunctionalization purposes to markedly improve bone tissue regeneration performance. A potential risk of increasing the surface area is that bacteria contaminating the surgical site at implantation may have a higher chance of adhering to the surface and initiate biofilm formation. It is therefore vital to protect this increased surface area against infecting bacteria.

In this study, we aimed to develop rationally designed and additively manufactured porous metallic implants equipped with antimicrobial functionality to prevent implant-associated infection (IAI), including infections by worst case pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA). The use of porous metallic biomaterials provides the benefit of a superior mechanical support relative to the biodegradable candidate materials. Furthermore, for applications such as spinal cages or parts for reconstruction surgery in orthopedics and dentistry, permanent metallic porous implants are needed. However, the presence of such implants in the body for the rest of the patients' lifetime poses challenges with regard to cytotoxicity and IAI.

Long (i.e. high aspect-ratio) implants were rationally designed and additively manufactured using selective laser melting (SLM) from Ti-6Al-4V to increase the surface area. The surface of the implants was then biofunctionalized using plasma electrolytic oxidation (PEO), chosen because of its great potential for inducing multiple functionalities in a fast single-step process. When performed in presence of silver nanoparticles (AgNPs), PEO not only results in a bioactive surface with interconnected micro-/nano-porosity that can improve implant osseointegration [28–31] but also dopes the surface of the implants with fully dispersed and firmly attached AgNPs all within the span of a few minutes. AgNPs were chosen because, when oxidized, they release Ag ions that are known to be potent antimicrobial agents and have shown strong bactericidal behavior against a wide spectrum of bacteria including MRSA [32–37] through multiple mechanisms such as damage to bacterial membranes and production of reactive oxygen species [38].

The most important feature of PEO-treated surfaces that sets them apart from other antimicrobial surfaces based on AgNPs is the fact that in PEO the AgNPs are entrapped in an in-depth growing oxide layer which fully immobilizes them and prevents them from freely circulating through the blood stream, thereby preventing any potential nanotoxic effects [39,40]. At the same time, AgNPs are fully dispersed within the huge and hierarchical surface area of additively manufactured porous implants which greatly facilitates oxidation of the AgNPs and, thus, the release of Ag ions. Following biomaterials synthesis and characterization, their antimicrobial activity and cytotoxicity were assessed.

2. Materials and methods

2.1. Rational design and additive manufacturing

We designed implants suitable for implantation in a mouse femur model, which therefore needed to simultaneously meet multiple design criteria. First, the entire volume of the implant was designed to be porous with the aim of substantially increasing the surface area while providing ample space for bone ingrowth and implant fixation. Second, the pore size was required to be larger than 300 μm to maximize the bone regeneration performance of the porous biomaterials [22]. Third, the diameter of the implant was defined not to exceed 0.5 mm to make sure the implants can be fit in murine femurs used to assess *ex vivo* antimicrobial activity. Fourth, the porous structure had to be designed for SLM additive manufacturing. Since the laser spot in metal additive manufacturing techniques is between 100 and 150 μm, not every porous structure with the desired diameter of 500 μm (only 3–4 times the laser spot size) can be additively manufactured. Various designs of unit cells were evaluated against the above-mentioned criteria using design and computational software. Two-dimensional cross-section drawings were first produced in SolidWorks (Dassault Systèmes, Vélizy-Villacoublay, France) to evaluate the porosity and pore size of the porous structure. The two-dimensional drawings were then used to create the required three-dimensional geometries based on a vector space generated using a MATLAB (MathWorks, Natick, Massachusetts, United States) script. Moreover, design constraints were imposed on the coordinates of the top and bottom planes of the unit cell to ascertain the designed unit cells were space-filling, i.e. they could be repeated along the required directions to give rise to the desired porous structure. The unit cell fulfilling all the above-mentioned criteria, i.e. hexagonal unit cell, was selected for SLM manufacturing of the implants.

Implants of 4 cm in length were produced at the Additive Manufacturing Lab (TU Delft, Delft, The Netherlands) using an SLM machine (SLM-125, Realizer, Borchem, Germany) with YLM-400-AC Ytterbium fibre laser (IPG Photonics Corporation, Oxford, United States), under inert atmosphere (i.e. Argon) with an oxygen content below 0.2%. Medical-grade (grade 23, ELI) Ti-6Al-4V powder (AP&C, Boisbriand, Quebec, Canada) with particle sizes between 10 and 45 μm and spherical particle morphology was used. The laser spot size and layer thickness were 145 μm and 50 μm, respectively. A parametric study was performed to determine the optimum laser processing parameters, which resulted in an exposure time of 300 μs, a wavelength of 1070 ± 10 nm and laser power of 96 W. After the SLM procedure, the loose powder was removed by vacuum cleaning followed by ultrasonication in acetone, 96% ethanol, and demineralized water for 5 min each. For comparison with SLM implants, solid annealed Ti-6Al-4V implants (Goodfellow, Cambridge, England) with a diameter of 500 μm were used.

2.2. Surface biofunctionalization

The surface of the implants was biofunctionalized using an electrochemical process, namely PEO. The PEO electrolyte consisted of 0.02 M calcium glycerophosphate and 0.15 M calcium acetate as well as AgNPs. The AgNPs (Sigma-Aldrich, St. Louis, Missouri, United States) ranging in size between 7 and 25 nm and with a spherical shape were dispersed in the PEO electrolyte at a concentration of 3.0 g/l. To obtain a homogeneous dispersion of AgNPs, the electrolyte was ultrasonicated 2 times 3 min. In between the sonication steps, the electrolyte was stirred at 500 rpm for 5 min using a magnetic stirrer (IKA-Werke GmbH & Co. KG, Staufen, Germany) with a stir bar of 40 × 8 mm (Radnor, Pennsylvania,