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Bone regeneration performance of surface-treated porous titanium

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ABSTRACT

The large surface area of highly porous titanium structures produced by additive manufacturing can be modified using biofunctionalizing surface treatments to improve the bone regeneration performance of these otherwise bioinert biomaterials. In this longitudinal study, we applied and compared three types of biofunctionalizing surface treatments, namely acid–alkali (AcAl), alkali–acid–heat treatment (AlAcH), and anodizing-heat treatment (AnH). The effects of treatments on apatite forming ability, cell attachment, cell proliferation, osteogenic gene expression, bone regeneration, biomechanical stability, and bone-biomaterial contact were evaluated using apatite forming ability test, cell culture assays, and animal experiments. It was found that AcAl and AnH work through completely different routes. While AcAl improved the apatite forming ability of as-manufactured (AsM) specimens, it did not have any positive effect on cell attachment, cell proliferation, and osteogenic gene expression. In contrast, AnH did not improve the apatite forming ability of AsM specimens but showed significantly better cell attachment, cell proliferation, and expression of osteogenic markers. The performance of AlAcH in terms of apatite forming ability and cell response was in between both extremes of AnH and AsM. AcAl resulted in significantly larger volumes of newly formed bone within the pores of the scaffold as compared to AnH. Interestingly, larger volumes of regenerated bone did not translate into improved biomechanical stability as AnH exhibited significantly better biomechanical stability as compared to AcAl suggesting that the beneficial effects of cell-nanotopography modulations somehow surpassed the benefits of improved apatite forming ability. In conclusion, the applied surface treatments have considerable effects on apatite forming ability, cell attachment, cell proliferation, and bone ingrowth of the studied biomaterials. The relationship between these properties and the bone-implant biomechanics is, however, not trivial.

1. Introduction

In clinical practice, bone is often substituted by biomaterials that fulfill (some of) its functions either temporarily or permanently. Autologous and allogeneic bone has traditionally been the most widely used bone substitutes with autologous iliac crest bone being

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the clinical gold standard [1]. However, there is often limited bone stock available for autologous bone grafting. Moreover, donor-site morbidity and complication rates of around 20% have been reported for iliac crest and intramedullary canal bone harvesting [2]. Synthetic bone substituting materials are therefore being continuously developed. Synthetic bone substituting biomaterials need to provide enough mechanical support without being overly stiff, and well integrate within the host bony tissue. Increasingly, it is important for bone substitutes to enhance bone regeneration [3] and improve the biomechanical stability of the treated bony defects [4].

In this study, we introduce and evaluate three variations of surface-modified porous titanium alloy biomaterials. Recent developments in additive manufacturing techniques such as selective laser sintering [5–8] and selective laser melting [9–12] have enabled production of highly porous titanium alloy biomaterials with precisely-controlled micro-architectures. One can therefore ensure that the porous structure is fully-interconnected [13], has a precisely-controlled pore size that can be optimized for cell attachment, proliferation, and migration [14], and possesses overall mechanical properties in the range of bone mechanical properties [15]. In addition, the ample pore space allows for incorporation of, for example, hydrogels that release growth factors [16] to maximize the bone regeneration performance of the biomaterial.

Another important feature of highly porous bone substitutes is their large surface area. It is known that titanium alloys are generally bioinert [17,18] and may be also hydrophobic [19]. Hydrophobicity could adversely affect cell attachment [20] while bioinertness means that the bioactivity potential of the highly porous biomaterials remains unused. One may therefore need to use bio-functionalization techniques to improve cell attachment and induce bioactivity on the surface of porous titanium bone substitutes. Since surface chemistry [21,22] and nanotopography [23–27] both play important roles, biofunctionalizing techniques could target both in order to achieve the best performance.

In this study, we used three surface treatment techniques to modify both surface chemistry and topography of highly porous titanium bone substitutes. The aim was to 1. improve cell attachment and proliferation, 2. induce a hierarchical micro- and nano-topography on the surface of the biomaterial, and 3. improve the osseointegration of the biomaterial through enhanced apatite formation. The surface modifications included two chemical surface treatment techniques, namely alkali–acid–heat treatment [17,28–32] and acid–alkali treatment [33–35], and one electrochemical surface treatment technique, namely anodizing [36–39]. The above-mentioned surface modifications were chosen, because they are known to induce one or more of the three above-mentioned effects and also because they can be applied on complex 3D surfaces. A comprehensive longitudinal *in vitro* and *in vivo* study was performed to evaluate the bone regeneration performance of the applied surface modification techniques and to benchmark the surface modifications techniques against each other.

2. Materials and methods

2.1. Materials and manufacturing

Spherical pre-alloyed Ti6Al4V ELI powder (ASTM B348, grade 23) was used for manufacturing porous titanium alloy structures using selective laser melting (Layerwise NV, Belgium) as detailed before [40]. The porous structures were based on dodecahedron unit cells with the following design (nominal) dimensions: strut size = 120 μm, pore size = 500 μm, porosity = 88%. The specimens were built on top of a solid titanium alloy substrate in an inert atmosphere and were subsequently removed from the substrate using wire electro-discharge machining (EDM). Disk-shaped samples (Ø8 mm × L3 mm) were used for *in vitro* assays (Fig. 1a). The samples used for *in vivo* implantation were based on a mid-diaphyseal segment of a rat femur (Fig. 1a). The actual micro-architectures of both as-built and surface-treated samples were characterized using micro-computed tomography (micro-CT). The micro-CT images were subsequently segmented using a global threshold for detecting the morphometric details of the titanium structure [40]. The morphometric parameters of the porous structure including pore size, strut size, and the average structure porosity were then determined using the segmented micro-CT images and 3D morphometry algorithms [40].

2.2. Surface treatments

For the alkali-acid-heat (AlAcH) treatment [32], the specimens were first immersed in 5 M NaOH (Sigma–Aldrich) solution (24 h, 60 °C) and were subsequently washed gently with distilled water. The specimens were then immersed in hot water (24 h, 40 °C) subsequently in 0.5 mM HCl (Sigma–Aldrich) (24 h, 40 °C). Afterwards, the specimens were dried in an oven (24 h, 40 °C). The dried specimens were heated with a rate of 5 °C/min to 600 °C and were kept at that temperature for 1 h after which they were allowed to cool down in the oven to the room temperature.

For the acid–alkali (AcAl) treatment [35], the specimens were first immersed in a mixture of 18% HCl (Sigma–Aldrich) and 48% H_2SO_4 (Sigma–Aldrich) aqueous solutions (1 h, 70 °C) followed by immersion in 6 M NaOH (Sigma–Aldrich) (5 h, 70 °C). The specimens were afterwards washed with distilled water and dried in an oven (24 h, 40 °C).

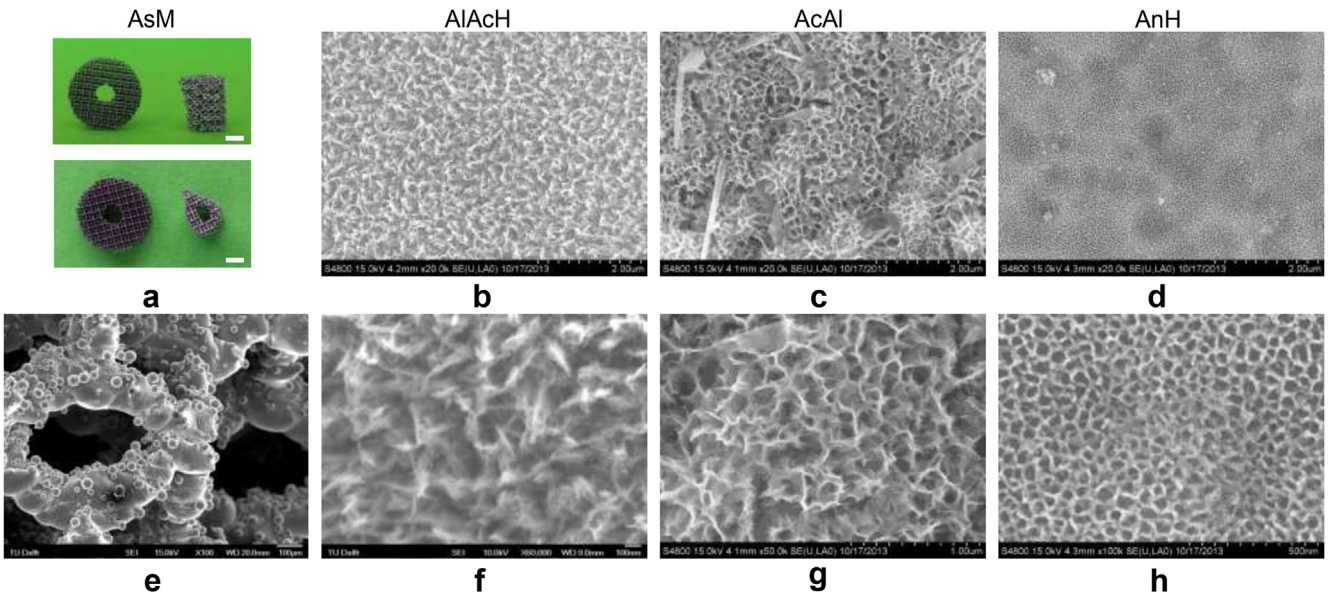

Fig. 1. Macrographs of *in vitro* and *in vivo* test specimens; scale bar: 2 mm (a) as well as the SEM pictures of AsM (e), AlAcH (b, f), AcAl (c, g), and AnH (d, h) specimens.

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