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Hydroxyapatite and bioactive glass surfaces for fiber reinforced composite implants via surface ablation by Excimer laser

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ABSTRACT

In skeletal reconstructions, composites, such as bisphenol-A-glycidyldimethacrylate resin reinforced with glass fibers, are potentially useful alternatives to metallic implants. Recently, we reported a novel method to prepare bioactive surfaces for these composites. Surface etching by Excimer laser was used to expose bioactive glass granules embedded in the resin. The purpose of this study was to analyze two types of bioactive surfaces created by this technique. The surfaces contained bioactive glass and hydroxyapatite granules.

The selected processing parameters were adequate for the creation of the surfaces. However, the use of porous hydroxyapatite prevented the complete exposure the granules. In cell culture, for bioactive glass coatings, the pattern of proliferation of MG63 cells was comparable to that in the positive control group (Ti6Al4V) while inferior cell proliferation was observed on the surfaces containing hydroxyapatite granules. Scanning electron microscopy revealed osteointegration of implants with both types of surfaces.

The technique is suitable for the exposure of solid bioactive glass granules. However, the long-term performance of the surfaces needs further assessment.

1. Introduction

Metals and their alloys are standard materials for load-bearing fracture fixation devices and joint replacement implants. However, the mismatch of the mechanical properties of metals and bone leads to the unfavorable load distribution, known as "stress-shielding". In addition, metallic implants are known to produce metallic debris and corrosion products. Stress-shielding and debris formation may result in periprosthetic bone loss and aseptic loosening of the implant. Due to the bone loss, the revision surgery could be complex with the increased risk of complications. Therefore, there is a need to develop advanced non-metallic load-bearing implants. These implants should have improved functional biocompatibility, mechanical properties and long-term stability as well as the ability to withstand the demanding physiological loading conditions while being permissive for the normal stress-related bone remodeling. In the past numerous attempts have been made to substitute metallic implants with composites (Evans and Gregson, 1998). Biostable fiber-reinforced composites (FRC) are potentially useful alternatives to metallic implants as they can be engineered to closely match the various moduli of bone at the same time retaining high strength and fatigue resistance (Evans and Gregson, 1998; Zhao et al., 2009; Moritz et al., 2014). In addition, while the FRC implants are visible in radiographs and computed tomography (CT), these implants produce clinically fewer artefacts with modern diagnostic imaging and do not interfere with radiotherapy (Kuusisto et al., 2015). Moreover, FRC implants allow the use of postoperative radiation therapy in the case of tumor surgery without the risk of radiation scattering.

Novel non-load bearing FRC implants based on bisphenol-A-glycidyldimethacrylate (BisGMA) and triethyleneglycoldimethacrylate (TEGDMA) resin matrix reinforced with E-glass fibers are being successfully used in the clinic for cranial reconstructions (Aitasalo et al., 2012). These implants contain bioactive glass as an osteopromotive and antibacterial agent. Recently, attempts were made to develop load-bearing implants using the same concept (Zhao et al., 2009; Moritz et al., 2014). The potential of these FRC implants was scrutinized within

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EU-funded NEWBONE-project (NMP3, contract number CT-2007-026279, 2006–2010) (NEWBONE, 2010). An intramedullary interlocking nail is one of the most promising implant types for the FRC technology (Zhao et al., 2009; Moritz et al., 2014; NEWBONE, 2010). One of the attractive features of this technology is the possibility to augment the implant surface with biologically active compounds such as BG and hydroxyapatite (HA). These surfaces can reduce the bacterial colonization of the implant and, at the same time, promote healing of the bone (Ballo et al., 2009, 2014).

During the past decades, HA-coated metallic implants have been used clinically on a routine basis. HA-coatings are to enhance osteointegration and improve fixation in the host bone (Cook et al., 1992a, 1992b; Thomas et al., 1987). Typically, HA-coatings are applied on the metal implants by plasma-spraying, sputter deposition, sol-gel coating, electrophoretic deposition and biomimetic precipitation (Ellingsen et al., 2000; Junker et al., 2009). BG was introduced by Prof. Hench in the early 1970s (Hench et al., 1971). Bone-bonding, a distinctive feature of BG is achieved via the formation of a bone-like hydroxyapatite layer on the material surface when in contact with aqueous solutions. In addition, BG possesses an antibacterial effect in vitro and in vivo. Theoretically, the advantages of a BG-coating on metallic implants are similar to those of a HA-coating (Wheeler et al., 2001). However, coating of metallic implants with BG is technically challenging (Moritz and Vallittu, 2017) and these types of implants have not succeeded to reach the clinic.

HA-coatings have been proposed for carbon fiber-reinforced epoxy-based FRCs (Evans and Gregson, 1998). However, to our best knowledge, there were no HA-coatings prepared on BisGMA-based FRCs. In our previous paper, we reported a novel method to prepare osteoconductive surfaces on BisGMA-based FRCs by means of surface etching by Excimer laser (Kulkova et al., 2016). In this paper we used this method to prepare HA-based surfaces for FRC implants and compared the BG-based and HA-based surfaces in terms of their biological response.

2. Materials and methods

2.1. Methodology of the study

Intramedullary nails were the targeted implants (Zhao et al., 2009; Moritz et al., 2014). As seen in Fig. 1A, long E-glass fibers are used as the reinforcing phase in these composite implants. This surface layer, which is around 1 mm in thickness, is not reinforced with glass fibers but contains only the resin and BG granules. Laser irradiation affects only the top of this surface layer as shown in Fig. 1B. Therefore, in our previous report on the surface etching by Excimer laser, we excluded the reinforcing phase from consideration and used unreinforced thermosets (Kulkova et al., 2016). The same assumption was made in this study to simplify the experimental setup.

Standard cell proliferation test was performed to study MG63 cell proliferation on the surfaces of the specimens to verify the absence of negative effects of laser radiation. In addition, to corroborate the results of the cell proliferation test, supplementary examinations were performed on a sub-set of ex vivo specimens originating from an experiment reported separately (NEWBONE, 2010).

2.2. Preparation of the specimens

Five experimental groups of specimens were prepared (Table 1). Materials used for the preparation of the specimens are listed in Table 2. The specimens were disc-shaped (Ø8.6 mm, thickness 3 mm). Micro-roughened Ra = 4 μm) made of titanium alloy (Ti6Al4V) (Medacta International SA, Switzerland) served as a positive control group.

The photopolymerisable resin was prepared by mixing BisGMA (70 wt%) and triethylene glycol dimethacrylate (TEGDMA) (30 wt%) with camphorquinone (CQ) (0.7 wt%) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) (0.7 wt%) (Kulkova et al., 2016).

The specimens termed “Thermoset” were made of the grit-polished resin. In brief, the resin was poured into the molds and pre-cured (40 s) by a hand-held light curing device (Optilux 501, Kerr, Danbury, USA) under ambient conditions. Next, to complete the curing process, the pre-cured thermoset specimens were taken out of the molds and placed in a vacuum light oven (Visio Beta vario, 3 M/ESPE, Germany) for 15 min at the ambient temperature. Subsequently, in order to reduce the amount of residual monomers, the specimens were placed in a light oven (Liculite, Dentsply De Trey GmbH, Germany) for 25 min at 90 °C. The wavelength of these devises is typically in the range of 400–500 nm. The thermoset specimens were grit-polished (granulometry: 1200). After the preparation, the specimens were cleaned ultrasonically in water.

The specimens with bioactive ceramic coatings, Thermoset-S53P4, Thermoset-45S5 and Thermoset-HA, were prepared using the technique reported earlier (Kulkova et al., 2016). In brief, after pre-curing by a hand-held curing device, the thermoset specimens were taken out of the molds and a thin layer of resin was applied to the pre-cured surface of the specimens. The specimens were then pressed against BG or HA granules spread over a flat surface. Thereafter, the final resin layer was applied on top of the granules. All these steps were performed under ambient conditions. There after the specimens were further cured first in a vacuum light oven for 15 min at the ambient temperature, and then a light oven for 25 min at 90 °C.

The specimens termed “Thermoset-S53P4” contained glass S53P4 granules in the fraction of 90–315 μm (Vivoxid Ltd, Turku, Finland). The specimens termed “Thermoset-45S5” contained glass 45S5 granules in the fraction of 100–250 μm (Mo-Sci Corp, Rolla, USA). The specimens termed “Thermoset-HA” contained HA granules in the fraction of 100–300 μm (Berkeley Advanced Biomaterials, Inc, Berkeley, USA). The Thermoset-S53P4, Thermoset-45S5 and Thermoset-HA specimens were not grit-polished.

Excimer (XeCl) laser (ASX-750, MPB Technologies Inc, Estonia) with a wavelength of 308 nm and a pulse width of 28 ns (FWHM) was used for the ablation of the thermoset resin covering the granules. The laser beam remained static during the experiment while the specimen was moved by a computer-controlled stage. The ablation process was performed under ambient conditions as reported earlier in detail (Kulkova et al., 2016). The processing parameters were: energy of 175 mJ, energy density of 0.32 J/cm^2, 30 pulses. The specimens were cleaned with compressed air after laser etching.

The surfaces of the specimens were analyzed using a scanning electron microscope (SEM) (JEOL JSM-5500, Japan) and a profilometer (Mitutoyo Surftest – 301, Mitutoyo Corp, Japan). The crystallographic properties of the specimens were determined by x-ray diffraction (XRD) measurements with a diffractometer (Philips X'Pert Pro MPD, Philips Analytical, The Netherlands) using Cu Kα radiation. In these measurements, possible structural changes in the HA and BG due to the Excimer laser ablation were of primary interest.

2.3. Cell proliferation test

In the standard cell proliferation test six replicates were used for each group of specimens. In addition, in each group, two cell-free specimens were used as blank to subtract the average signal. The specimens were sterilized by UV light for 1 h on both sides. Approximately 20,000 cells (Osteosarcoma MG-63 cell line, ATCC number: CRL-1427) were suspended in 30 μL of culture medium (Dulbecco's modified Eagle's medium, Sigma-Aldrich) supplemented with inactivated fetal bovine serum (10%), penicillin (100 units/mL), streptomycin (100 μg/mL), and L-glutamine (2 mM). The cell suspension was placed in the center of the specimens. Thereafter, the specimens were incubated for 4 h at 37 °C at 5% pCO_2. After 4 h of incubation, a total of 0.85 mL of fresh culture medium was added and the specimens were further incubated for 18 h under the same conditions. To stain the cells, 0.1 mL of