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Multi-layer porous fiber-reinforced composites for implants: *In vitro* calcium phosphate formation in the presence of bioactive glass

Sara Nganga[a,b,*], *Di Zhang*[c], *Niko Moritz*[a,b], *Pekka K. Vallittu*[a,b], *Leena Hupa*[c]

[a] *Department of Biomaterials Science, University of Turku, Turku, Finland*
[b] *Biocity, Turku Biomaterials Research Program, Turku Clinical Biomaterial Centre – TCBC, Turku, Finland*
[c] *Process Chemistry Centre, Åbo Akademi University, Piispankatu 8, FI-20500 Turku, Finland*

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ABSTRACT

Objectives. Glass-fiber-reinforced composites (FRCs), based on bifunctional methacrylate resin, have recently shown their potential for use as durable cranioplasty, orthopedic and oral implants. In this study we suggest a multi-component sandwich implant structure with (i) outer layers out of porous FRC, which interface the cortical bone, and (ii) inner layers encompassing bioactive glass granules, which interface with the cancellous bone.

Methods. The capability of Bioglass® 45S5 granules (100–250 μm) to induce calcium phosphate formation on the surface of the FRC was explored by immersing the porous FRC-Bioglass laminates in simulated body fluid (SBF) for up to 28 d.

Results. In both static (agitated) and dynamic conditions, bioactive glass granules induced precipitation of calcium phosphate at the laminate surfaces as confirmed by scanning electron microscopy.

Significance. The proposed dynamic flow system is useful for the *in vitro* simulation of bone-like apatite formation on various new porous implant designs containing bioactive glass and implant material degradation.

1. Introduction

Durable, biostable fiber-reinforced composites (FRC), which were originally started to be developed for applications in dentistry in early 1960s took until mid-1990s until they made their way into the routine clinical use in various types of dental restorations [1,2]. Among the applications of prosthetic and restorative dentistry, FRC has been tested as implant material in craniofacial, orthopedic and oral implantology. The implant material is based on co-polymer matrices out of polymethyl methacrylate (PMMA) or bisphenol-A-dimethacrylate (BisGMA) and triethyleneglycoldimethacrylate (TEGDMA) reinforced with E-glass fibers. The potential of FRCs as implant material for craniofacial bone reconstructions has also been demonstrated [3,4]. In cell culture and *in vivo* studies, the photopolymerized BisGMA-TEGDMA resin system has shown good biocompatibility and bone formation capability [5].

Further enhancement of the FRC biocompatibility can be attained by mimicking fibrous structure of bone at the implant–bone interface [6]. To achieve this goal, we suggest

* *Corresponding author at*: Turku Clinical Biomaterials Centre – TCBC, Itäinen Pitkäkatu 4 B, FI-20520 Turku, Finland. Tel.: +358 2 333 8527; fax: +358 2 333 8390.
E-mail address: sarsch@utu.fi (S. Nganga).
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a sandwich implant structure based on multiple components. The outer implant layers, which interface the cortical bone, are made of porous FRC, while the inner layers, which interface the cancellous bone, comprise a biologically active component. This implant structure would allow attachment, proliferation, and migration of cells leading to vascularization and rapid bone in-growth for the stabilization of the implant in medium and long terms [7,8]. 45S5 Bioglass® (BG), a biocompatible and osteoconductive bone graft substitute material [9,10], is approved by the U.S. Food and Drug Administration for certain clinical applications in orthopedic and craniomaxillofacial surgery [11–18]. For this reason this bioactive glass was chosen to be used as biologically active component in the FRC implant structures.

The formation of a bone-like apatite layer on the implant surface is essential for the achievement of bone-bonding and osteoconductivity [19]. In contact with body fluids BG is capable of forming such an apatite layer [20]. The layer formation can also be simulated *in vitro* [21]. In addition, it has been demonstrated that the presence of BG can lead to apatite formation on the surface of adjacent bioinert polymer materials [22–24]. The induction of this so called "halo" effect to the sandwich FRC implant structures is expected to enhance the implant attachment and also to improve the bone ingrowth into the porous FRC layers [25].

The goal of this work was to study the calcium phosphate (CaP) formation induced on the surfaces of the FRC implant by BG granules. Therefore, experimental *in vitro* set-ups were developed to describe the reactions taking place upon immersion of composite structures in simulated body fluid (SBF) in both static and dynamic conditions with various arrangements of the bioactive component. Detailed knowledge of the *in vitro* and *in vivo* reactions is essential for the development of different implant structures for craniofacial applications.

2. Materials and methods

2.1. Experimental arrangements

The capability of bioactive glass granules to induce CaP formation on the composite surfaces during immersion in SBF [21] was studied by using two different experimental conditions, static and dynamic. In the static tests, FRC specimens were incubated in SBF in the presence and in the absence of separate BG granules. Incubated specimens included (1) *FRC1*: control polished resin specimens without reinforcement, (2) *FRC2*: porous FRC, and (3) *FRC3*: laminate FRC structures. Specimen types *FRC1* and *FRC2* were included to study the influence of the distance between the composite surfaces and BG granules on the *in vitro* behavior. *FRC3* was a surrogate of a cranial implant. In the dynamic test, a flow of SBF was passed through the *FRC3* specimens. Details of experimental set-ups are shown in Fig. 1.

FRC2 was made of a randomly oriented chopped silanized E-glass veil impregnated within the BisGMA/TEGDMA polymer matrix. This composite was used to create multicomponent laminates (*FRC3*) which had two layers of BG granules entrapped between three layers of porous FRC. The BG granules were retained by the polymer, but not embedded into it.

2.2. Preparation of the specimens

The *FRC1* specimens were prepared by pouring resin mixture consisting of BisGMA (50 wt%), TEGDMA (50 wt%), camphorquinone (0.7 wt%) and DMAEMA (0.7 wt%) into putty molds and then polymerized by light curing. Subsequently, the specimens were ground (LaboPol-21 Grinding Machine, 300 rpm, Struers A/S, Rødovre, Denmark) to the final dimension of 9 mm × 59 mm × 2.9 mm (each ±0.2 mm) and the surfaces were polished with grit 4000 SiC paper. The photo-initiated polymerization of the specimens was carried out in three stages to ensure minimal content of residual monomers. First, the specimens were pre-cured by a dental hand cure device (Elipar® S10 LED curing light, 3M/ESPE, Seefeld, Germany) with an exposure time of 120 s (intensity: 1200 mW/cm^2, wavelength: 430–480 nm). Second, the specimens were post-cured in a vacuum light oven (Visio Beta vario, 3M/ESPE, Seefeld, Germany) for 15 min in ambient temperature and finally in a light oven (Targis Power, Ivoclar Vivadent AG, Schaan, Lichtenstein) for 25 min at a temperature of 95 °C.

The *FRC2* specimens were prepared from 1 mm thick E-glass fiber sheets (Ahlstrom Glassfiber Oy, Kotka, Finland). The randomly oriented fibers in the sheets had a diameter of 10 μm and a length of 8 mm, and were bound together by an acrylic binder with a methacrylate silane coupling agent. Pieces of 9 mm × 60 mm in size were cut from these sheets and scaled. The cut pieces were then impregnated with 1–1.5 ml of the BisGMA/TEGDMA resin mixture, and were left for 48 h in the dark. After the impregnation, the excessive resin was removed by compressing and the specimens were then light cured as described above. These procedures ensured that 75–81 wt% resin remained in the *FRC2* specimen structure, which was expected to result in a total porosity of 39–49 vol% with more than 90% of functional (open) porosity [26]. The average pore size was larger than 100 μm. The reinforcing E-glass fibers in the specimens were covered by polymer resin.

The *FRC3* specimens were prepared by assembling two pieces of *FRC2* with fiber bundles (everStick® Ortho, Ø 0.75 mm, StickTech Ltd., Turku, Finland) serving as spacers (Fig. 2) between the layers to allow the incorporation of the BG granules. Each of the two BG layers contained 45S5 Bioglass® granules of the 100–250 μm fraction (composition in wt%: SiO_2: 45, Na_2O: 24.5, CaO: 24.5, P_2O_5: 6). The two sides of the *FRC2* pieces along the spacers were sealed with additional resin prior to final light curing (Fig. 2). Smaller specimens (10 mm × 10 mm × 4 mm), containing 60 ± 5 mg of BG per layer were used in the static test. Larger specimens (9.3 mm × 30 mm × 4 mm), comprising 200 ± 10 mg of BG per layer were applied in the dynamic test. The BG content of both smaller and larger *FRC3* was around 38% of the total specimen weight. In previous tests, the packed arrangement of the granules in between the *FRC2* layers enabled wetting throughout the composite structure [27].

After preparation the specimens were washed in ultrapure water (milliQ quality), then washed with ethanol, dried at ambient temperature and stored in a desiccator. Specimens