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Functionally graded bioactive coatings: Reproducibility and stability of the coating under cell culture conditions ☆

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Abstract

This work sought to provide a basic protocol for treatment of functionally graded bioactive glass coatings (FGC) that reliably adhere to titanium alloy (Ti6Al4V) prior to in vivo evaluation. The effect of the fabrication process on glass structure and reproducibility of the coating's properties, and the effect of cell culture conditions on the integrity of the coating were assessed. The structure of FGCs was compared to that of the as cast glass used as a top coating. X-ray diffraction (XRD) showed that the fabrication process resulted in 5.9 ± 3.0 vol.% crystallization, while glass as cast was amorphous. Glass as cast and coatings behaved similarly in simulated body fluid (SBF): an amorphous layer rich in phosphate formed, and it crystallized, over 4 weeks, into apatite-like mineral (Fourier transform infrared spectroscopy (FTIR), XRD, scanning electron microscopy (SEM)). Reproducibility of the fabrication process was tested from three batches of coatings by measuring thickness and crystallinity. MC3T3-E1.4 mouse pre-osteoblast cells were cultured and induced to mineralize on FGCs, either as made or pre-conditioned in SBF. The sub-surface glass silica network in FGCs was compromised by cell culture conditions. A crystalline phosphate was formed during pre-conditioning (XRD, FTIR, and SEM). SBF-pre-conditioning stabilized the coatings. Thus incubation in SBF is recommended to produce a stable coating.

Keywords: Bioactive glass; Osteoblasts; Coating; Simulated body fluid

1. Introduction

Implants for load-bearing orthopedic or dental applications face the dual challenge of osteointegration while withstanding complex forces. Bioinert metal alloys (Ti6Al4V, Co–Cr and others) have been used as implant materials because they can support loads while provoking only minor reactions in the body [1–5]. The degree of the reactions can vary depending not only on intrinsic properties of bioinert materials but also on the site of implantation, degree of trauma and implant fit [6,7]. Bioactive materials that elicit formation of normal tissue and form a direct bond with bone, have been extensively investigated to improve osteointegration [7]. Bioactive titanium and its alloys can be fabricated through the combination of specific chemical and thermal treatments. As a result the metal surface is covered with amorphous calcium phosphate which is later transformed into apatite [8]. Bioactive metal implants are currently undergoing clinical trials in total hip replacement, showing promising results [8]. Bioactive glasses of specific compositions such as Bioglass®, and calcium phosphate-containing materials such as hydroxyapatite (HA) can provide the desired bioactivity but cannot be

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used for load-bearing applications [9]. Additionally to bioactive metals [8] a bioactive material coating on a bioinert metal alloy may allow osteointegration and load-bearing capability. Several different techniques have been developed and/or applied to yield such coatings, with plasma sprayed HA being the most common and currently used to fabricate commercially available implants [10,11]. Plasma sprayed HA does not retain the original characteristics of HA and yields unreliable coatings prone to delamination [12,13]. Bioglass® cannot be used to fabricate FGCs because of its coefficient of thermal expansion (CTE) mismatch with Ti6Al4V and high crystallization upon firing [14,15]. Functionally graded coatings (FGC) of bioactive glasses on titanium alloy that retain bioactivity after the fabrication process and provide enhanced glass–metal adhesion [15–17] have the potential for use as load-bearing implants. The term "graded" is hereby used for consistency with previous work in which the silica gradient created at the interface between glass 6P61 and 6P55 was shown to be a continuum across an interface of about 15 μm created between the two glasses [15–17]. Although macroscopically the change in glass composition from the coating's surface to the interface with titanium alloy is not a continuum, the nomenclature functionally graded coatings (FGC) is retained for consistency.

In this study functionally graded coatings on titanium alloy were fabricated using a glass containing 61 wt.% silica in contact with the alloy and a bioactive glass containing 55 wt.% silica as surface coating. A recent in vitro study showed good cytocompatibility of the latter glass [18]. The surface reactions occurring on bioactive glasses have been studied extensively [19–22]. An important property of Bioglass® and other bioactive glasses is the reactivity when exposed to simulated body fluid. Initial reactions involve the exchange of alkali ions with hydronium ion (H_3O^+ or H^+). This causes a rise in pH in the immediate environment [5]. It is common practice to pre-condition bioactive glasses in SBF or similar solutions to initiate the surface reactions and avoid the rise in pH during the assessment of cytocompatibility and osteogenic potential [23–28]. There is a significant difference in the reaction kinetics leading to the formation of apatite, depending on the presence or absence of proteins in the "conditioning" solution. In vivo and in vitro the presence of proteins, combined with periodic solution exchange, appears to cause a delay in apatite formation, accelerating the dissolution of Si species [28–31]. A proposed mechanism involves the absorption of proteins within the surface silica-gel reaction layer, and its consequent increased permeability. This would allow further release of Si and delay apatite formation [28,31]. When bioactive glass powder was tested in the presence of proteins and periodically exchanging the solutions, the particles were excavated, yielding hollow Ca P-rich shells [32]. The sub-surface silica network degradation that occurred in the excavated particles suggests that bioactive glass coatings may undergo a similar degradation that could result in delamination. Evidence shows that glass silica network dissolution occurs also in the absence of proteins [33]. In vitro experiments showed that in the absence of proteins the sub-surface silica dissolution is significantly slowed by a calcium phosphate-rich reaction layer which forms on the surface [28,31]. The presence of proteins yielded higher rates of silica dissolution regardless of the mode of immersion (static vs. dynamic) [32]. Cell culture conditions include the presence of proteins (fetal bovine serum) and periodic exchange of solution. In this study the hypothesis that the integrity of the coatings may be compromised by cell culture conditions was tested using mouse pre-osteoblastic cell (MC3T3-E1.4) culture on FGC with or without pre-conditioning in SBF. The cell line chosen is a well established in vitro bone model [34–39]. To determine a possible 'excavation' below the coating surface we applied suction (low vacuum) to evaluate the integrity of the coatings after "in vitro" tests and possibly identify the weakest interface. As a basic requirement prior to any further investigation we also assessed the effect of the coating fabrication process on microstructure and bioactivity (in vitro, in SBF) of the top surface glass as well as the reproducibility of the fabrication process. Reproducibility was evaluated by determining if there was batch to batch variation in coating thickness and microstructure. The objective of this work was to provide a basic protocol for treatment of functionally graded coatings prior to in vivo evaluation.

2. Materials and methods

2.1. Glass and coating fabrication

Glasses in the SiO_2–Na_2O–K_2O–CaO–MgO–P_2O_5 system were prepared by mixing the reagents in propanol using a high speed stirrer for 1 h and drying at 80 °C for 12 h as previously described [15]. Table 1 gives the glass compositions by wt.%. Reagents: SiO_2 (99.5% Cerac Inc., Milwaukee, WI), $CaCO_3$ (99.9%, J.T. Baker, Phillipsburg, NJ), MgO (98.6%, J.T. Baker, Phillipsburg, NJ), K_2CO_3 (99.4% Mallinckrodt, Paris, KY), $NaHCO_3$ (99.5% J.T. Baker, Phillipsburg, NJ), and Na_2HPO_4 (99% Sigma, St. Louis, MO). The mixture was dried and fired in air in a Pt crucible for 5 h at 1400 °C (glass 6P55) or 1450 °C (glass 6P61). The melt was cast in a pre-heated (200 °C) graphite mold yielding glass bars ($\sim 2 \times 2 \times 8$ cm). Functionally graded coatings were prepared as previously described [15]. Briefly, the glasses (6P61 and 6P55) were separately milled in a planetary agate mill (speed: 3000 rpm). Titanium alloy (Ti6Al4V, Goodfellow Ltd., Huntingdon, UK) was cut into plates ($1.5 \times 1.5 \times 0.1$ cm) with a low

Table 1
Glass compositions in wt.%

	SiO_2	Na_2O	K_2O	CaO	MgO	P_2O_5
6P61	61.1	10.3	2.8	12.6	7.2	6.0
6P55	54.5	12.0	4.0	15.0	8.5	6.0

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