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Bioactivity and biocompatibility of two fluoride containing bioactive glasses for dental applications

T. De Caluwé[a,*], C.W.J. Vercruysse[a], H.A. Declercq[b], D. Schaubroeck[c], R.M.H. Verbeeck[a], L.C. Martens[d]

[a] Biomaterials Group, Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, Bdg B, 4th Floor, 9000 Ghent, Belgium
[b] Tissue Engineering Group, Ghent University, De Pintelaan 185, 6B3, 9000 Ghent, Belgium
[c] Center for Microsystems Technology (CMST), IMEC and Ghent University, Technologiepark 15, B-9052 Ghent, Belgium
[d] Department of Paediatric Dentistry and Special Care Dentistry, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium

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ABSTRACT

Objective. Bioactive glasses (BAG) form, in contrast to formerly used implant materials, a stable bond with tissues, especially bone, when implanted. Nowadays BAGs are often mixed with a cement/composite that hardens *in situ* to broaden its applications in dentistry or orthopedics. The bioactivity and biocompatibility of possible BAG candidates for BAG-cement/composite development were evaluated.

Methods. Two fluoride containing BAGs were tested: a Na^+-containing (45S5F), based on the first commercial BAG, and a Na^+-free BAG (CF9), with a higher Ca^{2+} and PO_4^{3-} content. BAGs were tested on their bioactivity upon immersion in SBF for 7 days by evaluating the surface changes by FT-IR, SEM, EDS and PO_4^{3-} and Ca^{2+} uptake and/or release from SBF. Moreover, the biocompatibility of the BAGs was investigated with a direct contact cell viability study with HFF cells and a cell adhesion study with MG-63 cells.

Results. The Na^+-free BAG, CF9, showed the highest potential to bioactivate cements because of its high Ca^{2+}-release and apatite (Ap) formation, as evidenced by SEM pictures and corresponding EDX patterns. FT-IR confirmed the formation of an Ap layer. Moreover CF9 had a higher biocompatibility than 45S5F.

Significance. For the bioactivation of GICs/composites in order to enhance bonding and remineralization of surrounding tissues, fluoride containing BAG may have advantages over other BAGs as a more stable fluorapatite can be formed. CF9 may be an excellent candidate therefore.

1. Introduction

Bioactive glass (BAG) was invented by Larry Hench in 1969 and was the first implant material that could form a stable interface or bond with tissues, such as bone or muscle. Until

* *Corresponding author.*
E-mail address: tamara.decaluwe@ugent.be (T. De Caluwé).
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then implants were merely bioinert and evoked a non-wanted fibrous encapsulation of the material [1]. The first bioactive glass consisted of 46.1 mol% SiO_2, 24.4 mol% Na_2O, 26.9 mol% CaO and 2.6 mol% P_2O_5 and was called 45S5 or Bioglass® [1,2].

A bioactive material can be defined as a material that stimulates a beneficial response from the body, particularly bonding to host tissue [1]. This bonding to for example bone is in BAG achieved by the formation of hydroxyapatite (HAp) as an interconnective layer on the dissolving glass and in the near environment of the glass in humid or aqueous environments [1–3]. The surface reactions taking place upon the immersion of BAG in aqueous solutions are illustrated in Fig. 1 [1,2,4,5]. In a first phase, due to hydrolysis, rapid cation exchange of Na^+ and/or Ca^{2+} from the glass with H^+ from the solution occurs. In addition, phosphate is also released from the glass. The dissolution of these ions creates silanol bonds (Si—OH) on the glass surface. Silanol groups condensate and repolymerization of the silica-rich layer occurs. Meanwhile, Ca^{2+} and PO_4^{3-} migrate from the solution to the surface, forming a film rich in amorphous calciumphosphate (CaP) on the silica-rich layer [1,2,4]. Hydroxyl groups and carbonate from solution are incorporated and the CaP-film crystallizes to hydroxyapatite (HAp) [1,2]. This process can be mimicked by the immersion of a sample in simulated body fluid (SBF) and as such *in vitro* tests can predict the bioactivity of materials [3]. However, some researchers are critical about this method, as both false positive as false negative results can occur. So after positive results in SBF, *in vivo* tests should be conducted to validate the results [6]. The actual interconnection of the glass with bone namely occurs due to proteins, such as growth factors (*e.g.*, bone morphogenic protein (BMP)), fibronectin and collagen that easily bind with the formed HAp and in this way attract for example mesenchymal stem cells (MSCs) and enhance them to differentiate [1,5].

Until now, 45S5 is the most used commercial BAG. This BAG regenerates bone better than commercially used HAp [2]. It dissolves easier and therefore improves remineralization. A direct relationship between bioactivity (apatite formation) and glass network dissolution has been shown [1,7]. This can be explained by the network connectivity (NC). NC is calculated as the relative amount of bridging oxygens per network forming element in the glass. A NC between 1.8 and 2.7 is described to be favorable to induce apatite (Ap) formation. A higher NC impedes the dissolution of the glass [7–9]. Phosphate, present in the BAG is also favorable to induce apatite formation [7,10–14]. But care should be taken when increasing the phosphate content as the NC also increases, except when simultaneously network balancing ions are added [7]. Apart from the NC and phosphate content, the amount of Ca^{2+} available to be released should also be taken into account. Substitution of Ca^{2+} by Na^+ in the BAG leads to low NC and therefore has high reactivity, but decreases bioactivity by the low amount of Ca^{2+} present in the BAG [7,15].

Although bioactive glasses are very promising, their commercial success is concentrated on incorporation in toothpaste as remineralizing agents. Their use in restorative applications has never reached its full potential. In surgery they can be used as a bulk scaffold, fitting large defects. But then the disadvantage is that the scaffolds are mostly non-porous and brittle. Moreover, these bulk glasses show low degradability and hence not much ingrowth of cells and blood vessels. Another way to use BAG is as glass particles, often mixed with the patient's own blood to form a putty [1]. In this case, nearly no initial strength exists. BAG in the form of an injectable paste could broaden the possible applications of this material, provided that the paste hardens *in situ*. For this reason, bioactive glasses have been combined with polymers such as PLA, PDLLA, PGA or chitosan, but compressive strength results are limited [1]. Also, BAG has been incorporated in calcium phosphate cements (CPCs), resulting in good bioactivity but also increased degradability, which can hamper the mechanical properties of these cements [16]. Recently, BAGs are also combined with resin based composites in order to minimize marginal leakage as it is shown that BAG included in these composites may act as an antibacterial and remineralizing agent [17,18].

Research groups have already shown that bioactive glasses can be incorporated in glass ionomer cement (GIC) formulations [19]. GICs were invented in the 70s and are commonly used in restorative dentistry and more recently as medical applications for ENT surgery for bonding cochlear implants in place and repairing the occicular chain [20–22]. GICs are formed by an acid–base reaction between a polyalkenoic acid and an aluminosilicate glass (ASG) [21]. They bind directly with the apatite in dentin, enamel and bone [23,24]. Due to the incorporation of fluoride in the glass network, GICs have the possibility to release fluoride, which leads to a continuous protection against caries by the formation of fluorapatite (FAp) and the anti-bacterial effect [23,25–27]. Despite the advantages, their clinical use is relatively restricted because of their inferior mechanical properties. This drawback has already been tackled by the invention of resin modified GICs. The latter cements come however with higher sensitivity to moisture and contain toxic monomers [28,29]. The mechanical properties of conventional GIC can also be enhanced by incorporating apatite particles in the GIC [27,30]. As BAG form an apatite layer upon immersion, bioactivation of GIC by the incorporation of BAG could further improve the mechanical properties. The formation of an Ap layer in time on BAG containing GICs (BAG-GICs) may enhance the interaction of the cement with bone or pulpal cells and consequently mechanical interlocking may occur in addition to the normal chemical bonding of GICs to dental/bone tissue [31].

In order for a material to be biocompatible and allow cell growth, dissolution and precipitation reactions have to take place, preferably forming an interfacial hydroxyapatite layer on which biological molecules can be adsorbed [34]. Since of as conventional GICs do not release high amounts of Ca^{2+} and PO_4^{3-} and do not form apatite on their surfaces in SBF [12,35], they have no inherent bioactivity according to the previously discussed definition [36]. Moreover, they even decrease the pH in the surrounding tissues, release F^- and Al^{3+} which can make them cytotoxic in certain applications, leading to a restricted use in dental applications and ENT surgery [22,37]. Incorporation of Ap crystals, devitrification of ASG to Ap or incorporation of highly bioactive and biocompatible BAGs may thus overcome these problems [22,30].

However, the hypothesis that BAG-GICs improve mechanical properties seems to be false, at least on the short term, since research conducted by Yli-Urpo et al. showed the incor-