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Original Article

The effect of fibrin sealant on bioactive glass S53P4 particles – pH impact and dissolution characteristics *in vitro*

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ABSTRACT

Fibrin glue, a two-component tissue adhesive, has a range of clinical indications. Bioactive glass (BG) S53P4 has been approved for clinical use in several craniomaxillofacial and orthopedic applications. Although sometimes used simultaneously, there is no data available regarding the possible interaction of these two biocompatible substances. In this *in vitro* study, using a BG particle concentration of 4 mg/ml, a 0.4 unit pH increment ($p < 0.001$) was observed in simulated body fluid (SBF) after a 7-day incubation period. The addition of fibrin glue (0.13 g, SD 0.04; or 3.7 mg/ml) on top of the BG particles raised further the pH by 0.5 units ($p < 0.001$). The difference between these groups was statistically significant ($p = 0.008$). With a BG concentration of 25 mg/ml and a fibrin glue concentration of 18 mg/ml during a 14-day incubation period, a pH increment of 0.6 units and SBF ion concentration change of Ca, K, Mg, Na, P and Si ions was seen. Moreover, a penetration depth between 4 and 6 mm was observed when fibrin glue was applied on top of a bed of BG particles. Conclusions: Fibrin glue is not likely to have a distracting effect on BG-induced pH increase of the SBF although it might delay early BG surface reactions based on ion concentration measurements. Fibrin glue penetrated to the interparticle space to some extent, binding the particles together for easy clinical use of BG.

1. Introduction

Fibrin sealant or fibrin glue, is a two-component tissue adhesive consisting of fibrinogen and thrombin. It has a variety of clinical indications including hemostasis, colonic sealing and skin graft attachment [1]. Additional, clinical and experimental uses are being continuously developed, and the current literature on fibrin sealant exceeds 4900 indexed articles.

Various types of bioactive glass (BG) and bioactive glass ceramics have been in clinical use since the 1980's [2,3]. BG S53P4 has been approved for clinical use in Europe (European conformity CE-mark) and in USA (US Food and Drug administration approval, 510k clearance) for several craniomaxillofacial and orthopedic applications. As a bone cavity filling material, bioactive glass is biocompatible [4], induces new bone formation [5] and has significant antibacterial effects [6–9]. Antibacterial properties are related to the pH increase near the BG particles as well as increased alkali metal and alkali earth metal ion concentration, released from the BG particles [8].

When the treatment of a challenging chronic middle ear infection requires canal wall-down mastoidectomy [10], it is sometimes necessary to fill the bony cavity with a suitable material. When bioactive glass S53P4 particles are used as a filling material, BG particles can be used as such moistened with physiological saline solution before application, or in tandem with fibrin glue. The latter method is currently preferred by ear surgeons, head and neck surgeons in order to bind the BG particles and allow an easy clinical application of the material [11–14].

Although a favourable osteoinductive interaction between fibrin glue and one type of BG has been shown by Abiraman et al. in a mouse model [15], the data collected via *in vivo* experiments

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examining whether using BG together with fibrin glue is or isn't of benefit, has been controversial [16]. Only recently, new data has been published by Zazgyva et al. to support the use of fibrin glue with BG S53P4 [17]. To our knowledge, there is no data available addressing the absorption characteristics of fibrin sealant used on top of BG particles. Also, considering that BG slowly dissolves, creating a rise in pH of the surrounding liquid environment which facilitates its antibacterial effects [18], there is a need to understand the possible impact of using fibrin glue simultaneously with BG not only on pH values but also on ion concentration of the surrounding environment. The purpose of this study was to test a hypothesis that the use of fibrin glue with BG S53P4 particles does not have a negative influence on the pH change of simulated body fluid.

2. Materials and methods

The fibrin glue (Tisseel Duo Quick, Baxter AG, Vienna, Austria) used in this study is a two-component sealant made of pooled human plasma. The active ingredients consist of human fibrinogen with fibrinolysis-delaying synthetic aprotinin (sealer protein solution) and thrombin (thrombin solution). After the frozen, pre-filled syringes are warmed, preferably to a 33–37 °C temperature using a water bath or an incubator, the product is ready for application.

The BG S53P4 particles used in this study were produced by BonAlive Biomaterials Ltd., Turku, Finland. Particle size Varies between 0.5 and 0.8 mm and the manufacturer lists the following composition by weight for BG S53P4 particles: silicon dioxide 53%, sodium oxide 23%, calcium oxide 20%, and phosphate pentoxide 4%.

2.1. Absorption test

The absorption depth of fibrin glue in between BG particles was studied by first creating a solid polyvinyl siloxane mould (Coltène® Lab-Putty, Coltène/Whaledent AG, Altstätten, Switzerland) for BG particles with a cylindrical hole of 5.0 mm in diameter and 10 mm in depth. Exactly 0.14 g of BG granules, moistened with a physiological saline solution, were applied tightly inside each mould. Fibrin glue was then incubated in water to temperature of either 9, 21 or 37 °C and a drop of fibrin glue was applied on top of the BG-particle layer covering the whole particle bed surface. As BG particles filled the moulds completely, the thickness of each particle bed before fibrin glue application was approximately 10 mm. The BG–fibrin glue-combination was left to solidify for 24 h at 21 °C room temperature. The penetration depth of fibrin glue was assessed by removing the solid BG–fibrin glue-piece from the mould and releasing all loose particles from the piece, followed by the height measurement of the solid piece. This measurement was used to indicate the penetration depth of fibrin glue into the interparticle space of BG particles. These solid particles were then examined and photographed using light microscopy, and at this stage, the maximum BG–fibrin glue-combination thickness was measured. The penetration depth was measured for fibrin glue temperatures of 9, 21 and 37 °C, using two samples at each temperature.

2.2. pH test

To investigate the effect of fibrin glue on pH values in a liquid environment, a two-stage-protocol was used. Using a XS105 Dur–laboratory scale (Mettler Toledo, United States), 0.14 g (SD 0.00) of BG S53P4 particles were weighed for each six test tubes. Then 35 ml of simulated body fluid (SBF) was added, prepared according to the Kokubo protocol [19], with a seventh control test tube containing only SBF without BG. Using a Grant OLS200 shaking incubator (Grant Instruments, United Kingdom) test tubes were kept at 37 °C with a 100 rpm shaking frequency. For each test tube, SBF pH values were measured at 21 °C room temperature after 1, 2, 3, 4 and 7 days, with a PHM220 Lab pH Meter (Radiometer, Copenhagen, Denmark). This protocol represented a control series, where only BG was tested in SBF without fibrin glue.

In a second series, six samples of BG S53P4 particles, weighing 0.14 g (SD 0.00) per sample as well, in tandem with 0.13 g (SD 0.04) of fibrin glue, were tested as in the previous description. A feature of fibrin glue is its rapid coagulation on the tip of the application cannula, and consequently the exact dosing and direct weighing presented a challenge. The amount of used fibrin glue was determined by weighing the two-syringe system before and after application and thus calculating the weight difference. The temperature of the SBF test tubes was also maintained at 37 °C and pH values were measured accordingly. In addition, as a control, two samples of fibrin glue alone without BG were incubated in the same manner to see whether the glue by itself had any effect on SBF pH.

2.3. Ion dissolution test

Dissolution characteristics of BG–fibrin glue-combination were determined for 22 BG–fibrin glue-samples. Using a similar protocol as described above, a cylindrical mould was created for each sample, 8 mm in diameter and 6 mm in height, and 0.25 g of BG S53P4 particles (SD 0.00) were weighed into each mould. An average of 0.18 g of fibrin glue (SD 0.04), warmed to 37 °C temperature was applied on top of each sample and these BG–fibrin glue-mixtures were left to solidify under a 0.125 mm thick Mylar® polyester film for 18 h at 21 °C room temperature. Each sample was then immersed in 10 ml of SBF and kept in shaking incubator for up to 14 days in the same manner previously described.

After incubation, concentration of calcium, potassium, magnesium, sodium, phosphorus and silicon ions in the solution were measured for samples immersed for 2, 5, 9 and 14 days, using inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 5300 DV, United States). Weights of the BG–fibrin glue-combinations were measured by collecting the solid sample and any detached BG particles from SBF for this purpose. In addition, pH values (37 °C) were measured at 0, 1, 2, 4, 5, 6, 7, 8, 12 and 14 days.

2.4. Statistical analysis

Statistical analysis was performed using SPSS Statistics software (IBM Corporation, New York, United States). The comparison of the daily SBF pH change within and between the two groups (BG only versus BG and fibrin glue) in the 7-day-incubation experiment was performed with repeated measures analysis of variance (rm ANOVA). The statistical significance was set at the $p < 0.05$ level.

3. Results

The measured penetration depths of fibrin glue within BG particles were 4.2 and 6.4 mm for 9 °C fibrin glue; 3.2 and 5.6 mm for 21 °C fibrin glue and 3.72 and 4.05 mm for 37 °C fibrin glue, respectively (Fig. 1).

When only fibrin glue was kept in SBF, no change in pH was observed as pH values stayed between 7.48 and 7.49 for both samples at 1, 2, 3, 5 and 7 days. In contrast, when immersing only BG S53P4 particles in SBF, the pH rose continuously from the initial average value of 7.6–8.0 (SD 0.1), measured after seven days of incubation (Table 1). The pH change was statistically significant ($p < 0.001$).

When incubating BG S53P4 particles together with fibrin glue, the average pH of the solution increased after seven days from 7.5