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Bovine serum albumin release from novel chitosan-fluoro-aluminosilicate glass ionomer cement: Stability and cytotoxicity studies

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ABSTRACT

Objective: This study aimed to evaluate the effect of adding chitosan (CS) to conventional glass ionomer cement (GIC) on protein release and its cytotoxicity.
Methods: Bovine serum albumin (BSA) was used as the released protein from two glass ionomer formulations. One (GIC + BSA) contained fluoro-aluminosilicate glass mixed with BSA, and another (GIC:CS + BSA) used a similar glass and BSA with 20% chitosan. Six disc specimens per group (10 mm in diameter, 2 mm in height) were prepared and placed in phosphate buffer saline, which was replaced at various times over 2 weeks. The released protein was determined by a BCA assay. Cytotoxicity of the extracts from these materials for 1, 2 and 7 days to dental pulp cells was evaluated using MTT assay.
Results: The GIC:CS + BSA released a burst of BSA in the first 6 h, and slowly released at different rates over the 2 weeks. GIC + BSA showed a similar result, but protein could not be detected at the 12 h. The protein release rate of GIC:CS + BSA was significantly greater than GIC + BSA ($P < 0.01$); nearly three times higher. The released BSA had the same molecular weight as evaluated by SDS-PAGE. From the MTT assay, the percentages of viable cells were significantly different and can be arranged as: GIC:CS + BSA > GIC:CS > GI + BSA > GI and the cytotoxicity was increased by time of extraction.
Conclusion: Chitosan added in glass ionomer cement can prolong release of BSA as well as not increasing the toxicity to pulp cells. This material may be useful for protein delivery.

1. Introduction

Glass ionomer cements (GIC) have been used in various dental and medical applications,[1] primarily due to their biocompatibility, antibacterial properties, ion leachability, and capacity to bond to bone, enamel, dentine and metals.[2–4] GIC are products of an acid–base reaction between polyalkenoic acids, mainly poly (acrylic acid), and fluoro-aluminosilicate glass.[5] This biomaterial has long been known to prolong the release of fluoride[6]; however, it's potential to increase the property of sustained release of protein has not been previously investigated. Biomaterials that can sustain the release of protein such as growth promoting factors or bioactive molecules may have a number of applications, including tissue engineering and vital pulp therapy.[7,8] There have been some studies involving material that can control the release of growth

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factor; for example, fibroblast growth factor 2 (FGF2) from hydrogel[9] and poly lactic-co-glycolic acid (PLGA) microparticles containing transforming growth factor beta1 (TGF β1), as pulp capping to promote dentin–pulp complex regeneration.[10]

Many studies have shown that the interpolymer complex from the reaction of poly (acrylic acid) and chitosan can generate polyionic complexes, which can be used to prolong drug delivery.[11–13] Chitosan (CS) is a deacetylation product of chitin, a high molecular weight natural polymer found in shells of marine crustaceans and cell walls of fungi. Chitosan is a co-polymer of glucosamine and N-acetyl-D-glucosamine. It is a biodegradable polymer used in various biomedical and pharmaceutical applications due to its biocompatibility and the slow release of active molecules.[14–17] This polymer is one of the materials that is also widely used in controlled drug release.[18] Chitosan might have the potential to increase the release of proteins. Recently, a study examined the addition of a minute amount of chitosan in the liquid part of GIC and its investigated flexural strength and fluoride release. It was found that adding only 0.0044 wt% of chitosan can increase flexural resistance and increase the amount of fluoride released compared to commercial GIC.[19]

The hypothesis of this study is that because poly (acrylic acid) can react both with chitosan and fluoro-aluminosilicate glass, the reaction of these three components will lead to the new material that may have both the properties of GIC and the prolonged release from the polyelectrolytes complexes. Therefore, it would be interesting to incorporate chitosan into conventional GIC in order to potentially increase the property of sustained release of proteins. Bovine serum albumin (BSA) was used as a model for this protein delivery system, because of its safety and ease of evaluation; many studies involving protein release from polymer materials also selected BSA.[20,21] BSA was also suggested for sustained release of growth factors,[22] so that any material introduced can control the release of BSA, may prolong the release of growth factors or other bioactive molecules. The aim of this study was to investigate the pattern of BSA release from chitosan modified glass ionomer cement, as a model of protein release, and also to evaluate the cytotoxicity of this material to dental pulp cells.

2. Materials and methods

2.1. Materials and specimen preparation

The specimens were divided into four groups (GIC + BSA, GIC:CS + BSA, GIC, GIC:CS) according to various formulations of the materials. Each group composed of six specimens, except GIC, which had three specimens. Two experimental glass ionomer formulations were used throughout this study. One (GIC + BSA) contained standard fluoro-aluminosilicate glass mixed with 1.5% of BSA by weight (analytic grade, Sigma Chemical, St Louis, MO, USA) and another formula (GIC:CS + BSA) used the same glass and BSA, to which was added 20% of chitosan by weight (Fluka, Steinhein, Switzerland). This chitosan was of a low molecular weight type (470 kDa) with 80% deacetylation. Glass ionomer cement used in this study was a conventional type III material (GC lining™, GC Corporation, Tokyo, Japan). The initiation of the chemical reaction was achieved by the addition of 40% poly (acrylic acid). An original commercial glass ionomer cement (GIC) acted as control material, while GIC added with 20% of chitosan (GIC:CS) was used as the comparator.

Disc specimens (10 mm in diameter, 2 mm in height) of the glass ionomer cement were prepared using a polytetrafluoroethylene (PTFE) mould. The powder was mixed with poly (acrylic acid) at the P/L ratio (1.16:1 by weight) and at a room temperature of 22 ± 3 °C. The cement was then packed into the PTFE mould using a mixing spatula. A polythene sheet and glass slide was then placed over the filled mould after which light hand pressure was applied. Specimens were retained in the mould during storage in an incubator at 37 °C for 1 h. After storage in an incubator, the specimens were removed from their moulds and then each specimen was weighed to an accuracy of 0.0001 g using a digital balance (Sartorius MC210, Germany) and the mean weight of each group was calculated. After weighing, each specimen was stored at 37 °C in individual pots containing 1 mL of phosphate buffer saline (PBS) of pH 7.4. The storage pot was continually shaken (50 rpm) in an incubator. All storage solution was replaced with a similar volume of fresh PBS at 1, 6, 12, 24, 48, 168 and 336 h. The protein concentration of the storage solution was measured at the time of commencing.

2.2. Protein analysis

Prior to protein release measurement, the stored PBS solution was concentrated 10 times using a freeze dryer (Freeze dryer ALPHA 2-4, Martin Christ, German), and protein concentration was determined by a BCA protein assay kit (Pierce Biotechnology Inc., Rockford, IL, USA). This method was based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. The assay was performed using a 96-well microplate. Briefly, 10 μL of each sample, as well as standard BSA ranging from 25 to 2000 μg/mL in PBS, was added in each well and mixed with 200 μL of the working reagent, which was prepared following the procedure provided by the company and incubated for 30 min at 37 °C before reading the absorbance at 570 nm with a microplate reader (Titertek Multiscan Plus MK II, Flow Laboratories International SA).

The molecular weight of the released protein was also investigated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with 12.5% (v/v) of acrylamide for separating gel and 4.5% (v/v) of acrylamide for stacking gel. Protein was stained with 0.1% (w/v) of Coommasie Brilliant Blue R250. SDS-PAGE molecular weight standard (Gibco, Invitrogen Corporation, Grand Island, NY, USA) ranging from 2.5 to 200 kDa and BSA standard were used as a marker and control, respectively.

2.3. Cell culture

Pulp cells were cultured from a normal human third molar from an adolescent patient aged about 18 years at the Dental Hospital, Faculty of Dentistry, Prince of Songkla University, with the approval of the Research Ethics Committee, Faculty of Dentistry, Prince of Songkla University (No. 0521103/0710). All patients gave informed consent for donation of cells. Primary culture of pulp cells was performed using an enzymatic