

# A glass polyalkenoate cement carrier for bone morphogenetic proteins

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**Abstract** This work considers a glass polyalkenoate cement (GPC)-based carrier for the effective delivery of bone morphogenetic proteins (BMPs) at an implantation site. A 0.12 CaO–0.04 SrO–0.36 ZnO–0.48 SiO<sub>2</sub> based glass and poly(acrylic acid) (PAA, Mw 213,000) were employed for the fabrication of the GPC. The media used for the water source in the GPC reaction was altered to produce a series of GPCs. The GPC liquid media was either 100 % distilled water with additions of albumin at 0, 2, 5 and 8 wt% of the glass content, 100 % formulation buffer (IFB), and 100 % BMP (150 µg rhBMP-2/ml IFB). Rheological properties, compressive strength, ion release profiles and BMP release were evaluated. Working times ( $T_w$ ) of the formulated GPCs significantly increased with the addition of 2 % albumin and remained constant with further increases in albumin content or IFB solutions. Setting time ( $T_s$ ) experienced an increase with 2 and 5 % albumin content, but a decrease with 8 % albumin. Changing the liquid source to IFB containing 5 % albumin had no significant effect on  $T_s$  compared to the 8 % albumin-containing BT101. Replacing the albumin with IFB/BMP-2 did not

significantly affect  $T_w$ . However,  $T_s$  increased for the BT101\_BMP-2 containing GPCs, compared to all other samples. The compressive strength evaluated 1 day post cement mixing was not affected significantly by the incorporation of BMPs, but the ion release did increase from the cements, particularly for Zn and Sr. The GPCs released BMP after the first day, which decreased in content during the following 6 days. This study has proven that BMPs can be immobilized into GPCs and may result in novel materials for clinical applications.

## 1 Introduction

Bone morphogenetic proteins (BMPs) are a group of growth factors (GFs) that are members of the transforming growth factor  $\beta$  (TGF $\beta$ ) superfamily [1]. BMPs can also be defined as cytokines that are significant in bone formation and healing. An example includes the recombinant human BMPs (rhBMPs) which are widely used in tissue engineering for the complete regeneration of cartilage or bone [2, 3]. Several BMPs influence osteoprogenitor cell migration, proliferation and differentiation [1, 4]. Among them, BMP-2 has been identified as the most potent and has been approved for clinical use in spinal fusion, long bone non-union healing and alveolar ridge augmentation [5–10].

Carriers, also known as scaffolds, can be used to deliver BMPs into a targeted area in order to aid bone healing. Seeherman and Wozney [11] define successful carriers in terms of their biocompatibility, resorbability and ability to allow regenerative tissue forming cells into the area to proliferate and differentiate. Ideally when a carrier is implanted, the exogenous BMP concentration needs to be above the critical minimum required for a positive host response for a sufficient time, as to allow rapid cell

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population and to generate a positive tissue response [11, 12]. The exogenous BMP release profiles are dependent on the properties of the carrier, and as numerous release profiles exist; the carrier design is important.

BMPs can be immobilized into different carriers by three main techniques: (1) adsorption, (2) entrapment or (3) covalent bonding. The latter is the most preferred technique since adsorption results in conformational changes associated with less sustained release while entrapment may result in denaturation of the protein (disrupting the structure of the protein and un-coiling it into a random shape) due to pH or temperature changes during material processing [13, 14]. Current carriers can be divided into natural polymers, inorganic materials, synthetic polymers and their co-polymers with some examples of these being calcium phosphates [15, 16], phosphate-based cements [17], poly(lactic-co-glycolic acid) [18], lipid based composites [19], bio gelatin microparticles [20] and silica nanotubes [21]. Additionally, allograft and autograft materials have also been used [22, 23]. The most common carriers are collagen sponges, however they are unable to retain their shape against the compressive forces that they experience after implantation, preventing cell ingrowth and only weakly binding to the BMP which may result in a large bolus release of the BMP from the carrier within hours. This “burst profile” results in the need to apply large amounts of BMP to ensure sufficient concentrations remain during the healing period [10]. These supraphysiological doses have been reported to elevate cancer risk when released in very large amounts [24–26], alongside stimulating an immune response [27, 28]. However initial burst profiles have been shown to aid in the signalling of BMP response cells [29].

Glass polyalkenoate cements (GPCs) were first developed by Wilson and Kent in the 1970s. The original GPCs consisted of a matrix of *fluoro-alumino-silicate* glass, a base, mixed with an aqueous solution of poly(acrylic acid) (PAA), the acid phase. The matrix is obtained by an acid–base reaction between both components in the presence of water, where the protons from the acid rapidly attack the glass network resulting in degradation and release of the glass ionic components. Thereafter, the released cations chelate and crosslink the polymer chains to form a complex composite (cement) that matures with the presence of water as a medium [30, 31].

It is proposed that an injectable GPC may have the potential to deliver BMPs to a specific surgical site and subsequently release them while the GPC is setting, and throughout the cement’s maturation. However only a limited number of studies investigating the effects of incorporating organic molecules on the properties of GPCs have been conducted. Wren et al. [32] investigated the effects of the addition of biological substances including chitin,

collagen, cysteine and keratin to an aluminium free Ca–Sr–Zn–SiGPC and reported that these proteins had little influence on the working and setting times of the GPCs; although compressive strength was found to decrease post incorporation. Furlan et al. [33] investigated the effects of grafting PAA carboxylic groups with organic molecules such as chitin on the metal binding ability of calcium ( $\text{Ca}^{2+}$ ) ions. They have recommended the use of chitin-PAA copolymer in the preparation of GPCs due to the improved ability of such polymer to adsorb and bond to  $\text{Ca}^{2+}$  ions.

The work contained herein investigates the feasibility of a CaO–SrO–ZnO–SiO<sub>2</sub> based GPC as a carrier for BMPs by evaluating the time dependent physical, mechanical and biological properties of composites formulated from this GPC loaded with BMP-2 ligands.

## 2 Materials and methods

### 2.1 Glass synthesis

A 0.12 CaO–0.04 SrO–0.36 ZnO–0.48 SiO<sub>2</sub> glass, hereby known as BT101, was formulated by weighing out appropriate amounts of analytical grade reagents (Sigma-Aldrich, Canada) and ball milling (1 h). The mixture was then oven dried (100 °C, 1 h), fired in a platinum crucible (1500 °C, 1 h) and shock quenched in water. The resulting frit was dried, ground and sieved to retrieve a glass powder with a maximum particle size of 45 µm. The glass was then annealed (640 °C, 3 h to relieve internal stresses within the glass network) and used for cement production.

### 2.2 Cement preparation

PAA (Mw, 213,000) was supplied by Advanced Healthcare Limited (Kent, UK). The cements were formulated in a powder: liquid (P/L) ratio of 1:0.75, i.e. 1 g of glass powder was mixed with 0.37 g PAA and 0.37 ml of liquid. The liquid portion was varied between 100 % distilled water, 100 % formulation buffer (IFB; 2.5 % glycine, 0.37 % glutamic acid, 0.01 % sodium chloride, 0.5 % sucrose and 0.01 % Tween 80, pH 4.5) and 2, 5 and 8 % (of the total glass content) albumin in IFB and 100 % BMP (150 µg rhBMP-2/ml IFB). Thorough mixing of these samples was achieved within 35 s in ambient temperature (23 ± 1 °C). Table 1 outlines the formulations tested.

### 2.3 Working time

The working time ( $T_w$ ) of five samples per formulation were measured in ambient air using a technique outlined in ISO 9917-1:2007. The  $T_w$  is defined as the time from the

**Table 1** Formulation table showing the changing liquid portion of the cement samples

Formulation number	Albumin (%)	Distilled water (%)	IFB (%)	BMP-2 (%)
1 (Control)	0	100	0	0
2	2, 5, 8	100	0	0
3	5	0	100	0
4	0	0	0	100

*IFB solution:* 5.0 mg sucrose, NF; 25 mg glycine, USP; 3.7 mg L-glutamic acid, FCC; 0.1 mg sodium chloride, USP; 0.1 mg polysorbate 80, NF; 1.0 mL of sterile water (WFI) and a pH of  $4.4 \pm 0.1$

start of mixing, through which the material can be manipulated without having an adverse effect on its properties.

## 2.4 Net setting time

The net setting time ( $T_s$ ) of five samples per formulation were tested in ambient air according to ISO 9917-1:2007.

## 2.5 Compressive strength

The compressive strength ( $\sigma_c$ ) of five samples per formulation, one day post mixing, was evaluated in ambient air according to ISO 9917-1:2007. Five cylindrical samples (6 mm height, 4 mm diameter) were tested after being incubated in distilled water (37 °C, 1 day). Testing was undertaken on a United Universal Tester (STM-50KN, United Testing Systems, Inc., Huntington Beach, CA, USA) using a  $\pm 2$  kN load cell at a crosshead speed of  $1 \text{ mm} \cdot \text{min}^{-1}$ .

## 2.6 Statistical methods

One-way analysis of variance (ANOVA) was used to analyze the data. *Post-hoc* Bonferroni test was used to compare the relative means and to report the statistically significant differences when  $P < 0.05$ . Statistical analysis was performed using statistical package for the social sciences (SPSS) software (IBM SPSS statistics 21, IBM Corp., Armonk, NY, USA).

## 2.7 Ion release

Ion release studies were performed according to a method described by Wren et al. [34]. The ion release profiles ( $n = 5$  samples) were measured after 1-day maturation using an Agilent 4100 (Agilent Technologies, Inc., Santa Clara, CA, USA) microwave plasma-atomic emission spectrometer (MP-AES). MP-AES calibration standards for Si, Sr, Ca, and Zn were prepared from a stock solution on a gravimetric basis. Three target calibration standards were prepared for each ion with 0.3, 0.5 and 1.0 parts per million (ppm) concentrations while distilled water was

used as a *blank*. Samples for Ca, Sr, and Zn analysis were diluted in a ratio of 1:10 while samples for Si analysis were diluted in a ratio of 1:30. A pilot study was conducted to determine the appropriate ratio for dilution of all elements.

## 2.8 BMP release studies

Samples of the GPCs loaded with BMP were placed into an Eppendorf tube ( $n = 5$ ). 1 mL of phosphate buffered saline +0.1 % bovine serum albumin (PBS + BSA) was added to each tube and the tubes were closed and held at 38 °C. After 1 day the PBS + BSA was removed and replaced with a further 1 mL of fresh PBS + BSA. This was incubated for a further 6 days and then removed. The PBS + BSA samples were stored at  $-20$  °C until ready for analysis.

BMP-2 concentrations were determined using an enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Quantikine ELISA Kit; R&D Systems Inc. Minneapolis, MN). The amount of BMP released was normalized to the weight of GPC in each tube.

## 3 Results

GPCs were formulated in line with the methods section and evaluated physically, mechanically and biologically.

### 3.1 Working and setting times

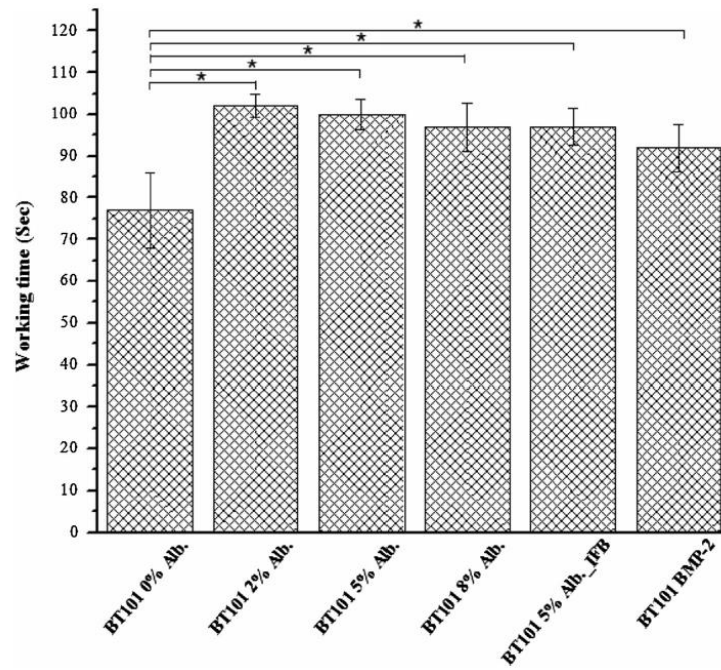
The  $T_w$  and  $T_s$  for BT101 cement formulations with PAA and distilled water (i.e. 0 % albumin (Alb.)), distilled water with 2, 5 and 8 % Alb., IFB with 5 % Alb. and BMP-2 solution are presented in Figs. 1 and 2, respectively.

$T_w$  shows a statistical increase in the BT101\_2 % Alb samples, with an increase from 77 to 102 s. Increasing the albumin content to 5 and 8 %, and subsequently changing the water component to IFB with albumin and then IFB with BMP-2 resulted in no statistical difference ( $P < 0.05$ ) in  $T_w$ . However all samples are statistically different compared to the *Control* sample, BT101 0 % Alb.

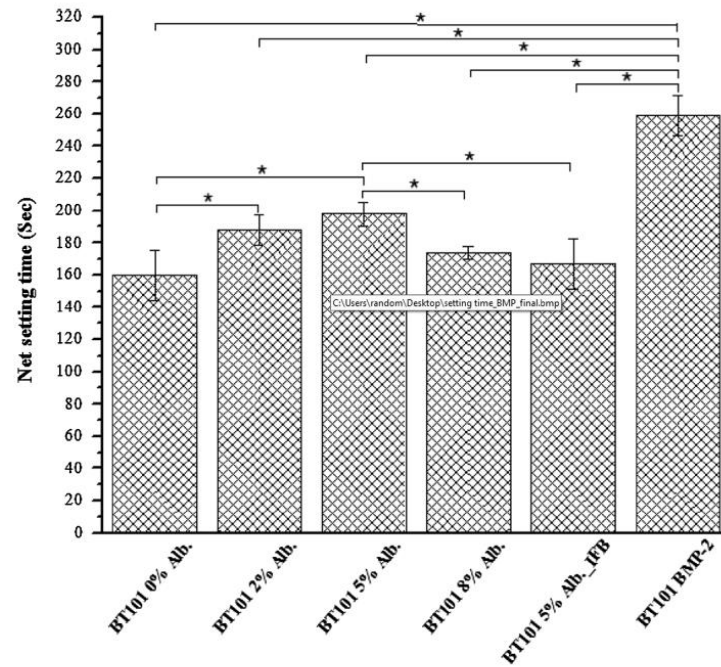
$T_s$  experiences a statistical increase from 160 to 188 s with 2 % albumin additions to the water source, which



**Fig. 1** Working times for BT101 glasses mixed with different albumin (Alb.) loadings (0, 2, 5, 8 wt%), IFB with 5 % Alb. and BMP-2 solution. *Stars* and *bars* show statistical significance ( $P < 0.05$ )



**Fig. 2** Setting times for BT101 glasses mixed with different albumin (Alb.) loadings (0, 2, 5, 8 wt%), IFB with 5 % Alb. and BMP solution. *Stars* and *bars* show statistical significance ( $P < 0.05$ )



increases to 198 s for the BT101 5 % Alb samples, although there is no statistical difference between the 2 and 5 % Alb samples. Introducing 8 % albumin results in a decrease in  $T_s$  down to 174 s which is significantly different to the previous BT101 5 % Alb samples, but not compared to the Control BT101 0 % Alb. This decrease in  $T_s$  continues when the water source is replaced with 5 % Alb in 100 % IFB solution, giving a  $T_s$  of 167 s. Removing the 5 % albumin from the IFB and replacing with BMP-2 proteins results in a statistically significant jump in  $T_s$  to 259 s.

### 3.2 Compressive strengths

The compressive strength data for BT101 cements mixed with 0 % Alb., 5 % Alb., IFB with 5 % Alb., and BMP-2 are presented in Fig. 3. Testing was conducted 1 day post cement preparation.

BT101 samples displays a mean strength of ~24 MPa, which decreases down to ~15 MPa with the addition of 5 % Albumin. Replacing the water source for the cement reaction with IFB solution increases the mean strength to ~22 MPa. This mean strength increases to ~30 MPa with the replacement of the 5 % albumin with BMP-2 in the IFB solution. One-way ANOVA shows that there is only a significant difference between the BT101 5 % Alb samples and the BT101\_BMP-2 samples.

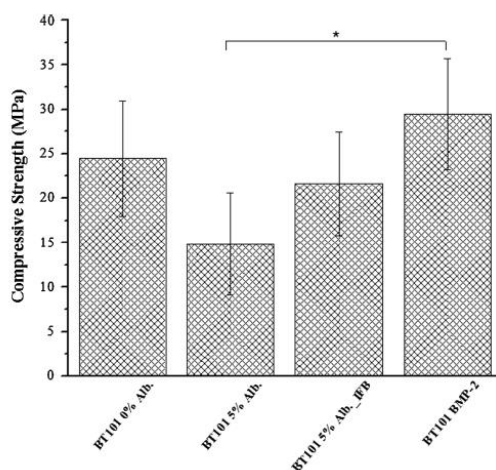


Fig. 3 One day compressive strengths of BT101 glasses mixed with different albumin (Alb.) loadings (0, and 5 wt%), IFB with 5 % Alb. and BMP solution. Stars and bars show statistical significance ( $P < 0.05$ )

### 3.3 Ion release profiles

The ion release profiles for the BMP-2-containing BT101 GPCs were tested, 1d post cement preparation and compared to BT101 GPCs and blank water samples. Figure 4 presents the results obtained. Figure 4 shows that the incorporation of BMP-2 into BT101 GPCs resulted in increased dissolution of all cations when compared to the BT101 or water blank samples. Si levels were substantially higher when released from the BMP-2 containing samples (19.6 ppm) compared to the blank (1.9 ppm). Sr was released at a rate five times higher in the BMP containing samples (3.9 ppm) than the blank (0.1 ppm). Ca also released at slightly higher rates from the BMP-2 containing samples (3.6 ppm) than the blank (0.54 ppm). Similarly, Zn released at slightly higher rates from the BMP-2 containing samples (3.85 ppm) when compared to the blank (0.19 ppm).

### 3.4 BMP release

Table 2 displays the amounts of BMP released at 1 and 7 days, post cement preparation and incubation with PBS + BSA at 37 °C. After 1d the GPCs released a mean amount of  $1.01 \pm 0.36$  ng BMP-2/g GPC. Over the following 6 days a further 1.67 ng/g were released.

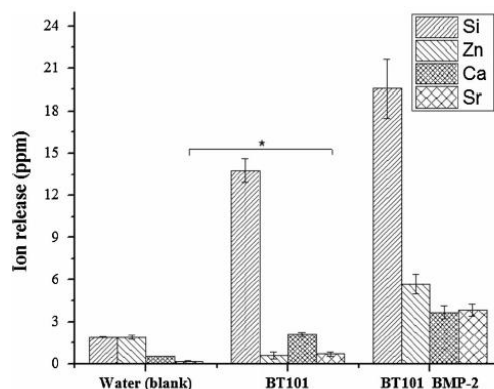


Fig. 4 One day ion release profiles for water (blank sample), Control BT101 and BMP-2 loaded BT-101 cements (ppm). Stars and bars show statistical in-significance ( $P < 0.05$ ). All  $P$  values were  $< 0.05$  except for Sr tested for water (blank) and BT101 cement samples

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