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## Compositional changes to brushite cements to improve mechanical and antibiotic-delivery properties



Claudia Morilla Espino <sup>a</sup>, Louise van der Weerd <sup>a,d</sup>, Lioe-Fee de Geus-Oei <sup>a,e,f</sup>, Jeroen JJP van den Beucken <sup>b,c,\*</sup>

<sup>a</sup> Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands

<sup>b</sup> Radboudumc, Department of Dentistry - Regenerative Biomaterials, Nijmegen, the Netherlands

<sup>c</sup> Research Institute for Medical Innovation, Radboudumc, Nijmegen, the Netherlands

<sup>d</sup> Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands

<sup>e</sup> Biomedical Imaging Group, University of Twente, Enschede, the Netherlands

<sup>f</sup> Department of Radiation Science and Technology, Delft University of Technology, Delft, the Netherlands

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### ABSTRACT

Brushite calcium phosphate cements (bCPCs) are promising synthetic bone substitutes due to their bioactivity, osteoconductivity, and controlled degradation. However, their clinical use is limited by poor handling, low mechanical strength, and insufficient antimicrobial capability. This study explores the incorporation of  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) and silk fibroin (SF) to enhance bCPC performance and enable localized antibiotic delivery using tetracycline (TC). Adding  $\alpha$ -TCP improved compressive strength (up to 2.65 MPa), while SF in liquid or fiber form reinforced the matrix. The inclusion of TC and SF extended setting times (4–7 min initial; 7–30 min final) and maintained injectability (up to 50 %). Drug release studies showed a 10 % burst in the first 24 h, with a sustained release of 77 % over 14 days. These multifunctional bCPCs offer injectable, resorbable, and antibacterial properties, making them suitable for non-load-bearing bone repair applications.

### 1. Introduction

The global demand for dental, craniofacial, and orthopedic bone repair and regeneration has grown due to population aging and the increasing incidence of bone diseases and trauma-induced bone defects [1,2]. For instance, bacterial infections of bone tissue in the form of osteomyelitis or orthopedic implant-related infections account for substantial clinical cases requiring therapeutic interventions annually [3–5]. Especially orthopedic implant-related infections are likely to become a burden in the near future due to the increasing popularity of orthopedic implants and complexity of the involved surgical procedures. In case these bone conditions result in bone defects that do not heal spontaneously, effective treatment strategies are required. For this, the tissue engineering paradigm has emerged as a ‘toolbox’ to select scaffolds, growth factors, stem cells, or a combination thereof to enhance bone regeneration [6]. Scaffolds, particularly calcium phosphate cements (CPCs), provide a robust scaffold component that can serve as a suitable environment for bone cell migration, proliferation, and differentiation while supporting defect reconstruction and promoting

osteogenesis [2]. CPCs are biocompatible, bioactive, injectable, and resorbable, making them especially suitable for complex crano- and maxillofacial applications where esthetics are critical. Conventional treatments for (local) bone infection extensive debridement combined with prolonged systemic antibiotic administration (via e.g. intravenous injection). However, these treatments often face challenges in terms of complete removal of bacteria and insufficient antibiotic levels at the infection site to effectively kill bacteria [7]. Emerging drug delivery systems utilizing CPCs offer sustained release of therapeutic agents while promoting osteointegration and reducing adverse side effects [7].

CPCs are mainly classified depending on the final product, i.e. apatitic CPCs (aCPCs) or brushite CPCs (bCPCs) [1]. Apatitic CPCs have  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) as the main precursors, and the reaction normally occurs under basic conditions ( $\text{pH} > 4.2$ ) resulting in the formation of apatite with high similarity to bone mineral [8]. However, the degradation of apatitic CPCs in physiological conditions is relatively slow [1,9]. In contrast, bCPCs have monocalcium phosphate monohydrate (MCPM) and  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) as their main precursors that form dicalcium phosphate dihydrate (DCPD; brushite) as

\* Corresponding author.

E-mail address: [jeroen.vandenbeucken@radboudumc.nl](mailto:jeroen.vandenbeucken@radboudumc.nl) (J.J. van den Beucken).

their end-product [9,10]. Both these types of CPCs have garnered attention for their use in orthopedic and craniofacial surgeries due to their distinctive properties. During their formation process, a CPC paste is obtained that solidifies in seconds to minutes [10]. bCPCs are known for their injectability, self-setting nature, high biocompatibility, osteoconductivity, and resorbability, which make them particularly suitable for precise applications like craniofacial defect repairs [1,11]. bCPCs normally form under acidic conditions ( $\text{pH} < 4.2$ ) and at physiological temperature ( $37^\circ\text{C}$ ), making them thermodynamically metastable and appealing for applications requiring material resorption and replacement by new bone [1]. The rapid resorption of bCPCs within the first weeks post-implantation is driven by disintegration, dissolution, and macrophage activity, which facilitates the removal of bCPC crystals and promotes osteoblast-driven bone formation [11].

Despite their appealing premises, bCPCs face several limitations that hinder their broad application. One significant limitation is the short setting time, which limits the time available for surgeons to apply and manipulate the material for adequate bone defect filling during surgical procedures [12]. Furthermore, their injectability is hindered by issues like liquid phase separation during injection [13,14]. Regarding mechanical properties, their compressive strength of  $\sim 1 \text{ MPa}$  [10] is much weaker than cortical ( $\sim 300 \text{ MPa}$ ; [15]) and even cancellous ( $\sim 1-15 \text{ MPa}$ ; [16,17]) bone; this mechanical mismatch limits their application in load-bearing scenarios [18]. To address these challenges, researchers have explored the incorporation of biopolymers such as chondroitin sulphate [19], chitosan [20], gelatine [21], and alginate [22,23]. These additives, even in small amounts (e.g. less than 1 wt %), have demonstrated improvements in the mechanical properties and anti-washout characteristics of bCPCs [22]. Further advancements include the addition of natural polymers like silk fibroin, which has shown significant potential in enhancing the mechanical properties of bCPCs [24]. Silk fibroin's robust mechanical properties, biocompatibility, and adjustable bioresorbability make it an attractive additive for modifying mechanical properties of bCPCs [24]. Its unique  $\beta$ -sheet structure introduces surface potentials that promote hydroxyapatite (HA) crystallization, mimicking the natural bone environment and improving the molecular, structural, and biological compatibility of the material [24]. In previous work, it was observed that silk fibroin accelerates the transformation of DCPD into HA [25], which could in principle further improve mechanical properties and promote cell growth [26]. More straightforward, also the addition of  $\alpha$ -TCP into a bCPC formulation would result in apatite formation and improvements of mechanical properties.

In view of bacterial bone infections, bCPCs are appealing due to their potential as delivery systems for drugs. Their intrinsic micro- and sub-microporous structure makes them appropriate carriers for active therapeutic agents. Consequently, previous work has explored bCPCs for the local delivery of drugs [27], antioxidants [28], growth factors [29], and antibiotics [27], offering a localized and controlled release of these agents [22]. Antibiotics have a pivotal role in the prevention of bacterial infections after any surgical intervention, particularly when applied locally. Notably, the conventional systemic administration of antibiotics has several side effects and complications. For this reason, utilizing bCPCs as a drug delivery system for antibiotics presents a promising solution, allowing for minimal, localized dosing while reducing systemic exposure [27,30].

We herein aim to improve the handling and mechanical properties of bCPC by adding  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) and silk fibroin (SF) into bCPC formulations, and explore different bCPC formulations regarding their function as a drug delivery system for the broad-spectrum antibiotic tetracycline (TC).

## 2. Materials and methods

### 2.1. bCPCs preparation

A basic bCPC formulation (monocalcium phosphate monohydrate,

**Table 1**  
Composition of experimental bCPC formulations.

Formulations (L/P = 0.35mg/ml of 8 wt. % solution of $\text{Na}_2\text{HPO}_4$ )	SF proportions (wt. %; added in liquid phase, fibers and microparticles, added in solid phase)	Tetracycline proportions ( $\text{mg}/\text{cm}^3$ ) (added in liquid phase)	Proportions of $\alpha$ -TCP (%) (added in solid phase)
M $\beta$	0	0	0
M $\beta$ -SO-T1	0	50	
M $\beta$ -S1-T0	1.50	0	
M $\beta$ -S1-T1	1.50	50	
$\alpha$ 10	0	0	10
$\alpha$ 10-SO-T1	0	50	
$\alpha$ 10-S1-T0	1.50	0	
$\alpha$ 10-S1-T1	1.50	50	
$\alpha$ 40	0	0	40
$\alpha$ 40-SO-T1	0	50	
$\alpha$ 40-S1-T0	1.50	0	
$\alpha$ 40-S1-T1	1.50	50	

MCPM, Sigma-Aldrich, Germany;  $\beta$ -tricalcium phosphate,  $\beta$ -TCP, CaP Biomaterials LLC, United States) was modified by the addition of  $\alpha$ -TCP (CaP Biomaterials LLC, United States) in the powder phase. SF was extracted from silk cocoons as described previously [31] and added into the liquid phase, when in liquid form. For the mechanical properties test the SF was also added as microparticles, obtained following the procedure described elsewhere [32], and fibers, obtained after lyophilization [31], and were added in the solid phase of the bCPC. These constituents were used to prepare a range of bCPC formulations (Table 1).

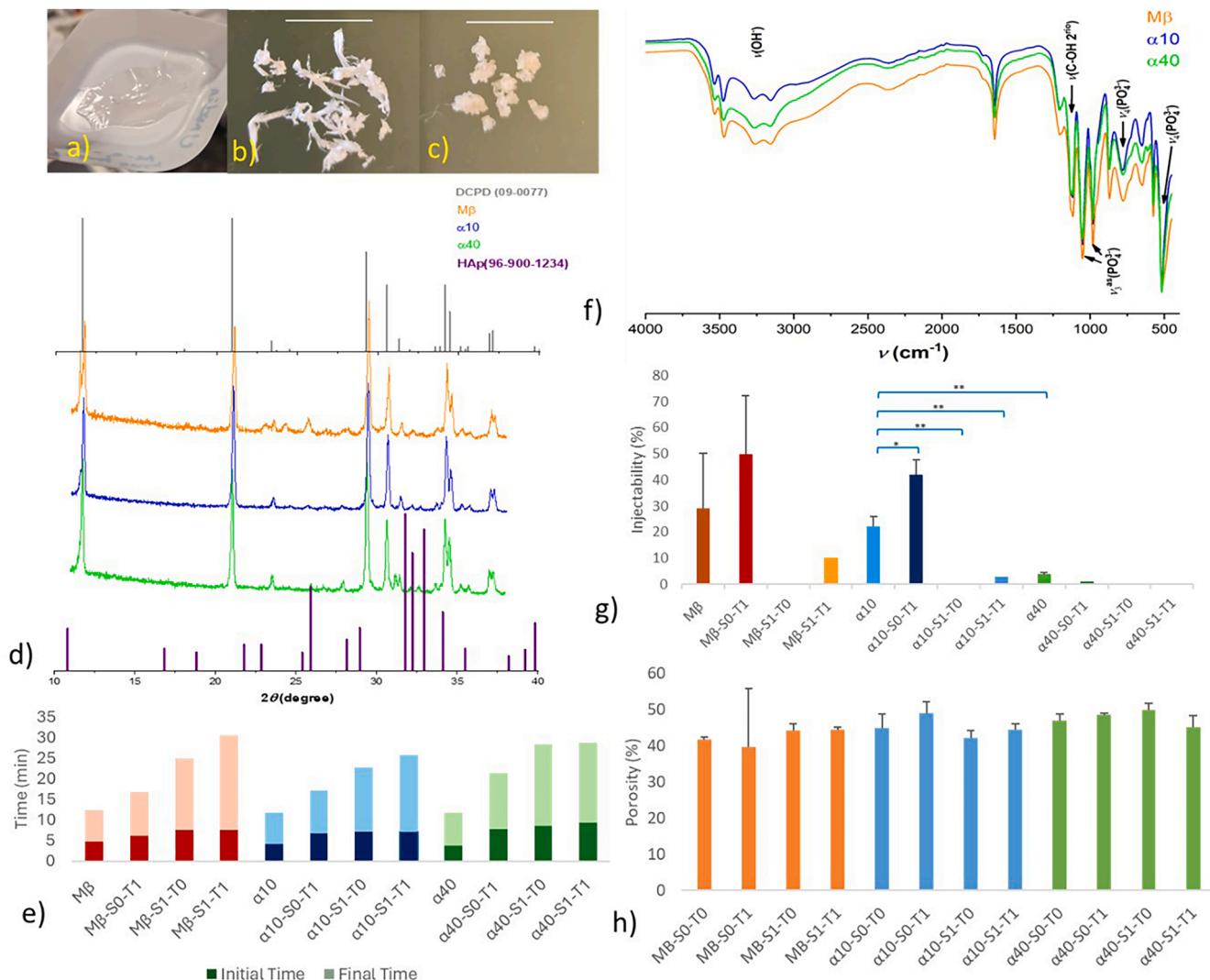
The formulations shown in Table 1 were designed as part of a full factorial experimental setup to systematically evaluate the individual and combined effects of adding  $\alpha$ -TCP, SF, and TC on the performance of the bCPCs. Three  $\alpha$ -TCP levels (0 %, 10 %, and 40 %) were selected to study its influence on mechanical reinforcement and phase transformation. SF was incorporated at 1.5 wt % [24] based on literature demonstrating its ability to enhance matrix cohesion and strength. TC was added at  $50 \text{ mg}/\text{cm}^3$  to assess the potential for local antibiotic delivery. The M $\beta$  formulation (lacking  $\alpha$ -TCP, SF, and TC) serves as the negative control, while the remaining formulations include all relevant single-variable and combination groups. This design ensures that the impact of each component can be evaluated independently and in synergy. Sample codes follow a consistent logic: “ $\alpha$ ” refers to  $\alpha$ -TCP percentage, “S” to the presence (S1) or absence (SO) of SF, and “T” to the presence (T1) or absence (T0) of TC. For example,  $\alpha$ 10-S1-T1 represents a formulation with 10 %  $\alpha$ -TCP, 1.5 % SF, and  $50 \text{ mg}/\text{cm}^3$  TC.

### 2.2. Physico-chemical material characterization

Crystallographic phase characterization was carried out by means of X-ray diffraction, in a Philips X'pert Modular Powder Diffractometer (PANalytical), RU200B diffractometer with  $\text{Cu-K}\alpha$  radiation ( $1.54059 \text{ nm}$ ). The scans were made in a  $2\theta$  angular interval of  $10-40^\circ$  and scanning speed of  $0.02^\circ/\text{min}$ . The results were interpreted using the X'Pert HighScore PANalytical program database, version 3.0 (PANalytical B. V. Almelo, The Netherlands).

Infrared spectra (FTIR) were obtained in a Fourier transform infrared spectrometer Shimadzu IR Tracer 100 (Japan), with a resolution of  $4 \text{ cm}^{-1}$  and 21 scans per sample in a range of  $400-4000 \text{ cm}^{-1}$ .

The total porosity of the bCPC formulations was determined by assessing the volume of water contained in the bCPCs pores relative to the total sample volume. The bCPCs were prepared in Teflon cylindric molds of 12 mm height by 6 mm diameter. Once the bCPCs were fully set, the wet samples were weighed. The samples were then completely dried by placing them under vacuum at room temperature for 24 h. After drying, the samples were weighed again. Porosity was then determined as the ratio of the pore volume (the volume of water removed during



**Fig. 1.** Characterization of constituents, bCPC formulations, and handling properties. (a) SF liquid. (b) SF fibers (scale bar=1 cm). (c) SF microparticles (scale bar=1 cm). (d) XRD spectra of bCPC formulations and standards (DCPD, ICPD PDF 9-0077; HA, 96-900-1234). (e) Initial and final setting times of the bCPC formulations (f) FTIR spectra of the bCPCs (g) Injectability of bCPC formulations (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ). (h) Porosity of the bCPC formulation samples ( $p > 0.05$ ). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

drying) to the total sample volume. The pore volume was calculated as the difference between the wet and dry weights, assuming the density of water is  $1 \text{ g/cm}^3$ . The total volume of the sample was calculated using the formula  $\pi r^2 h$ . The porosity is then expressed as:

$$\text{Porosity} = \frac{\text{Pore Volume}}{\text{Total Volume}} = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Total Volume}}$$

### 2.3. Handling properties

The setting time of bCPCs was determined using a Gillmore needle protocol according to a voluntary consensus technical international standard (American Society for Testing and Materials; ASTM C266-89). The lighter-weighted needle (100 g in weight and 2 mm in diameter) was used to determine the initial setting time, while the heavy-weighted needle (300 g in weight and 1 mm in diameter) for the final setting time [33]. After the homogeneous paste of bCPC was inserted into the mold, the setting time was quantified by measuring the timepoint when the needles did not make visible indentations anymore in the bCPCs surface.

The injectability of the bCPCs was determined by extruding a certain quantity of the paste placed in a commercial plastic syringe of 3 mL capacity and with an exit diameter in the nozzle of 2 mm. The extrusion

was performed by placing the syringe in a universal testing machine (ESM 303, Mark 10, New York, USA) using a compression speed of 15 mm / min until reaching a maximum load of 100 N [34]:

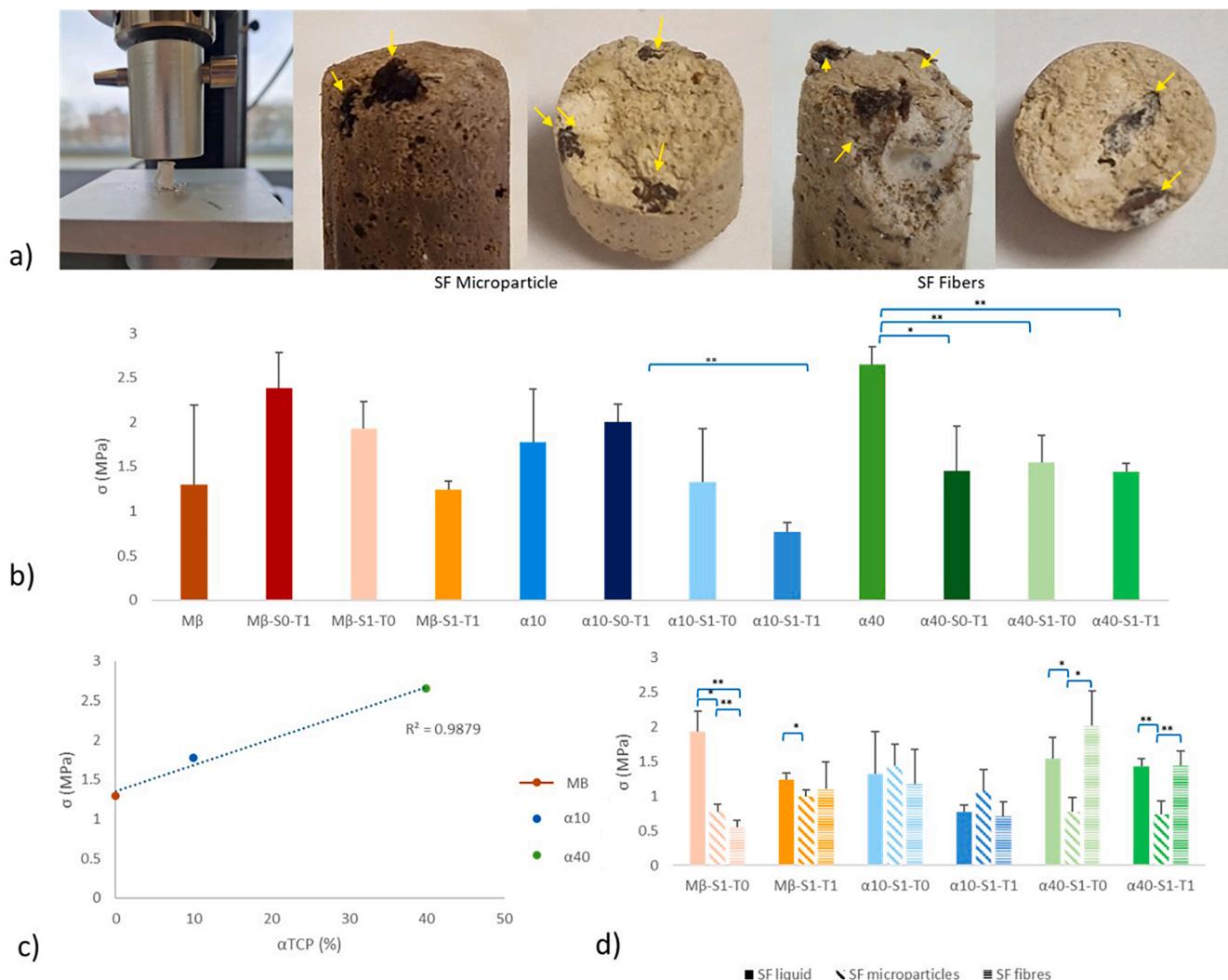
$$\text{Injectability} = \frac{\text{mass of injected material}}{\text{total mass of material}} \cdot 100\%$$

### 2.4. Mechanical characterization

The compressive strength of bCPC formulations was assessed as described previously [35]. In brief, samples (12 mm height, 6 mm diameter,  $n = 3$ ) were immersed in water at  $37^\circ\text{C}$  and tested after 24 h of sample preparation, immediately after being extracted in order to maintain hydration. The study was carried out in a universal testing machine (ESM 303, Mark 10, New York, USA) with a load cell of 200 N at  $1 \text{ mm min}^{-1}$  load application speed. The compressive strength ( $\sigma_c$ ) in MPa was determined by the following formula:

$$\sigma_c = \frac{F}{A_0} = \frac{4P}{\pi d^2} \cdot 10^{-6}$$

where  $P$  is the maximum breaking load (N) and  $d$  the diameter of the specimen (m). Three samples were tested for each bCPCs formulation.



**Fig. 2.** Mechanical properties of bCPC formulations. (a) Experimental set-up of the test system used for compressive strength evaluation. SF microparticles and fibers were observed at the plane of fracture of samples after compressive strength (yellow arrows). (b) Compressive strength of bCPC formulations (SF in liquid form added in the liquid phase; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ). (c) Compressive strength of bCPC formulations depending on αTCP addition. (d) Compressive strength depending on SF addition in different forms. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

## 2.5. Drug release kinetics

bCPC samples ( $n = 3$ ) loaded with tetracycline were immersed in 2 mL of MES buffer (pH 4.6) at  $37.0 \pm 0.5$  °C throughout the study. Samples representing 1 mL of the supernatant were taken and replaced with an equal volume of fresh MES buffer to maintain submerged conditions; sampling was done at 24 h, 72 h, 120 h, 168 h and 336 h. The concentration of released TC was measured using a UV-Visible Spectrophotometer SpectraMax® iD3 Multi-Mode Microplate Reader (Molecular Devices, USA) at a wavelength of 276 nm. Results were calculated as cumulative release over time [36].

## 2.6. Antibacterial efficacy

For the evaluation of antibacterial efficacy [37], *Staphylococcus aureus* strain ATCC 29,213 Agar Tripton was used at a strain concentration adjusted with a turbidimetric method employing as a reference a 0.5 McFarland standard ( $1 \times 10^8$  CFU mL<sup>-1</sup>). Subsequently, 200 µL of a previously prepared culture medium of 90,922-500 G Mueller Hinton Broth 2 agar (Merck) was inoculated in Petri dishes. After a period of 1 hour, the bCPCs samples were placed on top of the plates containing the culture medium and the bacterial suspension and were incubated at 37

± 1 °C for a period of 168 h. Three samples were tested for each formulation and the zone of inhibition was measured using APP Interscience scan 500 colony counter (diameters of inhibition zone less than 10 mm were recorded as non-active antibacterial activity [38]).

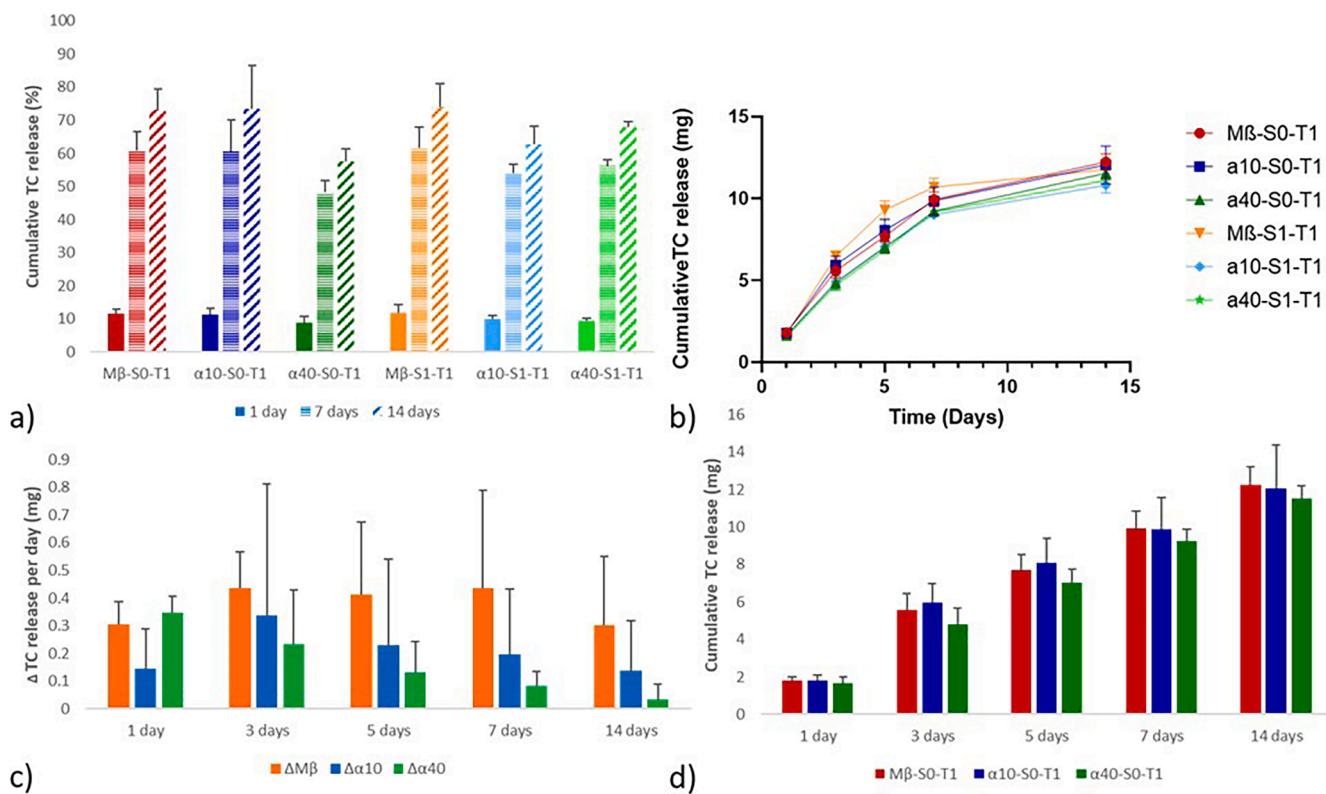
## 2.7. Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using OriginPro 2018 (OriginLab, USA) and GraphPad Prism 8.0.1 (GraphPad Software, USA). Differences between groups were evaluated using one-way ANOVA followed by appropriate post-hoc tests. A  $p$ -value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Characterization of constituents, bCPC formulations, and handling properties

SF was used in different forms: dissolved in water, as fibers, and as particles (Fig. 1a-c). The liquid SF (Fig. 1a) appeared smooth and homogeneous, indicating its suitability for uniform mixing with the bCPCs



**Fig. 3.** Evaluation of tetracycline release from bCPC formulations. (a) Cumulative TC release ( %) after immersion of samples in MES buffer for 1, 7, and 14 days. (b) Cumulative TC release (mg) after immersion of samples in MES buffer for 1, 3, 5, 7, and 14 days. (c) Effects of SF addition on TC release ( $\Delta$ TC release = release formulation with SF – release formulation without SF;  $p > 0.05$ ). (d) Effects of  $\alpha$ -TCP addition on TC release ( $p > 0.05$ ). Statistical analyses were done using a one-way ANOVA followed by Tukey's post-hoc test.

components, while the SF fibers (Fig. 1b) displayed an elongated structure, and the SF microparticles (Fig. 1c) appeared irregular. X-ray diffraction (XRD) analysis (Fig. 1d) confirmed DCPD (brushite, ICDD PDF 9-0077) as the dominant phase across all bCPC formulations ( $M\beta$ ,  $\alpha 10$ ,  $\alpha 40$ ). bCPCs exhibited initial setting times between 4 and 7 min, and final setting times ranging from 7 to 30 min (Fig. 1e). The setting time data shows that adding TC and/or SF to the baseline bCPC formulations ( $M\beta$ ,  $\alpha 10$ ,  $\alpha 40$ ) tends to prolong both initial ( $T_i$ ) and final ( $T_f$ ) setting times. FTIR spectra of the bCPCs (Fig. 1f) revealed the presence of characteristic phosphate bands  $\nu_3(\text{PO}_4^{3-})$  and the double signal of  $\nu_4(\text{PO}_4^{3-})$ , across all groups, confirming the formation of calcium phosphate phases. Injectability of the bCPC formulations reached values up to 50 % (Fig. 1g), the  $\alpha 10$  group showed significant differences upon addition of TC and SF. Comparisons between  $\alpha 10$  and  $\alpha 40$  groups also revealed formulation-dependent variability.

Porosity ranged from 39.6 % to 49.9 % (Fig. 1h), and did not differ significantly across groups ( $p > 0.05$ ), suggesting that the addition of SF or TC did not markedly alter the internal microstructure of the set bCPCs.

### 3.2. Mechanical evaluation of bCPC formulations

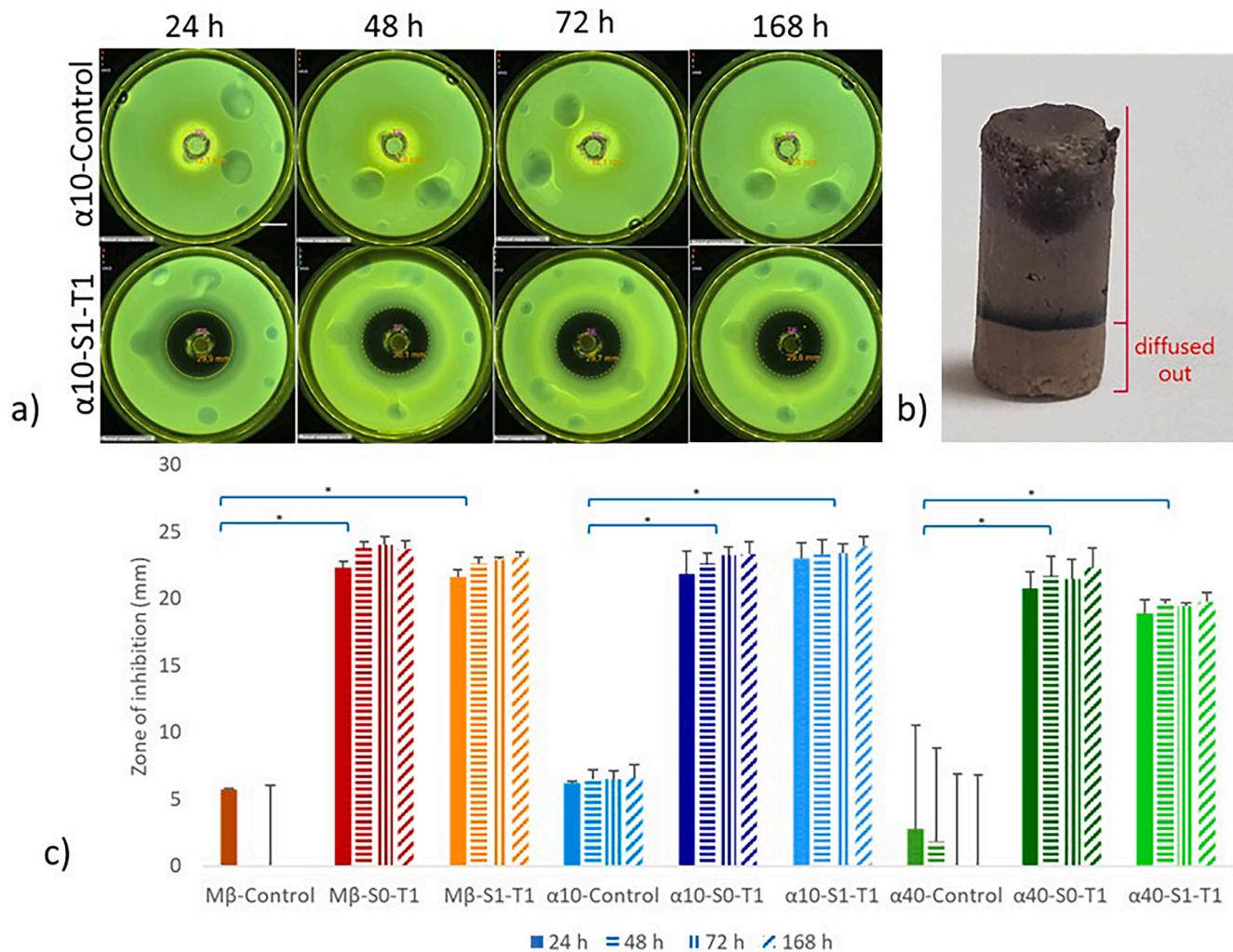
Mechanical properties of the bCPC formulations were tested with an experimental set-up (Fig. 2a). Macroscopic evaluation of fractured samples (after compressive strength testing) showed presence of SF as fibers or microparticles in the plane of fracture (Fig. 2a). The compressive strength of the samples (Fig. 2b) showed a range between 0.77 and 2.65 MPa, with statistically significant differences observed in several  $\alpha 10$  formulations, where the addition of SF to the samples also containing TC decreased mechanical strength ( $p < 0.01$ ). For the  $\alpha 40$  formulations, all additives significantly decreased mechanical strength. Fig. 2c displays the correlation between the  $\alpha$ -TCP content and the compressive

strength. Notably, the inclusion of  $\alpha$ -TCP correlated with increased compressive strength. Comparisons between SF forms (Fig. 2d) indicated that SF microparticles reduce compressive strength compared to SF liquid and fibers for  $\alpha 40$  formulations, while SF liquid and fibers showed comparable effects on mechanical strength ( $p > 0.05$ ). For  $M\beta$  and  $\alpha 10$  formulations, no clear effects on mechanical strength were detected upon addition of any type of SF. Overall, the results indicate that  $\alpha 40$  formulations outperform  $M\beta$  and  $\alpha 10$  in terms of compressive strength, and SF microparticles consistently decrease mechanical performance compared to SF liquid and fibers in these formulations.

### 3.3. Tetracycline release and antibacterial efficacy

Fig. 3a show the cumulative TC release ( %) after immersion of samples in MES buffer for 1, 7, and 14 days. On the first day, all samples showed a burst release of approximately 10 %. Thereafter, TC release continued to reach approximately 56 % in the first 7 days, increasing to approximately 77 % cumulative release after 14 days. The release profiles (Fig. 3b) show a steady and comparable release profile for all formulations over time. Examination of the influence of SF addition (Fig. 3c) indicated no statistically significant differences between groups at any time point ( $p > 0.05$ ). Similarly, the addition of  $\alpha$ -TCP (Fig. 3d) did not affect TC release across groups ( $p > 0.05$ ).

Fig. 4 shows the evaluation of antibacterial efficacy of bCPC formulations against *Staphylococcus aureus*. In Figure a, the zone of inhibition (ZOI) for  $\alpha 10$ -S1 samples demonstrates significant antibacterial activity when TC is loaded, with consistent ZOI values observed over time. For  $\alpha 10$ -S1-T1 samples, the sample cylinder shows a clear line related to TC diffusion, with the top portion of the cylinder visibly darker, indicating undiffused TC (Fig. 4b). The ZOI measurements for the bCPC formulations over 24, 48, 72, and 168 h are displayed in Fig. 4c. The controls show negligible ZOI values (<10 mm), confirming



**Fig. 4.** Antibacterial efficacy of bCPC formulations. (a) Representative visual aspect of Zone of Inhibition (ZOI) from  $\alpha$ 10-S1 samples (+/- TC) over a period of 168 h (scale bar=10 mm). (b) Representative  $\alpha$ 10-S1-T1 sample after 168 h showing colour differences along the height of the sample (high in the cylinder the TC remains undiffuse). (c) Quantitative ZOI values against *Staphylococcus aureus* at 24, 48, 72, and 168 h. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. Significant differences between T0 controls and most T1 groups are marked with brackets (\*  $p < 0.05$ ) in the graph, confirming enhanced antibacterial activity due to TC addition.

no inherent antibacterial effect without TC. In contrast, TC-loaded samples exhibit significantly larger ZOIs (20–25 mm) that remain consistent over time.

#### 4. Discussion

In this study, we set out to develop a multifunctional bone graft based on brushite calcium phosphate cement (bCPC) that integrates  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) and silk fibroin (SF) with the antibiotic tetracycline (TC) to achieve a balance of mechanical performance and sustained drug release. Our experimental set-up involved characterizing the different forms of SF (liquid, fibers, and microparticles), evaluating setting times, compressive strength, and assessing both drug release profiles and antibacterial efficacy. The main findings were that (i) adding SF and TC significantly prolonged setting times, (ii) adding  $\alpha$ -TCP markedly increased compressive strength, and (iii) adding SF in liquid or fiber form reinforced the matrix. For all bCPC formulations, the TC release profile showed an initial burst followed by sustained release over 14 days, and TC-loaded formulations exhibited robust antibacterial efficacy against *Staphylococcus aureus*. bCPC formulations clearly showed effects of additives on setting times. Our results indicated that the addition of TC and SF extends both the initial and final setting times

of the bCPC formulations. This extended setting time is likely due to TC's ability to chelate calcium ions, thereby delaying brushite crystallization [30,36]. From a clinical standpoint, these extended setting times can be beneficial, as they allow surgeons more flexibility during implantation [39]. However, overly extended setting times may be impractical in emergency surgical situations, such as cranioplasty [40]. Consequently, specific clinical applications might require individual optimization setting times to strike a balance between workability and efficiency.

Mechanical testing revealed that  $\alpha$ -TCP plays a pivotal role in enhancing compressive strength, with bCPC formulations comprising 40 wt %  $\alpha$ TCP achieving values up to 2.65 MPa, a 2.65-fold increase over the 1 MPa strength of the unmodified (bare) bCPC [10]. Notably, incorporation of SF in liquid or fiber form resulted in maintained or improved mechanical performance [41,42], whereas SF microparticles consistently reduced compressive strength—most likely due to irregular shapes acting as stress concentrators [43]. Although prior studies have reported higher compressive strengths for pure  $\alpha$ -TCP-based CPC systems [44], the multi-component nature of our bCPC formulations explains the lower absolute values. These observations corroborate recent literature. For instance, Roshanfar et al. [41] demonstrated that incorporating electrospun SF fibers into CPC increased compressive strength significantly by bridging microcracks and arresting crack propagation,

while discrete polymer particles sometimes served as weak points in the matrix. Similarly, Cassel et al. [45] reported that even low concentrations of SF, when well-dispersed, can refine the crystallization of the CPC, resulting in a denser microstructure with enhanced load-bearing capability. These studies support our findings and underscore the importance of not only the presence but more importantly the form of SF in determining mechanical performance.

TC release profiles demonstrated a predictable burst-sustained release pattern, with approximately 10 % release on the first day and up to 77 % cumulative release at 14 days. This release profile suggests that the initial burst release is related to diffusion of TC through the intrinsic porosity of the bCPC formulations [27], and corresponds to the Higuchi model [46]. Importantly, neither SF addition nor  $\alpha$ -TCP significantly altered TC release kinetics ( $p > 0.05$ ). Antibacterial tests against *Staphylococcus aureus* confirmed the efficacy of the TC-loaded bCPCs, with inhibition zones sustained for up to 168 h, demonstrating prolonged diffusion of TC out of the cylindrical samples. Comparisons with recent literature further elucidate these findings and bCPCs are known to exhibit burst release due to their high solubility. In studies by Fosca et al. [47], pure bCPCs systems were shown to release a majority of their drug payload in a rapid initial phase, whereas incorporating a secondary phase such as HA (or partially converted  $\alpha$ -TCP) moderated the burst, shifting the release profile toward a more sustained pattern. Similarly, Rödel et al. [21] incorporated gelatin into bCPCs, which transformed the typical burst release into a more prolonged, controlled delivery, following a near-linear release over several days. In work by Dong et al. [48], the integration of SF as a hydrogel network moderated the initial burst, allowing for sustained release without compromising mechanical integrity. Our findings align with literature reporting that while bCPC composition impacts initial burst magnitude, the microstructural porosity ultimately controls sustained drug diffusion [23,30].

Collectively, these results indicate that SF (particularly in liquid or fiber form) and  $\alpha$ -TCP can be effectively combined within bCPC matrices to improve both mechanical integrity and therapeutic functionality without negatively affecting drug release behaviour. The observed improvements in compressive strength as well as the predictable, sustained drug release provide a robust foundation for further optimization of bCPC-based drug delivery systems. However, several challenges remain. First, our experiments were conducted exclusively *in vitro*, which does not fully capture the complexities of the *in vivo* environment, such as variations in fluid dynamics, local tissue interactions, long-term biodegradation behaviour, and effects of proteins in bodily fluids. In addition, although our data demonstrate clear effects of SF morphology on mechanical properties, the current study does not fully elucidate the optimal size, shape, or distribution of SF microparticles to minimize stress concentration without sacrificing their bioactive benefits. Finally, additional drug-loading strategies should be explored to further enhance and tailor the antibiotic release profile for different clinical scenarios. By integrating these strategies and leveraging recent advances in multi-functional bCPCs, future research can pave the way for clinically effective, economically viable materials for bone regeneration.

## 5. Conclusions

Our study demonstrates that bCPC systems are promising multi-functional platforms for bone regeneration, effectively balancing mechanical strength, injectability, and sustained antibiotic delivery. Incorporating SF (in liquid or fiber form) and  $\alpha$ -TCP allows for tuneable setting times and improved compressive strength without compromising porosity or drug diffusion. These effects of integrating these additives in bCPC provide a robust foundation for optimizing bCPC formulations for clinical applications in bone repair. Future studies should focus on *in vivo* validation, long-term degradation, and advanced drug-loading strategies to expand the therapeutic reach of these biomaterials.

## CRediT authorship contribution statement

**Claudia Morilla Espino:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Louise van der Weerd:** Writing – review & editing, Supervision, Project administration. **Lioe-Fee de Geus-Oei:** Writing – review & editing, Supervision. **Jeroen JJP van den Beucken:** .

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT, an AI language model developed by OpenAI in order to improve readability and language of the work. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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