Advancements in Brushite cement formulations for bone repair

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Chapter 6: SUMMARY AND DISCUSSION

Summary

Bone regeneration remains a critical challenge in clinical orthopaedics and dentistry, requiring the development of biomaterials that combine structural support with bioactivity and drug delivery capabilities to expedite the clinical availability of synthetic bone graft materials. Brushite calcium phosphate cements (bCPCs) have emerged as promising bone graft candidates due to their biocompatibility, bioresorbability, and ability to serve as localized drug delivery systems. bCPCs are prepared from an acidic calcium phosphate and a basic one, which upon mixing with a liquid phase form a mouldable paste that easily adapt to the bone defect shape. Furthermore, bCPCs can be injected, which facilitates the use of minimally-invasive surgical techniques. However, optimizing the mechanical properties, handling characteristics, and controlled release mechanisms of bCPCs is essential to enhance their clinical applicability and efficacy.

To address these limitations, this thesis aimed to enhance the mechanical and handling properties of bCPCs, and evaluate the effectiveness of bCPCs as a local antibiotic delivery system, focusing on their potential for improved clinical applications in bone regeneration therapies.

Chapter 1 offered an overview of the current state-of-the-art for the different limitations of bCPCs and the possible strategies for their improvement. The subsequent chapters each addressed distinct research questions and the obtained results to answer these. In brief, these research questions, the experimental results toward answering these, and the conclusions are summarized below:

1) How can the synergy between bCPCs and drug delivery mechanisms be optimized to advance bone regeneration therapies and improve clinical outcomes? *(Chapter 2)*

The synergy between bCPCs and drug delivery mechanisms can be optimized to advance bone regeneration therapies and improve clinical outcomes by addressing key challenges, while leveraging their unique advantages. bCPCs are highly valued for their biocompatibility, bioactivity, and rapid resorption rates, which facilitate integration with host tissue and enhance long-term (complete) regeneration. However, challenges such as rapid setting times, suboptimal mechanical properties, and limited handling characteristics must be mitigated to fully unlock their clinical potential. Strategies like the use of setting retarders to modulate hardening and the integration of polymers to improve mechanical strength and injectability have shown promise in overcoming these limitations.

Additionally, bCPCs' capability as localized drug delivery systems offers a significant advantage in addressing post-surgical complications, particularly bacterial infections. By

enabling targeted antibiotic release directly at the surgical site, bCPCs reduce the need for systemic antibiotic therapy, minimizing adverse side effects and contributing to the global effort to combat antibiotic resistance. Optimizing this synergy involves fine-tuning the formulation of bCPCs to maintain bCPC handling properties, control drug release kinetics, enhance porosity for improved drug loading, and ensure sustained therapeutic action. Incorporating materials such as silk fibroin or other polymers could further improve the mechanical and handling properties, while supporting controlled drug release.

In summary, by optimizing the composition, mechanical performance, and drug delivery mechanisms of bCPCs, their role in bone regeneration therapies can be significantly enhanced. This not only improves immediate post-surgical outcomes by preventing complications but also ensures long-term defect regeneration and tissue integration, addressing critical clinical needs for effective and reliable bone repair solutions.

2) What is the effect of varying precursor proportions and the addition of collagen on the formulation, biocompatibility, and bioactivity of bCPCs? *(Chapter 3)*

Varying precursor proportions and incorporating collagen significantly influence the formulation, mechanical performance, and bioactivity of bCPCs. Six formulations of a dicalcium phosphate dehydrate (DCPD) were prepared, in which a malleable paste that sets within about 2 or 3 min was obtained. The addition of collagen emerged as a key factor in improving the mechanical properties of the bCPCs, particularly increasing compressive strength to a range of 0.8–1.7 MPa. A higher proportion of β-tricalcium phosphate (β-TCP) enhanced the release of tetracycline, highlighting the role of precursor proportions in modulating drug delivery kinetics. This understanding enables better prediction of drug release behaviour within the complex bCPC matrix, particularly when reinforced with polymers like collagen. From a bioactivity perspective, the bCPC formulations demonstrated effective antimicrobial activity against *Escherichia coli* strains over a period of 24 to 72 hours, further underlining their suitability as drug delivery systems with antimicrobial properties.

3) How does the incorporation of sodium alginate in bCPC formulations influence tetracycline release and cell viability? *(Chapter 4)*

The incorporation of sodium alginate in bCPC formulations influenced tetracycline release and cell viability in several ways. This combination of sodium alginate and tetracycline led to a decrease in compressive strength, primarily due to the antibiotic's effect on setting times and porosity. Tetracycline forms chelates with calcium ions (Ca^{2+}), delaying primary crystal

nucleation, which increases porosity and setting time, negatively impacting the bCPCs mechanical properties. However, this effect also improves injectability, as the delayed setting time allows the material to remain fluid(ic) for longer periods, facilitating easier handling and application. Tetracycline release from the bCPC matrices followed a diffusion mechanism in the initial hours, driven by drug solubility and proximity to the matrix surface. Exploring pH fluctuations highlighted a decrease in pH due to the hydroxyl groups from sodium alginate, which can impact cell survival in *in vitro* conditions. Despite this, sodium alginate, as a biocompatible polymer, supported cell growth and improved cell viability by providing a favourable substrate. Tetracycline, on the other hand, evoked a bacteria-free environment, promoting safer conditions for cell growth within the implant. Generally, sodium alginate enhanced the controlled release of tetracycline and improved biocompatibility, while its combination with tetracycline positively influenced cell viability.

4) What strategies can improve the handling characteristics, mechanical strength, and antimicrobial efficacy of bCPCs, and how does the addition of α-tricalcium phosphate (α-TCP) and silk fibroin (SF) impact these properties along with tetracycline (TC) release dynamics? *(Chapter 5)*

The incorporation of α-TCP and SF into bCPCs demonstrated to be an effective strategy to enhance bCPC performance. α-TCP improved compressive strength, particularly at higher concentrations (40wt%), without compromising injectability or porosity. SF, especially in liquid or fiber form, contributed to structural reinforcement, while microparticles reduced mechanical performance due to their irregular morphology. Handling characteristics were influenced by the presence of TC and SF, with extended setting times observed for dual-loaded formulations. This effect, linked to calcium chelation by TC and polymer interactions, highlights the importance of balancing additive concentrations for optimal clinical usability. Antimicrobial efficacy was consistently achieved across all TC-loaded formulations, with sustained inhibition of *Staphylococcus aureus* growth for up to 1 week. The TC release profile followed a typical burst/sustained pattern (~77% release over 14 days), and was not significantly affected by SF or α-TCP content—suggesting diffusion was primarily driven by intrinsic porosity. Together, these results demonstrate that α-TCP and SF can be used to tune the mechanical and handling properties of bCPCs without negatively impacting drug release or antibacterial performance, offering a promising approach for multifunctional, resorbable bone graft materials.

General Discussion

The development of bCPCs represents a significant stride in the pursuit of effective, resorbable bone graft substitutes with multifunctional properties. This thesis explored several modification strategies to address bCPCs' inherent limitations, such as poor mechanical strength, (too) rapid setting times, and limited control over drug delivery, with the ultimate goal of enhancing their clinical applicability and efficacy. This general discussion provides a critical reflection on the broader scientific and clinical implications of the findings and evaluates how these results align with previous research within the context of current literature.

A key takeaway message from the presented studies is that material composition and additive choice exert a profound influence on the handling properties and physicochemical characteristics of bCPCs. For instance, the inclusion of collagen and sodium alginate showed a clear trade-off between mechanical performance and biological functionality. Collagen improved compressive strength, but introduced variability in injectability, while sodium alginate enhanced biocompatibility and sustained drug release, but reduced mechanical strength due to increased porosity. These findings are consistent with prior research, which suggests that the integration of biopolymers apparently requires a careful balance to preserve critical handling properties and clinical needs [1].

Furthermore, the incorporation of tetracycline into bCPCs reaffirmed its dual role in antimicrobial defence and modulation of cement setting behaviour. The observed chelation of calcium ions and resultant delay in brushite crystallization are well-documented phenomena [2, 3], but the practical implications, such as enhanced injectability or the risk of compromised mechanical strength, require further exploration. The sustained release profile of tetracycline in this thesis (~77% over 14 days) is in line with other calcium phosphate drug delivery systems [2], reinforcing the viability of bCPCs for localized, controlled antibiotic administration.

Another important insight relates to the role of precursor ratio and phase selection, particularly the use of α-TCP. α-TCP not only improved compressive strength, but also contributed to a more stable material matrix that tolerated the integration of silk fibroin with minimal disruption to the setting process. However, the morphology of the polymer additive—liquid vs. fibers vs. microparticles—proved to be crucial. While liquid silk fibroin and fibers reinforced the cement structure, microparticles led to mechanical degradation, likely due to heterogeneity-induced stress concentration. This highlights the critical role of not only chemical composition but also the physical form of additives during formulation development [4].

From a translational perspective, these findings together suggest that bCPCs can be engineered to suit specific clinical scenarios. For example, formulations prioritizing injectability and antimicrobial function could be used in maxillofacial surgeries or minimally-invasive orthopedic procedures, while those enhanced for mechanical integrity might find application in non-load-bearing defects. Nevertheless, many of these conclusions are drawn from in vitro data. While these systems showed promise in antimicrobial testing and preliminary cytocompatibility assessments, in vivo studies will be essential prior to potential clinical applications to validate degradation kinetics, immunogenic response, and long-term therapeutic performance in conditions with physiological resemblance to clinical conditions.

In alignment with the overarching aim of this thesis—to develop clinically relevant, multifunctional bCPCs—this body of work offers foundational insights into formulation optimization and translational potential.

Closing remarks and future perspectives

The research presented in this thesis reinforces the concept that bCPCs offer a promising platform for the development of multifunctional synthetic bone graft materials. By systematically evaluating different strategies—including the use of α-TCP, silk fibroin, collagen, sodium alginate, and the antibiotic tetracycline—the work has expanded our understanding of how material design can influence both structural performance and biological function.

One of the central contributions of this thesis lies in demonstrating that multifunctionality in biomaterials does not necessarily come at the expense of mechanical or handling properties. Through careful selection of precursor ratios and additives, it is possible to simultaneously enhance mechanical strength, injectability, and bioactivity. This nuanced control over multiple parameters supports the vision of adaptable bCPC systems that can be tailored for specific clinical applications, such as bone defect fillers with antimicrobial protection or scaffolds for dental bone regeneration in guided tissue regeneration treatments.

Nevertheless, the work also underscores critical trade-offs. For instance, the improved injectability associated with tetracycline and sodium alginate coincides with reductions in compressive strength. These opposing trends highlight the importance of formulation balance and the need for integrated testing approaches that consider mechanical, biological, and practical requirements in tandem. In this context, the adoption of machine learning and high-throughput screening may help predict optimal compositions more efficiently in future studies.

Looking ahead, several avenues are poised for further exploration. First, (long-term) in vivo studies are essential to confirm the biocompatibility, degradation behaviour, and therapeutic efficacy of the developed bCPC formulations. While in vitro antimicrobial testing demonstrated sustained tetracycline release and effective bacterial inhibition, the impact on host immune response and bone remodelling dynamics remains unknown. Incorporating advanced in vivo imaging techniques and longitudinal histological analyses will be crucial in validating the translational potential of these materials. Second, the field may benefit from exploring multifunctional additives that offer both mechanical and biological enhancements. Polymers such as gelatin methacryloyl (GelMA) [5], hyaluronic acid derivatives [6], or bioinspired peptides [7] could offer new mechanisms for promoting osteogenesis while maintaining desirable physical properties. The emergence of "smart" and multifunctional biomaterials is another key trend. Studies have shown that integrating controlled drug delivery into scaffold systems can significantly modulate local inflammation and promote tissue regeneration [8, 9]. For example, stimuli-responsive scaffolds have been engineered to release therapeutic agents on demand—triggered by environmental cues such as pH changes common in injured or infected tissues [10]. Moreover, integrating additive manufacturing and 3D bioprinting with bCPC formulations can unlock the potential for personalized implants with anatomically precise geometries [11, 12]. Custom 3D-printed scaffolds, for example, can be made to precisely fit a bone defect while incorporating porous networks that promote cell infiltration and nutrient flow. Researchers are already leveraging biofabrication techniques to integrate cells and growth factors into printed scaffolds, essentially building living implants that encourage tissue integration from the moment of implantation. Such personalized, cell-seeded constructs blur the line between an implant and a tissue graft, and they hold promise for treating critical-size bone defects that cannot heal on their own. These emerging technologies (from 3D bioprinting to smart surface chemistry) are highlighted as promising strategies for next-generation implants that meet complex clinical needs and push the boundaries of regenerative medicine [13].

Translating these advanced biomaterials from lab to clinic, however, presents significant challenges that the field is actively working to overcome. One issue is scalability and manufacturing consistency: many of the most advanced materials (especially those combining living cells, growth factors, or intricate architectures) are difficult to produce in large quantities under the strict quality controls required for medical products. Automated, standardized fabrication methods will be crucial so that implants can be made reliably [14]. Alongside manufacturing, regulatory approval is a major hurdle. Regulatory agencies have well-defined pathways for drugs and conventional devices, but a multi-component bone-regenerating

scaffold with cells or novel biomolecules does not fit neatly into existing medical class categories. New biomaterials must be rigorously tested for biocompatibility, long-term stability, and safety (i.e. absence of toxicity or adverse immune reactions). For example, a complex scaffold that degrades in the body must prove that its degradation products are harmless and that it degrades at the appropriate rate to allow natural bone to replace it [15].

Data-driven approaches are also beginning to accelerate medical device development [16, 17]. The rise of artificial intelligence (AI) and machine learning provides powerful methods to design and test biomaterials in silico before they ever enter the stage of animal experimentation or clinical trials. Researchers can now train models on large datasets of material properties and biological outcomes, enabling predictive modelling, for example, predicting which material composition or scaffold structure will best induce bone formation [18]. These techniques can dramatically cut down on trial-and-error, optimizing material properties and design parameters much faster than traditional R&D.

On a personal note, I believe that the real breakthrough in this field will emerge from a tighter integration of interdisciplinary collaboration and a willingness to embrace novel regulatory standards. In my view, researchers must actively engage with clinical practitioners earlier in the development process to ensure that the materials we design address tangible, unmet clinical needs. Equally, I feel that fostering partnerships with industry and regulators from the outset will be essential to navigate the complexities of product approval and commercialization. This proactive approach, combined with the exciting technological advances we are witnessing, such as AI-driven material optimization and 3D bioprinting, will accelerate the translation of laboratory innovations into real-world therapies.

In addition to the impressive technical advances in multifunctional biomaterials, I consider it vital to address their affordability and accessibility, particularly for low-resource settings. Coming from a third-world country, I have witnessed firsthand that many of the techniques and materials we discuss in high-end research are often out of reach for nations facing economic challenges or recovering from conflict. Therefore, while continuing to push the envelope in terms of innovation, we must also invest in developing cost-effective, scalable technologies and simplified production methods that can be adapted to meet the urgent needs of poor and war-devastated regions, where the prevalence of bone defects is alarmingly high. Addressing these challenges will not only broaden the clinical impact of advanced biomaterials but also contribute to more equitable global healthcare outcomes.

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