Advancements in Brushite cement formulations for bone repair

Morilla Espino, C.

Citation

Morilla Espino, C. (2025, November 11). *Advancements in Brushite cement formulations for bone repair*. Retrieved from https://hdl.handle.net/1887/4282795

Chapter 1: GENERAL INTRODUCTION

Introduction

Bone regeneration is a complex process involving well-orchestrated biological events, including bone induction and conduction, that mimic skeletal development and repair [1]. While bone has an intrinsic capacity for regeneration, large defects caused by trauma, infection, tumour resection, or skeletal abnormalities often exceed the body's self-healing potential and require external intervention [1]. Additionally, compromised conditions such as osteoporosis and avascular necrosis further hinder regeneration [1]. The reconstruction of bone defects, whether congenital or acquired, poses significant challenges due to the lengthy and unpredictable nature of the healing process [2]. Factors influencing the success of bone repair include the size and location of the defect, the soft tissue environment, and patient-specific circumstances such as age and chronic disease [3]. Despite advancements, there is no consensus on the diagnosis and treatment of "critical-size bone defects," (i.e. the smallest size intra-osseous wound in a particular bone and species that will not heal spontaneously during the lifetime of the organism [4]) reflecting the complexity and variability of these cases in clinical practice [3].

Composition of bone tissue

The bone extracellular matrix (ECM) and its associated cells play critical roles in bone formation and regeneration (Figure 1) [5]. During bone regeneration, mesenchymal stem cells (MSCs) home to the site of injury and differentiate into osteoblasts, which produce the ECM and facilitate osteoid mineralization, leading to the formation of terminally differentiated osteocytes [5]. The ECM is a non-cellular, three-dimensional structure secreted by cells into the extracellular space, composed of proteins and polysaccharides, with a unique composition and topology that supports tissue development, integrity, and homeostasis [5]. In bone tissue engineering, the ECM is considered a critical component for development [6].

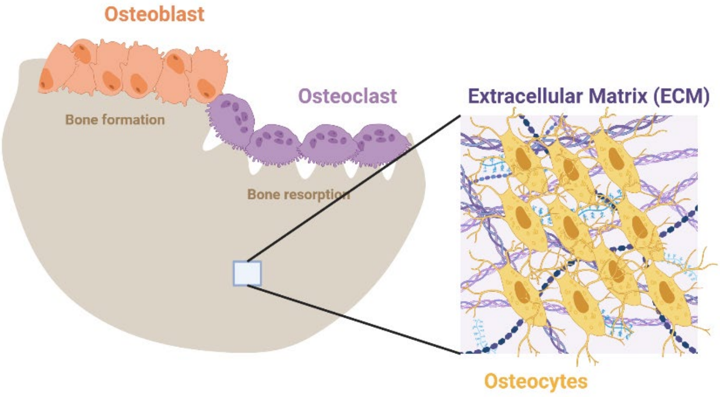

Figure 1. The bone extracellular matrix (ECM) and its associated cells osteoblasts, osteoclasts and osteocytes. (Created in https://BioRender.com)

The bone ECM consists of organic (40%) and inorganic (60%) components, with its exact composition varying by sex, age, ethnicity, and health conditions. The main inorganic elements include calcium-deficient apatite and trace minerals [5]. Osteoblasts, derived from MSCs, play a vital role in bone remodelling by synthesizing the ECM and ensuring its proper mineralization. These cells depend on collagen-based surfaces to produce mechanically stable and structured bone tissue [5]. Osteocytes, the terminally differentiated cells embedded within the bone matrix, communicate with each other and with bone lining cells to support bone growth and repair through processes like perilacunar/canalicular remodelling, which involves continuous resorption and deposition of the matrix [7]. Osteoclasts, which are multinucleated cells formed by the fusion of monocyte/macrophage precursors, are responsible for bone resorption. Their activity is regulated by macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor κB ligand (RANKL), which are secreted by osteoblasts [5]. Together, the interactions between ECM components and cellular activities ensure bone regeneration, homeostasis, and repair.

Regeneration of bone tissue

Bone tissue regeneration relies on various grafting techniques, with autografts currently considered the gold standard due to their superior osteogenic potential [8]. Autografts involve harvesting bone from the patient, typically from sites like the iliac crest, and are highly effective in promoting bone regeneration. However, they present significant drawbacks, including donor-site morbidity, increased risk of wound infection, prolonged hospital stays, and higher costs, making them less favourable in some cases [9]. These challenges have driven the development and use of alternative grafting methods, including allogeneic, xenogeneic, and alloplastic scaffolds.

Allogeneic bone grafts, derived from human donors other than the patient, are the most commonly used alternative to autografts. While they eliminate the need for a second surgical site, allogeneic grafts pose risks of disease transmission, immune rejection, and resorption [9]. Alloplastic grafts, composed of synthetic materials, and xenogeneic scaffolds, derived from animal sources, offer additional alternatives in regenerative dentistry and bone reconstruction. These substitutes reduce the reliance on autografts, but ongoing research aims to address their limitations, such as varying osteogenic capabilities and integration within the host tissue [8]. Despite advancements, no single substitute has yet fully matched the effectiveness and reliability of autografts.

Calcium phosphate cements (CPCs)

Powder and liquid composition of CPCs

Calcium phosphate cements (CPCs) are biocompatible materials made from calcium phosphate powders that, when combined with a liquid phase, create a mouldable paste capable of hardening at room or body temperature. Once set, CPCs form a solid structure resembling the mineral makeup of natural bone, which makes them ideal for applications in bone repair and regeneration [10, 11]. The powders, often including alpha-tricalcium phosphate (α-TCP) or β-tricalcium phosphate (β-TCP), determine the CPC's reactivity and final composition based on their crystallinity and particle size [12-14]. Lower crystallinity and smaller particle sizes increase reactivity, enhancing the CPC setting properties [12, 14, 15]. The liquid phase typically contains water, but additives like organic acids (e.g., citric acid, sodium citrate) are frequently incorporated to regulate the setting reaction, handling properties, or injectability [16, 17]. These components enable the formulation of injectable CPC pastes that adapt perfectly to bone defect dimensions, facilitating minimally invasive surgical applications [18-20].

Unlike many other biomaterials, CPCs can support long-term bone defect repair by promoting new bone formation in harmony with their own gradual degradation [21-23]. CPCs are also biocompatible and osteoconductive [24-26]. The most common CPCs on the market include those based on apatite (aCPCs) (Norian SRS® (Synthes, USA) [27], Kyphos® (Kyphon, USA) [28], or Biopex-R® (Hoya Corp., Japan) [29]) [30, 31] and those based on brushite (bCPCs) [18] (ChronOs® Inject (Synthes, USA) [32], Eurobone (Groupe FH ORTHO, France) (Figure 2) [33]).

Figure 2. Commercial bCPC Eurobone (Groupe FH ORTHO, France). This is a 2-chamber device to separate powder and liquid; the plunger allows to break the separation and mix the components into a paste that then can be injected via the needle. (Reprinted from the "Eurobone 2 Injectable macroporous substitutes to fill bone defects. Documentation" provided by the company web page. [34])

Setting Reaction

The setting reaction of CPCs is a dissolution-precipitation process occurring in three stages: (1) dissolution of reactants to release calcium and phosphate ions; (2) nucleation of a new phase (brushite or apatite crystals, depending on pH); and (3) growth of these crystals to form hardened cement [21, 35]. bCPCs form when the pH is below 4.2, while aCPCs (e.g., hydroxyapatite or calcium-deficient hydroxyapatite) form at pH levels above 4.2 [16, 21, 36]. For clinical use, the setting time is crucial: initial setting time (3–8 minutes) allows for manipulation, and final setting time (15–18 minutes) ensures proper hardening within a realistic timeframe for a clinical procedure [37-40].

Properties of set CPCs

Set CPCs exhibit biocompatibility, osteoconductivity, and the ability to degrade at rates conducive to new bone formation [23-25]. bCPCs are metastable under physiological conditions and resorb faster than aCPCs, which are more stable and degrade more slowly [35, 41]. aCPCs, characterized by low crystallinity, mimic the bone mineral phase and are preferred for long-term stability [35, 42]. bCPCs, while less mechanically robust, are advantageous for applications requiring rapid resorption [36, 41] (Table 1). Injectability and mechanical properties of CPCs are properties of variable magnitude depending on the clinical application, but it is evident that these are critical factors for clinical success.

Table 1. Comparison between bCPCs and Apatite Calcium Phosphate Cements (CPCs), highlighting their differences in chemical composition, formation conditions, solubility, resorption rates, mechanical properties, and clinical applications

Aspect	**Brushite (bCPCs)**	**Apatite (e.g., HA, CDHA)**
pH of Formation	Formed when pH < 4.2	Formed when pH > 4.2
Main Composition	Dicalcium phosphate dihydrate (DCPD)	Hydroxyapatite (HA), calcium-deficient hydroxyapatite (CDHA), or carbonated apatite
Solubility	Metastable under physiological conditions; resorbed faster than apatite	More stable and less soluble under physiological conditions
Setting Kinetics	Faster precipitation; brushite forms more rapidly than monetite	Slower precipitation compared to brushite
Resorption Rate	Faster resorption, which can promote faster remodelling	Slower resorption, ensuring longer stability in bone voids
Transformation	Can transform into apatite in vivo under physiological conditions	Does not transform; remains stable as apatite
Mechanical Properties	Generally weaker and more brittle	Stronger and more durable
Clinical Use	Preferred when faster resorption or remodelling is needed (e.g., temporary fillers)	Preferred for applications requiring long-term stability
Key Reference Studies	[43-45]	[43, 46, 47]

Brushite calcium phosphate cements (bCPCs)

bCPC is a type of calcium phosphate cement that forms dicalcium phosphate dihydrate (DCPD) (Table 1), commonly known as brushite, upon setting. It is created by mixing calcium phosphate powders, such as β-tricalcium phosphate (β-TCP) and monocalcium phosphate monohydrate (MCPM), with water. [16, 21, 48].

Disadvantages of Brushite CPCs

bCPCs, while advantageous for fast bone resorption, have significant limitations. Their short setting time (30–60 seconds) is often impractical for surgical applications and requires the use of additives like citric acid to delay hardening [16, 17]. They exhibit lower mechanical strength (Figure 3) compared to cancellous bone, restricting their use in load-bearing applications [49]. Additionally, injectability issues, such as liquid phase separation during injection, result in uneven material composition and compromised in vivo behaviour [36, 50]. These challenges, combined with brittleness, limit their use despite their favourable resorption properties.

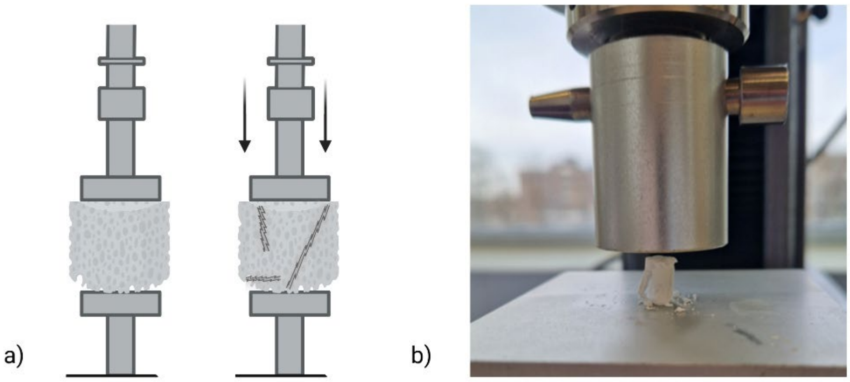

Figure 3. Mechanical test: a) Schematic representation of a compressive strength test, the cement is compressed until the point of fracture; b) Real picture of the compression strength set-up (Created in BioRender.com).

Brushite CPCs as drug-delivery systems

bCPCs have shown significant potential as drug-delivery systems, particularly for treating bone infections such as osteomyelitis. Osteomyelitis, caused by bacteria, mycobacteria, or fungi, is often challenging to treat due to the inability to maintain high antibiotic concentrations at the infection site. Incorporating antibiotics directly into CPCs for localized delivery has emerged as a promising approach to overcome this challenge and ensure effective treatment [51] (Figure 4). One key advantage of CPCs is their low setting temperature, which enables the incorporation of heat-sensitive drugs without denaturation. Furthermore, the intrinsic porosity of CPCs facilitates drug elution, enhancing the release profile to achieve the desired clinical outcomes [20]. This porosity, combined with isothermal setting, helps maintain the structural integrity of loaded drugs. Additionally, CPCs are evaluated for their ability to enhance bone regeneration and osseointegration, critical for restoring mechanical function, reducing failure risks, and promoting long-term stability of the material [20].

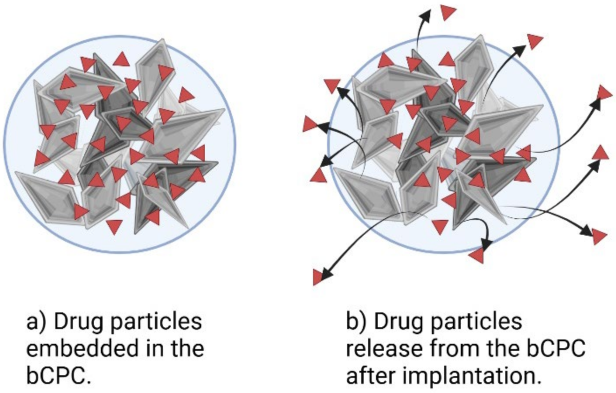

Figure 4. bCPCs as drug delivery systems: a) Drug molecules loaded in the bCPC are embedded in the matrix of the cement; b) After implantation, the drug starts to release from the bCPC mainly by diffusion [35] (Created in BioRender.com).

Objectives of the thesis

bCPCs are advantageous due to their rapid bone resorption; however, they are hindered by several significant drawbacks. Their short setting time (30–60 seconds) poses challenges during surgical procedures. Additionally, bCPCs exhibit low mechanical strength, limiting their suitability for load-bearing applications. Furthermore, issues with injectability, such as liquid phase separation, can lead to inconsistent material properties.

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

In more detail, this thesis addressed the following research questions:

1) How can the synergy between brushite calcium phosphate cements (bCPCs) and drug delivery mechanisms be optimized to advance bone regenerative therapies and improve clinical outcomes? *(Chapter 2)*
2) What is the effect of varying precursor proportions and the addition of collagen on the formulation, biocompatibility, and bioactivity of bCPCs? *(Chapter 3)*
3) How does the incorporation of sodium alginate in bCPC formulations influence tetracycline release and cell viability? *(Chapter 4)*
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 release dynamics? *(Chapter 5)*

References

[1] R. Dimitriou, E. Jones, D. McGonagle, and P. V. Giannoudis, "Bone regeneration: current concepts and future directions," *BMC medicine,* vol. 9, pp. 1-10, 2011.

[2] G. Arealis and V. S. Nikolaou, "Bone printing: new frontiers in the treatment of bone defects," *Injury,* vol. 46, pp. S20-S22, 2015.

[3] A. Nauth, E. Schemitsch, B. Norris, Z. Nollin, and J. T. Watson, "Critical-size bone defects: is there a consensus for diagnosis and treatment?," *Journal of orthopaedic trauma,* vol. 32, pp. S7-S11, 2018.

[4] A. Vajgel, N. Mardas, B. C. Farias, A. Petrie, R. Cimões, and N. Donos, "A systematic review on the critical size defect model," *Clinical oral implants research,* vol. 25, no. 8, pp. 879-893, 2014.

[5] X. Lin, S. Patil, Y.-G. Gao, and A. Qian, "The bone extracellular matrix in bone formation and regeneration," *Frontiers in pharmacology,* vol. 11, p. 757, 2020.

[6] S. Ravindran *et al.*, "Biomimetic extracellular matrix-incorporated scaffold induces osteogenic gene expression in human marrow stromal cells," *Tissue Engineering Part A,* vol. 18, no. 3-4, pp. 295-309, 2012.

[7] N. S. Dole *et al.*, "Osteocyte-intrinsic TGF-β signaling regulates bone quality through perilacunar/canalicular remodeling," *Cell reports,* vol. 21, no. 9, pp. 2585-2596, 2017.

[8] M. Heitzer *et al.*, "In vitro comparison of the osteogenic capability of human pulp stem cells on alloplastic, allogeneic, and xenogeneic bone scaffolds," *BMC Oral Health,* vol. 23, no. 1, p. 56, 2023.

[9] H. G. Moghadam, G. K. Sándor, H. H. Holmes, and C. M. Clokie, "Histomorphometric evaluation of bone regeneration using allogeneic and alloplastic bone substitutes," *Journal of oral and maxillofacial surgery,* vol. 62, no. 2, pp. 202-213, 2004.

[10] M. P. F. Graça and S. R. Gavinho, "Calcium phosphate cements in tissue engineering," *Contemporary Topics about Phosphorus in Biology and Materials,* vol. 1, 2020.

[11] F. Ozdemir, I. Evans, and O. Bretcanu, "Calcium phosphate cements for medical applications," *Clinical applications of biomaterials: state-of-the-art progress, trends, and novel approaches,* pp. 91-121, 2017.

[12] G. Lewis, "Injectable bone cements for use in vertebroplasty and kyphoplasty: State-of-the-art review," *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials,* vol. 76, no. 2, pp. 456-468, 2006.

[13] M. Bohner, U. Gbureck, and J. Barralet, "Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment," *Biomaterials,* vol. 26, no. 33, pp. 6423-6429, 2005.

[14] T. J. Brunner, R. N. Grass, M. Bohner, and W. J. Stark, "Effect of particle size, crystal phase and crystallinity on the reactivity of tricalcium phosphate cements for bone reconstruction," *Journal of Materials Chemistry,* vol. 17, no. 38, pp. 4072-4078, 2007.

[15] M. Bohner, T. J. Brunner, and W. J. Stark, "Controlling the reactivity of calcium phosphate cements," *Journal of Materials Chemistry,* vol. 18, no. 46, pp. 5669-5675, 2008.

[16] K. Hurle *et al.*, "Osteogenic lithium-doped brushite cements for bone regeneration," *Bioactive Materials,* vol. 16, pp. 403-417, 2022.

[17] F. Tamimi, Z. Sheikh, and J. Barralet, "Dicalcium phosphate cements: Brushite and monetite," *Acta Biomaterialia,* vol. 8, no. 2, pp. 474-487, 2012, doi: 10.1016/j.actbio.2011.08.005.

[18] L. Ding *et al.*, "Preparation and characterizations of an injectable and biodegradable high-strength iron-bearing brushite cement for bone repair and vertebral augmentation applications," *Biomaterials Science,* vol. 11, no. 1, pp. 96-107, 2023.

[19] M. Parent, H. Baradari, E. Champion, C. Damia, and M. Viana-Trecant, "Design of calcium phosphate ceramics for drug delivery applications in bone diseases: A review of the parameters affecting the loading and release of the therapeutic substance," *Journal of Controlled Release,* vol. 252, pp. 1-17, 2017.

[20] G. Mestres *et al.*, "Changes in the drug release pattern of fresh and set simvastatin-loaded brushite cement," *Materials Science and Engineering,* vol. 58, pp. 88-96, 2016.

[21] A. A. Mirtchi, J. Lemaitre, and N. Terao, "Calcium phosphate cements: study of the b-tricalcium phosphate - monocalcium phosphate system," *Biomaterials,* vol. 10, pp. 475-480, 1989, doi: 10.1016/0142-9612(89)90089-6.

[22] L. Borkowski *et al.*, "Fluorapatite ceramics for bone tissue regeneration: Synthesis, characterization and assessment of biomedical potential," *Materials Science and Engineering: C,* vol. 116, p. 111211, 2020.

[23] L. Cui *et al.*, "A novel tissue-engineered bone graft composed of silicon-substituted calcium phosphate, autogenous fine particulate bone powder and BMSCs promotes posterolateral spinal fusion in rabbits," *Journal of Orthopaedic Translation,* vol. 26, pp. 151-161, 2021.

[24] C.-L. Ko, J.-C. Chen, C.-C. Hung, J.-C. Wang, Y.-C. Tien, and W.-C. Chen, "Biphasic products of dicalcium phosphate-rich cement with injectability and nondispersibility," *Materials Science and Engineering: C,* vol. 39, pp. 40-46, 2014, doi: 10.1016/j.msec.2014.02.033.

[25] D. L. Alge, W. S. Goebel, and T.-M. Gabriel Chu, "In vitro degradation and cytocompatibility of dicalcium phosphate dihydrate cements prepared using the monocalcium phosphate monohydrate/hydroxyapatite system reveals rapid conversion to HA as a key mechanism," *J Biomed Mater Res Part B: Appl Biomater,* vol. 100B, pp. 595-602, 2012, doi: 10.1002/jbm.b.31938/full.

[26] C.-H. D. Chen, C.-C. Chen, M.-Y. Shie, C.-H. Huang, and S.-J. Ding, "Controlled release of gentamicin from calcium phosphate/alginate bone cement," *Materials Science and Engineering: C,* vol. 31, no. 2, pp. 334-341, 2011.

[27] D. Apelt *et al.*, "In vivo behavior of three different injectable hydraulic calcium phosphate cements," *Biomaterials,* vol. 25, no. 7-8, pp. 1439-1451, 2004.

[28] R. Gumpert *et al.*, "Demineralization after balloon kyphoplasty with calcium phosphate cement: a histological evaluation in ten patients," *European Spine Journal,* vol. 23, pp. 1361-1368, 2014.

[29] M. Yomoda, S. Sobajima, A. Kasuya, and M. Neo, "Calcium phosphate cement–gelatin powder composite testing in canine models: Clinical implications for treatment of bone defects," *Journal of Biomaterials Applications,* vol. 29, no. 10, pp. 1385-1393, 2015.

[30] T. Kurien, R. G. Pearson, and B. E. Scammell, "Bone graft substitutes currently available in orthopaedic practice," *The Bone & Joint Journal,* vol. 95-B, pp. 583-597, 2013.

[31] L. Schröter, F. Kaiser, S. Stein, U. Gbureck, and A. Ignatius, "Biological and mechanical performance and degradation characteristics of calcium phosphate cements in large animals and humans," *Acta Biomaterialia,* vol. 117, pp. 1-20, 2020.

[32] F. Theiss *et al.*, "Biocompatibility and resorption of a brushite calcium phosphate cement," *Biomaterials,* vol. 26, no. 21, pp. 4383-4394, 2005.

[33] G. Cama, "Calcium phosphate cements for bone regeneration," in *Biomaterials for Bone Regeneration*, S. V. Vlierberghe Ed.: Woodhead Publishing, 2014, pp. 3-25.

[34] F. ORTHO. "Eurobone 2." https://www.fhortho.com/en/our-products/bone-substitutes/eurobone-2/ (accessed 05-11, 2024).

[35] M.-P. Ginebra, C. Canal, M. Espanol, D. Pastorino, and E. B. Montufar, "Calcium phosphate cements as drug delivery materials," *Advanced Drug Delivery Reviews,* vol. 64, pp. 1090-1110, 2012.

[36] S. V. Dorozhkin, "Self-Setting Calcium Orthophosphate Formulations," *J. Funct. Biomater,* vol. 4, no. 4, pp. 209-311, 2013, doi: https://doi.org/10.3390/jfb4040209.

[37] I. Khairoun, M. Boltong, F. Driessens, and J. Planell, "Limited compliance of some apatitic calcium phosphate bone cements with clinical requirements," *Journal of Materials Science: Materials in Medicine,* vol. 9, no. 11, pp. 667-671, 1998.

[38] A. Vezenkova and J. Locs, "Sudoku of porous, injectable calcium phosphate cements–Path to osteoinductivity," *Bioactive Materials,* vol. 17, pp. 109-124, 2022.

[39] B. van Oirschot, A. G. Mikos, Q. Liu, J. J. van den Beucken, and J. A. Jansen, "Fast Degradable Calcium Phosphate Cement for Maxillofacial Bone Regeneration," *Tissue Engineering Part A,* vol. 29, no. 5-6, pp. 161-171, 2023.

[40] Q. Liu *et al.*, "Tunable calcium phosphate cement formulations for predictable local release of doxycycline," *Materialia,* vol. 28, p. 101769, 2023.

[41] S. Vahabzadeh, M. Roy, and S. Bose, "Effects of silicon on osteoclast cell mediated degradation, in vivo osteogenesis and vasculogenesis of brushite cement," *Journal of Materials Chemistry B,* vol. 3, no. 46, pp. 8973-8982, 2015.

[42] B. R. Constantz *et al.*, "Skeletal repair by in situ formation of the mineral phase of bone," *Science,* vol. 267, no. 5205, pp. 1796-1799, 1995.

[43] M. Bohner, "Reactivity of calcium phosphate cements," *Journal of Materials Chemistry,* vol. 17, no. 38, pp. 3980-3986, 2007.

[44] G. Vereecke and J. Lemaître, "Calculation of the solubility diagrams in the system Ca (OH) 2-H3PO4-KOH-HNO3-CO2-H2O," *Journal of Crystal Growth,* vol. 104, no. 4, pp. 820-832, 1990.

[45] A. Gisep, R. Wieling, M. Bohner, S. Matter, E. Schneider, and B. Rahn, "Resorption patterns of calcium-phosphate cements in bone," *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials,* vol. 66, no. 3, pp. 532-540, 2003.

[46] S. Larsson and T. W. Bauer, "Use of injectable calcium phosphate cement for fracture fixation: a review," *Clinical Orthopaedics and Related Research®,* vol. 395, pp. 23-32, 2002.

[47] J. Luo, I. Ajaxon, M. P. Ginebra, H. Engqvist, and C. Persson, "Compressive, diametral tensile and biaxial flexural strength of cutting-edge calcium phosphate cements," *Journal of the mechanical behavior of biomedical materials,* vol. 60, pp. 617-627, 2016.

[48] S. H. Dabiri, A. Lagazzo, B. Aliakbarian, M. Mehrjoo, E. Finocchio, and L. Pastorino, "Fabrication of alginate modified brushite cement impregnated with antibiotic: Mechanical, thermal, and biological characterizations," *Journal of Biomedical Materials Research Part A,* vol. 107, no. 9, pp. 2063-2075, 2019.

[49] Q. Liu, W. F. Lu, and W. Zhai, "Toward stronger robocast calcium phosphate scaffolds for bone tissue engineering: A mini-review and meta-analysis," *Biomaterials Advances,* vol. 134, p. 112578, 2022.

[50] A. T. Saleh, L. S. Ling, and R. Hussain, "Injectable magnesium-doped brushite cement for controlled drug release application," *Journal of materials science,* vol. 51, no. 16, pp. 7427-7439, 2016.

[51] K. Hurle, J. Oliveira, R. Reis, S. Pina, and F. Goetz-Neunhoeffer, "Ion-doped brushite cements for bone regeneration," *Acta Biomaterialia,* vol. 123, pp. 51-71, 2021.