REVIEW ARTICLE

Current Use of Calcium Sulfate Bone Grafts

Authors

Andrew W. Barone, DDS, Sebastiano Andreana, DDS, MS, Rosemary Dziak, Ph.D

Affiliation

University at Buffalo, School of Dental Medicine, Buffalo, NY, USA, 14214

Correspondence

Rosemary Dziak

Email: rdziak@buffalo.edu

Abstract

Bone graft placement is the most widely used therapeutic strategy for the surgical correction of osseous defects. In recent years, increasing attention has been given to the development of synthetic bone grafts. Of those currently available, calcium sulfate materials exhibit several unique properties that warrant discussion. These include their intrinsic osteogenic potential, their stimulatory effect on angiogenesis, the fact that they are fully biodegradable, the lack of proinflammatory responses following their placement in situ, and their lost cost of production. However, despite the attractiveness of these features, the use of calcium phosphate materials for bone grafting continues to be more widespread. This review examines the current use of calcium sulfate bone grafts in regenerative medicine. It also considers their clinical drawbacks before providing insight into the development of new calcium sulfate grafting constructs that might address these concerns.

Introduction

Bone is representative of a highly dynamic living tissue with an intrinsic capacity for self-repair after injury.[1] However, the regenerative capacity of host bone tissue is subject to limitation in cases of extensive skeletal breakdown. Such cases often necessitate the placement of bone grafts to achieve complete osseous regeneration. Bone grafts serve multiple purposes.[2-4] They serve to uphold the structural integrity of damaged osseous tissues by maintaining their skeletal architecture during host bone remodeling. They also prevent against further skeletal breakdown by buttressing the surrounding walls of osseous defects that might otherwise collapse under mechanical load. In addition, bone grafts can improve the osseointegration of implanted devices by reducing void formation at the tissue/prosthesis interface, thereby improving the functional stability of prostheses *in situ*. For these reasons, bone graft placement represents the most widely used therapeutic strategy for the surgical correction of osseous defects.[4]

At present, host-derived autografts represent approximately 58% of all bone grafts in current use.[5] Their harvesting, however, requires patients to undergo an additional surgical procedure, thereby placing them at risk for morbidity at a second surgical site. In addition, the amount of autogenous bone available for grafting is limited and may be insufficient for filling large scale osseous defects. When autograft harvesting is contraindicated or declined by patients, allografts are the most common alternative.[6] Allografts though tend to be expensive and pose risks for viral transmission. The transplantation of allogenous bone also carries a risk for the development of graft versus host disease. Furthermore, it is not uncommon for patients to express reservations against receiving bone grafts from unknown donors. The same can be said if not more so for xenografts. Most xenografts are harvested from bovine bone. Despite their reported safety, the presence of bovine prion proteins and the possibility for violent rejection reactions cannot be disregarded.[7-8] To these ends, alternative sources for bone grafts deserve exploration.

With the potential to circumvent some of the drawbacks associated with bone grafts harvested from living tissues, a preponderance of US Food and Drug Administration (FDA) approved alloplastic bone grafts have emerged on the market in recent years.[9-10] Many of these synthetic constructs are purported to have widespread application in bone tissue engineering. As a result, clinicians are faced with a diverse array of alloplastic materials from which to choose. In short, alloplastic materials used as bone grafts should be capable of promoting three processes.[11-12] First, they should promote bone formation over their surfaces via direct bone bonding (osteoconduction). Second, they should be capable of inducing the differentiation of osteoprogenitor cells toward the osteoblast phenotype (osteoinduction). Lastly, they should have a stimulatory effect on the activity of bone-forming osteoblasts (osteogenesis).

Alloplastic bone grafts are designed to function as scaffolding units capable of housing the cellular regulators of bone remodeling and supporting the deposition of osseous matrix by resident osteoblasts.[11] Several biochemical and physical parameters must be considered in the course of their development. Foremost, any biomaterial contemplated for human implantation must be entirely non-toxic. Their biomechanical strength, moreover, needs be carefully assessed to determine whether their use is suitable for the correction of load bearing osseous defects. They should also be fully biodegradable. This last feature is particularly important because the presence of residual graft material in the bone fill can adversely affect the architecture of regenerated osseous tissues.[12-15] For this reason, the biodegradation rate of alloplastic bone grafts should closely match the rate at which new bone is formed. If it is too slow, it becomes embedded in the bone fill and can interfere with the otherwise normal secretion and cross-linking of osseous matrix proteins. On the other hand, if it is too fast, the graft may not afford adequate structural support time for complete bone regeneration to occur. This is undesirable as it increases the likelihood of voids being present within the bone fill.

Currently, calcium phosphate (CP) bone grafts are the most used alloplastic materials. The most common variants of which are β-tricalcium phosphate (β-TCP) and synthetic hydroxyapatite (HA).[6, 16] Over the past 40 years, significant research efforts have been made to develop effective CP based bone grafting systems. As recently reviewed, however, their clinical efficacy continues to be hindered, owing to their limited osteogenic potential and low tensile strength.[17] In addition, a recurrently cited limitation of CP bone grafts is the unpredictability surrounding their biodegradation profile. Most exhibit slow resorption rates that are not commensurate with the rate of host bone regeneration *in vivo*.[16-17] Their slowness to resorb is likely due to their limited porosity and low water solubility. The former feature is undesirable in that it can impede the diffusion of nutrients needed by the cellular inhabitants of the grafting system. As for the latter, several authors have commented on the adverse consequences of having non-resorbed CP particles present in the bone fill.[16-18] Some of these include the incitement of proinflammatory responses and the disruption of osteoclastic processes that mediate bone remodeling.[18] Impaired resorptive processes warrant concern since these processes are coupled to the ossification of inorganic osseous matrix by bone-forming osteoblasts.[19] In their absence physiological bone regeneration would not occur. On a final note, impaired resorptive processes are also problematic since the degradation of alloplastic materials *in situ* is ultimately dependent on their uptake and metabolism by bone-resorbing osteoclasts.[20-21]

Considering the prognostic complications associated with CP bone grafts, there has been considerable effort devoted to the exploration of alternative synthetic constructs. Recently, there has been a resurgence of interest in the use of calcium

sulfate (CS) for bone grafting.[22-24] CS bone grafts hold a unique position with respect to other regenerative biomaterials. The raw material from which they are synthesized tends to be inexpensive and relatively abundant. They are also fully biodegradable and can function as a barrier membrane supportive of guided tissue regeneration.[25] The latter feature is noteworthy as it has motivated recent increases in the use of CS by dentists for bone augmentation procedures prior to implant placement.[26] Aside from this, evidence suggests that CS bone grafts are promotive of angiogenesis. Strocchi and colleagues found greater microvascular vessel density in the bone fills of osseous defects in rabbits treated with CS bone grafts versus those treated with autografts.[27] On a different note, some of the most cited disadvantages of CS bone grafts is their rapid resorptive rate and their poor biomechanical strength.[28] This has limited their use primarily to the correction of small non-load bearing osseous defects. This paper reviews the current use of CS in the surgical correction of osseous defects. It also considers recent research into new CS based bone grafting systems that might address some of the limitations associated with conventional CS bone grafts.

Clinical Applications

From a historical perspective, reports on the clinic use of CS (Plaster of Paris) for bone grafting date as far back as 1892 where it was used by Dressman of the Trendelenburg clinic to fill osseous defects in patients with tuberculosis osteomyelitis.[29] Further reports exist on its successful use by surgeons during the Vietnam War for filling craniofacial bone defects.[30] In 1980, Coetzee described 110 patients that were treated with CS bone grafts for filling craniofacial bone defects.[31] He found that CS bone grafts ensured complete bone regeneration with results comparable to autografts, if not better. With the progress of time, CS has been used in a wide range of clinical procedures that include osseous augmentation for dental implant placement, orthopedic surgeries, and for the purposes of bone regeneration following oncologic resections.[13, 23-25] CS can be manipulated into different forms such as hard pellets or an injectable paste that hardens *in situ*.[32] Compared to autografts, injectable CS bone graft cements have the advantage of not requiring bone resection to accommodate their implantation. Other reasons underlying the attractiveness of CS for bone grafting include its low risk for inducing a proinflammatory response to its established potential to promote osteogenesis.[13] Evidence also shows that CS can be treated with various pharmacologic agents and osteogenic growth factors prior to its placement into wound sites and can effectively release these agents from its inner constructs.[33-35] For instance, studies indicate that the incorporation of antibiotics into CS can reduce the risk of post implantation infections in patients with chronic osteomyelitis.[36-37] In addition, CS has been shown to be a suitable carrier of morphogens and growth factors that promote the differentiation of mesenchymal stem cells toward the osteoblast phenotype and enhance the metabolic activity of human osteoblastic cells *in vitro* and *in vivo*.[38-39] It is beyond the scope of this review to provide a full description of the many studies detailing the

successful surgical application of CS for bone tissue engineering. This discussion is therefore limited to the clinical arenas wherein CS has shown the most success as a grafting material for bone repair. These include dentistry and skeletal tumor surgery.

Extraction Socket Preservation

In dentistry, partial or complete resorption of the residual alveolar ridge is an undesirable sequelae of tooth extractions. To this end, multiple studies support the use of CS bone grafts for alveolar ridge preservation.[40-44] A study conducted by Aimetti et al evaluated the healing of human extraction sockets filled only with CS in patients awaiting maxillary dental implant placement.[42] These authors report that filling the sockets with injectable CS cement effectively reduced alveolar ridge resorption and accelerated bone maturation. The bone regenerative potential of CS bone grafts can be further enhanced by the addition of osteogenic growth factors; a common source of which is platelet-rich plasma (PRP). When lysed, platelets release growth factors capable of stimulating the activity of bone-forming osteoblasts. In this regard, an attractive feature of injectable CS cements is that they undergo an exothermic crystallization reaction upon their implantation in situ. When mixed with PRP, the heat released from this setting reaction is sufficient to induce platelet lysis.[33] A clinical study performed by Kutkut et al examined the amount of bone regeneration in sixteen patients who underwent dental extractions.[43] Eight of the patients received CS mixed with PRP in their extraction sockets, while the other eight received collagen resorbable plugs. At the conclusion of the 3-month study, the sockets of the patients who received CS mixed with PRP demonstrated greater vital bone volume and improved wound healing. More recently, a study by Cheah et al examined alveolar ridge preservation in twelve patients who underwent non-molar extractions followed by placement of either CS alone or CS mixed with PRP in the sockets. After a 4-month healing period, the six patients who had received CS bone grafts mixed with PRP demonstrated greater mineralized bone volume than the six who that received CS bone grafts alone.

Maxillary Sinus Augmentation

Another common use of CS bone grafts in dentistry is for maxillary sinus floor augmentation. This surgical procedure is known as a sinus-lift or sinus floor elevation. It is used to increase the vertical dimension of maxillary alveolar bone. The main indication for performing a sinus-lift is when the amount of bone in the posterior maxilla in inadequate to support dental implant placement. Another indication is when the quality of maxillary alveolar bone is prone to compromise the stability of dental implants after their placement. In a longitudinal prospective study conducted by De Leonardis and Pecora, the histologic characteristics of bone formed *in situ* after sinus augmentation procedures done with CS bone grafts were investigated.[46] Histologic biopsies from the participants were harvested at 6 and 9 months post-operatively. Examination of the specimens revealed that the placement of CS into surgical sites facilitated the formation of vital trabecular bone that was suitable for

accommodating the integration of dental implants. On a side note, residual CS particles were found to be present in radiographs taken at 6-months but not at 9-months. Furthermore, the quality of the bone formed was found to correlate with time elapsed. A study carried out by Dasmah et al evaluated the effectiveness of using CS for augmenting posterior maxillary bone in ten patients with edentulous maxillae.[47] During the first phase of this study, the patients underwent sinus lifts followed by CS bone graft placement. After a 4-month healing period, the authors observed new bone formation with a mean value of 22.1%. In the second phase of the study, 40 dental implants were placed. Of the 40 implants placed, only 1 was lost at the one-year follow-up. The authors concluded that CS bone grafts can be used successfully for osseous augmentation in the endentulous posterior maxilla, promoting the generation of vital bone supportive of dental implant placement.

Bone Tumor Surgery

The surgical resection of bone tumors often leaves behind a large contained osseous defect.[48-52] Emerging evidence suggests that CS bone grafts can be successfully used to repair these defects. In support of this, a study by Gitelis et al reports that CS is an effective alternative to autogenous bone grafts for the surgical correction of osseous defects generated from the curettage of benign bone lesions.[49] Furthermore, Clayer reported that CS bone grafts can be used successfully for the regeneration of bone in osseous defects produced after the excision of aneurismal bone cysts. This author found that defects filled with CS cements showed favorable healing rates and radiological responses with a low incidence of complications. In a later study performed by Kim et al, 56 patients with either benign or low-grade malignant bone tumors underwent surgical curettage.[51] The bony defects produced following the tumor resections were then filled with either injectable CS or allogenic demineralized bone matrix (DBM). The radiographic results of the study showed that bone regeneration in defects filled injectable CS was comparable to those filled with DBM. However, the mean healing time of patients who received injectable CS tended to be more delayed versus those who received DBM.

Clinical Drawbacks

As mentioned, the most cited limitations to the clinical application of CS bone grafts are their rapid resorptive rate and lack of biomechanical strength. The rapidity of their biodegradation profile relative to CP-based bone grafts can be attributed to the greater solubility of CS in physiological body fluids.[53-55] This warrants attention in clinical cases where bone healing might be compromised or delayed, such as in elderly patients or those who are immunocompromised. To this end, Jepeganam and von Schroeder reported on early implant failure in the cases of 2 elderly patients who received CS bone grafts for the management of malunited distal radius fractures.[56] CS bone grafts have been further criticized for having a resorptive rate that is higher than new bone growth.[52] In this regard, Glazer et al found that CS bone grafts resorbed too quickly to support spinal fusion in a rabbit model.[57]

The lower biomechanical strength of CS versus CP-based bone grafts can be attributed to the greater porosity of their crystalline architectures *in situ*.[53] Moreover, the hardening of CS cements *in situ* is often accompanied by volumetric expansion, thereby contributing to the generation of a scaffold substructure with a lesser material density than would be the case otherwise.[58] These features have limited the use of CS primarily to the filling of small non-load bearing osseous defects. On a final note, some studies could not find evidence supportive of the purported intrinsic osteogenic potential of CS bone grafts.[59-60] Shaffer and App used CS to fill periodontal defects in a small number of patients.[59] In postoperative radiographs taken at 6-months, these authors observed complete resorption of the graft without any indication of new bone formation.

Future Directions

Although the results of selective studies do not advocate the use of CS bone grafts in skeletal reconstructive surgery, reports published within the past year provide evidence to the contrary.[23-24, 61] For instance, Tamboowalla et al retrospectively assessed the clinical outcomes in patients that received antibiotic impregnated CS bone grafts for the treatment of either osteomyelitis or non-union long bone fractures.[61] At a 1-year postoperative follow-up, this group found that 9 of the 10 patients with osteomyelitis demonstrated good radiographic healing of the osseous defects and complete resolution of infection. With respect to the patients being treated for nonunion long bone fractures, most demonstrated evidence of union at an average of 3.75 months after surgery.

CS bone graft composites

Some studies have provided insight into ways in which CS bone grafting systems can be modified to enhance their bone regenerative potential. Tan et al retrospectively reviewed the clinical outcomes of 25 patients who underwent treatment with CS/CP bone graft composites following bone tumor resection.[62] These authors found that CS/CP bone grafts exhibit a characteristic resorption rate and demonstrate a familiar radiographic pattern of graft dissolution in combination with new bone ingrowth. In this regard, knowledge of the radiographic appearance of CS bone grafts and their changing appearance overtime owing to their resorption can allow clinicians to discriminate between the dissolution of the graft *in situ* versus tumor recurrence.

When comparing CS to synthetic HA bone grafts, the consensus seems to be that CS grafts have greater osteogenic potential and HA grafts provide longer-term space-maintenance in lieu of their slower resorption rates *in situ*. Crespi et al assessed the amount of bone formed in tooth extraction sockets filled with either CS or magnesium-enriched HA grafts (MHA) at 3-months.[63] Their research group used a split mouth design that involved 15 patients who required the extraction of three teeth on each side of the jaw. Extraction sockets on one side were filled with CS and those on the other were filled with MHA grafts. The results of this study revealed that sockets filled with CS demonstrated a greater reduction in the

vertical bone height of the alveolar ridge versus those filled with MHA grafts. On the other hand, histologic examination indicated more bone formation and faster graft resorption in the CS group and more residual graft material in the MHA group. To these ends, some studies have focused on developing bone grafting systems that combine the osteogenic potentiating effects of CS bone grafts with the slower resorption times of synthetic HA grafts.[23-24]

At present, there is increasing attention being given to slowing the biodegradation rate of CS *in situ* by the addition of synthetic HA to the bone graft material. Hence, the bone graft is comprised of a combination of both CS and synthetic HA. Recent findings suggest that composite CS/HA bone grafts have slower resorption rates versus CS bone grafts, thereby imparting them with a longer-term space-maintaining ability.[23-24] With respect to the issue of having residual HA present in the bone fill, one research group evaluated the percentage of HA particles present in the bone fill of dental defects treated with CA/HA composite grafts. Analysis at 8 months revealed that HA residual graft particles constituted only 3% of the bone fill. A recent review by Barnes and Kurtzman discusses the successful use of a CS/HA composite graft product called Bond Apatite for the dental treatment of osseous defects in 454 clinical cases.[23] These included defects associated with extraction sockets, periodontal lesions, and infected apices of tooth roots. Of those, a failure rate of less than 2% was noted.

Nanocrystalline CS

Because large-scale osseous defects often require lengthy recovery times, grafting materials with slower resorption rates and higher compressive strengths are most suitable for their treatment. In a study by Hu et al, the authors studied the effect of particle size on the biophysical properties of CS bone grafts.[64] This group found that grafts comprised of smaller sized CS particles demonstrated increased compressive strength and a slower *in vitro* degradation rate. In this regard, various nano-sized bone grafting materials have received FDA approval in the past decade for osseous corrective surgeries.[65] Several groups have commented on the advantages of nanomaterials compared to conventional-sized bulky constructs.[66-67] Examples include superior mechanical strength, exponentially higher surface area, increased porosity, and a biodegradation rate that is commensurate with new bone growth. Slower resorption rates are desirable as more time is allotted for cell proliferation and bone remodeling to occur. This feature among others are suggested to permit osteoconduction to occur at deeper levels within the three-dimensional architecture of the scaffolding system, thus leading to improved bone formation.[65]

An ideal nanomaterial for bone grafting should be capable of releasing growth factors or drug content in a controlled fashion, thereby keeping it localized to the wound site.[69-70] In this regard, the following properties of nanomaterials deserve additional comment: exponentially increased surface area and nanoporous architectures. An increased surface area provides more space for growth factor adsorption and

therefore increases the amount of biologic agent available for delivery. A nanoporous architecture, on the other hand, can effectively slow the outward diffusion of pre-loaded growth factor content from its inner constructs.[71] Timeframe of release is a critical parameter because different growth factors have characteristic half-lives that effectively dictate the duration of time in which they remain biologically active.

Recent translational studies provide evidence supportive of the clinical use of nanocrystalline CS (nCS) for the reconstruction of osseous defects.[72-77] Compared to CS, nCS offers many advantages. These include superior mechanical strength, increased resistance to fracture, higher surface area, and a slower resorptive rate. Moreover, unlike conventional sized CS, nCS can release growth factors and various pharmacologic in a sustained fashion overtime, thereby keeping these therapeutic agents confined to wound sites and preventing their diffusion outward. Accumulating evidence indicates that nCS is virtually non-toxic and may represent a functional vehicle for the delivery of osteogenic factors, such as platelet-derived growth factor (PDGF-BB) and melatonin.[74, 77] In an *in vitro* study conducted by Barone et al, the cellular viability of mesenchymal stem cells and human osteoblastic cells cultured on solid discs fabricated from nCS mixed with human platelet lysate (hPL) were measured.[74] The authors found that both cell types demonstrated increased metabolic activity when cultured on nCS/hPL discs versus nCS discs alone. In a study using a rat model, nCS scaffolds containing PRP and BMP-2 modified mesenchymal stem cells were shown to successfully promote bone regeneration in critical-sized cranial vault defects.[75] Additional evidence of the bone regenerative ability of nCS was observed in a preclinical critical size canine mandibular bone defect model in which nCS filled sites displayed significantly greater bone yield in comparison to unfilled control sites.[78] Based on these findings, there is a strong basis for further development of nCS as a clinical regenerative therapeutic.

Conclusion

Based on a long history of published research and clinical reports, there appears to be distinct advantages of the use of CS in bone augmentation procedures, particularly in dentistry as well as bone tumor surgical procedures. Safety and efficacy has been consistently demonstrated and clinically experienced. These studies, reviewed here, also provide some insight into the need for further investigations to improve on the physical properties of CS to optimize bone fill with the use of nanosizing the material and to fabricate composites with other materials to enhance the bone augmentation properties of CS.

References:

[1] Rios HF, Giannobile WV. Principles of bone biology and regeneration. *Implant Site Development*. 2015;30:1-3

[2] Salinas AJ, Vallet-Regí M. Bioactive ceramics: from bone grafts to tissue engineering. *RSC advances*. 2013;3:11116-11131

[3] Kuznetsova DS, Timashev PS, Bagratashvili VN, Zagaynova EV. Scaffold-and cell system-based bone grafts in tissue engineering. *Medical Technologies in Medicine/Sovremennye Tehnologii v Medicine*. 2014;6:201-211

[4] Griffin KS, Davis KM, McKinley TO, Anglen JO, Chu TM, Boerckel JD, et al. Evolution of bone grafting: bone grafts and tissue engineering strategies for vascularized bone regeneration. *Clinical Reviews in Bone and Mineral Metabolism*. 2015;13:232-44

[5] Samartzis D, Shen FH, Goldberg EJ, An HS. Is autograft the gold standard in achieving radiographic fusion in one-level anterior cervical discectomy and fusion with rigid anterior plate fixation?. *Spine*. 2005;30:1756-1761

[6] Zimmermann G, Moghaddam A. Allograft bone matrix versus synthetic bone graft substitutes. *Injury*. 2011;42:S16-S21

[7] Platt JL. A perspective on xenograft rejection and accommodation. *Immunological reviews*. 1994;141:127-149

[8] Rodriguez AE, Nowzari H. The long-term risks and complications of bovine-derived xenografts: A case series. *Journal of Indian Society of Periodontology*. 2019;23:487

[9] Carson JS, Bostrom MP. Synthetic bone scaffolds and fracture repair. *Injury*. 2007;38:S33-S37

[10] Liu Y, Lim J, Teoh SH. Development of clinically relevant scaffolds for vascularised bone tissue engineering. *Biotechnology advances*. 2013;31:688-705

[11] Moore WR, Graves SE, Bain GI. Synthetic bone graft substitutes. *ANZ journal of surgery*. 2001;71:354-361

[12] Janicki P, Schmidmaier G. What should be the characteristics of the ideal bone graft substitute? Combining scaffolds with growth factors and/or stem cells. *Injury*. 2011;42:S77-S81

[13] Thomas MV, Puleo DA, Al-Sabbagh M. Calcium sulfate: a review. *Journal of long-term effects of medical implants*. 2005;15:599-607

[14] Laurencin C, Khan Y, El-Amin SF. Bone graft substitutes. *Expert review of medical devices*. 2006;3:49-57

[15] Kumar P, Vinitha B, Fathima G. Bone grafts in dentistry. *Journal of pharmacy & bioallied sciences*. 2013;5:S125

[16] Bohner M, Galea L, Doebelin N. Calcium phosphate bone graft substitutes: Failures and hopes. *Journal of the european ceramic society*. 2012;32:2663-2671

[17] Ambard AJ, Mueninghoff L. Calcium phosphate cement: review of mechanical and biological properties. *Journal of Prosthodontics*. 2006;15:321-328

[18] Hing KA, Wilson LF, Buckland T. Comparative performance of three ceramic bone graft substitutes. *The Spine Journal*. 2007;7:475-490

[19] Gonda Y, Ioku K, Shibata Y, Okuda T, Kawachi G, Kamitakahara M, et al. Stimulatory effect of hydrothermally synthesized biodegradable hydroxyapatite granules on osteogenesis and direct association with osteoclasts. *Biomaterials*. 2009;30:4390-4400

[20] Xia Z, Grover LM, Huang Y, Adamopoulos IE, Gbureck U, Triffitt JT, et al. In vitro biodegradation of three brushite calcium phosphate cements by a macrophage cell-line. *Biomaterials*. 2006;27:4557-4565

[21] Rumpel E, Wolf E, Kauschke E, Bienengräber V, Bayerlein T, Gedrange T, et al. The biodegradation of hydroxyapatite bone graft substitutes in vivo. *Folia morphologica*. 2006;65:43-48

[22] Chen CC, Wang CW, Hsueh NS, Ding SJ. Improvement of in vitro physicochemical properties and osteogenic activity of calcium sulfate cement for bone repair by dicalcium silicate. *Journal of alloys and compounds*. 2014;585:25-31

[23] Baranes D, Kurtzman GM. Biphasic Calcium Sulfate as an alternative grafting material in various dental applications. *Journal of Oral Implantology*. 2019;45:247-255

[24] Yahav A, Kurtzman GM, Katzap M, Dudek D, Baranes D. Bone Regeneration: Properties and Clinical Applications of Biphasic Calcium Sulfate. *Dental Clinics*. 2020;64:453-472

[25] Pecora G, Andreana S, Margarone III JE, Covani U, Sottosanti JS. Bone regeneration with a calcium sulfate barrier. *Oral surgery, Oral medicine, Oral pathology, Oral radiology, and Endodontology*. 1997;84:424-429

[26] Rodríguez-Sendra J, Jiménez N, Picó R, Faus J, Camarena F. Monitoring the Setting of Calcium Sulfate Bone-Graft Substitute Using Ultrasonic Backscattering. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*. 2019;66:1658-1666

[27] Strocchi R, Orsini G, Iezzi G, Scarano A, Rubini C, Pecora G, et al. Bone regeneration with calcium sulfate: evidence for increased angiogenesis in rabbits. *Journal of oral implantology*. 2002;28:273-278

[28] Beuerlein MJ, McKee MD. Calcium sulfates: what is the evidence?. *Journal of orthopaedic trauma*. 2010;24:S46-S51

[29] Peltier LF. The use of plaster of Paris to fill defects in bone. *Clinical Orthopaedics and Related Research*®. 1961;21:1-31

[30] Tay BK, Patel VV, Bradford DS. Calcium sulfate–and calcium phosphate–based bone substitutes: mimicry of the mineral phase of bone. *Orthopedic Clinics*. 1999;30:615-623

[31] Coetzee AS. Regeneration of bone in the presence of calcium sulfate. *Archives of Otolaryngology*. 1980;106:405-409

[32] Chen Z, Liu H, Liu X, Cui FZ. Injectable calcium sulfate/mineralized collagen-based bone repair materials with regulable self-setting properties. *Journal of Biomedical Materials Research Part A*. 2011;99:554-563

[33] Intini G, Andreana S, Margarone Iii JE, Bush PJ, Dziak R. Engineering a bioactive matrix by modifications of calcium sulfate. *Tissue engineering*. 2002;8:997-1008

[34] Intini G, Andreana S, Intini FE, Buhite RJ, Bobek LA. Calcium sulfate and platelet-rich plasma make a novel osteoinductive biomaterial for bone regeneration. *Journal of translational medicine*. 2007;5:13

[35] Tuzuner T, Uygur I, Sencan I, Haklar U, Oktas B, Ozdemir D. Elution characteristics and mechanical properties of calcium sulfate-loaded bone cement containing teicoplanin. *Journal of Orthopaedic Science*. 2007;12:170-177

[36] Gitelis S, Brebach GT. The treatment of chronic osteomyelitis with a biodegradable antibiotic-impregnated implant. *Journal of orthopaedic surgery*. 2002;10:53-60

[37] Qin CH, Zhou CH, Song HJ, Cheng GY, Zhang HA, Fang J, et al. Infected bone resection plus adjuvant antibiotic-impregnated calcium sulfate versus infected bone resection alone in the treatment of diabetic forefoot osteomyelitis. *BMC musculoskeletal disorders*. 2019;20:246

[38] Qi Y, Wang Y, Yan W, Li H, Shi Z, Pan Z. Combined mesenchymal stem cell sheets and rhBMP-2-releasing calcium sulfate–rhBMP-2 scaffolds for segmental bone tissue engineering. *Cell transplantation*. 2012;21:693-705

[39] Aquino-Martínez R, Angelo AP, Pujol FV. Calcium-containing scaffolds induce bone regeneration by regulating mesenchymal stem cell differentiation and migration. *Stem cell research & therapy*. 2017;8:1-10

[40] Guarnieri R, Pecora G, Fini M, Aldini NN, Giardino R, Orsini G, et al. Medical grade calcium sulfate hemihydrate in healing of human extraction sockets: clinical and histological observations at 3 months. *Journal of periodontology*. 2004;75:902-908

[41] Shi B, Zhou Y, Wang YN. Alveolar ridge preservation prior to implant placement with surgical-grade calcium sulfate and platelet-rich plasma: a pilot study in a canine model. *International Journal of Oral & Maxillofacial Implants*. 2007;22:656-665

[42] Aimetti M, Romano F, Griga FB, Godio L. Clinical and histologic healing of human extraction sockets filled with calcium sulfate. *International Journal of Oral & Maxillofacial Implants*. 2009;24:902-909

[43] Kutkut A, Andreana S, Kim HL, Monaco Jr E. Extraction socket preservation graft before implant placement with calcium sulfate hemihydrate and platelet-rich plasma: A clinical and histomorphometric study in humans. *Journal of periodontology*. 2012;83:401-409

[44] Toloue SM, Chesnoiu-Matei I, Blanchard SB. A clinical and histomorphometric study of calcium sulfate compared with freeze-dried bone allograft for alveolar ridge preservation. *Journal of periodontology*. 2012;83:847-855

[45] Cheah CW, Vaithilingam RD, Siar CH, Swaminathan D, Hornbuckle GC. Histologic, histomorphometric, and cone-beam computerized tomography analyses of calcium sulfate and platelet-rich plasma in

socket preservation: A pilot study. *Implant dentistry*. 2014;23:593-601

[46] De Leonardis D, Pecora GE. Prospective study on the augmentation of the maxillary sinus with calcium sulfate: histological results. *Journal of periodontology*. 2000;71:940-947

[47] Dasmah A, Hallman M, Sennerby L, Rasmusson L. A clinical and histological case series study on calcium sulfate for maxillary sinus floor augmentation and delayed placement of dental implants. *Clinical implant dentistry and related research*. 2012;14:259-265

[48] Kelly CM, Wilkins RM, Gitelis S, Hartjen C, Watson JT, Kim PT. The use of a surgical grade calcium sulfate as a bone graft substitute: results of a multicenter trial. *Clinical Orthopaedics and Related Research®*. 2001;382:42-50

[49] Gitelis S, Piasecki P, Turner T, Haggard W, Charters J, Urban R. Use of a calcium sulfate-based bone graft substitute for benign bone lesions. *Orthopedics*. 2001;24:162-166

[50] Clayer M. Injectable form of calcium sulphate as treatment of aneurysmal bone cysts. *ANZ journal of surgery*. 2008;78:366-370

[51] Kim JH, Oh JH, Han I, Kim HS, Chung SW. Grafting using injectable calcium sulfate in bone tumor surgery: comparison with demineralized bone matrix-based grafting. *Clinics in orthopedic surgery*. 2011;3:191-201

[52] Evaniew N, Tan V, Parasu N, Jurriaans E, Finlay K, Deheshi B, et al. Use of a calcium sulfate–calcium phosphate synthetic bone graft composite in the surgical management of primary bone tumors. *Orthopedics*. 2013;36:216-222

[53] Ricci JL, Weiner MJ, Mamidwar S, Alexander H. Calcium sulfate. *In Bioceramics and their Clinical Applications*. Woodhead Publishing. 2008:302-325

[54] Podaropoulos L, Veis AA, Papadimitriou S, Alexandridis C, Kalyvas D. Bone regeneration using B-tricalcium phosphate in a calcium sulfate matrix. *Journal of Oral Implantology*. 2009;35:28-36

[55] Zima A, Paszkiewicz Z, Siek D, Czechowska J, Ślósarczyk A. Study on the new bone cement based on calcium sulfate and Mg, CO3 doped hydroxyapatite. *Ceramics International*. 2012;38:4935-4942

[56] Jepegnanam TS, von Schroeder HP. Rapid resorption of calcium sulfate and hardware failure following corrective radius osteotomy: 2 case reports. *The Journal of hand surgery*. 2012;37:477-480

[57] Glazer PA, Spencer UM, Alkalay RN, Schwardt J. In vivo evaluation of calcium sulfate as a bone graft substitute for lumbar spinal fusion. *The Spine Journal*. 2001;1:395-401

[58] Singh NB, Middendorf B. Calcium sulphate hemihydrate hydration leading to gypsum crystallization. *Progress in crystal growth and characterization of materials*. 2007;53:57-77

[59] Shaffer CD, App GR. The use of plaster of paris in treating infrabony periodontal defects in humans. *Journal of periodontology*. 1971;42:685-690

[60] Petruskevicius J, Nielsen S, Kaalund S, Knudsen PR, Overgaard S. No effect of Osteoset®, a bone graft substitute, on bone healing in humans: A prospective randomized double-blind study. *Acta orthopaedica Scandinavica*. 2002;73:575-578

[61] Tamboowalla KB, Thomas J, Madan Mohan M, Pilar A, Belliappa CP, Amaravathi RS. The Results of Synthetic Pure Calcium Sulfate Impregnated with Antibiotic in the Management of Bone Infections and Non-unions. *Journal of Karnataka Orthopaedic Association*. 2019;7:22-26

[62] Tan V, Evaniew N, Finlay K, Jurriaans E, Ghert M, Deheshi B, et al. Chronology of the radiographic appearances of the calcium sulfate-calcium phosphate synthetic bone graft composite following resection of bone tumors: a follow-up study of postoperative appearances. *Canadian Association of Radiologists' Journal*. 2016;67:21-27

[63] Crespi R, Capparè P, Gherlone E. Magnesium-enriched hydroxyapatite compared to calcium sulfate in the healing of human extraction sockets: *Radiographic and histomorphometric evaluation at 3 months. Journal of periodontology*. 2009;80:210-218

[64] Hu P, Lun DX, Wang PS, Tu ZM. Effect of Particle Size Ratios on the Physical and Chemical Properties of Surgical-Grade Calcium Sulfate Hemihydrate. *Orthopaedic Surgery*. 2020;12:295-303

[65] Dziak R, Barres L, Andreana S. Recent patents on nanoceramics and bone regeneration and repair. *Recent Patents on Regenerative Medicine*. 2014;4:94-102

[66] Padmanabhan J, Kyriakides TR. Nanomaterials, inflammation, and tissue engineering. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*. 2015;7:355-370

[67] Rogel MR, Qiu H, Ameer GA. The role of nanocomposites in bone regeneration. *Journal of Materials Chemistry*. 2008;18:4233-4241

[68] Duan B, Wang M. Customized Ca–P/PHBV nanocomposite scaffolds for bone tissue engineering: design, fabrication, surface modification and sustained release of growth factor. *Journal of the Royal Society Interface*. 2010;7:S615-S629

[69] Tayalia P, Mooney DJ. Controlled growth factor delivery for tissue engineering. *Advanced materials*. 2009;21:3269-3285

[70] Whitaker MJ, Quirk RA, Howdle SM, Shakesheff KM. Growth factor release from tissue engineering scaffolds. *Journal of Pharmacy and Pharmacology*. 2001;53:1427-1437

[71] Rahman SU, Nagrath M, Ponnusamy S, Arany PR. Nanoscale and macroscale scaffolds with controlled-release polymeric systems for dental craniomaxillofacial tissue engineering. *Materials*. 2018;11:1478

[72] Park YB, Mohan K, Al-Sanousi A, Almaghrabi B, Genco RJ, Swihart MT, et al. Synthesis and characterization of nanocrystalline calcium sulfate for use in osseous regeneration. *Biomedical Materials*. 2011;6:055007

[73] He X, Dziak R, Mao K, Genco R, Swihart M, Li C, et al. Integration of a novel

injectable nano calcium sulfate/alginate scaffold and BMP2 gene-modified mesenchymal stem cells for bone regeneration. *Tissue Engineering Part A*. 2013;19:508-518

[74] Barone A, Morrell A, Dziak R. Fabrication and Characterizatio n of NanoCalcium Sulfate and Human Platelet Lysate as a Growth Factor Delivery System. *International Journal of Dentistry and Oral Health*. 2016;2:doi http://dx.doi.org/10.16966/2378-7090.172

[75] Liu Z, Yuan X, Fernandes G, Dziak R, Ionita CN, Li C, et al. The combination of nano-calcium sulfate/platelet rich plasma gel scaffold with BMP2 gene-modified mesenchymal stem cells promotes bone regeneration in rat critical-sized calvarial defects. *Stem Cell Research & Therapy*. 2017;8:1-9

[76] Fageeh HN, Moussa H, Maddi A, Ciancio S, Dziak R. Nano-Calcium Sulfate as a Local Delivery System for Antibiotics. *International Journal of Dentistry and Oral Health*. 2017;3: doi http://dx.doi.org/10.16966/2378-7090.234

[77] Barone AW, Pringle M, Nguyen D, Dziak R. Dose-Related Effects of Melatonin on Human Osteoblastic Cells *via in vitro* Controlled Release from Nanoscale Calcium Sulfate International Journal of Dentistry and Oral Health. 2020;6:doi http://dx.doi.org/10.16966/2378-7090.325

[78] Khobragade P, Jain A, Nagesh SS, Andreana S, Dziak R, Sunkara SK, et al. Micro-Computed tomography (CT) based assessment of dental regenerative therapy in the canine mandible model. *In Medical Imaging 2015: Biomedical Applications in Molecular, Structural, and Functional Imaging*. International Society for Optics and Photonics. 2015:9417:94171

