General Orthopaedics

EOR | VOLUME 6 | MAY 2021
DOI: 10.1302/2058-5241.6.200083
www.efortopenreviews.org

EFORT open reviews

Review of calcium-sulphate-based ceramics and synthetic bone substitutes used for antibiotic delivery in PJI and osteomyelitis treatment

Razvan Ene[1,2]
Mihai Nica[1,3]
Dragos Ene[1,2]
Adrian Cursaru[1,3]
Catalin Cirstoiu[1,3]

- Infection in orthopaedic and trauma surgery remains a destructive complication with particularly challenging diagnosis and treatment due to bacterial antibiotic resistance and biofilm formation.
- Along with surgical debridement and systemic antibiotics, an important type of adjuvant therapy is local antibiotic delivery, with the purpose of eliminating bacterial colonization and biofilm development.
- Calcium sulphate, as a synthetic absorbable biomaterial used for local antibiotic delivery, has experienced an increasing popularity during the last decade, with multiple promoted advantages such as predictable antibiotic elution kinetics, complete and quick biodegradation, good biocompatibility, and limited associated complications.
- A series of commercially available antibiotic-delivery systems based on calcium sulphate are under investigation and in clinical use, with different presentations, compositions, and application techniques.
- The current article presents the main available calcium-sulphate-based products and the existing data about the clinical and preclinical research results, stemming from their implementation as local antibiotic carriers for surgical site and implant-associated infections treatment and prevention.

Keywords: antibiotic delivery; biofilm; biomaterial; calcium sulphate; infection

Cite this article: *EFORT Open Rev* 2021;6:297-304.
DOI: 10.1302/2058-5241.6.200083

Introduction

Infection associated with orthopaedic implants or surgical site infections is a deleterious complication, resulting in considerable rates of morbidity and mortality. Furthermore, management of this complication is incredibly challenging, with exceedingly high associated financial and psychological burden.[1,2] Despite the progress in prevention, a sustained increase in the number of orthopaedic traumatology and joint replacement surgeries has also increased the total tally of cases complicated by infection. The incidence of infection associated with primary hip or knee arthroplasty interventions can reach up to 1–2%, with even higher numbers after revision surgery.[3] Surgical site infections incidence following fracture fixation reaches considerable percentages, especially in the setting of open fracture treatment.[4,5] Tremendous improvements have been added to the understanding and treatment principles of infection in orthopaedics but the race to mitigate it still has a long way to go.

Treatment principles

A particularly important principle in the etiopathogenesis of implant-associated infections provides the rationale for the difficulty of achieving the best treatment strategy. The ‘race to the surface’ concept, states that bacteria and host tissue compete for the surface of the implants. If bacterial adherence and colonization ensues first, then the eradication of infection becomes difficult.[6] This is due to biofilm which is a conglomeration of bacteria embedded in a self-synthesized mass composed of extracellular polymeric substances (EPSs), very adherent to the underlying surface. Biofilms provide infection persistence by shielding the microorganisms from the action of the host’s immune system and antibiotics (antibiotic tolerance) and allowing phenotypic and genotypic changes (antibiotic resistance).[7] It has been stipulated that tolerance to antibiotics of bacteria included in this layer is one hundred to one

thousand times higher compared to the free-floating, so-called planktonic bacteria. Furthermore, after maturation, the biofilm can become a source of septic emboli which can seed other distant locations.[8,9] Not only is the treatment complicated by the presence of the biofilm but so is the diagnostic process, which depends on a specific and sensitive microbiological identification. For this purpose, novel techniques such as implant sonication have been developed which can break down the biofilm using low-intensity ultrasound waves and which allow isolation of pathogens.[10]

Treatment strategy is based on antibiotic use alone or in conjunction with surgical debridement. Myriad factors influence the overall management algorithm including patient-specific factors, accuracy of diagnosis, type of microorganism and susceptibility profile to antibiotics, location, extension, implant loosening and most importantly the type of infection defined by time of occurrence (acute or chronic). Timing is of great significance because, in conjunction with the type of causative bacteria, it can predict the formation of biofilm and thus the indication for implant removal.[11,12] Once the biofilm has occurred, systemic antibiotic therapy becomes ineffective, especially when poorly vascularized scar tissue is also present at the infection site.

Great efforts have been made to define acute and chronic implant-related infections, especially when it comes to arthroplasty, to guide the decision of retaining or changing the components during surgical treatment. The latest consensus states that the infectious process, and the biofilm formation, is a continuum and a clear-cut time for defining an acute or chronic stage cannot be based on duration of symptomatology or time from initial surgery. For establishing the best management strategy some other variables such as bacterial virulence, implant stability and status of the patient must be taken into consideration.[13]

When the situation is favourable a Debridement Antibiotics and Implant Retention (DAIR) procedure can be implemented, which consists of surgical debridement, antibiotic therapy, and implant retention (with exchange of the modular components). Its success is based on the absence of a mature biofilm which is produced at specific rates by different species of bacteria. The indication for such an approach is a case of early postoperative or acute haematogenous implant-related infection, with symptomatology lasting no more than 30 days.[14] Most of the cases demand revision of all components in either a one-stage or a two-stage procedure. Despite its increasing acceptance during the last decade, the one-stage exchange is suited only for immunocompetent patients, without critical soft tissue or bone damage and for whom the causative bacteria and its susceptibility have been determined.[15] The two-stage approach usually implies the use of articulating or non-articulating spacers, loaded with antibiotics adjusted to the bacterial susceptibility or a broad-spectrum antibiotic when the causative microorganism has not been identified.[16]

Biomaterials used for local antibiotic delivery

Even though, in some cases, the systemic administration of antibiotics is enough for infection eradication, prevention and most treatment strategies rely on concomitant local antibiotic delivery. This is based on the principle that local minimal inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) of antibiotics cannot be achieved only by systemic treatment due to biofilm formation and poor vascular supply.[17] For this purpose different biomaterials have been used and/or are under investigation, which can deliver antibiotics locally, fill the 'dead space' and, when needed, provide reconstruction material for bone defects. Based on their characteristics the two main categories of materials used for local antibiotic treatment are non-degradable and biodegradable.

The most prevalent non-biodegradable material used for decades in orthopaedic surgery, not only for antibiotic delivery, but also for reconstruction and endoprosthetic component fixation, is polymethyl methacrylate (PMMA).[18,19] It has a good biocompatibility and high versatility, and by combining it with different types of antibiotics it can be used for local dispatch, in the form of preformed beads or spacers. The main disadvantage is that after completely eluting the antibiotic it needs to be removed during a second surgical intervention because it can become a substrate for bacterial colonization like the osteosynthesis or arthroplasty implants.[20] Other drawbacks of PMMA are suboptimal drug elution kinetics and limitation to only heat-resistant antibiotics because of its highly exothermal setting reaction (most commonly Vancomycin, Gentamycin and Tobramycin).[21] Therefore the attention has shifted towards the biodegradable delivery alternatives comprised of three main groups: *polymers, natural bone grafts* and *synthetic bone graft substitutes*.

In the management of implant-related infections the choice of a specific antibiotic treatment is based on identification of the causative pathogen and type of infection, acute or chronic. For local antibiotic treatment, a few principles are particularly important: maintaining a concentration higher than MIC/MBC which tackles the planktonic bacteria for at least four to six weeks and a suitable tissue penetration in order not to cause local and systemic toxicity. When biofilm has already formed, tremendously higher antibiotic concentrations (up to 1000-fold increase) are necessary for eradicating the embedded microorganisms and the biofilm per se. This has been termed minimum biofilm eradicating

concentration (MBEC) and it must be maintained by the antibiotic eluting carrier for an extended time to counteract the extracellular polymeric layer. All this can be achieved through a good drug-release profile of the delivering material. The elution kinetics of a biomaterial is highly related to its characteristics provided by composition, surface area, porosity, and affinity for water.[22] Furthermore, the degradation process must not only ensure an optimal antibiotic release, but it should be complete so it does not leave any substrate for bacterial colonization and needs to favour host tissue integration. The antibiotic release of biodegradable materials occurs by three means: surface erosion layer by layer, bulk erosion and diffusion.[23,24]

Autologous or allogenic bone grafts are not usually used for antibiotic drug delivery due to unpredictable elution profiles and because of the infectious risk shown by inappropriately processed grafts. Using a rigorous surgical protocol in combination with adequately prepared bone grafts in terms of purification, and efficient antibiotic impregnation techniques can be a safe and promising adjunctive option for local antibiotic treatment.[25] Beside their extensive use in manufacturing of arthroscopic fixation devices, polymers have been studied as antibiotic carriers in the form of synthetic materials such as polylactic acid (PLA) or polyglycolic acid (PGA) or natural polymers such as chitosan, collagen, thrombin, or gelatin.[26] In clinical practice collagen type I, in the form of a mesh, sponge or fleeces, is the most used as local antibiotic delivery system. However, the literature is not conclusive on the ability of collagen to maintain a sustainable and predictable release of antibiotics and this, along with specific observed complications such as prolonged wound drainage, does not recommend them for implant-related infection treatment.[27]

Synthetic bone graft substitutes studied for local antibiotic delivery include bioactive glass, calcium phosphate and calcium sulphate ceramics. Bioactive glass is one of the first synthetic biomaterials developed, with good bone integration, osteoconductive and osteoinductive capabilities. Beside its high potential to form a strong physical bond to host tissue, it can provide mechanically sound grafting material. The high bioactivity results from the development of a silica gel layer on the glass, due to the release of soluble ions. On this newly formed layer calcium phosphate precipitation ensues, which then is transformed to hydroxyapatite.[28–30] Furthermore, during the last decade, bioactive glass has gained momentum in the treatment of implant-related bone infection. For local antibiotic delivery, it seems that only glass composites (CaP-borate glass composite; chitosan-borate glass composite) can achieve acceptable elution kinetics. Degradation speed depends on the composition and it has been proven that further investigation in this area is needed for establishing an ideal antibiotic delivery system based on bioactive glass. Until solid data are provided by research into this synthetic biomaterial as an antibiotic delivery system its use for this purpose remains experimental and off-label.[31–34]

Calcium phosphate ceramics are probably the most popular synthetic graft substitutes investigated and implemented for bone defect reconstruction. Their chemical structure resembles the mineral stage of bone, composed of calcium apatite and with particularly good osteoconductivity. The mechanical properties and biodegradation profile of this type of ceramics are determined by their calcium to phosphate ratio.

The natural form of calcium apatite, hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) presents with superior osteoconduction potential, stimulatory effect on angiogenesis and bone-binding capability. Unfortunately, the extremely high calcium content and crystalline structure impairs the mechanical strength and biodegradation process, which can extend over a long period. Even with the development of innovative nanocrystalline hydroxyapatite the antibiotic elution profile is not optimal and predictable enough for efficient local drug release.[35] Tricalcium phosphate ($Ca_3(PO_4)_2$), in its betta variant (β-TCP) is another form of calcium apatite with orthopaedic applications that does not have a stable enough biodegradation profile to be used for local delivery of antibiotics.[36,37] Compared to other delivery materials, calcium phosphate ceramics can only bind antibiotics on the surface, which does not provide the ideal application requirements. Nonetheless, the calcium phosphate cements which require a liquid hardening agent can fully incorporate antibiotics and the setting reaction of the cement is isothermal, so it does not restrict the use to only thermoresistant drugs. The problem comes yet again from the slow and sometimes incomplete degradation of the material, which can affect elution kinetics and promote local bacterial colonization.[38,39] In order to tackle these disadvantages of calcium phosphate ceramics, a new wave of research in focused on development of composite biomaterials.[40–42]

Calcium sulphate as antibiotic delivery device

Also known as Plaster of Paris, calcium sulphate (CS) has been used routinely in orthopaedics for an exceptionally long time as a manufacturing material for external fracture fixation devices. Its use inside the human body is a relatively novel implementation and the early research and development was aimed at bone defect reconstruction and treatment of nonunion. Medical-grade calcium sulphate displays excellent biocompatibility and a degradation profile remarkably close to bone formation, lasting between four to eight weeks.[43,44] Furthermore, it possesses good osteoconductive and osteointegration

Table 1. Commercially available calcium-sulphate-based products for musculoskeletal infection treatment

Product	Composition	Available configuration	Preloaded antibiotic	Approved antibiotics for mixing	Suggested optimal elution time (> MIC)
Stimulan®	calcium sulphate hemihydrate	beads (3 mm, 4.8 mm, 6 mm); paste; bullets (7 mm; 9 mm)	–	Vancomycin, Gentamicin, Tobramycin	Over 40 days
OsteoSet-T®	calcium sulphate hemihydrate	beads (3 mm, 4.8 mm)	4% Tobramycin	–	30–60 days
Herafill-G®	calcium sulphate; calcium carbonate; triglycerides	beads	1% Gentamicin	–	3 days
Cerament-G®	60% calcium sulphate; 40% hydroxyapatite	paste	Gentamicin 17.5 mg/mL	–	28 days
Cerament-V®	60% calcium sulphate; 40% hydroxyapatite	paste	Vancomycin 66 mg/mL	–	28 days
PerOssal®	48.5% calcium sulphate; 51.5% nanocrystalline hydroxyapatite	pellets (6 mm)	–	Vancomycin, Gentamicin, Tobramycin, Rifampicin	10 days

capabilities which have made it extremely popular for a multitude of medical applications, leading with bone defect reconstruction, drug delivery, guided tissue regeneration, endodontic surgery, sinus augmentation and ridge preservation.[45]

Calcium sulphate is a naturally occurring mineral as CS dihydrate ($CaSO_4 2H_2O$) which must be purified and processed into calcium hemihydrate for medical utilization. This is achieved through calcination, which involves dehydration under intense heat. Even though it comes in two variations, an alpha and a beta form, mainly the α-hemihydrate is best suited for orthopaedic application due to better physical properties and a complete and predictable biodegradation profile, with minimal local inflammation.[46]

Medical CS comes as preformed products (pellets) or as a compound (cement) that must be mixed and allowed to set at time of use. The setting reaction of CS hemihydrate, after mixed with water, is a marginally exothermic reaction which is greatly influenced by contaminating inorganic or biological molecules. This is the reason why preset forms of CS are to be preferred, especially when used as drug delivery devices. Furthermore, an abnormal setting process will affect the final composition and proprieties of the compound and thus its biodegradation profile. As a delivery system for different therapeutic agents, CS has been used for some time in combination with growth factors, antibiotics, or other drugs.[47–50]

It was suggested that pure calcium sulphate has antimicrobial activity by creating an acidic microenvironment on its surface during in vivo degradation.[51] For musculoskeletal infection treatment, CS has been combined with a variety of antimicrobial agents and used locally to mitigate bacterial colonization and biofilm formation. Management of osteomyelitis benefits not only on the antimicrobial effect of local antibiotic release, but also on the bone regeneration potential provided by implanted calcium sulphate. Another advantage is that, due to its mildly exothermic setting reaction, CS can incorporate a larger diversity of antibiotics, not only thermoresistant agents. To improve the dissolution characteristics and physical proprieties numerous investigations of calcium sulphate composites have been implemented. Suggested combinations of CS with other materials such as autogenous or allogenic bone, calcium phosphate, hydroxyapatite, polylactic acid, bioactive glass, chitosan, gelatin or Platelet-Rich Plasma (PRP) have been analysed for bone regeneration and antibiotic delivery purposes. All the water-soluble antibiotics like aminoglycosides and vancomycin can be incorporated into calcium sulphate compounds and, because the in vivo dissolution is quick and complete, the release of the integrated antibiotics is quick. Nonetheless, even with a variable percentage of the total antibiotic quantity being released, an effective elution with concentrations higher than the MIC is maintained over a few weeks.[52,53]

Nowadays, the commercially available calcium sulphate products, used for musculoskeletal infection treatment are based either on pure CS or composites made by combining CS with other biomaterials. Also, these compounds can be preloaded with a certain antibiotic or can be charged with a drug of choice (Table 1).

Composite materials have been developed to improve mechanical characteristics of the final product. Good physical integrity is important for preventing the ingrowth of soft tissue, providing structural support and a scaffold for osteoconduction when used not only for bone defect reconstruction but also for osteomyelitis or implant-associated infections. Nevertheless, by varying the composition, biodegradation profile (complete/incomplete; speed) and antibiotic elution kinetics (percentage, local concentration, time) are also changed. Surface area of the device determined by its shape and size, porosity of the material and its affinity for water also impact the release of antimicrobial agents.

When it comes to setting reaction of these products, for the majority this takes place ex vivo, except for when they

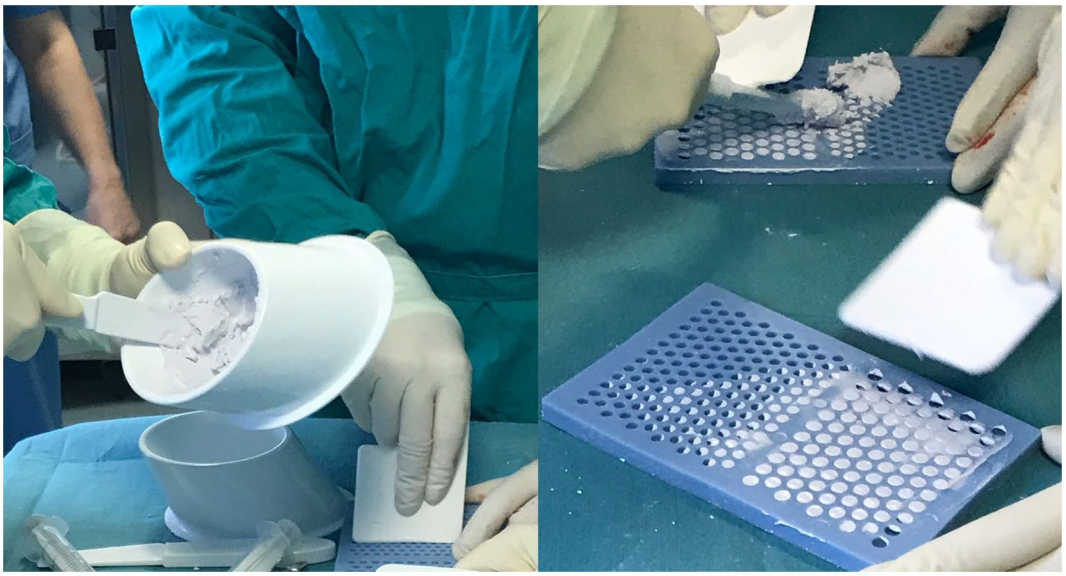

Fig. 1 Preparation of calcium sulphate (CS) beads (Stimulan®) by combining the antibiotic-loaded aqueous solution with CS hemihydrate powder.

are used as an injected paste (Cerament-V®; Cerament-G®). Even though rapid setting compounds are available, and the reaction is just mildly exothermic, the possible contamination and the unpredictable conditions of the internal environment may alter the whole process and the final structure of the material. Custom antibiotic loading is achieved by adding the agent during the preparation of the setting compound (Stimulan®) or by soaking of the preformed material (PerOssal®) (Fig. 1).

The results documented by clinical research articles are derived mostly from osteomyelitis and surgical site infection (SSI) treatment with only three studies using control groups for comparison. Out of these only one was based on a randomized control trial, comparing the use of OsteoSet-T® with loaded PMMA beads, generally accepted as the gold standard for local antibiotic delivery. At the two ends of the spectrum one study compared the use of adjunctive OsteoSet-T® with only local debridement and another included multiple biomaterials for antibiotic delivery used for treating osteomyelitis. All publications reported good rates of eradication, ranging from 80% to 100%, complete or almost-complete degradation of the biomaterial and new bone formation. The most common complication reported by the research evaluating calcium sulphate ceramics for antibiotic delivery is prolonged wound drainage. Be that as it may, this complication cannot be directly corelated with infection recurrence.[54,55] Another valid observation is that composite materials exhibit longer biodegradation times when compared to pure calcium sulphate compounds. The time until complete resorption of the calcium sulphate material, reported by the available studies in the literature, ranges between 3 and 12 weeks after implantation.

As an adjuvant treatment in periprosthetic joint infection management, good research data are limited and inconsistent (Fig. 2). One study published in 2017 by Flierl et al concluded that application of antibiotic-impregnated CS beads does not appear to improve outcomes after analysing 32 DAIR procedures in the setting of acute hematogenous or acute postoperative periprosthetic joint infection.[56] The only other study focused on assessment of only septic revision procedures augmented with local antibiotic delivery by CS beads was Kallala et al 2015, with a reported rate of 6.7% reinfection but a small number of

Fig. 2 Antibiotic-loaded calcium sulphate beads (Stimulan®) implanted around the endoprosthesis for infection prophylaxis.

cases (15 patients).[57] Another study by the same author published in 2018, based on a much higher number of cases, does not explicitly report the reinfection rate, which is 6.5% (23 out of 356 cases of septic revision). Nonetheless, the 2018 article included a mix of cases, with revision surgery performed for aseptic complications also. The same situation of mixed cases of septic and aseptic revision cases are presented by McPherson et al in 2013 and Lum and Pereira in 2018, with reinfection rates of 2.4% (six patients out 250) and respectively no cases of reinfection out of 56 cases.[58,59]

Regarding specific complications of CS bead use for antibiotic delivery, probably the most exhaustive and relevant article is the one published by Kallala et al in 2018, based on a methodical analysis of a large case series of 755 patients who underwent hip or knee arthroplasty revision surgery with implantation of pure CS beads loaded with vancomycin and tobramycin. It reports three specific complications as prolonged wound drainage (4.2%; with a 3.2% to 51.0% range reported in the literature), transient hypercalcemia (5.4%) and heterotopic ossification (1.7%). A pertinent observation is that total volume of implanted beads directly correlates with the incidence of complications, especially for wound leakage and hypercalcemia. This brings the recommendation for precautionary measures against hypercalcemia, such as monitoring serum calcium levels, screening for specific comorbidities and limiting the volume of beads to 40 cc per intervention (80 cc if used intramedullary).[60] Another possible deleterious effect of implanted CS beads which is under investigation refers to potential increased wear of the prosthetic joint articular surfaces by the interposition of the material beads and the mechanical abrasion during joint movement.

Going back to the basic principles, the main objectives for local antibiotic release provided by synthetic ceramics are to suppress the planktonic bacterial colonies, prevent biofilm formation on the implant surface and on their own surface, and, if possible, eliminate already formed biofilms. This can be achieved by ensuring local MIC/MBC and MBEC of antibiotics and having a predictable and complete biodegradation profile. A study published in 2015 by Howlin et al used experimental research for evaluation of pure calcium-sulphate 4.8-mm beads, loaded with tobramycin, vancomycin, or vancomycin-tobramycin in their ability to eradicate planktonic methicillin-resistant Staphylococcus aureus (MRSA) and Staphylococcus epidermidis, prevent biofilm formation over multiple days and to eradicate preformed biofilms. The results showed that antibiotic elution from the CS beads was highly effective in eradicating the planktonic bacteria up to 39 days, when tailored to the microbial susceptibility profile. Furthermore, the comparison with loaded PMMA beads, under same conditions, revealed shorter elution time up to 12 days. Using confocal microscopy, scanning electron microscopy and cell counts it was revealed that the loaded beads have the potential to suppress bacterial colonization, mitigate biofilm formation and partially eliminate established biofilm for up to 7–14 days. Nevertheless, this study provides consistent data but also highlights the challenge of biofilm eradication and the importance of prevention measures and further in vivo investigations.[61,62]

Summary

Bacterial resistance to antibiotics, and biofilm formation are the greatest challenges for eradication of musculoskeletal infections. Implant-associated infections are presumably underreported and undertreated because of the great difficulties in diagnosis, management, and the existence of very low-grade infections. Perfecting the prevention principles remains of utmost importance, but when infection occurs the need for implant removal must be mitigated and the most effective treatment applied, with systemic and local antibiotics. Even if, in many views, PMMA beads are the gold standard for local antibiotic delivery, modern biomaterials such as CS preparations and composites provide viable treatment options with comparable results. Nonetheless, in some situations when structural resistance is a crucial requirement, for spacers or component fixation, calcium sulphate ceramics cannot replace PMMA. This brings into discussion the idea of combining the two systems to benefit from a synergistic therapeutic effect.

Calcium sulphate, as a drug delivery biomaterial, has demonstrated optimal degradation, mechanical and antibiotic elution characteristics and ensured its place in the future treatment strategies of musculoskeletal infection management. Nevertheless, there is a low level of evidence and a limited number of studies published that can unequivocally prove its superiority over other materials. Maybe future research will provide revolutionary materials based on CS which can incorporate new antimicrobials and delivery techniques.

AUTHOR INFORMATION

[1]Carol Davila University of Medicine and Pharmacy, Orthopedics and Traumatology Department, Bucharest, Romania.
[2]Bucharest Emergency Clinical Hospital, Romania.
[3]University Emergency Hospital Bucharest, Romania.

Correspondence should be sent to: Mihai Nica, Orthopedics and Traumatology Department, Carol Davila University of Medicine and Pharmacy, Bulevardul Eroii Sanitari 8, București 050474, Romania.
Email: mikx99n@gmail.com

ICMJE CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest relevant to this work.

FUNDING STATEMENT

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES

1. de Lissovoy G, Fraeman K, Hutchins V, Murphy D, Song D, Vaughn BB. Surgical site infection: incidence and impact on hospital utilization and treatment costs. *Am J Infect Control* 2009;37:387–397.

2. Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J. Infection burden for hip and knee arthroplasty in the United States. *J Arthroplasty* 2008;23:984–991.

3. Bohl DD, Sershon RA, Fillingham YA, Della Valle CJ. Incidence, risk factors, and sources of sepsis following total joint arthroplasty. *J Arthroplasty* 2016;31:2875–2879.e2.

4. Oliveira PR, Carvalho VC, da Silva Felix C, de Paula AP, Santos-Silva J, Lima AL. The incidence and microbiological profile of surgical site infections following internal fixation of closed and open fractures. *Rev Bras Ortop* 2016;51:396–399.

5. Hu Q, Zhao Y, Sun B, Qi W, Shi P. Surgical site infection following operative treatment of open fracture: incidence and prognostic risk factors. *Int Wound J* 2020;17:708–715.

6. Gristina A. Biomaterial-centered infection: microbial adhesion versus tissue integration. 1987. *Clin Orthop Relat Res* 2004;427:4–12.

7. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 2001;358:135–138.

8. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol* 1999;37:1771–1776.

9. Costerton JW. Biofilm theory can guide the treatment of device-related orthopaedic infections. *Clin Orthop Relat Res* 2005;437:7–11.

10. Rothenberg AC, Wilson AE, Hayes JP, O'Malley MJ, Klatt BA. Sonication of arthroplasty implants improves accuracy of periprosthetic joint infection cultures. *Clin Orthop Relat Res* 2017;475:1827–1836.

11. Zimmerli W, Ochsner PE. Management of infection associated with prosthetic joints. *Infection* 2003;31:99–108.

12. Parvizi J, Zmistowski B, Berbari EF, et al. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. *Clin Orthop Relat Res* 2011;469:2992–2994.

13. Elkins JM, Kates S, Lange J, et al. General assembly, diagnosis, definitions: proceedings of international consensus on orthopedic infections. *J Arthroplasty* 2019;34:S181–S185.

14. Argenson JN, Arndt M, Babis G, et al. Hip and knee section, treatment, debridement and retention of implant: proceedings of international consensus on orthopedic infections. *J Arthroplasty* 2019;34:S399–S419.

15. Bialecki J, Bucsi L, Fernando N, et al. Hip and knee section, treatment, one stage exchange: proceedings of International Consensus on Orthopedic Infections. *J Arthroplasty* 2019;34:S421–S426.

16. Abdel MP, Barreira P, Battenberg A, et al. Hip and knee section, treatment, two-stage exchange spacer-related: proceedings of international consensus on orthopedic infections. *J Arthroplasty* 2019;34:S427–S438.

17. McConoughey SJ, Howlin R, Granger JF, et al. Biofilms in periprosthetic orthopedic infections. *Future Microbiol* 2014;9:987–1007.

18. Popescu D, Ene R, Popescu A, Cîrstoiu M, Sinescu R, Cîrstoiu C. Total hip joint replacement in young male patient with osteoporosis, secondary to hypogonadotropic hypogonadism. *Acta Endocrinol (Bucur)* 2015;11:109–113.

19. Ene R, Sinescu RD, Ene P, Popescu D, Cîrstoiu MM, Cîrstoiu FC. Proximal tibial osteosarcoma in young patients: early diagnosis, modular reconstruction. *Rom J Morphol Embryol* 2015;56:413–417.

20. Nica M, Cretu B, Ene D, Antoniac I, Gheorghita D, Ene R. Failure analysis of retrieved osteosynthesis implants. *Materials (Basel)* 2020;13:1201.

21. McConoughey SJ, Howlin RP, Wiseman J, Stoodley P, Calhoun JH. Comparing PMMA and calcium sulfate as carriers for the local delivery of antibiotics to infected surgical sites. *J Biomed Mater Res B Appl Biomater* 2015;103:870–877.

22. Hanssen AD. Local antibiotic delivery vehicles in the treatment of musculoskeletal infection. *Clin Orthop Relat Res* 2005;437:91–96.

23. van Vugt TA, Geurts JA, Arts JJ, Lindfors NC. Biomaterials in treatment of orthopedic infections. Arts JJ, Geurts JA, editors. In: *Management of periprosthetic joint infections (PJIs)*. Amsterdam: Woodhead Publishing, 2017:41–68.

24. Cirstoiu C, Ene R, Panti Z, Ene P, Cirstoiu M. Particularities of shoulder recovery after arthroscopic Bankart repair with bioabsorbable and metallic suture anchors. *Mater Plast* 2015;52:361–363.

25. Winkler H, Haiden P. Allograft bone as antibiotic carrier. *J Bone Jt Infect* 2017;2:52–62.

26. Garvin K, Feschuk C. Polylactide-polyglycolide antibiotic implants. *Clin Orthop Relat Res* 2005;437:105–110.

27. Chang WK, Srinivasa S, MacCormick AD, Hill AG. Gentamicin-collagen implants to reduce surgical site infection: systematic review and meta-analysis of randomized trials. *Ann Surg* 2013;258:59–65.

28. Välimäki VV, Aro HT. Molecular basis for action of bioactive glasses as bone graft substitute. *Scand J Surg* 2006;95:95–102.

29. Gaisser DM, Hench LL. Clinical applications of bioactive glass: orthopaedics. Helch LL, editor. In *An introduction to bioceramics* London: Imperial College Press 2013:151–158.

30. Rahaman MN, Bal BS, Huang W. Review: emerging developments in the use of bioactive glasses for treating infected prosthetic joints. *Mater Sci Eng C* 2014;41:224–231.

31. Van Gestel NA, Geurts J, Hulsen DJ, Van Rietbergen B, Hofmann S, Arts JJ. Clinical applications of S53P4 bioactive glass in bone healing and osteomyelitic treatment: a literature review. *BioMed Res Int* 2015;2015. doi:10.1155/2015/684826

32. Luo SH, Xiao W, Wei XJ, et al. In vitro evaluation of cytotoxicity of silver-containing borate bioactive glass. *J Biomed Mater Res B Appl Biomater* 2010;95:441–448.

33. Xie Z, Liu X, Jia W, Zhang C, Huang W, Wang J. Treatment of osteomyelitis and repair of bone defect by degradable bioactive borate glass releasing vancomycin. *J Control Release* 2009;139:118–126.

34. Jia WT, Zhang X, Zhang CQ, et al. Elution characteristics of teicoplanin-loaded biodegradable borate glass/chitosan composite. *Int J Pharm* 2010;387:184–186.

35. Kattimani VS, Kondaka S, Lingamaneni KP. Hydroxyapatite: past, present, and future in bone regeneration. *Bone Tissue Regen Insights* 2016;7:BTRI-S36138.

36. Barrère F, van Blitterswijk CA, de Groot K. Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics. *Int J Nanomedicine* 2006;1:317–332.

37. Ogose A, Kondo N, Umezu H, et al. Histological assessment in grafts of highly purified beta-tricalcium phosphate (OSferion) in human bones. *Biomaterials* 2006;27:1542–1549.

38. Aiken SS, Cooper JJ, Florance H, Robinson MT, Michell S. Local release of antibiotics for surgical site infection management using high-purity calcium sulfate: an in vitro elution study. *Surg Infect (Larchmt)* 2015;16:54–61.

39. Wichelhaus TA, Dingeldein E, Rauschmann M, et al. Elution characteristics of vancomycin, teicoplanin, gentamicin and clindamycin from calcium sulphate beads. *J Antimicrob Chemother* 2001;48:117–119.

40. Lodoso-Torrecilla I, Grosfeld EC, Marra A, et al. Multimodal porogen platforms for calcium phosphate cement degradation. *J Biomed Mater Res A* 2019;107:1713–1722.

41. Roy A, Jhunjhunwala S, Bayer E, Fedorchak M, Little SR, Kumta PN. Porous calcium phosphate-poly (lactic-co-glycolic) acid composite bone cement: a viable tunable drug delivery system. *Mater Sci Eng C* 2016;59:92–101.

42. Tang Y, Wu C, Zhang P, Zhao K, Wu Z. Degradation behavior of non-sintered graphene/barium titanate/magnesium phosphate cement bio-piezoelectric composites. *Ceram Int* 2020;46:12626–12636.

43. Peltier LF. The use of plaster of Paris to fill large defects in bone. *Am J Surg* 1959;97:311–315.

44. Kumar YC, Nalini KB, Menon J, Patro DK, Banerji BH. Calcium sulfate as bone graft substitute in the treatment of osseous bone defects, a prospective study. *J Clin Diagn Res* 2013;7:2926–2928.

45. Thomas MV, Puleo DA. Calcium sulfate: properties and clinical applications. *J Biomed Mater Res B Appl Biomater* 2009;88:597–610.

46. Orsini G, Ricci J, Scarano A, et al. Bone-defect healing with calcium-sulfate particles and cement: an experimental study in rabbit. *J Biomed Mater Res B Appl Biomater* 2004;68:199–208.

47. 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. *Clin Orthop Relat Res* 2001;382:42–50.

48. Lioliou MG, Paraskeva CA, Koutsoukos PG, Payatakes AC. Calcium sulfate precipitation in the presence of water-soluble polymers. *J Colloid Interface Sci* 2006;303:164–170.

49. Ricci J, Alexander H, Nadkarni P, Hawkins M, Turner J, Rosenblum S, Brezenoff L, DeLeonardis D, Pecora G. Biological mechanisms of calcium sulfate replacement by bone. Davies JE, editor. In *Bone engineering*. Toronto: Em2 Inc, 2000:332–344.

50. Walsh WR, Morberg P, Yu Y, et al. Response of a calcium sulfate bone graft substitute in a confined cancellous defect. *Clin Orthop Relat Res* 2003;406:228–236.

51. Wichelhaus TA, Dingeldein E, Rauschmann M, et al. Elution characteristics of vancomycin, teicoplanin, gentamicin and clindamycin from calcium sulphate beads. *J Antimicrob Chemother* 2001;48:117–119.

52. Roberts R, McConoughey SJ, Calhoun JH. Size and composition of synthetic calcium sulfate beads influence dissolution and elution rates in vitro. *J Biomed Mater Res B Appl Biomater* 2014;102:667–673.

53. Ferguson JY, Dudareva M, Riley ND, Stubbs D, Atkins BL, McNally MA. The use of a biodegradable antibiotic-loaded calcium sulphate carrier containing tobramycin for the treatment of chronic osteomyelitis: a series of 195 cases. *Bone Joint J* 2014;96-B:829–836.

54. McNally MA, Ferguson JY, Lau AC, et al. Single-stage treatment of chronic osteomyelitis with a new absorbable, gentamicin-loaded, calcium sulphate/hydroxyapatite biocomposite: a prospective series of 100 cases. *Bone Joint J* 2016;98-B:1289–1296.

55. Romanò CL, Logoluso N, Meani E, et al. A comparative study of the use of bioactive glass S53P4 and antibiotic-loaded calcium-based bone substitutes in the treatment of chronic osteomyelitis: a retrospective comparative study. *Bone Joint J* 2014;96-B:845–850.

56. Flierl MA, Culp BM, Okroj KT, Springer BD, Levine BR, Della Valle CJ. Poor outcomes of irrigation and debridement in acute periprosthetic joint infection with antibiotic-impregnated calcium sulfate beads. *J Arthroplasty* 2017;32:2505–2507.

57. Kallala R, Haddad FS. Hypercalcaemia following the use of antibiotic-eluting absorbable calcium sulphate beads in revision arthroplasty for infection. *Bone Joint J* 2015;97-B:1237–1241.

58. McPherson E, Dipane M, Sherif S. Dissolvable antibiotic beads in treatment of periprosthetic joint infection and revision arthroplasty-the use of synthetic pure calcium sulfate (Stimulan®) impregnated with vancomycin & tobramycin. *Reconstr Rev* 2013;3.

59. Lum ZC, Pereira GC. Local bio-absorbable antibiotic delivery in calcium sulfate beads in hip and knee arthroplasty. *J Orthop* 2018;15:676–678.

60. Kallala R, Harris WE, Ibrahim M, Dipane M, McPherson E. Use of Stimulan absorbable calcium sulphate beads in revision lower limb arthroplasty: safety profile and complication rates. *Bone Joint Res* 2018;7:570–579.

61. Howlin RP, Brayford MJ, Webb JS, Cooper JJ, Aiken SS, Stoodley P. Antibiotic-loaded synthetic calcium sulfate beads for prevention of bacterial colonization and biofilm formation in periprosthetic infections. *Antimicrob Agents Chemother* 2015;59:111–120.

62. Butini ME, Cabric S, Trampuz A, Di Luca M. In vitro anti-biofilm activity of a biphasic gentamicin-loaded calcium sulfate/hydroxyapatite bone graft substitute. *Colloids Surf B Biointerfaces* 2018;161:252–260.