■ ASPECTS OF CURRENT MANAGEMENT

Biodegradable antibiotic delivery systems

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Bacterial infection in orthopaedic surgery can be devastating, and is associated with significant morbidity and poor functional outcomes, which may be improved if high concentrations of antibiotics can be delivered locally over a prolonged period of time. The two most widely used methods of doing this involve antibiotic-loaded polymethylmethacrylate or collagen fleece. The former is not biodegradable and is a surface upon which secondary bacterial infection may occur. Consequently, it has to be removed once treatment has finished. The latter has been used successfully as an adjunct to systemic antibiotics, but cannot effect a sustained release that would allow it to be used on its own, thereby avoiding systemic toxicity.

This review explores the newer biodegradable carrier systems which are currently in the experimental phase of development and which may prove to be more effective in the treatment of osteomyelitis.

Bacterial infection in orthopaedic surgery can be devastating, and is associated with significant morbidity and poor functional outcomes.¹ The reported rate of infection varies from 1% after primary joint replacement² to 23% after open fractures.³

Traditionally, the management of post-operative infection includes surgical debridement, removal of all necrotic tissue and implants, and the administration of systemic antibiotics. This is successful in up to 90% of cases.^{4,5} Antibiotic treatment may be inadequate or ineffective in patients with poorly vascularised infected tissues and osteonecrosis, which is often present in cases of osteomyelitis. Moreover, normal doses of systemic antibiotics may be insufficient to breach the glycocalyx or biofilm produced by the infecting bacteria.

Although antibiotic-loaded cement spacers have been widely used in the past,⁶ there has, over the last three decades, been an effort to develop biodegradable antibiotic carrier systems. This article explores a number of these and considers other technologies that may yet supersede them.

Rationale for biodegradable carriers

There has been interest in the development of carriers of local antibiotics since 1970, when Buchholz and Engelbrecht⁷ reported that bone cement mixed with antibiotics was effective in

the prophylaxis and treatment of infection in total hip replacement. However, non-biodegradable carriers such as polymethylmethacrylate (PMMA) can attract glycocalyx-producing bacteria despite the presence of local antibiotics. The resulting biofilm acts as a foreign body and a surface for bacterial colonisation, leading to secondary infection and the necessary removal of the cement in a further procedure.⁸ The development of biodegradable carriers is seen as theoretically advantageous, because of the potential reduction in the risk of secondary infection and the need for removal of the implant. This is particularly useful in conditions such as osteomyelitis, where maintenance of a soft-tissue space is not necessarily required.

Collagen fleece

The most widely used and established biodegradable carrier is collagen fleece. It is a solid mesh of collagen-based spongy material produced from sterile animal skin or the tendo Achillis. Since collagen is a major component of connective tissue and the main structural protein of all organs, it has several desirable biological properties, including both biocompatibility and non-toxicity. Its ability to release drugs can be modified by changing the porosity of the matrix or by treating it with chemicals.⁹ It can also attract and stimulate the proliferation of osteoblasts,

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Table I. Clinical studies detailing the efficacy of gentamicin-loaded collagen fleece in treating osteomyelitis

Author/s	Number of patients	Rate of eradication of infection (%)	Follow-up (yrs)
Ascherl et al ¹⁵	67	94	< 1
von Hasselbach ¹⁶	58	93.1	< 1
Ipsen et al ¹⁷	10	100	1
Wernet et al ¹⁸	47	93.6	2

thereby promoting mineralisation and the production of collagenous callus tissue, which aids the formation of new bone.¹⁰

Because of the distinctive structure of the collagen matrix, an antibiotic may undergo rapid, intermediate and prolonged release. Rapid release of the drug occurs soon after its insertion into the tissues because of the partially open porous structure of the fleece. Intermediate release occurs due to the partially closed pores in which the antibiotic is encased. Subsequently, prolonged release occurs due to the partial immobilisation of the drug within the fibrillar collagen structure. As the fleece undergoes resorption, further antibiotic is released which had previously been encased within the collagen matrix.¹¹

Collagen fleece is usually impregnated with an aminoglycoside, such as gentamicin, because of its broad spectrum of bactericidal properties.

Despite a number of advantages over more traditional carriers such as PMMA, some doubts remain over the suitability of collagen fleece. Although it is bioabsorbable and provides a three-phase release of antibiotics, kinetic studies have shown that 95% of the gentamicin can be released in the first 1.5 hours, compared with 8% from PMMA beads over the same period of time.¹²

Diffusion from the fleece occurs as its collagen is degraded by macrophages which is usually complete within eight weeks.¹³ Antibiotic elution can be prolonged by using hydrophilic gentamicin sulphate in combination with hydrophobic gentamicin crobafate. This mixture results in a constant gentamicin level of 1000 µg/ml over the first 40 hours and then a prolonged release of around 100 g/ml over 10 days.¹⁴

Unlike more experimental biodegradable carriers, gentamicin-loaded collagen fleece has been evaluated in a number of clinical studies. In the first of these, 67 patients with post-traumatic and post-operative osteomyelitis were treated. The infection resolved in 94% of cases.¹⁵ Subsequent studies have demonstrated rates of efficacy between 93% and 100%, although additional intravenous antibiotics were used in all of these (Table I).¹⁶⁻¹⁸ The evidence suggests that antibiotic-loaded collagen fleece is a useful adjunct to systemic antibiotic treatment.

It is worth noting that some clinical evidence exists to suggest that collagen fleece is more effective than PMMA in the prophylactic setting. In a comparative study, Hettfleisch and Schöttle¹⁹ implanted either collagen fleece or PMMA

cement into patients undergoing intramedullary fixation of a tibial fracture. They showed that gentamicin-loaded collagen fleece had a superior pharmacokinetic profile with complete elution of the antibiotic.

Collagen fleece is a reliable and biodegradable method of delivering antibiotics locally, and its use is likely to continue.

Polyesters

Although collagen fleece is an established method of managing infection, there is great interest in developing a carrier with longer lasting effects and better penetration. Biodegradable polyesters have been used in surgery since the 1950s as suture materials. Advances in processing have generated stronger, more reliable polyester-based implants for consideration as carriers.

In vivo, widely used polymers such as polyglycolic acid (PGA) and poly-L-lactic acid (PLLA) are broken down into their monomeric forms and are then metabolised into either oxalic acid or carbon dioxide and water (Fig. 1). It is the breakdown of these polymers over several weeks or months that allows for the slow release of antibiotics and enables their delivery above breakpoint sensitivity (the level at the transitional point between bacterial killing and antibiotic resistance) for eight weeks.^{20,21}

Although lactide/glycolide polymers were suggested as carriers for antibiotics in 1982,²² it was ten years before linked lactic acid chains were proposed as a drug delivery system for the treatment of osteomyelitis. Wei et al²³ implanted moulded rods, made by heating a mixture of lactic acid oligomer and dideoxykanamycin B, into rabbit models. They showed that the minimum inhibitory concentration (MIC) of dideoxykanamycin B for the common causative organisms of osteomyelitis was exceeded for six weeks in the cortex, the cancellous bone, and in the bone marrow. Furthermore, the majority of the implant material had been absorbed, and the bone marrow had returned to a nearly normal state within nine weeks of implantation.

Sampath, Garvin and Robinson²⁴ demonstrated an alternative method of delivering gentamicin locally using polymers. They prepared microcapsules of between 125 µm and 400 µm in diameter composed of a PLLA shell containing gentamicin which was then compressed into the desired shape. It was noted that more than 80% of the gentamicin was released in the first three weeks *in vitro*. The efficacy of microcapsules in osteomyelitis has also been demonstrated in a study by Garvin et al.²⁵ Using a canine model with osteomyelitis caused by *Staphylococcus aureus*, the authors completely eradicated the infection with rods made of moulded polylactide:PGA microcapsules containing gentamicin. They achieved a rate of eradication of infection of 89% in dogs implanted with an antibiotic-loaded cement spacer, and of 63% in a further group given intramuscular antibiotics.

There is evidence that *Staph. aureus* can adhere to and persist within osteoblasts,^{26,27} which may partly explain why osteomyelitis can be resistant to antibiotic treatment. It is

Fig. 3

Picture showing moulded Septacin beads containing gentamicin (reproduced by permission from Li et al³⁰).

wound leakage, implying that it was being released locally by the polyanhydride carrier. However, no data were recorded on clinical outcomes, complications, or rates of ongoing infection. Further work is required to evaluate their efficacy in clinical practice.

Amylose starch

Amylose starch was first used in 1991 as an oral drug carrier and is of potential use in orthopaedics since it is both biodegradable and biocompatible, and has controlled local delivery properties.^{31,32} It is prepared by cross-linking and chemical modification of high amylose starch, thermal gelatinisation, and drying.

Désévaux et al³² investigated increasing concentrations of ciprofloxacin loaded onto cross-linked amylose starch both *in vitro* and in a rabbit model of osteomyelitis. *In vitro* evaluation showed that it was possible to achieve concentrations of ciprofloxacin in excess of 100 times the MIC for *Staph. aureus* in local muscle and bone over four weeks. The MIC for *Staph. aureus* was also achieved *in vivo* for 28 days, when ciprofloxacin constituted 20% of the total weight of the implant.

In 2004, the same group investigated the effect of ciprofloxacin-loaded amylose starch in a canine model of femoral osteomyelitis, comparing it with one group receiving oral ciprofloxacin and a further control group receiving no treatment.³³ All groups underwent surgical debridement. After four weeks, significantly higher rates of eradication were seen in the amylose starch group with 62% and 75% of cases demonstrating eradication of infection at the proximal and distal femoral sites respectively. These were significantly higher rates than those seen in the oral treatment and control groups.³³ Despite promising experimental data, further work is required to evaluate the efficacy of this carrier in humans.

Alginates

Alginates are derived from marine algae and are composed of linear polyuronate containing a co-polymer of D-mannuronic acid and L-gulonic acid. These two monosaccharides undergo reversible gelation when alginate in solution interacts with calcium ions.^{34,35}

In 1990, Plunkett and Hailey³⁶ developed alginate beads containing tumour cells, and demonstrated in mice that neovascularisation may occur following the production of tumour cell growth factors, without the host attacking the tumour cells.³⁷ Although these compounds were developed for use in other medical fields, they represented a new type of biological delivery system, and have since been developed as antibiotic carriers.

In 1996, Chun et al³⁸ described the release patterns of ampicillin from sodium alginate microspheres. They showed a cumulative release of 90% of the antibiotic within six hours of implantation. Subsequently, sustained-release beads were produced by Ueng et al³⁹ by coating them with the synthetic polypeptide poly-L-lysine, which aimed to decrease leakage of antibiotic by creating a secondary semipermeable membrane. The authors reported that *in vitro* antibiotic release ranged from nine to 17 days, confirming successful sustained release. Binding of poly-L-lysine, and therefore the rate of antibiotic release, was found to be strongly influenced by the composition of the alginate core. The higher the content of mannuronic acid, the higher the binding of poly-L-lysine. Increasing the concentration of poly-L-lysine and the time of exposure enhanced this effect. Additionally, lyophilisation (freeze-drying) was used to ensure stability during storage, which further prolonged antibiotic release.

The addition of mesenchymal stem cells (MSCs) in combination with antibiotics has proved promising in the management of the bony defects seen in osteomyelitis after surgical debridement. In one study, alginate beads that had been combined with vancomycin and MSCs cultured in an osteogenic medium for 14 days, then inserted into a rabbit model of osteomyelitis, produced multiple positive findings.⁴⁰ Osteogenic differentiation of the cultured stem cells in the alginate carrier was seen, while the *in vivo* findings included a sustained elution of vancomycin, above the MIC for *Staph. aureus* for 14 days, and generation of new bone attributable to the MSCs.^{40,41}

In order to enhance both osteoinductivity and osteoconductivity, Hou et al⁴² embedded vancomycin-loaded alginate beads in a fibrin gel seeded with MSCs. They hypothesised that the fibrin gel assisted the final stage of the coagulation cascade in which fibrinogen molecules contained within the gel are cleaved by thrombin, converted into fibrin, and form fibres which will fill a bony cavity. Fibrin gel is biodegradable and porous, and is therefore suitable as a scaffold for bone tissue engineering. *In vitro* evaluation of this carrier showed that vancomycin release stayed above the MIC for 22 days. Further *in vivo* assessments are required to evaluate the efficacy of fibrin gels in humans.

Fig. 4

Scanning electron microscopy image showing vancomycin-loaded chitosan microspheres (reproduced with permission from Cevher et al⁴¹).

Chitosans

Although collagen sponges, polymers and amylose starch have been used as local antibiotic carriers, they do not possess any bactericidal properties themselves. Chitosan, a polymerised D-glucosamine polysaccharide, is able to act as a drug carrier with the additional benefits of antibacterial and antifungal activity.⁴³ Chitosan alone has been shown to reduce the rate of infection of *Staph. aureus* in experimental models of osteomyelitis.⁴⁴

Chitosan is obtained by alkaline deacetylation of chitin and is one of the most abundant polysaccharides in nature, second only to cellulose. It has two types of antibacterial property. Firstly, its positive charge reacts with negatively charged molecules at the surface of cells, altering cell permeability and thereby preventing material from entering the intracellular space. Secondly, chitosan is able to bind to DNA to inhibit RNA synthesis.⁴⁵

Drug elution from chitosans is determined by the amount of cross-linkage, the size of the implant, and the initial drug content. Indeed, the speed at which cross-linked chitosan gel degrades has been shown to be several times slower than that of non-cross-linked chitosan.⁴⁶ Although chitosan loaded with gentamicin has delivered effective concentrations of antibiotic for approximately eight weeks in animal models (Fig. 4),⁴⁴ this technology has been developed much further as a composite carrier, and will be discussed later.

Calcium-based carriers

Calcium sulphate hemihydrate in the form of Plaster of Paris (POP) was first used in 1892 by Dreesmann⁴⁷ to pack bone defects. Water-soluble antibiotics can be incorporated into the crystalline structure, although they tend to be delivered at an uncontrolled rate unless coated.

In addition to POP, pellets of calcium sulphate are also available for clinical use (Fig. 5). One case series has reported

Fig. 5

Photograph showing pellets of borate glass (BG), borate glass mixed with vancomycin (BG + VCM) and calcium sulphate mixed with vancomycin (CS + VCM) (reproduced with permission from Xie et al⁶⁰).

promising results in six cases of osteomyelitis treated with antibiotic-loaded calcium sulphate in addition to intravenous antibiotics, but the series was small and there were no controls.⁴⁸ The disadvantages of calcium sulphate include cytotoxicity, an insufficient ability to stimulate bone regeneration, a very high rate of resorption, and rapid elution *in vitro*. Additionally, its low mechanical strength and friability, especially in wet conditions, prevents its application in locations where load-bearing is required.⁴⁹⁻⁵¹ Furthermore, Mackey, Varlet and Debeaumont⁵² found that when POP was implanted into the bones of rabbits and dogs, the levels of serum calcium rose as it was absorbed. This does not appear to occur in humans during the post-operative period, and favourable results have been seen in the treatment of post-operative infection.⁵³

POP has also been combined with calcium hydroxyapatite (HA) as a composite carrier. The ability of resorbable ceramics to promote regeneration of tissue depends on how fast the ceramic dissolves and how quickly it is replaced by the host tissues. A small initial pore size confers high mechanical strength, but as it dissolves, it becomes more porous, allowing the ingrowth of tissue. The mixture of POP and HA is therefore interesting, as if mechanical integrity can be maintained, then stress concentrations are minimised. Sato, Koshino and Saito⁵⁴ used equal quantities of POP and HA to form a composite delivery system, and found that the presence of HA slowed down the resorption of the POP. The addition of HA to POP in a rabbit model has been shown to maintain the serum levels of antibiotic at four weeks better than bioglass or POP alone.⁵⁵

The advantage of HA over other biodegradable carriers is that it is slowly replaced by new-forming bone, thereby reducing the need for additional forms of reconstruction.

Cornell et al⁵⁶ treated osteomyelitis in a rabbit model with antibiotic-impregnated HA and found that it cleared infection of *Staph. aureus* in 72.7% of cases. Despite its

initial promise, the results of using PMMA have been varied. Shirtliff, Calhoun and Mader⁵⁷ showed that the implantation of HA (impregnated with vancomycin) into the dead space after debridement for methicillin-resistant *Staphylococcus aureus* (MRSA) osteomyelitis cleared the infection in 81.8% of cases, compared with PMMA beads impregnated with vancomycin, which had a success rate of 70%. This accorded with Korkusuz et al⁵⁸ who used a model of rat osteomyelitis to show that infection could be eradicated within seven weeks of implantation of a HA-ceramic composite with the production of consistently higher serum levels of gentamicin than antibiotic-loaded PMMA. By contrast, Zelken et al⁵⁹ showed inferior results for antibiotic-loaded HA when compared with PMMA, citing difficulties in handling the material due to its texture as a possible reason for this disparity.

Borate glass

One of the most recent carrier systems is borate glass, which is produced by replacing the SiO₂ in bioactive glass with B₂O₃. This technology has advanced rapidly, as borate glass has the advantage that it degrades readily into HA, thereby promoting the formation of new bone without the need for stimulation by MSCs. Furthermore, it has been suggested that borate glass, because of its compressive strength, can be used to fill a defect in cancellous bone where load-bearing is required.⁶⁰ Xie et al⁶⁰ recently compared vancomycin-loaded borate glass to vancomycin-loaded calcium sulphate in an MRSA-infected rabbit model (Fig. 5). They showed that not only did vancomycin-loaded borate glass clear the infection faster, but it also generated more new bone formation.

Composite carriers

There is increasing interest in the production of hybrids of biodegradable carrier systems which take advantage of the properties of the different components to improve the release of antibiotics. There are a range of these, each with a different composition, and some show particular promise. Most composite carriers include HA, since its microporous structure allows greater antibiotic absorption and a more prolonged antibacterial activity, in addition to its inherent osteogenic properties.⁶¹

Borate glass in combination with chitosans has provided some interesting data. Jia et al⁶² implanted a teicoplanin-loaded borate glass-chitosan composite in rabbits with MRSA osteomyelitis, and showed that infection was eradicated in 85%, compared with 43% in those treated with a four-week course of intravenous teicoplanin. Of note is the fact that a borate glass-chitosan composite without antibiotic eradicated infection in only 21% of cases, adding weight to the argument that antibiotics and chitosan work synergistically.

Prolonged drug elution is a primary goal of composite carriers. The combination of chitosans, nanohydroxyapatite and ethyl cellulose microspheres has been shown to release

gentamicin above the MIC for 45 days in a rabbit osteomyelitis model.⁶³ It is clear that the combination of HA and chitosans is of particular interest. Furthermore, combination with POP has been shown to provide even longer drug elution times. Buranapanitkit et al⁶⁴ designed an HA and POP carrier in the ratio of 85:10, adding either vancomycin, fosfomycin or fusidate, before mixing it with chitosan gel. They found that the release of antibiotics from the hybrid carrier continued for three months with vancomycin and fosfomycin, and for three weeks for fusidate. Such sustained release, compared with that seen with carriers in isolation, may be due to the porous structure of HA, allowing the infiltration of POP. Additionally, this effect may also be partly attributable to chitosan, which acts as a binder for the two materials.

Conclusions

There is considerable interest in finding methods of delivering effective doses of antibiotics locally, not only in orthopaedics, but across a range of specialties. While most of the antibacterial agent contained within a biodegradable system may be eluted, only 25% is actually released from PMMA beads. The sheer diversity of available systems and the lack of suitable trials comparing them *in vivo* makes their evaluation difficult. Nonetheless, it is apparent that while collagen fleece is currently the most widely used antimicrobial carrier system, the duration of its antibiotic delivery is the shortest. Other delivery systems have shown greater promise, and those that are able both to stimulate the formation of new bone and provide a scaffold, such as composite antibiotic carriers, are most likely to gain widespread acceptance in the future.

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References

1. Haddad FS, Muirhead-Allwood SK, Manktelow AR, Bacarese-Hamilton I. Two-stage uncemented revision hip arthroplasty for infection. *J Bone Joint Surg [Br]* 2000;82-B:689-94.
2. Phillips JE, Crane TP, Noy M, Elliott TS, Grimer RJ. The incidence of deep prosthetic infections in a specialist orthopaedic hospital: a 15-year prospective survey. *J Bone Joint Surg [Br]* 2006;88-B:943-8.
3. Patzakis MJ, Wilkins J. Factors influencing infection rate in open fracture wounds. *Clin Orthop* 1989;243:36-40.
4. Insall JN, Thompson FM, Brause BD. Two-stage reimplantation for salvage of infected total knee arthroplasty. 1983. *J Bone Joint Surg [Am]* 2002;84-A:490.
5. Hanssen AD, Spangehl MJ. Treatment of the infected hip replacement. *Clin Orthop Relat Res* 2004;420:63-71.
6. Biring GS, Kostamo T, Garbuz DS, Masri BA, Duncan CP. Two-stage revision arthroplasty of the hip for infection using an interim articulated Prostalac hip spacer: a 10- to 15-year follow-up study. *J Bone Joint Surg [Br]* 2009;91-B:1431-7.
7. Buchholz HW, Engelbrecht H. Depot effects of various antibiotics mixed with Palacos resins. *Chirurg* 1970;41:511-15 (in German).
8. Rushton N. Applications of local antibiotic therapy. *Eur J Surg Suppl* 1997;578:27-30.
9. Rao KP. Recent developments of collagen-based materials for medical applications and drug delivery systems. *J Biomater Sci Polym Ed* 1995;7:623-45.
10. Reddi AH. Implant-stimulated interface reactions during collagenous bone matrix-induced bone formation. *J Biomed Mater Res* 1985;19:233-9.
11. Uludag H, Friess W, Williams D, et al. rhBMP-collagen sponges as osteoinductive devices: effects of in vitro sponge characteristics and protein pl on in vivo rhBMP pharmacokinetics. *Ann NY Acad Sci* 1999;875:369-78.

12. Sørensen TS, Sørensen AI, Merse S. Rapid release of gentamicin from collagen sponge: in vitro comparison with plastic beads. *Acta Orthop Scand* 1990;61:353-6.
13. Stemberger A, Grimm H, Bader F, Rahn HD, Ascherl R. Local treatment of bone and soft tissue infections with the collagen-gentamicin sponge. *Eur J Surg Suppl* 1997;578:17-26.
14. Moser A. Comparative human pharmacokinetics of two gentamicin-containing collagen carriers. In: Walenkamp GHM, ed. *Biomaterials in surgery*. Stuttgart: Thieme, 1998:21-5.
15. Ascherl R, Stemberger A, Lechner F, et al. Treatment of chronic osteomyelitis with a collagen-antibiotic compound: preliminary report. *Unfallchirurgie* 1986;12:125-7 (in German).
16. von Hasselbach C. Clinical aspects and pharmacokinetics of collagen-gentamicin as adjuvant local therapy of osseous infections. *Unfallchirurgie* 1989;92:459-70 (in German).
17. Ipsen T, Jørgensen PS, Damholt V, Tørholm C. Gentamicin-collagen sponge for local applications: 10 cases of chronic osteomyelitis followed for 1 year. *Acta Orthop Scand* 1991;62:592-4.
18. Wernet E, Ekkernkamp A, Jellestad H, Muhr G. Antibiotic-containing collagen sponge in therapy of osteitis. *Unfallchirurgie* 1992;95:259-64 (in German).
19. Hettfleisch J, Schöttle H. Local preventive antibiotic treatment in intramedullary nailing with gentamycin impregnated biomaterials. *Aktuelle Traumatol* 1993;23:68-71 (in German).
20. Ueng SW, Yuan LJ, Lee N, et al. In vivo study of hot compressing molded 50:50 poly (DL-lactide-co-glycolide) antibiotic beads in rabbits. *J Orthop Res* 2002;20:654-61.
21. Liu SJ, Wen-Neng Ueng S, Lin SS, Chan EC. In vivo release of vancomycin from biodegradable beads. *J Biomed Mater Res* 2002;63:807-13.
22. Thies C. Microcapsules as drug delivery devices. *Crit Rev Biomed Eng* 1982;8:335-83.
23. Wei G, Kotoura Y, Oka M, et al. A bioabsorbable delivery system for antibiotic treatment of osteomyelitis: the use of lactic acid oligomer as a carrier. *J Bone Joint Surg [Br]* 1991;73-B:246-52.
24. Sampath SS, Garvin K, Robinson DH. Preparation and characterization of biodegradable poly(L-lactic acid) gentamicin delivery systems. *Int J Pharmaceutics* 1992;78:165-74.
25. Garvin KL, Miyano JA, Robinson D, et al. Polylactide/polyglycolide antibiotic implants in the treatment of osteomyelitis: a canine model. *J Bone Joint [Am]* 1994;76-A:1500-6.
26. Jevon M, Guo C, Ma B, et al. Mechanisms of internalization of *Staphylococcus aureus* by cultured human osteoblasts. *Infect Immun* 1999;67:2677-81.
27. Ellington JK, Reilly SS, Ramp WK, et al. Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts. *Microb Pathog* 1999;26:317-23.
28. Pillai R, Somayaji SN, Rabinovich M, Hudson MC, Gonsalves KE. Nafcillin-loaded PLGA nanoparticles for treatment of osteomyelitis. *Biomed Mater* 2008;3:034114.
29. Jain JP, Chitkara D, Kumar N. Polyanhydrides as localized drug delivery carrier: an update. *Expert Opin Drug Deliv* 2008;5:889-907.
30. Li LC, Deng J, Stephens D. Polyanhydride implant for antibiotic delivery: from the bench to the clinic. *Adv Drug Deliv Rev* 2002;54:963-86.
31. Désévaux C, Dubreil P, Lenaerts V, Girard C. Tissue reaction and biodegradation of implanted cross-linked high amylose starch in rats. *J Biomed Mater Res* 2002;63:772-9.
32. Désévaux C, Lenaerts V, Girard C, Dubreuil P. Characterization of crosslinked high amylose starch matrix implants. 2: in vivo release of ciprofloxacin. *J Control Release* 2002;82:95-103.
33. Huneault LM, Lussier B, Dubreuil P, Chouinard L, Désévaux C. Prevention and treatment of experimental osteomyelitis in dogs with ciprofloxacin-loaded cross-linked high amylose starch implants. *J Orthop Res* 2004;22:1351-7.
34. Aydelotte MB, Thonar EJ, Mollenhauer J, Flechtenmacher J. Culture of chondrocytes in alginate gel: variations in conditions of gelation influence the structure of the alginate gel, and the arrangement and morphology of proliferating chondrocytes. *In Vitro Cell Dev Biol Anim* 1998;34:123-30.
35. Binette F, McQuaid DP, Haudenschild DR, et al. Expression of a stable articular cartilage phenotype without evidence of hypertrophy by adult human articular chondrocytes in vitro. *J Orthop Res* 1998;16:207-16.
36. Plunkett ML, Hailey JA. An in vivo quantitative angiogenesis model using tumor cells entrapped in alginate. *Lab Invest* 1990;62:510-17.
37. Downs EC, Robertson NE, Riss TL, Plunkett ML. Calcium alginate beads as a slow-release system for delivering angiogenic molecules in vivo and in vitro. *J Cell Physiol* 1992;152:422-9.
38. Chun KH, Kwon IC, Kim YH, et al. Preparation of sodium alginate microspheres containing hydrophilic β -lactam antibiotics. *Arch Pharm Res* 1996;19:106-11.
39. Ueng SW, Lee SS, Lin SS, et al. Biodegradable alginate antibiotic beads. *Clin Orthop* 2000;380:250-9.
40. Ueng SW, Lee MS, Lin SS, Chan EC, Liu SJ. Development of a biodegradable alginate carrier system for antibiotics and bone cells. *J Orthop Res* 2007;25:62-72.
41. Cevher E, Orhan Z, Mülazimoglu L, et al. Characterization of biodegradable chitosan microspheres containing vancomycin and treatment of experimental osteomyelitis caused by methicillin-resistant *Staphylococcus aureus* with prepared microspheres. *Int J Pharm* 2006;317:127-35.
42. Hou T, Xu J, Li Q, Feng J, Zen L. In vitro evaluation of a fibrin gel antibiotic delivery system containing mesenchymal stem cells and vancomycin alginate beads for treating bone infections and facilitating bone formation. *Tissue Eng Part A* 2008;14:1173-82.
43. Muzzarelli R, Tarsi R, Filippini O, et al. Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrob Agents Chemother* 1990;34:2019-23.
44. Aimin C, Chunlin H, Juliang B, Tinyin Z, Zhichao D. Antibiotic loaded chitosan bar: an in vitro, in vivo study of a possible treatment for osteomyelitis. *Clin Orthop* 1999;366:239-47.
45. Illum L. Chitosan and its use as a pharmaceutical excipient. *Pharm Res* 1998;15:1326-31.
46. Tikhonov VE, Yamskov IA, Varlamov VP, Ilyina AV, Davankov VA. Chitinolytic digestibility of cross-linked chitosan gels by an enzyme complex from *Streptomyces kurssanovii*. *Biotechnol Appl Biochem* 1993;17:251-6.
47. Dreesmann H. Über knochenplombierung. *Beitr Klin Chir* 1982;9:804-10 (in German).
48. Gitelis S, Brebach GT. The treatment of chronic osteomyelitis with a biodegradable antibiotic-impregnated implant. *J Orthop Surg (Hong Kong)* 2002;10:53-60.
49. Chang W, Colangeli M, Colangeli S, et al. Adult osteomyelitis: debridement versus debridement plus Osteoset T pellets. *Acta Orthop Belg* 2007;73:238-43.
50. Sanicola SM, Albert SF. The in vitro elution characteristics of vancomycin and tobramycin from calcium sulfate beads. *J Foot Ankle Surg* 2005;44:121-4.
51. Rauschmann MA, Wichelhaus TA, Stürmal V, et al. Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier material for local delivery of antibiotics in bone infections. *Biomaterials* 2005;26:2677-84.
52. Mackey D, Varlet A, Debeaumont D. Antibiotic loaded plaster of Paris pellets: an in vitro study of a possible method of local antibiotic therapy in bone infection. *Clin Orthop* 1982;167:263-8.
53. McKee MD, Wild LM, Schemitsch EH, Waddell JP. The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: early results of a prospective trial. *J Orthop Trauma* 2002;16:622-7.
54. Sato S, Koshino T, Saito T. Osteogenic response of rabbit tibia to hydroxyapatite particle-Plaster of Paris mixture. *Biomaterials* 1998;19:1895-900.
55. Heybeli N, Oktar FN, Ozyazgan S, Akkan G, Ozsoy S. Low-cost antibiotic loaded systems for developing countries. *Technol Health Care* 2003;11:207-16.
56. Cornell CN, Tyndall D, Waller S, Lane JM, Brause BD. Treatment of experimental osteomyelitis with antibiotic-impregnated bone graft substitute. *J Orthop Res* 1993;11:619-26.
57. Shirliff ME, Calhoun JH, Mader JT. Experimental osteomyelitis treatment with antibiotic-impregnated hydroxyapatite. *Clin Orthop* 2002;401:239-47.
58. Korkusuz F, Uchida A, Shinto Y, et al. Experimental implant-related osteomyelitis treated by antibiotic-calcium hydroxyapatite ceramic composites. *J Bone Joint Surg [Br]* 1993;75-B:111-14.
59. Zelken J, Wanich T, Gardner M, Griffith M, Bostrom M. PMMA is superior to hydroxyapatite for colony reduction in induced osteomyelitis. *Clin Orthop* 2007;462:190-4.
60. Xie Z, Liu X, Jia W, et al. Treatment of osteomyelitis and repair of bone defect by degradable bioactive borate glass releasing vancomycin. *J Control Release* 2009;139:118-26.
61. Chai F, Hornez JC, Blanchemain N, et al. Antibacterial activation of hydroxyapatite (HA) with controlled porosity by different antibiotics. *Biomol Eng* 2007;24:510-14.
62. Jia WT, Zhang X, Luo SH, et al. Novel borate glass/chitosan composite as a delivery vehicle for teicoplanin in the treatment of chronic osteomyelitis. *Acta Biomater* 2010;6:812-19.
63. Shi P, Zuo Y, Li X, et al. Gentamicin-impregnated chitosan/nanohydroxyapatite/ethyl cellulose microspheres granules for chronic osteomyelitis therapy. *J Biomed Mater Res A* 2010;93:1020-31.
64. Buranapanitkit B, Srinilta V, Ingvis N, et al. The efficacy of a hydroxyapatite composite as a biodegradable antibiotic delivery system. *Clin Orthop* 2004;424:244-52.