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,
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.19,20

Although lactide/glycolide polymers were suggested as carriers for antibiotics in 1982,21 it was ten years before linked lactic acid chains were proposed as a drug delivery system for the treatment of osteomyelitis. Wei et al22 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
believed that nanoparticles tagged to drugs may facilitate the movement of antibiotics across the cell membrane, thereby ensuring a better delivery of the drugs to intracellular bacteria. This technology has a wide potential and a number of existing clinical applications, although there is little published data relating to the use of nanoparticles in the treatment of osteomyelitis. Pillai et al.,\(^\text{28}\) using cultured mouse osteoblasts \textit{in vitro}, have shown that poly-lactic-co-glycolic acid (PLGA) nanoparticles (Fig. 2) loaded with nafcillin can either kill or significantly reduce the levels of intracellular bacteria after an incubation of only 48 hours. This appears to be a promising area for future research.

**Polyanhydrides**

In addition to polyester implants, there are polyanhydride implants which differ from polyesters in their pattern of drug release. Polyesters undergo ‘bulk erosion’, whereby drug release occurs without significant loss of mass or volume of the polymer. In contrast, polyanhydrides degrade via ‘surface erosion’ where both degradation and erosion occur simultaneously.\(^\text{29}\) Proponents of this delivery system argue that it has a greater ability to achieve close to zero-order release kinetics (antibiotic elimination is independent of the antibiotic concentration), while over-exposure of the biomaterial to a particular type of cell is prevented if the polymer degrades at the same rate that the antibiotic elutes.\(^\text{29}\)

There is some evidence that polyanhydrides are effective in humans. Li, Deng and Stephens\(^\text{30}\) reported the use of Septacin (Nova Pharmaceutical Corporation, Baltimore, Maryland) (Fig. 3) after the removal of infected hip and knee prostheses. Septacin is a copolymer of dimeric erucic acid and sebacic acid (50:50), containing gentamicin. In conjunction with a non-gentamicin based antibiotic, the authors demonstrated that gentamicin was present in
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. It is prepared by cross-linking and chemical modification of high amylose starch, thermal gelatinisation, and drying.

Déséaux et al 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. 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. Despite promising experimental data, further work is required to evaluate the efficacy of this carrier in humans.

**Alginate**

Alginites 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.

In 1990, Plunkett and Hailey 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. 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 al 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 al 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. 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.

In order to enhance both osteoinductivity and osteoconductivity, Hou et al 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.
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. Chitosan alone has been shown to reduce the rate of infection of *Staph. aureus* in experimental models of osteomyelitis.

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.

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. Although chitosan loaded with gentamicin has delivered effective concentrations of antibiotic for approximately eight weeks in animal models (Fig. 4), 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 Dreesmann 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 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. 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. Furthermore, Mackey, Varlet and Debeaumont 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.

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 Saito 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.

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 al. 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. Shirtsiff, Calhoun and Mader\(^7\) showed that the implantation of HA (impregnated with vancomycin) into the dead space after debridement for methicillin-resistant \textit{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 al\(^8\) 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 al\(^9\) 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 \text{SiO}_2 in bioactive glass with \text{B}_2\text{O}_3. 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.\(^{46}\) Xie et al\(^{40}\) 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 al\(^{62}\) implanted a teicoplanin-load 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 al\(^{64}\) 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 \textit{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**


