# Antibiotic-containing Bioceramics Can Provide Safe and Effective Treatment in Bone and Joint Infections

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## Abstract

Osteomyelitis is a deep bone infection. Treatment options available may include a combination of surgical debridement, antibiotic therapy and in selected cases, bone repair. Local antibiotic delivery has the advantage of providing sustained therapeutic drug concentrations at the infected site while maintaining relatively low serum levels, minimising the potential for systemic toxicity. Gentamicin drug delivery and release systems using mechanical pumps, non-biodegradable and biodegradable implants and bioceramic cements have been studied to evaluate the safe and effective management of bone infections. The purpose of the current review was to evaluate the *in vitro* and *in vivo* drug delivery characteristics, osteoconductivity, biophysiological compatibility, antimicrobial activity and elution kinetics of gentamicin and its effectiveness in local carrier systems for the treatment of osteomyelitis.

## Keywords

Gentamicin, osteomyelitis, bone and joint infections, biocompatibility, osteoconductive, local antibiotic drug delivery

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Deep bone and joint infections leading to osteomyelitis are a severe complication, often with persistent sequelae.[1] The main risk factors include: penetrating trauma, open fractures, joint replacement surgery and operation with internal fixation devices. Open fractures are especially vulnerable as they are associated with a soft tissue defect that permits contamination from the outside. Some 60 to 70 % of open wounds have positive cultures already in the emergency department.[2] While most of the contaminating bacteria are derived from normal skin flora, more virulent bacteria can also gain entry through the wound. Hematogenous osteomyelitis from septicaemia is mainly seen in childhood and among elderly. The presence of an untreated bone abscess leads to increased pressure and impaired blood flow with local vascular occlusion, the hallmarks of a chronic osteomyelitis or septic arthritis.

Although a broad range of bacterial species have been isolated in cases of acute and chronic osteomyelitis (see *Table 1*), *Staphylococcus aureus* still remains the most common pathogen and accounts for up to 80 % of all cases.[3–9] In one study, Bergman et al., reported that *Staphylococcus epidermidis* and various gram-negative bacilli were responsible for approximately one-third of chronic osteomyelitis cases.[10] Another study found gram-negative rods in half of the cases.[11]

At present, surgical intervention to eradicate the infection is considered in most cases of chronic osteomyelitis and includes debridement. In unhealed fractures stable fixation is a must as well as in most cases adding a bone substitute, eliminating dead space and stimulating healing. If the infected fracture is healed, removal of foreign material and sometimes reaming of the medullary canal to remove granulation tissue is necessary. Concomitant use of systemically administered antibiotics is also employed but often ineffective because of insufficient penetration into ischaemic or necrotic bone tissue.[12] Moreover, pathogens elude antimicrobial activity by secreting a biofilm, an extracellular polymeric polysaccharide, which protects the organism from phagocytosis and impedes delivery of the antibiotic.[13–17] This defensive mechanism is especially important in the pathogenesis of osteomyelitis, primarily due to the bacteria's ability to produce protein specific adhesive film onto prosthetic implants, acrylic cements and dead necrotic bone.[18] As result, systemic antibiotic treatment is often ineffective as an infected sequestrum forms a sessile matrix-protected community with increased antimicrobial resistance.[19–21]

To overcome these clinical issues high doses of systemic antibiotics are often administered, for longer periods. This methodology increases the risks of individual and ecological adverse reactions and systemic nephrotoxicity and hepatotoxicity.[22] Long term antibiotic treatment remains the standard of care[23,24] but newer methods of sustained local antibiotic delivery have been developed to minimise systemic complications, enhance clinical efficacy and improve cost-effectiveness.[25,26] A number of which include a broad array of gentamicin delivery systems exists, such as mechanical infusion pumps, biodegradable and non-biodegradable implants and coatings, polymeric cements, nanotubes and microspheres.[26–56]

**Table 1: Pathogens Isolated in Bacterial Osteomyelitis[5–9]**

| Organism | Comments |
|---|---|
| *Staphylococcus aureus* | Organism most commonly isolated in all types of osteomyelitis |
| *Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter spp.*, *Escherichia coli, Proteus mirabilis, Staphylococcus albus, Acinetobacter spp.*, Haemolytic *Streptococcus* | Organisms responsible for bone and joint infections |
| *Staphylococcus epidermidis* | Prosthesis infection |
| Coagulase-negative *Staphylococci* or *Propionibacterium* species | Foreign-body infection |
| *Enterobacteriaceae* species or *Pseudomonas aeruginosa* | Nosocomial infections |
| *Streptococci* or anaerobic bacterium | Associated with animal or insect bites, diabetic foot lesions, decubitus ulcers |
| *Salmonella* species or *Streptococcus pneumonia* | Sickle cell disease |
| *Bartonella henselae* | Human immunodeficiency virus infection |
| *Pasteurella multocida* or *Eikenella corrodens* | Animal or human bites |
| *Aspergillus* species, *Mycobacterium avium-intracellulare* or *Candida albicans* | Immunocompromised patients |
| *Mycobacterium tuberculosis* | Populations where tuberculosis is prevailing |
| *Brucella* species, *Coxiella burnetii* or other fungi | Populations where these pathogens are endemic |

**Table 2: Pathogens Encountered (*In Vitro* and *In Vivo* Data) in Various Studies[26,28,41–43,52,59] and their Sensitivity to Gentamicin[60–62]**

| Pathogen | Number of Cases[26,28,41–43,52,59] | Gentamicin Sensitivity | Stille[61] | Sweetman[62] | Grayson[60] |
|---|---|---|---|---|---|
| *Staphylococcus aureus* (including coagulase negative strains) | 69 | HIGH | 1 | 1 | 1 |
| *Staphylococcus epidermidis* | 29 | HIGH | 1 | 2 | 1 |
| *Pseudomonas aeruginosa* | 16 | MEDIUM | 2 | 2 | 2 |
| *Enterobacter cloacae* | 6 | HIGH | 1 | 2 | 1 |
| *Enterococcus faecalis* | 5 | LOW | 3 | 3 | 4 |
| Methicillin-resistant *Staphylococcus aureus* (MRSA) | 3 | NO | 4 | 4 | 4 |
| *Serratia marcescens* | 3 | HIGH | 1 | 2 | 1 |
| *Mycobacterium tuberculosis* | 2 | NO | N/A | 3 | 4 |
| *Enterococcus faecium* | 1 | LOW | 3 | 3 | 4 |
| *Micrococcus* | 1 | N/A | N/A | N/A | N/A |
| *Corynebacterium amycolstum* (presumably *C. amycolatum*) | 1 | MEDIUM | N/A | N/A | 2 |
| *Coryneybacterium* | 1 | MEDIUM | N/A | N/A | 2 |
| *Streptococcus* miller-group | 1 | N/A | N/A | 1 | N/A |
| *Neisseria weaver* | 1 | MEDIUM | N/A | 2 | 2 |
| *Streptococcus pyogens* | 1 | LOW | 3 | 3 | 4 |
| *Streptococcus viridans*-group | 1 | NO | – | 3 | 4 |
| *Streptococcus magnus* (probably *Peptostreptococcus magnus*?) | 1 | N/A | N/A | N/A | N/A |
| *Peptostreptococcus magnus* | 1 | N/A | N/A | N/A | N/A |
| *Peptostreptococcus anaerobius* | 1 | N/A | N/A | N/A | N/A |
| *Staphylococcus lugdunensis* | 1 | HIGH | 1 | N/A | N/A |
| *Enterococcus casseliflavus* | 1 | LOW | 3 | 3 | N/A |
| *Citrobacter* | 1 | HIGH | 1 | 2 | 1 |
| *Klebsiella pneumoniae* | 1 | HIGH | 1 | 2 | 1 |
| *Escherichia coli* | 1 | HIGH | 1 | 2 | 1 |
| *Morganella morganii* | 1 | HIGH | 1 | – | 1 |
| *Stenotrophomonas maltrophilia* | 1 | NO | 3 | – | 4 |
| *Acinetobacter anitratus* | 1 | HIGH | – | – | 1 |
| *Unidentified/vague identification* | 46 | N/A | N/A | N/A | N/A |

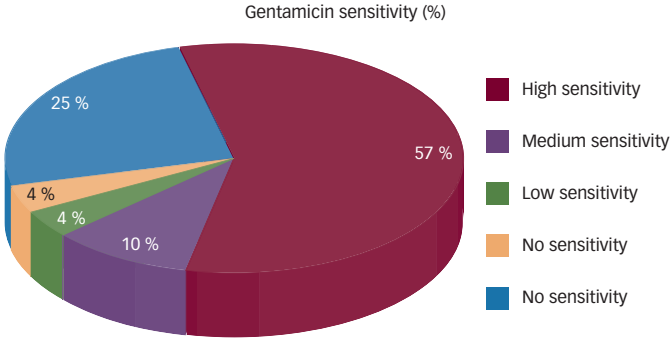
*Note: Gentamicin sensitivity classification (HIGH, MEDIUM, LOW, NO) were assigned from rounded mean values derived from the cumulative sensitivity classification scores assigned using the 'Stille', 'Martindale' and 'Kucer' sensitivity scale. Values from Stille et al. were assigned using the following grading system: good effectiveness = 1; moderate effectiveness = 2; relatively ineffective = 3; resistant to highly resistant = 4. Values from Sweetman were assigned using the following grading system: highly sensitive = 1; sensitive = 2; insensitive = 3; resistant = 4. Values from Grayson were assigned using the following grading system: highly susceptible/active = 1; susceptible to highly quite susceptible = 2; resistant to highly resistant/no activity = 4.*

Gentamicin is an aminoglycoside with broad-spectrum antibacterial activity against gram negative and gram positive organisms.[29] Its use is indicated in several serious bacterial infections, including acute and chronic osteomyelitis.[57–62] When it comes to choosing the most effective antibiotic agent for treating osteomyelitis, data is scarce.[58] In most cases, the appropriate drug is selected based on bacterial sensitivity and often given for a minimum of six months. We attempt to review the gentamicin used in a local delivery system for treatment of deep bone and joint infections.

## Bacterial Sensitivity

Most of the major causative bacteria of acute/chronic osteomyelitis are sensitive to gentamicin.[29] In addition, gentamicin has demonstrated good penetration into bone.[63,64] Several *in vitro* animal studies have examined gentamicin containing local delivery systems and have demonstrated effective eradication of the most prevalent pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*.[29,31,34,54]

**Figure 1: Percentage of Pathogens Identified in Osteomyelitis Studies[26,28,41–43,52,59] and their Respective Sensitivity to Gentamicin[60–62]**




In order to further increase our understanding of the pathogens most commonly identified in bone infections we assessed several human trials examining the use of antibiotic-loaded bone substitutes for the treatment of osteomyelitis and tuberculous spondylitis.[26,28,41–43,52,59] Of the 154 patients evaluated in the identified studies, 69 (45 %) cases were infected with *Staphylococcus aureus*, while 29 (19 %) were infected with *Staphylococcus epidermidis* and 16 (10 %) with *Pseudomonas aeruginosa* (see *Table 2*) (most authors reported several cases of mixed infections). The most recent data corroborates previously reported series regarding *Staphylococcus aureus* and *Staphylococcus epidermidis* implications in bone infections.[65]

As shown in *Table 2*, we attempted to illustrate the degree of activity of gentamicin to the pathogens encountered in the cited studies. Although the investigators did not specifically test for gentamicin sensitivity, we correlated the data from the pathogens encountered to the gentamicin sensitivity scales recorded by Stille[61] et al., Grayson[60] and Sweetman.[62] The pie chart in *Figure 3* illustrates gentamicin as exerting a high to medium activity to 2/3 of the relevant pathogens. Some caution is advocated since gentamicin sensitivity and resistance changes over time and varies between hospitals and countries, partly because of differences in the use and misuse of antimicrobial agents.[60-62]

Some infections are polymicrobial and the expected efficacy of gentamicin may be significantly reduced. Despite these warnings and based on the results of available reports,[3–9,58,65] we may assume that a majority of deep bone infections are caused by pathogens that have a medium to high sensitivity to gentamicin.

## Minimum Inhibitory Concentrations and Elution Kinetics

After an extensive review of the available literature we identified 10 animal studies that examined the minimum inhibitory concentrations (MIC) of gentamicin and its respective elution profiles in local drug delivery systems.[27,29,31,33,34,37,44,45,66,67] Unfortunately, there were no human trials to evaluate or compare with results of animal models. The latter have been useful in studying this complex disease, but do not correlate well with the many characteristics of human bone infection.[64] Nonetheless, the evaluated studies did report MIC levels in a range of 0.5–6 µg/ml for the *Staphylococcus aureus* strains. Gollwitzer et al.,[34] reported an MIC of 2 mg/l for the *Staphylococcus epidermidis* strain, while Aimen et al.[29] and Sanchez et al.,[67] cited gentamicin MICs of ≤6 µg/ml for *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Using the breakpoints (see *Table 3*) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[69] for the systemic use of gentamicin, these render most of the relevant microorganisms listed above susceptible to gentamicin, Because of the well-known toxicity of gentamicin (otovestibular side effects and nephrotoxicity) the systemic dosing of gentamicin (and other aminoglycosides) is limited. The breakpoints are pertinent to systemic infections and systemic administration of gentamicin and do not automatically translate well to local treatment where much higher concentrations will be attained. However, the breakpoints are also in most cases good predictors of the presence of resistance mechanisms and they may be difficult to overcome with any type of administration. However, taking everything into account, it is reasonable to assume that the most common microorganisms (*Staphylococci* and *Enterobacteriaceae*) will when exhibiting a MIC ≤4 mg/l lack resistance mechanisms and be possible to treat with a tissue concentration of 4 mg/l or more.

Elution profiles provided by the animal studies are more difficult to interpret, but existing data reveal a compelling consistency in gentamicin elution profiles that cannot be dismissed. Using different mechanisms of local delivery, both Aimen et al.[29] and Barro et al.,[31] reported *in vivo* gentamicin concentrations at or near the infected bone above the MICs of relative pathogens for up to four weeks. The two studies further demonstrated serum concentration levels (0.5–1 µg/ml) well below the limits for any increased risk of systemic toxicity. Garvin et al.,[33] compared gentamicin impregnated polymethylmethacrylate (PMMA) implants (100 mg gentamicin) with gentamicin infused polylactide/polyglycolide implants (100 mg gentamicin) to treat a subset of dog tibias experimentally infected with *Staphylococcus aureus*. Concentrations of gentamicin in the recovered bone samples at six weeks post implantation were 10.3 ± 2.6 (PMMA treated) and 20.1 ± 21.5 µg/ml (polylactide/polyglycolide implant), well above the MIC for *S. aureus*. Not surprisingly, eight of the nine (88.9 %) PMMA-gentamicin implants and all nine of the polylactide/polyglycolide-gentamicin implants eradicated the infections completely, clearly demonstrating that *S. aureus* infections of the dog tibia can successfully be treated with a local gentamicin application. Elution profiles were not determined, but local gentamicin concentrations remained at therapeutic levels after six weeks.

Another animal study, Nelson et al.[27] in rabbits experimentally infected with *S. aureus* and treated with a bioabsorbable composite impregnated with 20 or 10 % gentamicin sulphate beads, separately analysed, gave similar results as those cited earlier. The osteomyelitis was eradicated in 93 % of the rabbits treated with the 20 % gentamicin (116 mg) impregnated composite, while the corresponding figure for 10 % gentamicin (58 mg) was 67 %. Gentamicin concentrations in the bone closest to the implant for the 20 % gentamicin infused composite were 0.8, 1.2, 0.4 and 0.2 µg/ml at one, two, 3 and four weeks, and the corresponding concentrations for the 10 % gentamicin were 0.25, 0.4, 0.3 and 0.2 µg/ml, respectively.

Although the studies we reviewed do not provide sufficient information regarding optimal elution profiles in humans, they do provide some understanding of the gentamicin concentrations near the infection site over time and the efficacy of the respective regimen. Some investigators have proposed that one should try to achieve higher concentrations and try to exceed the MICs of relevant pathogens for prolonged periods of time. However, there is no evidence to suggest that eradication would be more effective. Further studies in humans are necessary to further the understanding of the elution kinetics in gentamicin infused carrier systems.

**Table 3: The Table Summarises the Gentamicin Minimum Inhibitory Concentrations Breakpoints – European Committee on Antimicrobial Susceptibility Testing[69]**

| Bacterium | MIC Breakpoint for Susceptibility S≤ Value (mg/l) | MIC Breakpoint for Resistance R> Value (mg/l) |
|---|---|---|
| *Enterobacteriaceae* | 2 | 4 |
| *Pseudomonas spp* | 4 | 4 |
| *Acinetobacter spp* | 4 | 4 |
| *Staphylococcus aureus* | 1 | 1 |
| Coagulase-negative *Staphylococci* | * | * |
| *Enterococcus spp* | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| *Streptococcus* group A, B, C & G | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| *Streptococcus pneumoniae* | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| Other Streptococci | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| *Haemophilis influenza* | Insufficient evidence | Insufficient evidence |
| *Moraxella catarrhalis* | Insufficient evidence | Insufficient evidence |
| *Neisseria gonorrhoeae* | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| *Neisseria meningitidis* | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| Gram-positive anaerobes | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| Gram-negative anaerobes | Species is a poor target for therapy with the drug | Species is a poor target for therapy with the drug |
| Non-species related breakpoints | 2 | 4 |

**Isolates with gentamicin minimum inhibitory concentrations (MIC) >128 mg/l or an inhibition zone diameter <8 mm have acquired resistance mechanisms and can be reported as high-level aminoglycoside resistant (with the exception of streptomycin).*

**Table 4: Overview of the Studies Investigating the Impact of Gentamicin on the Key Properties Involved in Osteogenesis**

| Ref | Concentrations of Gentamicin | Impact on Cellular Proliferation | ALP | Cell Viability | Protein Synthesis | Cell Morphology | Bone Growth | Measured Time | Test Model |
|---|---|---|---|---|---|---|---|---|---|
| 95 | >10 µg/ml<br><5,000 µg/ml | Negative | Negative | N/A | N/A | N/A | N/A | 10 days<br>14 days | *In vitro*<br>*In vivo* |
| 54 | 7–50 µg/ml | Neutral | Neutral | N/A | Neutral | N/A | N/A | 5, 10, 15 days | *In vitro* |
| 40 | 100 and 200 µg/ml | Neutral for days 3 and 5, decrease in cell proliferation at day 10 with 200 µg/mL | Neutral | N/A | N/A | N/A | N/A | 3, 5, 10 days | *In vitro* |
| 32 | Up to 1,000 µg/ml | Negative effect after 7–9 days of exposure | N/A | N/A | N/A | N/A | N/A | 0–17 days | *In vitro* |
| 97 | 25, 100, 400 µg/ml | Neutral | Negative | N/A | N/A | N/A | N/A | 6 hours | *In vitro* |
| 46 | 200, 400, 600 µg/ml | Neutral | Neutral | N/A | N/A | N/A | N/A | Cell count: 1 and 7 days<br>ALP: 1 and 3 weeks | *In vitro* |
| 97 | 12.5–800 µg/ml | Neutral | Negative | Positive | N/A | Neutral | N/A | 6 hours | *In vitro* |
| 39 | 10, 100, 1,000 µg/ml | N/A | N/A | Neutral | N/A | Neutral | N/A | 48 hours | *In vitro* |
| 98 | 12.5–800 µg/ml | Neutral | Negative | Neutral <200 µg/ml<br>Negative 200–800 µg/ml | N/A | N/A | N/A | 48 hours | *In vitro* |
| 49 | 400 µg/ml | N/A | Neutral | N/A | N/A | N/A | Positive | 1, 7 days | *In vitro* and *in vivo* |
| 99 | 0–1,000 µg/ml | Negative | Neutral 100 µg/ml<br>Negative ≥100 µg/ml | N/A | Neutral <100 µg/ml<br>Negative ≥100 µg/ml | N/A | N/A | 4 days | *In vitro* |
| 100 | 3 g | N/A | N/A | N/A | N/A | N/A | Positive | 6 months | *In vivo* (human) |
| 30 | Not reported | N/A | N/A | N/A | N/A | N/A | Neutral | 4, 12 weeks | *In vivo* (rabbit) |
| 50 | 0, 10, 20 mg | N/A | N/A | N/A | N/A | N/A | Positive | 1, 3, 6, 12 weeks | *In vivo* (rat) |

*ALP = alkaline phosphatase activity; Ref = reference.*

## Biocompatibility

In animal studies, it has been possible to maintain gentamicin serum levels below the systemic toxicity threshold while local concentrations have been sufficient to effectively eradicate the infection.[27,29,31,33] However, none of these studies attempted to identify the acute, sub-acute or chronic toxicity that may present a risk with gentamicin infused drug delivery systems.

The *in vitro* experimental studies investigating gentamicin cytotoxicity evaluated a wide range of cell types (mouse and human fibroblasts, human keratinocytes and mononuclear cells and rabbit epithelial cells).[36,70–74] Differences in the cellular biology as well as in the test methods make it difficult to compare and draw conclusions. Yet, the findings illustrate one significant point; gentamicin can be cytotoxic to certain cell types in a dose dependent manner. No cytotoxicity was described in mouse fibroblasts or human mononuclear cells at concentrations in the range of 0.18–0.94 µg/ml and 0–6.6 µg/ml.[36,70] In contrast, gentamicin concentrations in the range of 0.25–1.0 mg/ml were identified to be cytotoxic in human keratinocytes,[71,72,74] while concentrations as high as 0.6 mg/ml were identified to be cytotoxic in human fibroblasts.[68] We found no studies which described cytotoxicity with gentamicin in local concentrations of up to 100 µg/ml; values well beyond the MIC for gentamicin sensitive bacterias (1–4 µg/ml).

Gentamicin toxicity can also include risks related to systemic allergic dermatitis. A retrospective study in the Finnish population reported a 4.6 % sensitisation rate to gentamicin.[75] Other documented series reporting the link between systemic allergic dermatitis and gentamicin have been less clear. Haeberle et al.,[35] examined a single case study linking systemically induced allergic dermatitis in a patient with chronic leg ulcers and stasis dermatitis with gentamicin released from the cement used in a total knee replacement. Another report by Liippo et al.,[76] determined that gentamicin sensitisation may result from occupational exposure to gentamicin infused bone cements or from systemic cross reactions with other aminoglycosides. The very limited published data that does exist implicates dermal contact and systemic gentamicin exposure as related causes of allergic dermatitis, albeit its occurrence is considered a rare phenomenon.[35] Local gentamicin delivery does not appear to present any auxiliary risk.

A more pressing concern relates to the associated risks of aminoglycosides and drug-induced nephrotoxicity[77]. The significant biochemical and morphological alterations elicited by aminoglycosides in the kidney cortex are beyond the scope of this article. However, nephrotoxicity induced by aminoglycosides manifests clinically as nonoliguric renal failure, with a slow rise in serum creatinine and a hypo-osmolar urinary output developing after several days of treatment. High doses (40 mg/kg or more for gentamicin) are necessary in animals to rapidly induce extended cortical necrosis and overt renal dysfunction.[78,79] As reported with animals, human nephrotoxicity is associated with long term use of gentamicin and high serum concentrations.[80] Therefore, locally applied gentamicin release mechanisms should deliver therapeutic doses with serum concentrations of gentamicin ≤2 µg/ml in order to minimise the risk for developing nephrotoxicity.

Ototoxicity, which is the second main adverse effect of aminoglycosides and which, in contrast to nephrotoxicity, is irreversible, is the most common single known cause of bilateral vestibulopathy.[81,82] The incidence of ototoxicity related to parenteral use of gentamicin in humans estimated to be 3 %.[82] Other authors have estimated that about 1 % of all two-week courses of gentamicin result in significant vestibular toxicity.[82,83] Recent studies suggest that gentamicin ototoxicity is most closely related to total dose rather than having inappropriately high serum concentrations. This line of rationale suggests that high-peak levels are not necessarily ototoxic, but rather it is the total dose or some other variable that is more significant. It is possible that toxicity might be related to a combination of peak dose and total dose, or that toxicity is a complex function of peak and total dose. More studies are needed to establish the importance of respective variables.

Gentamicin has also been studied in a range of *in vitro* genotoxicity studies (*Salmonella* microsomal assay, mitotic cross over, gene conversion, DNA repair, Rec-assay) that have demonstrated no genotoxic potential for gentamicin.[84] However, positive results were observed *in vitro* for forward mutation in *Escherichia coli* at a cytotoxic dose level in a test for chromosome aberrations in mouse L-cells and in a test for sister chromatid exchange in human fibroblasts.[84] The results, however, were derived from a questionable study design and should be viewed with caution. Other mutagenicity studies have been designed to evaluate the effects of gentamicin sulphate salts. One such study, examined gentamicin for cytotoxicity and mutagenicity in a chromosomal aberration assay in CKO-K1 cells in the presence and absence of metabolic activity.[84] The conclusions were definitive for both positive and negative controls; gentamicin sulphate did not induce chromosomal aberrations in Chinese hamster ovary cells. Similar results were achieved when gentamicin sulphate was tested for mutagenic activity in Chinese hamster ovary cells using a gene mutation assay;[84] gentamicin sulphate was negative for inducing a mutagenic response. From the data reviewed it can be safely concluded that gentamicin is unlikely to be genotoxic.

Another consideration that must be examined for a gentamicin carrier system is the environment the drug will be applied. An acidic environment is known to weaken the activity of many antimicrobials. Aminoglycosides, in particular, are affected by the changes in pH. For example, lowering the pH can significantly increase the aminoglycoside MIC in *Enterobacteriaceae*.[85–88] Several publications have clearly demonstrated the pH impact on MIC and MBC for gentamicin.[48,85,89,90] Evaluating these studies cumulatively, we observe moderate changes in gentamicin MIC against the most prevalent pathogens (*Staphylococcus aureus* and *Escherichia coli*) with a pH between 7.5 and 6, while a pH <6 will significantly increase MIC and MIB for the same pathogens. No changes were observed with a pH >7.5. Thus, for any gentamicin delivery system to maintain its expected efficacy, the pH at the delivery site would need to be above a value of 6, with an optimal pH considered at 7.5.

## Osteogenesis

It has been suggested that the ideal local antibiotic delivery system not only bind significant amounts of active antibiotics and gradually release them at the most efficient rate, time and amount, but it should also provide efficacy as a bone void filler, and most preferably stimulate osteoblast proliferation. Many products have attempted to meet the ideal requirements, such as hydroxyapatite blocks and beads, apatite-wollastonite glass ceramics and calcium hydroxyapatite bone-void cements.[91–94] Delivery devices that employ the use of hydroxyapatite poses an advantage over other materials because it provides osteoconductive scaffolding that helps fill dead space and encourage new tissue ingrowth which in turn leads to repair of osseous defects while maintaining the capacity to elute high concentrations of antibiotic.[42] The ambiguity surrounding these delivery mechanisms rests on the impact of gentamicin on osteogenesis.

Several *in vitro* and a few *in vivo* studies (see *Table 4*) have examined the effects of gentamicin on cellular and osteogenic activity.[95–99] Results from the *in vitro* studies are conflicting. Five studies reported no effect of gentamicin on osteoblast proliferation, but three others

reported reductions in both cell count and proliferation compared to controls. There appears to be a correlation between long exposures of the antibiotic (>5 days) and cell count. This does not appear to be dose dependent.

Regarding the effects of gentamicin on alkaline phosphatase activity (ALP), a key metabolic indicator of osteogenic activity, we found five studies reporting a negative impact and four reporting no impact (neutral). One of the publications[99] describe a dose dependency for concentrations > 100 µg/ml. The impact on cellular viability and protein synthesis also demonstrate inconsistencies in the results. Cellular morphology appears to be unaffected, but long-term data (>48 hours) is lacking. In contrast to the other variables, the bone growth data show some evidence of a neutral impact and in some cases a beneficial 'protective' influence (reduced re-occurrence of osteomyelitis in new bone formation) on bone in growth. In fact, in the only human trial[100] evaluated, 22 patients diagnosed with an infected tibia with non-unions secondary to fracture were treated with revision surgery including the use of intra-medullar allograft with 3 g gentamicin. All non-unions healed within six months (median: 4.5 months, range: 3.5–6 months), clearly demonstrating that the locally applied gentamicin did not negatively impact bone in growth.

In the final analysis, the lack of uniformity in the *in vitro* results leaves significant uncertainty in regards to the impact of gentamicin on the process of osteogenesis. However, there appears to be evidence, in both *in vivo* animal and human studies, that indicates a neutral impact on the bone repair process and a possible 'protective' effect in the presence of local infection. Therefore, a gentamicin coupled hydroxyapatite drug delivery device could provide a viable and effective instrument in ameliorating the infection while also aiding osteogenesis.

## Discussion

The use of gentamicin as an effective antimicrobial is well established. Local delivery using suitable carrier systems holds significant promise as an effective treatment for osteomyelitis. Although some of the pharmacokinetic properties of a locally delivered gentamicin composite need further explanation, the principle remains sound and viable. As previously noted, there have been extensive *in vitro* investigations evaluating gentamicin in various drug delivery devices, but only a few systems have been tested adequately in the treatment of established osteomyelitis. Sulo[101] reported on a series of 409 patients with confirmed chronic osteomyelitis treating them with plaster of Paris antibiotic beads impregnated with gentamicin. Ninety-five per cent of these patients were evaluated as cured of chronic osteomyelitis after a mean follow-up period of 37 months. In addition, 42 % of the patients had complete filling of the osseous defect with this technique. He concluded that the procedure allowed immediate filling of the bone loss and led to high antibiotic concentrations which aided in resolving the chronic infection. Other authors have reported similar findings. Walenkamp et al[102]., treated 100 patients diagnosed with osteomyelitis with debridement and gentamicin-PMMA beads and followed them for a mean of five (range: 1–12) years. The study reported 92 % of the patients treated were free of infection at the end of the follow-up period. The major drawback of using antibiotic loaded PMMA beads is the required removal of the beads resulting in a 'two-stage' operative procedure.

Among the newer biodegradable carrier systems that have been recently developed and studied are those that are composed of a combination of hydroxyapatite and calcium sulphate.[47] Hydroxyapatite has been extensively used as an artificial bone substitute and provides a natural bone-like structure that is non-immunogenic, nontoxic, osteoconductive, biocompatible and bioactive. Meanwhile the calcium sulphate can act as an osteoconductive scaffolding aiding in the growth of new osseous tissue. A water soluble antibiotic, such as gentamicin, can be incorporated into the crystalline structure of the calcium sulphate, thus providing an optimal carrier system that meets two of the most important objectives in a successful clinical strategy: treatment/prevention of the infection and osseous repair. Further development of such biodegradable systems will provide a novel approach for the treatment of acute/chronic osteomyelitis.

In summary, the available evidence suggests that a gentamicin-impregnated local delivery systems can provide an effective therapeutic choice for patients with osteomyelitis. Gentamicin has an acceptable safety profile (at the appropriate concentrations), can effectively eradicate the most prevalent pathogens and does not appear to effect osteogenesis, and may, in fact, have a favourable effect.

Since 50 years, gentamicin has been used for local prophylaxis in millions of patients through incorporation in PMMA mainly for primary cemented joint replacements.[103] When coupled with an appropriate biologic delivery system, such as a hydroxyapatite/calcium sulphate composite, the gentamicin-impregnated carrier could both eradicate the infection and provide osseous repair and thus reduce the risks of recurrence. To what extent this approach is valid in the everyday treatment of human bone infections is not yet known. Further studies determining the optimal dosage, the elution profile and the biocompatibility of locally delivered gentamicin for patients with osteomyelitis are needed. ■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