**KNEE: RESEARCH**

Does cyclical loading affect the elution of antibiotics from articulating cement knee spacers?

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Two-stage revision surgery for infected total knee replacement offers the highest rate of success for the elimination of infection. The use of articulating antibiotic-laden cement spacers during the first stage to eradicate infection also allows protection of the soft tissues against excessive scarring and stiffness. We have investigated the effect of cyclical loading of cement spacers on the elution of antibiotics. Femoral and tibial spacers containing vancomycin at a constant concentration and tobramycin of varying concentrations were studied *in vitro*. The specimens were immersed and loaded cyclically to 250 N, with a flexion excursion of 45°, for 35 000 cycles. The buffered solution was sampled at set intervals and the antibiotic concentration was established so that the elution could be calculated. Unloaded samples were used as a control group for statistical comparison.

The elution of tobramycin increased proportionately with its concentration in cement and was significantly higher at all sampling times from five minutes to 1680 minutes in loaded components compared with the control group (p = 0.021 and p = 0.003, respectively). A similar trend was observed with elution of vancomycin, but this failed to reach statistical significance at five, 1320 and 1560 minutes (p = 0.0508, p = 0.067 and p = 0.347, respectively). However, cyclically loaded and control components showed an increased elution of vancomycin with increasing tobramycin concentration in the specimens, despite all components having the same vancomycin concentration. The concentration of tobramycin influences both tobramycin and vancomycin elution from bone cement. Cyclical loading of the cement spacers enhanced the elution of vancomycin and tobramycin.

Infection after total knee replacement (TKR) is an infrequent but devastating complication which is difficult to treat. The poor availability of antibiotics at the site of infection, the presence of a biofilm reducing the exposure of bacteria to the antibiotics and the relative immunodeficient zone around an implant are all factors which influence the efficacy of treatment.1-3

There are considerable financial costs and clinical implications from infection including increased morbidity and prolonged or repeated hospital admissions.4,5 Most studies report an incidence of infection of 1% to 2% after primary TKR,6-8 but this may increase to over 4% in patients with rheumatoid arthritis and an incidence of over 12% has been reported in certain groups of patients.9,10 The cost of revision knee surgery as a result of infection is more than twice the cost of an aseptic revision, and several times that of a primary TKR.4,5

Two-stage revision TKR, with the use of antibiotic-impregnated polymethylmethacrylate (PMMA) cement spacers is a widely practised method of managing this problem.11,12 After the removal of the primary prosthesis with thorough debridement, the cement spacer is implanted followed by an extended course of antibiotics. A second-stage procedure is subsequently performed when the patient is considered to be free of infection.13,14 Cement spacers assist in the delivery of antibiotics, maintain limb length and therefore tissue tension, and reduce the formation of soft-tissue contracture or arthrofibrosis, thereby simplifying re-implantation of the new components at the second stage.15-17 Spacers can be articulating or non-articulating (static) and there is debate regarding the benefits of each type.18 The non-articulating type provides a high concentration of antibiotics locally, maintains the joint space and limits the possible risk of introducing the inoculum further into the surrounding tissues by restricting movement of the knee. Several studies have shown their effectiveness.19,20 By contrast, articulating spacers allow joint movement and help to maintain soft-tissue function.21,22 Arthrofibrosis is minimised and function is better than that following the use of...
non-articulating spacers. Several forms of spacer have been described and they can be custom-made during the operation or commercially available as prefabricated components.

The addition of antibiotics to cement, especially in liquid form, adversely affects tensile and compressive strength. Ideal properties of an antibiotic which is mixed with cement include minimal adverse biomechanical effects on PMMA, water solubility; a broad spectrum of antimicrobial action, thermal stability and low allergenicity. As a consequence, there are only a few which fit these criteria. They include tobramycin, vancomycin, gentamicin and cephalosporins.

Since gentamicin is commonly a component of the cement used in primary TKR, organisms may acquire gentamicin-resistance. The release of antibiotics from cement has been studied both in vitro and with in vivo animal models. Static in vitro models have shown a high level of release of local antibiotic from cement which is affected by several factors including the surface area, porosity and the amount, type and number of antibiotics.

However, the effects of articulation, specifically cyclical fatigue loading, on the elution of antibiotics from cement knee spacers has not been clearly defined. The maintenance of joint movement is an advantage in maintaining the condition of the soft tissues, but the question arises as to whether the articulation per se influences the biology and pharmacokinetics of antibiotic elution. Tobramycin has a broad antimicrobial action with minimal effect on PMMA strength, while vancomycin is effective against methicillin-resistant Staphylococcus aureus and Staph. epidermidis with similarly low adverse biomechanical effects on PMMA.

Our in vitro study considered the differential effect of static or dynamic loading on the elution of vancomycin and tobramycin with a null hypothesis proposing that the cyclical loading of cement spacers has no significant effect on elution.

**Patients and Methods**

**Preparation of samples.** All the samples were prepared in an identical manner. Antibiotic-loaded spacers were prepared by the injection of Palacos R (Heraeus Kulzer GmbH, Hanau, Germany) standard radio-opaque cement into Biomet stage-one 70 mm silicone spacer moulds (Biomet Inc., Warsaw, Indiana). The addition of a constant concentration of vancomycin with incremental quantities of tobramycin was investigated (Table I). Vancomycin and tobramycin are two of the most commonly used antimicrobials partly because of their availability in powder form.

The dosing schedule used in our study correlated with that of admixtures reported in numerous clinical and in vitro studies. Before the polymerisation of cement, the cement monomer was chilled at -20°C for five minutes in order to extend the working time required to achieve a thorough mix of monomer and copolymer in the presence of added antibiotics. Vancomycin and tobramycin in their selected amounts and Palacos cement powder were blended for one minute in a small hand orthopaedic cement mixer (DePuy, Blackpool, United Kingdom) before polymerisation. The cement was mixed for one minute prior to being injected in the moulds, according to the manufacturer’s instructions, in air at room temperature (23°C ± 1°C), using a CemVac mixing system (DePuy CMW, Blackpool, United Kingdom). The cement was then injected into the silicone moulds and left to polymerise for a minimum of two hours. After curing, excess material, as a result of the moulding process, was removed from the femoral component taking care not to damage the surface of the condyles.

**Dynamic testing protocol.** Three sets of spacers were prepared for each of the three concentrations of antibiotics in the cement (Table I). The femoral component was attached to a polymer block conforming to the geometry of the spacer using waterproof silicone sealant (Figs 1 and 2). This was allowed to cure before testing. The femoral and tibial components were placed in the environmental chamber, maintained at 37°C and attached to the actuators as shown in Figure 2 (Instron 8874 servo hydraulic testing machine; Instron, Norwood, Massachusetts). The spacer was immersed in 1 l of phosphate-buffered saline (PBS). The femoral component was aligned relative to the tibial component in 22.5° of flexion. In order to reproduce the maximum tibiofemoral contact force of 2.5 times body-weight at 13% of the gait cycle, the tibial component was then loaded on to the femoral component with a force of 0.1375 kN. Dynamic loading was initiated as per the schedule detailed.
in Table II. The femoral component was cycled at a frequency of 1 Hz and an amplitude of 22.5° and the tibial component was loaded at a frequency of 1 Hz with an amplitude 0.1125 kN. The loading cycle (sinusoidal waveform, amplitude of 22.5° (maximum arc of movement of the femoral component), maximum load of 0.25 kN, R = 0.1, frequency (f) = 1 Hz) was selected to represent walking with crutches. The resistance value (R) is a material stiffness test. It expresses a material’s resistance to deformation as a function of the ratio of transmitted lateral pressure to applied vertical pressure. At each interval of the sampling, as indicated in Table II, cyclical loading was paused, the solution agitated to ensure an even distribution of the antibiotics, and three samples (5 ml) of solution collected from separated locations of the environmental chamber and stored at -20°C. The concentration of antibiotics, and hence elution, of these samples was measured by a TDx FLx Immunology Analyser (Abbott Laboratories, Abbott Park, Illinois).

As a control, two spacers for each concentration of antibiotic were prepared. The control samples were immersed in PBS and samples were taken in accordance with the schedule for dynamic testing. Thereafter, samples were taken twice daily, for four weeks. At the end of each day 30 ml of PBS were added to ensure that the sample remained fully immersed in solution.

**Statistical analysis.** The data points regarding the concentration of antibiotic, measured using enzyme-linked immunoassay, for each time interval were obtained from a mean of six samples from the two static controls per concentration (three per spacer) and the 12 samples for the dynamic testing from the four dynamic spacers per concentration (three per spacer; Table I).

Non-parametric statistical analysis of variance (ANOVA) was performed to compare the mean elution between the static and dynamic groups. A p-value ≤ 0.05 was considered to be statistically significant.

**Results**

**Control group.** The elution of both tobramycin and vancomycin, from all three concentrations of the static control samples, is shown graphically in Figure 3. There was a biphasic elution pattern with an initial rapid rate of antibiotic

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**Table II. Details of the sampling schedule for the dynamic and static groups**

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<tr>
<th>Sample number</th>
<th>Time elapsed (minutes)</th>
<th>Number of cycles</th>
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<td>Static control</td>
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</tr>
<tr>
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release which slowed to a steady state from 400 minutes. The interval in the data points after 400 minutes represented the overnight pause in the experiment (see Table II).

In the static control group vancomycin eluted at a higher rate than tobramycin. Furthermore, as the tobramycin concentration was increased in the cement mixture (the low, medium and high data sets), there was a corresponding increase in the release of vancomycin.

**Dynamic testing: tobramycin elution.** The rates of elution of tobramycin from the different concentrations of cement undergoing dynamic testing are shown in Figure 4 with comparison with the corresponding static control group.

Dynamic testing in comparison with the control group increased the elution of tobramycin. The release was dose-related, with an increasing concentration of tobramycin in the cement affording a corresponding increase in its elution. Furthermore, the dose-related elution response was exaggerated by dynamic testing. The mean elution of tobramycin between the static and dynamic groups was examined by ANOVA (Table III). Dynamic cyclical loading was associated with a significantly higher tobramycin elution at all sampling points.

Therefore, with respect to tobramycin elution, we can reject the null hypothesis and state that there is a statistically significant higher elution under dynamic loading compared with static loading.

**Dynamic testing: vancomycin elution.** The rates of elution of vancomycin in the different cement concentrations undergoing dynamic testing in comparison with the corresponding static group are shown in Figure 5.

Similarly to tobramycin, there was a dose-related increase in the elution of vancomycin as the concentration...
of tobramycin within the cement was increased. Thus, increasing the concentration of tobramycin in the cement augmented the release of vancomycin during dynamic testing in a dose-related manner.

There was a clear trend of increasing the elution of vancomycin with dynamic testing (Fig. 5) and this increase over the static samples was statistically significant (Table III) with the exception of the sampling time points of five, 1320 and 1560 minutes.

For the elution of vancomycin, the null hypothesis can also be rejected since, overall, higher elution occurred under dynamic loading which was statistically significant.

**Discussion**

Our results show that there was a biphasic elution of both vancomycin and tobramycin in the static control, with a rapid initial release followed by a plateau in the rate of concentration in cement (see Table I). The error bars show 95% confidence intervals.

For any concentration of antibiotic cement studied, dynamic loading increased elution compared with the corresponding static control group. Although dynamic loading improved elution *per se*, its effect on elution was further increased, in a dose-dependent manner, by the presence of tobramycin in the cement.

For tobramycin, a highly statistically significant dose-related increase was observed with dynamic loading compared with the control group (Fig. 5, Table III). Regarding vancomycin, a similar dose-related trend was seen, with statistical significance in all but three time intervals (Fig. 5, Table III). At five minutes, the p-value regarding vancomycin was 0.0508.

Therefore, by adding tobramycin to bone cement, there was enhanced static elution of vancomycin and tobramycin. This enhanced elution was significantly augmented by dynamic loading (Figs 4 and 5, Table III).

The mechanical benefits of maintaining joint movement during a two-stage revision TKR have been well documented. However, there is debate regarding the use of static or dynamic spacers. Static spacers have the theoretical advantage of reducing the inoculum being introduced into the surrounding soft tissues, which are also free from tension, augmenting antimicrobial action.

However, other reports have indicated a biomechanical advantage of dynamic over static knee spacers. The biological effect of dynamic cyclical loading *per se* on cement spacers, specifically differential antibiotic elution, has not previously been clarified since previous investigations have used static *in vitro* and animal *in vivo* models. Nevertheless, these previous non-dynamic studies have shown that the antibiotics are released in a biphasic manner in a dose-dependent way and that the presence of vancomycin increases the release of tobramycin.

It is uncertain how the addition of a second antibiotic enhances the release of another antibiotic, although it has been proposed that the second antibiotic increases the porosity of the cement thereby improving the release. Evidence elsewhere has shown that the addition of lactose to bone cement increases antibiotic release, as does increasing the surface roughness and surface area. This complementary release of antibiotic has been termed by Penner et al as passive opportunism. However, at present *in vitro* testing has not shown the ability of antibiotic bone cement to eradicate completely infection caused by bacteria which adhere to biomaterials.

Antibiotic elution characteristics have been quantified from studies on various mammalian species. A common pattern of a peak in the blood concentration after a few hours followed by a gradual reduction in concentration has been found, which was replicated in our study. These animal studies demonstrated that the local tissue concentration of antibiotic was considerably higher than that in the serum, and remained so for several weeks. However, the measured antibiotic concentration within the haematoma reduced within a matter of days.

For staphylococcal species, vancomycin has a minimum inhibitory concentration of 0.25 mg/l to 1.0 mg/l and a minimum bactericidal concentration of 0.25 mg/l, while tobramycin has a minimum inhibitory concentration of 0.12 mg/l to 1.0 mg/l and a minimum bactericidal concentration of 0.1 mg/l to 32.0 mg/l. It is accepted that a level of at least eight times the minimum inhibitory concentration is required for successful treatment, especially considering the *in vivo* dilution which occurs with time. The antibiotic elutions achieved in our study with dynamic

**Graph showing the comparison of vancomycin elution between static and dynamic (DYN) spacers at the low, medium and high concentrations in cement (see Table I). The error bars show 95% confidence intervals.**
loading were over ten times the respective minimum inhibitory concentration, 15 mg/l to 50 mg/l for vancomycin and 12 mg/l to 26 mg/l for tobramycin (Figs 4 and 5).

Masri et al using data obtained by joint aspiration before a second-stage revision total hip replacement or TKR have given the clinical guidelines for effective antibiotic concentration. Vancomycin had an inferior release rate if used in isolation, and again the combination of tobramycin with vancomycin gave encouraging results. The release of vancomycin, as assessed by joint aspiration, was found to reduce after four months to levels below the threshold for microbiological activity. However, the addition of tobramycin prolonged its time of beneficial vancomycin. On the strength of these clinical results, they recommended that tobramycin be used in a concentration of 3.6 g per 40 g of bone cement, provided that the articulating spacer did not remain in situ for longer than three to four months. Although providing guidelines, their study was limited by the lack of a control group, confounding patient factors, possible differences in the cement and antibiotic blending techniques and the inclusion of both hips and knees. Our dynamic in vitro study, with a static control group was not compromised by similar methodological limitations. Our results have shown a better elution of tobramycin from bone cement compared with vancomycin for both static and dynamic loading, consistent with the findings of other studies using static in vitro models.

The method of antibiotic mixing with cement was standardised in our study since there is some evidence that cement properties can be altered by the method of blending of vancomycin and tobramycin.

The addition of antibiotics to bone cement gives inferior mechanical properties, including reduced strength under both tensile and compressive loading. These properties have not been reported for the admixture of tobramycin and vancomycin. Although we have shown in vitro that a mixture of antibiotics under dynamic loading produces enhanced elution, the efficacy of low sustained release of antibiotics in a clinical setting remains unclear. Ideally, there should be an optimal antibiotic concentration at the site of the potential infection at the time of the surgery, and the benefit of continuing for a longer duration is not substantiated.

In the presence of renal impairment, extended release of either tobramycin (an aminoglycoside) or vancomycin (a glycopeptide) could potentially increase the risk of nephrotoxicity and otoxicity. The peak serum concentrations required for the treatment of infection in other organ systems range from 4 mg/l to 10 mg/l for tobramycin to 30 mg/ to 40 mg/l for vancomycin. Our results for the elution of tobramycin and vancomycin into a simulated synovial fluid were similar in range. It is unlikely therefore that concentrations would approach nephrotoxic serum levels.

An additional concern is that the widespread use of similar antibiotic mixtures may hasten the emergence of resistant strains. The clinician should always attempt therefore to adjust any antibiotic treatment, whatever the delivery method, in response to known tissue culture and sensitivity. The precise mechanism of antibiotic release from bone cement is uncertain. It is generally thought that it is directly released from the surface of the bone cement in the initial phase, and then subsequently released from a network of cracks and voids. Absorption of water to bone cement is thought to have a role in controlling the slow phase of antibiotic release. Our study suggests that cyclical dynamic loading enhances this process, probably by a mechanism of cyclical changes in the microstructure of the bone cement.

Our results provide evidence that, in addition to the benefit to the soft tissues claimed for articulating spacers, the dynamic loading of cement knee spacers per se affords a biological advantage by significantly enhancing antibiotic elution.

### References


