Antibiotic release from bone cement under simulated physiological conditions
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General Discussion

Chapter 7
On the mechanism of release of antibiotics from antibiotic-loaded bone cements

This thesis is concerned with gaining insight in the mechanism of action of antibiotic-loaded bone cement. The prerequisite for an antibiotic to act against bacteria is that it has to attain a sufficiently high concentration to be effective against bacteria. If the antibiotic is loaded in bone cement, it has to be released from the bone cement before this can occur. However, there are still many unanswered questions regarding the release component of the mechanism of action of antibiotic-loaded bone cements. Antibiotic release from bone cement can be considered to be composed of two steps. Firstly, the surrounding medium gains access to the antibiotic in the bone cement and secondly, the antibiotic dissolves in the medium and diffuses along the concentration gradient. All antibiotic particles on the surface of the bone cement rapidly dissolve in the surrounding medium, causing the initial burst release.

The origin of the subsequent low release, however, is less clear. One theory assumes a network of interconnecting cracks, pores and voids (i.e. the imprints of substances that have already been dissolved) in the bone cement. Antibiotic particles lining these ‘internal surfaces’ would dissolve in the medium filling this network and diffuse out of the bone cement. The slower kinetics could be explained by:

a) the time needed for the medium to penetrate the network
b) the slower dissolution of antibiotic due to a higher local concentration in the network
c) the time needed for the antibiotics to diffuse out of the network.

This assumption dictates that, unless the network is so widespread that antibiotic particles inside the bone cement have access to a surface (external or internal), a set proportion of antibiotic remains isolated in the bone cement indefinitely, whilst only the complementary proportion of antibiotic will be released from the bone cement.

An alternative theory is based on the fact that the matrix of PMMA, the basic polymer of bone cement, is permeable to water; much like rock or clay is permeable to rain or groundwater. Upon contact with water the antibiotic will dissolve and diffuse away from the antibiotic particle as dictated by the concentration gradient. Naturally, the amount of water
that accesses such a particle and that allows transport of dissolved molecules is microscopically little but, in this way, water will at some point in time gain access to all the antibiotic particles in the bone cement. Contrary to the network model, in this case eventually all antibiotic would be released from the bone cement.

These two theories are not mutually exclusive and it is not unlikely that both apply. The relative importance of both theories is subject to conjecture. Many reports have discredited use of antibiotic-loaded bone cement because of this long-term release although the mechanism behind the release remained unresolved and to date few attempts have been made to further understand this low release. During the course of this PhD project, an association with the Department of Physics was initiated to provide a theoretical background for the release of antibiotics from bone cement. The experiments described briefly below were performed within this framework.

The penetration of water into a porous substance can be revealed by addition of an indicator to the porous substance. The indicator, once reached by the penetrating water, will dissolve and change colour, indirectly revealing the presence of water. The indicator alizarin Red-S was added to three antibiotic-loaded bone cements (Figures 7-1 A 1 and B 1). Similarly sized samples as used in Chapter 6 were submersed in a buffered solution and examined periodically.

In the first hours, the red colour, revealing penetration of the buffer, was seen intensely only in regions that had contact with the external surface – both through direct association and through the presence of irregularities extending up to 100 µm into the bone cement (Figures 7-1 A 2 and B 2). After the first days, no intense colour changes were observed in deeper layers of the bone cement. Interestingly however, the indicator appeared to bleed out into the surrounding bone cement in the course of a few weeks. For CMW 1 Radiopaque G the whole of the bone cement was coloured evenly (Figure 7-1 A 3), but for Palacos R-G and Palamed G, the bleeding out occurred only in the neo-polymerisate, outside the polymer spheres that were present in the powder (Figure 7-1 B 3). In the first week, the colour change extended on average some 30 µm into the bone cement. Toward six weeks the colour gradient became too vague to allow adequate distinction.
These observations attest to both the first and the second model. The ‘shortcuts’ taken initially, by way of irregularities, could be interpreted as the network of cracks, pores and voids from the first model. The paucity of these shortcuts indicate that such a network has

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Figure 7-1. Schematic presentation of the penetration of an aqueous buffer into antibiotic-loaded bone cements, with colour indicators, with the top surface exposed to buffer.

A 1 through A 3: observations for a CMW bone cement
B 1 through B 3: observations for Palacos or Palamed bone cement
A 2 and B 2 show the situation during early exposure. The indicator is intensely coloured upon contact with the liquid, but has not yet entered the polymer matrix.
A 3 and B 3 show that after weeks the indicator has bled out into the matrix. For Palacos / Palamed (B 3) it is clear that the polymer spheres that were initially present do not take part in this process.

These observations attest to both the first and the second model. The ‘shortcuts’ taken initially, by way of irregularities, could be interpreted as the network of cracks, pores and voids from the first model. The paucity of these shortcuts indicate that such a network has
only very limited interconnectivity and cannot be assumed to be percolating. This was confirmed by mercury intrusion porosimetry, in which the volume of mercury that entered networks with dimensions ranging from 1 to 50 µm – the diameter of the pores observed in electron micrographs of bone cement – was hardly detectable. The ‘bleeding out’ in the indicator experiment could be seen as fluid entering the bone cement matrix and moreover transporting a tracer molecule as hypothesized in the second model. Since the dissolvability and molecular weight of alizarin Red S and gentamicin are comparable, it is likely that the same could hold for gentamicin.

Chapter 5 showed that cyclic loading increases the amount of gentamicin released from Palamed G bone cement initially, but not for CMW 1 G Radiopaque and Palacos R-G. This was not understood in terms of crack formation, as none of these bone cements showed a progressively increasing release upon cyclic loading as would be expected if a non-linear damage accumulation scenario were relevant for antibiotic release. These results can now be interpreted in the light of the synthesis of the two theories above. It would seem that the value of cracks in contributing to antibiotic release is secondary to the value of the transport mechanism that uses the PMMA matrix, particularly after the initial phase. Incidentally, the curves in Figure 5-3 show that the release has not yet come to an end by 3.5 weeks, as corroborated by others.\textsuperscript{3,4} This is also expected in the long run in the second theory of antibiotic release.

A secondary aim of this thesis was to access the proportion of the antibiotic that was isolated inside the bulk of the bone cement by means of ultrasound. In the case of a network of canals filled with a liquid, the pressure waves of low-frequency ultrasound might cause a micro-streaming or thermal effect in these canals that could add to diffusion effects, speeding up antibiotic release. However, the dense matrix of polymer chains would allow far less micro-streaming. The fact that ultrasound was only shown to have an effect in the fresh samples and not in post-elution samples in Chapter 6, is also in line with the synthesis model described above.

Ultrasound could speed up diffusion of the antibiotic in the initial phase when antibiotic release was taking place from surface irregularities (Figure 7-1). However, after three weeks this compartment can be considered to be empty and no further difference is noted due to ultrasound.
On the methodological aspects of this thesis

The title of this thesis contains the phrase ‘under simulated physiological conditions’. The methodology used to simulate two aspects of the physiological environment of bone cement is described in Chapters 2 through 4 for the prosthesis-related interfacial gap model and in Chapter 5 for the cyclic loading model. The lack of prior research on the influence of ultrasound on antibiotic release from bone cement necessitated more fundamental research before applying this new method to more physiological conditions.

There are two discrepancies between the clinical situation of bone cement and the simulated prosthesis-related interfacial gap model. First, the gap in the latter is lined on both sides with antibiotic-loaded bone cement, whereas in the body this is obviously the case on only one side. The width of the gap between bone cement and bone however, ranged from 50 to 500 µm.5 The simulated gaps have a width of 200 µm, so it may be clear that the area over volume ratio of the physiological situation and the simulation are similar. Secondly, in the clinical situation, the antibiotic is likely to diffuse out of the interfacial gap, resulting in a drop of the concentration. A high concentration can be reasonably assumed to persist for the first day or so, in keeping with the elevated antibiotic concentrations in the wound drainage fluids, although it is not known at what rate the antibiotic is drained from the gap.

Chapter 2 showed that the gentamicin concentrations inside the gap could not be reliably measured after one week of submersion. Nevertheless, Chapter 3 indicated that the concentrations inside a gap after 3 weeks of submersion were still sufficiently high to kill some bacterial strains. This could be due to the fact that in Chapter 2 the measurement was taken after only 30 s, whereas in Chapter 3 the gaps were left undisturbed for 24 h, in which time a higher concentration could result from the low release. Chapter 4 indicated that the efficacy of new developments in antibiotic-loaded bone cements could be tested in this gap model. Further comparative animal studies would help showing the validity of the gap model for bone cements loaded with two antibiotics.

Chapter 5 introduced a cyclic loading model of the femoral component of a hip replacement. To our knowledge, this model is the first that allows determination of gentamicin release due to the major stresses in the bone cement mantle. The determination of gentamicin release during loading required that a liquid had access to the bone cement.
Application of torsional loads, which have been considered to be of importance for bone cement mantle failure, would require a much more complicated model. In view of the apparent lack of influence of cyclic loading on the long-term antibiotic release described above, development of such a model would not appear to have priority as a research goal.

The gentamicin release from the bone cement mantles in the cyclic loading model showed considerable variation between individual specimens. This could not be correlated with the weight and dimensions of the bone cement mantles. The extent of debonding of the bone cement mantle from the aluminium support, however, may well have contributed to this variation. In fact, after removal from the support, the amount of red dye clinging to the free surface was quantified by means of counting the proportion of red pixels. This parameter generally correlated well with the total gentamicin release at 600 h. Since this debonding process influences both the area of bone cement exposed and the volume of the gap that is created, focus on the extent of debonding of antibiotic-loaded bone cement from its support due to cyclic loading, but also due to polymeric and thermal shrinkage, would seem to be in order.

The conclusion of Chapter 6 was that the insonation status and the average bath temperature were significant factors in the multiple linear regression model (Table 6-3). It was suggested that a local temperature rise at the sample due to insonation may be responsible for the ultrasound effect and elucidation of this matter could lead to further understanding of this ultrasound effect. This will require a further experiment in which the temperature in a sample can be measured by cementing the sample around a sensitive thermal probe. Monitoring the temperature during insonation would allow further estimation of the part of the increased gentamicin release due to ultrasound that can be ascribed to heating.

**On the clinical significance of this thesis**

The research described in this thesis has been demonstrated to have meaning for the understanding of the fundamental issues regarding antibiotic release from bone cement and for further developing new methods of study. It is also interesting to extrapolate from the conclusions from this work to the clinical situation. This has value in validating the experiments performed for this thesis, but also in better understanding some of the clinical debates.
The first debate is on the additional value of antibiotic-loaded bone cement over other methods of preventing and treating prosthesis-related infections. This has only scarcely been shown in a sound clinical study, although a host of publications based on limited surveys argue in favour of antibiotic-loaded bone cement. The difficulty in executing a proper randomized placebo-controlled clinical trial is that the infection rate is so low, that prohibitively large amounts of patients would need to be studied in order to reach a decisive conclusion. Added to this problem is the incongruity of the results from animal studies that generally show good efficacy of antibiotic-loaded bone cement and in vitro studies that tend to show bacterial survival on antibiotic-loaded bone cement. This has led to a poor understanding of the mechanism of action of antibiotic-loaded bone cement.

Chapter 3 offers an explanation for this discrepancy. The presence of a prosthesis-related interfacial gap can be assumed in the clinical and animal models, but not in the in vitro studies so far. Such a gap allows the build-up of a high local concentration of antibiotic, affecting the viability of bacteria. In addition, it was shown that the antibiotic concentration in a gap rises very quickly, leaving little or no time for bacteria to adopt a more resistant phenotype after adhesion to a surface. In this respect, it should also be borne in mind that the effect of aminoglycoside antibiotics, like gentamicin, relies more on the peak concentration achieved than the time during which a high concentration is maintained and that in this way gentamicin is a good choice of antibiotic to add to bone cement.

The second issue of controversy in clinical orthopaedics is the risk of antibiotic-loaded bone cement. Recently, antimicrobial resistance among bacteria found after use of antibiotic-loaded bone cement infections has been causatively ascribed to antibiotic-loaded bone cements.\textsuperscript{7-9} This conclusion may be premature, considering the lack of details on the fundamental background of the release pattern of antibiotics from bone cement and also on the origin of prosthesis-related infections as shown in Chapter 1. In view of the fact that up to 25% of bacterial strains can be gentamicin-resistant, the finding that only gentamicin-resistant bacteria are able to survive the gap environment offers a good alternative hypothesis explaining the correlation described. This line of reasoning is not a novelty in this thesis, since it has been voiced as early as 1983, although this was apparently based on an intuitive understanding of the presence of very high initial levels of gentamicin adjacent to the bone.\textsuperscript{10}
This hypothesis may further be supported by the fact that the relationship between gentamicin use and resistance has not been reported to be mutational and possibly selected for during therapy, as it has been shown for Enterobacter species and beta-lactam antibiotics.\textsuperscript{11,12} Clinical bacterial isolates of gentamicin-resistant staphylococci commonly show enzymatic modification of aminoglycosides.\textsuperscript{13} Adaptive resistance is also seen, particularly in bacteria that get a chance to downregulate its aminoglycoside uptake after surviving the first exposure.\textsuperscript{14,15} Resistance could theoretically also result from ribosomal mutations, but due to the fact that gentamicin appears to bind to multiple sites high level resistance is rare and cannot be selected by a single mutational step.

This thesis supports the use of antibiotic-loaded bone cement as an effective method in orthopaedic surgery. Nevertheless, the value of antibiotic-loaded bone cement to the clinician would seem to be confined to the first days after implantation. Years later, there may still be an ongoing low-level release of antibiotics that serves no purpose, but could have some side-effects. Therefore, there is still room for the development of a more optimal antibiotic carrier for orthopaedic surgery. Until such a time as this would be available, routine use of antibiotic-loaded bone cement remains a sub-optimal effort.

**General conclusion**

In conclusion, it has become clear that, over thirty years after the first use of antibiotic-loaded bone cement, this can still be considered to be a pragmatic approach. The work described in this thesis proved the possibility of a local build-up of high local antibiotic concentrations. These were found to be effective in killing relevant bacterial strains, provided that they were sensitive to the antibiotic in question. This offered an alternative explanation for the observation of increased resistance of bacteria after use of antibiotic-loaded bone cement, since only resistant strains could survive the initial burst release. Although there was a limited effect of cyclic compression on antibiotic release from one bone cement, this was not a general finding. Particularly, there was no progressive increase in release of antibiotics as was intuitively expected. Ultrasound is related to an increased release of antibiotics from fresh bone cement samples. Unfortunately, no access to the antibiotics isolated in the bone cement could be shown.
References
