Infection of orthopaedic implants is a significant problem, with increased antibiotic resistance of adherent ‘biofilm’ bacteria causing difficulties in treatment. We have investigated the in vitro effect of a pulsed electromagnetic field (PEMF) on the efficacy of antibiotics in the treatment of infection of implants.

Five-day biofilms of *Staphylococcus epidermidis* were grown on the tips of stainless-steel pegs. They were exposed for 12 hours to varying concentrations of gentamicin or vancomycin in microtitre trays at 37°C and 5% CO₂. The test group were exposed to a PEMF. The control tray was not exposed to a PEMF. After exposure to antibiotic the pegs were incubated overnight, before standard plating onto blood agar for colony counting.

Exposure to a PEMF increased the effectiveness of gentamicin against the five-day biofilms of *Staphylococcus epidermidis*. In three of five experiments there was reduction of at least 50% in the minimum biofilm inhibitory concentration. In a fourth experiment there was a two-log difference in colony count at 160 mg/l of gentamicin. Analysis of variance (ANOVA) confirmed an effect by a PEMF on the efficacy of gentamicin which was significant at p < 0.05. There was no significant effect with vancomycin.

Infection of implants is a significant problem in both elective and trauma surgery. Although recent series suggest that the rate of infection is only 1% for uncomplicated hip arthroplasty,¹ the costs of infection are high. The cost of revising an infected hip is six to seven times that of a primary operation,² not to mention the social costs to the patient, family and carers. The rates of infection after surgery for fractures vary significantly depending on the patient, the implant used and the type of fracture. Rates for intramedullary nailing of the tibia range from 1.5% to 12.5%.³ Often infection is not diagnosed quickly enough which allows adherence of bacteria to the implant and the development of a biofilm with increased resistance to antibiotics.⁴

The treatment of infection varies depending on the circumstances but diagnosis is often difficult. In elective surgery infection may be early or late, with treatment involving protracted courses of intravenous antibiotics and sometimes staged operative intervention. In surgery for trauma there are often attempts to control the infection by repeated washouts, the administration of high-dose antibiotics and quite commonly, replacement or removal of the implant. Infection of an implant is difficult to eradicate because of the adherent colonies of bacteria in a polysaccharide matrix, collectively called a biofilm. This mode of growth has been implicated in a range of infections of medical devices, its importance in infection of orthopaedic implants being first noted by Gris tina, Costerton and McGanity.⁵ Bacteria within biofilms are resistant to several hundred times the bactericidal concentrations of standard antibiotics.⁶ The exact mechanism of resistance is not fully known, but hypotheses vary from protective effects of the enveloping polysaccharide to phenotypic variation of bacteria.⁷ In simple terms, infecting bacteria can remain dormant on the surface of an implant for a variable length of time. When conditions are right, clinical symptoms can be caused directly or indirectly by the local proliferation of bacteria shed by the biofilm. This may occur acutely, such as after fixation of a fracture, or several months or years after joint replacement when septic loosening occurs.

Many attempts have been made to enhance the eradication of biofilms in the clinical infection of implants. A recent phenomenon has been the ‘bio-electric effect’ first described by Khoury et al,⁸ who showed increased susceptibility of biofilm bacteria to antibiotics in the presence of a very weak electric field of current density 15 µA/cm². Other groups, albeit with differing experimental arrangements, have produced similar results.⁹,¹⁰
The application of this technology to orthopaedics would involve some form of invasive technique. Much work, however, has been carried out over the last 40 years on the electrical stimulation of bone healing. Bassett, Pawluk and Pilla were pioneers in the use of a pulsed electromagnetic field (PEMF) delivered to the site of the fracture by externally placed electromagnetic coils. Calculations showed that it was possible to deliver a weak electric field in the \( \mu \text{A/cm}^2 \) range using very fast pulses of low-frequency electric current through electromagnetic coils. The rate of change of the magnetic field is proportional to the magnitude of the transient electric field. If current is pulsed very quickly through an electromagnetic coil, the magnetic field changes at the same rate and as a consequence the transient electric field generated is maximal.

Unfortunately, although much has been written about bone stimulation by a PEMF, most of the laboratory and clinical research has been criticised because of the weaknesses in the design of the studies with an absence of any clinical, randomised control trials in the literature. It is therefore not possible to dismiss or accept it as a valid technique, and certainly in the UK it has not found general acceptance. One review, however, has mentioned an improved outcome in infected fractures being treated by antibiotics, although no data were given.

We have therefore attempted to investigate the possible use of a PEMF in the generation of an electric field sufficient to enhance antibiotic efficacy in the treatment of biofilm-mediated infection of orthopaedic implants. We now describe our preliminary in vitro work.

Materials and Methods

A 100-turn solenoid of 0.1 m in diameter and 0.5 m in length was made using enamel-coated copper wire of 2 mm diameter around a heat-resistant Tufnol pipe (Tufnol Ltd, Birmingham, UK). This was connected in series to a Picker International gradient drive amplifier (Crown International Inc, Elkhart, USA), a Thurlby Thander TGP 100 pulse generator (Thurlby Thander Instruments, Huntingdon, UK) and a 40 ohm resistor. A standard oscilloscope was connected in parallel. Current pulses of 5A lasting for 380 \( \mu \text{s} \) were supplied to the coil at 72 Hz, one of the standard protocols used by Bassett. A search coil made of 30 turns of 21 gauge copper wire was wound around a 0.5 cm perspex rod and, by connecting to the second input port of the oscilloscope, used to measure the electric field indirectly at any point within the coil. The strength of the electric field at the points of the coil at the experimental zone was calculated to be 0.07 V/m at the maximum rate of change of the magnetic field within the solenoid. The latter was designed to fit inside a mu-metal shield (Magnetic Shields Ltd, Kent, UK) to screen out external sources of electromagnetic field, the effectiveness being confirmed with the search coil when no current was flowing through the coil. Mu-metal is an alloy of Ni (77%), Cr (0-5%), Cu (4.5%) and Fe as balance. It has high permeability at low field strengths, and is used as a shield against stray magnetic fields. A removable PVC drawer at the centre of the coil allowed accurate placement of a microtitre tray at the centre of the coil (see Fig. 2).

Biofilms were grown on the tips of identical, electrochemically polished, surgical-grade stainless-steel (SS316L) pegs suspended in a continuously stirred 300 ml reservoir at 37˚C. It contained tryptone soya broth (TSB) (Oxoid, Basingstoke, UK) inoculated with 3 ml of Staphylococcus epidermidis (F1481) (identified by APIStaph: Biomérieux, Basingstoke, UK) broth culture, isolated from a clinical infection of an implant. This particular Staphylococcus epidermidis was a known polysaccharide intercellular adhesion-positive strain which was sensitive to both gentamicin and vancomycin. Biofilms were grown for five days with the TSB changed continuously at a rate of 300 ml/day (Fig. 1). Growth of the biofilm was confirmed by removing bacteria from three randomly chosen pegs by sonication into 2 ml of sterile distilled water in disposable vials submerged in the water bath of an ultrasonicator.
Colony counts were performed on the bacterial suspension obtained. Environmental scanning electron microscopy (ESEM) was used to confirm the formation of a biofilm in some experiments but not performed routinely.

The minimum inhibitory concentration (MIC) of gentamicin and vancomycin against planktonic culture of *Staphylococcus epidermidis* (F1481) was determined by serial dilution. The biofilms were then exposed to a range of concentrations (e.g., 1x MIC, 16x MIC, etc.) of gentamicin or vancomycin in the TSB for 12 hours in microtitre trays at 37°C with a total CO₂ concentration of 5%. One microtitre tray was placed at the centre of the solenoid with pegs aligned in the outer wells of the tray’s long axis, so that all pegs were exposed to an equal electromagnetic field (Fig. 2). This was confirmed by placing a search coil at all four corners of the microtitre tray. The other microtitre tray was kept in the same incubator, but outside the mu-metal shield, isolated from the electromagnetic field.

After exposure, the pegs were removed. Biofilms continually shed bacteria, and therefore each multiwell contained bacteria shed from the surface of the biofilm. They retained the phenotypic characteristic of the biofilm bacteria rather than immediately reverting to the planktonic phenotype. The concentration of antibiotic required to inhibit their growth was, as expected, higher than that required for planktonic bacteria. The colony count in each of the multiwells was determined by a spread plate method, with greater growth of bacteria in the multiwells related to greater resistance of the biofilm bacteria to antibiotic.

Eleven experiments were carried out, with exposure to antibiotic performed in quadruplicate. In three of these the coils were switched off to check that there was no effect on the efficacy of gentamicin by being cultured inside the metal shield. Five experiments were performed with gentamicin and exposure to a PEMF, and three with vancomycin and exposure to a PEMF.

**Statistical analysis.** This was carried out by analysis of variance (ANOVA). Data were grouped with analysis performed on the effect of a PEMF on the efficacy of gentamicin, its effect on the efficacy of vancomycin and the effect of gentamicin inside or outside the mu-metal magnetic shield, but with the PEMF switched off. The colony counts were transformed using log (10), and the antibiotic dose by log (2) so that doses ranged from 0 to 8. The datasets were modelled using experiment as a factor (with five levels), log dose as a variate and power as a factor (with two levels).
Results

The MIC was 0.625 mg/l for gentamicin and 1.25 mg/l for vancomycin. ESEM showed formation of a biofilm at peg incubation of five days (Fig. 3). $10^6$ colony-forming units per ml (CFU/mL) of *Staphylococcus epidermidis* were sonicated from the three pegs randomly selected in each experiment except in one case in which $10^5$ CFU/ml were isolated. CFU/ml is a measure of the number of viable bacteria present in 1 ml of solution. There was no statistically significant effect of being inside or outside the mu-metal shield on the efficacy of antibiotic without a PEMF (Fig. 4) and no antibacterial effect of exposure of the biofilm to a PEMF alone.

Exposure to a PEMF increased the effectiveness of gentamicin against F1481 five-day biofilms in four of the five experiments where the biofilm was present. No statistically significant effect of being inside or outside the mu-metal shield was observed for gentamicin without a PEMF exposure. Analysis of variance of grouped data did not show any significant effect on the efficacy of gentamicin.

Fig. 4
Diagram of three experiments (a to c) showing CFU/ml of *Staphylococcus epidermidis* F1481 measured by plating out individual wells which contained biofilm on the tip of stainless-steel pegs exposed to gentamicin, as a shaded square (see legend). The four columns in each table represent experiments performed in quadruplicate in the same microtitre tray and the rows the concentration of antibiotic expressed as a multiple of the MIC for gentamicin against planktonic *Staphylococcus epidermidis* F1481. The table on the right in each pair represents the microtitre tray incubated inside the mu-metal magnetic shield but without the solenoid switched on. Analysis of variance of grouped data did not show any significant effect on the efficacy of gentamicin.

Fig. 5
Diagram of five experiments (a to e) showing CFU/ml of *Staphylococcus epidermidis* F1481 measured by plating out individual wells which contained biofilm on the tip of stainless-steel pegs exposed to gentamicin, as a shaded square (see legend). The four columns in each table represent experiments performed in quadruplicate in the same microtitre tray and the rows the concentration of antibiotic expressed as a multiple of the MIC for gentamicin against planktonic *Staphylococcus epidermidis* F1481. The table on the right in each pair represents the microtitre tray exposed to a PEMF. Analysis of variance of grouped data showed a significant effect of a PEMF on the efficacy of gentamicin.
experiments carried out (Fig. 5). In experiments (a) and (b) (Fig. 5) there was a reduction of at least 50% in the minimum biofilm inhibitory concentration (MBIC) of gentamicin after exposure to a PEMF. Experiment (c) (Fig. 5) showed a reduction in MBIC of 50% and in experiment (d) (Fig. 5) there was a 1 to 2 log difference in colony count on exposure to 256 times the MIC and a PEMF. The result in experiment (e) (Fig. 5) was unclear and it was difficult to draw a conclusion, although the general impression suggested that the trend was towards an effect of a PEMF. Overall, there was a significant effect of PEMF on gentamicin (p < 0.05). There was no significant effect of a PEMF on biofilms exposed to vancomycin (Fig. 6).

Discussion

There are many definitions of a biofilm. The critical aspect with respect to infection of an orthopaedic implant is the increased resistance of bacteria to the usual therapeutic concentrations of antibiotic. In the above experiments, the Staphylococcus epidermidis biofilms had greatly increased susceptibility to gentamicin. Our method of producing a model for the formation of orthopaedic biofilms is reproducible, with 10^6 CFU/ml per peg in most cases, and reliably produces biofilms of a similar size with randomly selected pegs always showing the same colony count in a particular experiment (data not shown). Not only can individual biofilms be directly visualised and bacterial counts performed, but it is also possible to specify accurately, and change, the biomaterial used in experiments, an important factor in the growth of biofilms. There are, however, limitations with such a model. It is clearly not possible to mimic the unique immune environment which exists around an implant in vivo. Secondly, a conditioning film of proteins derived from blood etc, forms on the surface of implants, which can alter the dynamics of the surface on which a biofilm forms. This can be simulated in vitro by more complex laboratory methods. The object of our experiment, however, was to determine the effect, if any, of a PEMF on the MBIC, not the dynamics of the formation of biofilms.

Naturally, every time a biofilm culture is begun its properties will be different, and this corresponds to the results presented, which show varying MBICs in the different experiments. In all experiments, however, the bacteria shed from the biofilms were resistant to the standard antibiotic doses. There is evidence that biofilm bacteria are not susceptible to certain antibiotics owing to down-regulation of metabolism and phenotypic change. Bacteria which are conserving energy will have a reduced active membrane transport and growth rate, both of which will hinder the activity of a range of antibiotics.

In three experiments we have shown a reduction of 50% or more in the MBIC for gentamicin in the presence of a pulsed electromagnetic field. In the remaining two experiments an effect was clearly present in one case, with only 1 log difference in colony count for the MBIC for biofilms exposed to gentamicin and a PEMF compared with gentamicin alone. The result was equivocal in the final experiment, although if a higher range of concentration of antibiotic had been used, the effect of a PEMF in augmenting the activity of gentamicin may have become apparent.

Of the hypotheses put forward to explain the bio-electric effect was the possibility that penetration of antibiotic through the biofilm and into bacteria was enhanced. There is some evidence in similar biological situations for this. Chang, Cho and Lim showed increased movement of cefotiam across the round window in the ear in the presence of an electric gradient. Berrier et al showed the presence of membrane-potential-sensitive ion channels in Escherichia coli.

Gentamicin, as with other aminoglycoside antibiotics, is a cationic antibiotic which binds reversibly to anionic sites

![Diagram of three experiments (a to c) showing CFU/ml of Staphylococcus epidermidis F1481 measured by plating out individual wells which contained biofilm on the tip of stainless-steel pegs exposed to vancomycin, as a shaded square (see legend). The four columns in each table represent experiments performed in quadruplicate in the same microtitre tray and the rows the concentration of antibiotic expressed as a multiple of the MIC for gentamicin against planktonic Staphylococcus epidermidis F1481. The table on the right in each pair represents the microtitre tray exposed to PEMF. Analysis of variance of grouped data showed no significant effect of a PEMF on the efficacy of vancomycin.](image)
of the bacterial cell membrane dependent on concentration. The second phase of uptake depends on the external concentration of aminoglycoside. Importantly, in the context of our research, the third phase requires bacterial energy from electron transport. An explanation of the effect seen in our experiments is the result of PEMF subtly altering the bacterial charge within the biofilm thereby encouraging a relatively higher accumulation of gentamicin. This in turn could influence the rate of uptake of the antibiotic. It may also be possible that the imparted electrical energy has an effect on the active transport mechanisms in the bacterial cell membrane. No effect was seen with vancomycin, probably because this is not a charged antibiotic and acts by inhibiting the synthesis of bacterial cell walls independently of the membrane-proton motive force.

A reduction of 50% in the MBIC for biofilm bacteria could have a significant impact on the success of treatment in many orthopaedic infections. While we have not yet tested these, there is no reason to believe that isolates of Staphylococcus aureus will not be affected similarly. Lowering the concentration at which an antibiotic inhibits bacterial growth may allow definitive treatment with combinations of drugs.

If shown to be effective in animal studies or well regulated clinical trials, this technology could be used as a non-invasive adjunct to current treatment protocols. PEMF enhancement of antibiotic activity could also be useful in the management of septic loosening. The diagnosis of infection as a cause of loosening is often under-diagnosed. Our study has shown that there was an effect with gentamicin, but not vancomycin. Gentamicin is an antibiotic present in some bone cements and also in antibiotic beads used to treat infection.

Further work is under way to clarify these early results in vitro and using animal studies with different antibiotics and different strains of bacteria. It is essential to establish the scope of this technique and hence its potential clinical value.

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References