Analysis of linezolid and tigecycline as candidates for local prophylaxis via antibiotic-loaded bone cement

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1   <u>Analysis of linezolid and tigecycline as candidates for local</u>

2   <u>prophylaxis via antibiotic-loaded bone cement</u>

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14

15   Running Title: Analysis of linezolid and tigecycline in bone cement

16

17   **Objective**: To assess the use of Gram-positive specific antibiotic linezolid and the broad-

18   spectrum antibiotic tigecycline, for use in local antibiotic delivery via antibiotic-loaded bone

19   cement.

20

21   **Methods**: Linezolid and tigecycline were added to Biomet bone cement at varying

22   concentrations. Antibiotic elution over one week was quantified by HPLC-MS. The effect of

23   wear on elution over 48 h was determined using a modified TE-66 wear tester. Eluted

24   antibiotics were used to determine MIC against a panel of clinically relevant bacteria. Impact

25   strength of antibiotic-loaded samples was determined using a Charpy-type impact testing

26   apparatus. Cytotoxicity of eluted antibiotics against MG-63 cells was evaluated using an

27   MTT assay.

28

29   **Results**: Linezolid and tigecycline eluted from bone cement to clinically relevant levels

30   within 1 hour and retained activity over 1 week. Mechanical wear significantly reduced

31   elution of tigecycline but had little effect on elution of linezolid. Linezolid showed low

32   cytotoxicity towards MG-63 cells with ≤ 300 mg/mL resulting in >50 % cell activity.

33   Cytotoxicity of tigecycline was higher, with an IC_{50} of 5-10 mg/L.

34

35   **Conclusions**: Linezolid and tigecycline retain activity after elution from bone cement. The

36   concentration of tigecycline may need to be carefully controlled due to cytotoxicity. The

37   effect of wear on bone cement may need to be considered if tigecycline is to be used for local

38   delivery. Up to 10% linezolid can be added without affecting the impact strength of the bone

39   cement. These results are promising indications for future investigation of these antibiotics

40   toward use in local antibiotic delivery strategies.

41

42   **<u>Introduction</u>**

43

44   Prosthetic joint infections present a rare but major complication in arthroplastic surgery. The

45   incidence of infection across all arthroplastic procedures has been reported as ranging from 1

46   – 3%.[1-3] Revision surgery to remedy an infected joint prosthesis is associated with increased

47   costs, longer stay in hospital and potential morbidity, compared to revision surgery after

48   aseptic failure.[4-6] The number of arthroplastic procedures and the incidence of infection have

49   increased over the last 10 years, as have the total costs associated with revision surgery.[4,5,7]

50   As the demand for arthroplastic surgery progressively rises, the costs associated with

51   prosthetic joint infection are set to increase greatly. This has led to perioperative antibiotic

52   prophylaxis strategies including the use of antibiotic-loaded bone cement becoming

53   routine.[8,9]

54

55   The management of a prosthetic joint infection involves removal of the infected prosthesis

56   and radical debridement of the surrounding infected tissue. This is followed by either a one-

57   stage revision where a new prosthesis is implanted in a single procedure or a two-stage

58   revision where a temporary spacer is used for several weeks before the new prosthesis is

59   implanted. In both procedures antibiotic therapy is standard practice, commonly combining

60   systemic antibiotic treatment with local delivery using antibiotic-loaded bone cement.

61   Antibiotic-loaded cement is used to cement the prosthesis into place and, in the two-stage

62   revision, is used to form the temporary spacer.[10]

63

64   Antibiotic-resistant organisms such as methicillin-, vancomycin- and multidrug resistant

65   strains are increasingly becoming associated with failure of revision surgery. More than 50%

66   of all prosthetic joint infections are caused by staphylococci such as *Staphylococcus aureus*

67   and *Staphylococcus epidermidis* and it has been estimated that around half of all *S. aureus*-

68   related periprosthetic joint infections are now methicillin resistant.[1,11-13] The ability of these

69   organisms to acquire antibiotic resistance requires the use of new antibiotics to be explored

70   for use in bone cement.

71

72   Here we evaluate linezolid and tigecycline for use in antibiotic-loaded bone cement systems

73   and assess their suitability for this application. There are few studies investigating the

74   inclusion of linezolid in bone cement[14,15] and, to our knowledge, there are no published data

75   on the inclusion of tigecycline in bone cement. Linezolid is a member of the oxazolidinone

76   family of antibiotics and is active against most Gram positive organisms including many

77   drug-resistant strains.[16] Tigecycline is a member of the glycylcycline family of antibiotics

78   and has good activity against both Gram negative and Gram positive organisms.[17]

79

80   **Materials and methods**

81

82   **Bacterial strains and growth conditions**

83

84   All strains were maintained on Mueller-Hinton agar or Mueller-Hinton broth and grown

85   overnight at 37°C. Clinical isolates of *S. aureus, S. epidermidis* and *Escherichia coli* were

86   isolated from infected prostheses at the Northern General Hospital, Sheffield. *S. epidermidis*

87   DSM 3269 was purchased from the Deutsche Sammlung von Mikroorganismen und

88   Zellkulturen (DSMZ, Braunschweig, Germany). The *S. aureus* strain SH1000 was provided

89   by Simon Foster, University of Sheffield.

90

91   **Antimicrobial susceptibility**

92

93   Serial dilutions of antibiotic standard solutions or serial dilutions of buffer from antibiotic

94   elution experiments were prepared in triplicate with fresh Mueller Hinton broth in 96

95   microtitre well plates. Wells were inoculated with each microorganism in triplicate to a final

96   density of 10^5 cfu/mL and incubated overnight at 37˚C. MICs were determined by eye and

97   were defined as the lowest concentration of antibiotic that showed complete inhibition of

98   growth.

99

100 **MG63 cell culture**

101

102 Cells were cultured on Eagles minimal essential medium (EMEM) containing 10 % fetal

103 bovine serum (v/v), 2 mM glutamine and 1 % non-essential amino acids (v/v). Cells were

104 incubated at 37˚C (5 % CO_2) and passaged three times a week.

105

106 **MTT assay**

107

108 MG63 cells were seeded at 2×10^3 cells per well in 100 µL of EMEM containing the

109 appropriate concentration of antibiotic. Cells were incubated at 37˚C (5 % CO_2) for 48 h.

110 After 48 h the medium was removed and fresh medium added. A 12 mM stock solution of

111 MTT was prepared and 10 µL added to each well before incubating at 37˚C (5 % CO_2) for 4

112 h. An SDS-HCl (100 mg/mL, 0.01M HCl) stock solution was prepared and 100 µL added to

113 each well before incubating for a further 4 h. Absorbance was measured at 570 nm and

114 compared to positive control cultures containing no antibiotic.

115

116 **Preparation of bone cement**

117

118 Linezolid, tigecycline and gentamicin-containing bone cement samples were prepared by

119 hand-mixing antibiotic powder (3% or 10% wt/wt) with Biomet Bone Cement R® powder

120 until a homogenous mix was produced. The antibiotic cement powder was then mixed with

121 the appropriate amount of polymethylmethacrylate (PMMA) monomer liquid in a Hi-Vac

122 bone cement mixing bowl (Biomet) as per the manufacturer's instructions. Refobacin Bone

123 Cement R® and Bone cement R (Biomet) were also prepared in a Hi-Vac bone cement

124 mixing bowl (Biomet) as per the manufacturer's instructions. The bone cement was placed

125   into the relevant mould and allowed to cure for 1 hour. Once removed from the mould,

126   antibiotic-loaded cement samples were stored at -20°C for up to 1 week until required in

127   order to preserve antibiotic activity. The storage of bone cement at this temperature was

128   shown to have no appreciable effect on elution of antibiotic (data not shown).

129

130   **Static elution of antibiotic from bone cement samples**

131

132   Antibiotic-loaded bone cement was placed in circular moulds and allowed to cure for 1 h to

133   produce a 31 mm diameter x 7 mm thick disc. The resulting bone cement discs were then

134   placed in 0.1 M ammonium acetate (pH 7.4) solution stirred at 300 rpm in a UV-opaque

135   container and 0.5 mL aliquots of solution taken over 1 week and stored at -20 °C until

136   analysed.

137

138   **Evaluation of the effect of wear on antibiotic elution**

139

140   Evaluation of the effect of wear on the rate of elution of antibiotics from the bone cement was

141   carried out via a procedure based on that described by Dodds et al.,[18] as follows. The

142   antibiotic-loaded bone cement was formed in an annulus-shaped mould and a 2 kg weight

143   placed on top. The resulting annular samples were 40 mm outer diameter, 8 mm inner

144   diameter and 10 mm thick. The sides of the annulus were coated with beeswax to ensure

145   antibiotic could only elute from the outer perimeter. Controlled wear was generated by use of

146   a HVOF-VPD hydroxyapatite (HA) coated 30 mm diameter x 3 mm thick Ti disc which was

147   placed onto the lever arm specimen holder of a TE-66 microabrasive wear tester.[16] The

148   sample was orientated so that the flat 10 mm thick outer perimeter was in contact with the

149   HA-coated counter-face and a 2.5 N force exerted by the counter-face onto the outer

150   perimeter of the wearing cement sample. A container was placed beneath the assembly and

151   filled with 0.1 M ammonium acetate solution (pH 7.4) until the lower portion of the cement

152   sample was submerged. A magnetic stirrer was used to mix the solution in the container at

153   300 rpm and samples were rotated against the HA counter-face at 60 rpm for 51 h. The HA

154   counter-face was repositioned every 10 - 12 h to ensure a sufficiently abrasive counter-face

155   throughout the experiment. An extension shaft was fitted to the TE-66 to allow simultaneous

156   rotation of an unworn control sample at the same speed. This sample was also partially

157   submerged in a separate container filled with 0.1M ammonium acetate solution (pH 7.4). The

158   experiment was placed in a UV-sealed air-tight container and the temperature and humidity

159   constantly measured during the experiment. At regular intervals, 200 µL aliquots of solution

160   were taken and stored at -20°C before analysis.

161

162   **Quantification of antibiotics by LC-MS**

163

164   Detection of linezolid was carried out on a Phenomenex Luna C_{18} reversed phase column

165   (150 mm x 1 mm) attached to a Finnigan LCQ ESI-MS. The isocratic mobile phase was 0.1%

166   aqueous trifluoroacetic acid (TFA)/acetonitrile (77:23) and the flow rate was 0.05 mL/min.

167   Measurement of linezolid concentration was carried out by monitoring the protonated parent

168   ion at m/z 338.2 and comparing the results to a standard curve. Quantification of tigecycline

169   was carried out as described above except the isocratic mobile phase was 0.1% aqueous

170   TFA/methanol (67:33) and monitoring the protonated parent ion at m/z 586.5.

171

172

173 **Impact strength analysis**

174

175 The impact testing was carried out as described by Barker et al.[19] using a Charpy-type impact

176 tester (Hounsfield Plastics impact testing apparatus). Antibiotic-loaded bone cement was

177 moulded into 44.45mm × 7.93mm × 7.93mm bars and notched using the Hounsfield notching

178 machine (notch tip radius 0.25mm). Impact analysis was carried out according to BS ISO

179 179-1:2010 specifications[20] with the exception of the specimen dimensions. For each sample

180 group 5 specimens were made and force applied to the un-notched side.

181

182 **Statistical analysis**

183

184 Statistical comparison of wear and non-wear samples was carried out by unpaired t-test. The

185 statistical analysis of impact testing samples was carried out by one way analysis of variance.

186 All statistical analysis was carried out using Microsoft Excel software

187

188 <u>**Results**</u>

189

190 **Elution of antibiotic from bone cement**

191

192 Elution of antibiotic from bone cement samples containing 3% (wt/wt) linezolid or 3%

193 (wt/wt) tigecycline was monitored over a 1-week period. The concentration of linezolid

194 eluted from the bone cement increased over the 1 week time period of the experiment (Fig 1).

195 A maximum concentration of 12.2 ± 2.9 mg/L of linezolid was reached after 168 h and the

196 initial elution rate of linezolid from bone cement was calculated as 213.4 ± 33.4 µg/hour/g

197 bone cement. The concentration of eluted tigecycline initially increased to a maximum

198 concentration of 0.66 ± 0.35 mg/L after one hour and then decreased to 0.084 ± 0.025 mg/L

199 after 24 h and 0.014 mg/L ± 0.013 after 168 h (Fig 2). The initial elution rate of tigecycline

200 from bone cement was calculated as 32.8 ± 17.2 µg/hour/g bone cement.

201

202 **Effect of wear on elution of bone cement**

203

204 The results from three separate experiments to investigate the effect of wear on elution

205 behaviour of cement containing 3 % (wt/wt) tigecycline are shown in Fig. 3. The samples

206 were collected over a 51 h period and the maximum concentration of eluted antibiotic was

207 reached between 5 h and 12 h. The highest concentration overall was seen in the unworn

208 sample 2 after 12 h with a concentration of 2.1 mg/L compared to 0.1 mg/L in the worn

209 counterpart (Fig 3b). Although there is some variability in the maximum concentrations

210 between the three experiments, in all cases a clear trend can be seen with the elution from

211 unworn samples being significantly higher than the worn bone cement samples (P < 0.05).

212 After 1 hour the elution of tigecycline from unworn samples was $9.4 \pm 2.6 \ \mu g/hour/cm^3$

213 surface and the rate of elution from the worn samples was $2.3 \pm 2.5 \ \mu g/hour/cm^3$ surface.

214

215 The results from three separate experiments to investigate the effect of wear on elution

216 behaviour of cement containing 3 % (wt/wt) linezolid are shown in Fig. 4. The samples were

217 collected over a 51 h period and the maximum concentration of eluted antibiotic was reached

218 between 24 h and 51 h with concentration continuing to increase in all but one sample. The

219 highest concentration overall was seen in the worn sample 2 after 51 h with a concentration

220 of 53.1 mg/L (Fig 4b). No significant difference can be seen in the elution kinetics between

221 the worn and unworn linezolid samples (P = 0.63). After 1 hour the rate of elution from

222   unworn linezolid samples was 232.5 ± 22.4 µg/hour/cm^3 surface and the rate of elution from

223   the worn linezolid samples was 242.4 ± 24.3µg/hour/cm^3 surface. The rates of antibiotic

224   elution from both unworn and worn linezolid samples were > 100-fold higher than that of the

225   worn tigecycline samples and 24.8 and 25.9-fold higher respectively than the unworn

226   tigecycline samples.

227   **Antimicrobial activity of eluted antibiotics**

228   *S. aureus* (SH1000), *S. epidermidis* (DSM 3269) and an *S. epidermidis* strain isolated from an

229   infected prosthesis were used as test organisms to investigate whether the eluted antibiotics

230   retained antimicrobial activity. The MICs of these strains with standard solutions of the

231   antibiotics are shown in Table S1 in the Supplementary material. Concentration of linezolid

232   and tigecycline eluted at various times from antibiotic-loaded cement samples were

233   determined via LC-MS and the MICs of the eluted antibiotics were determined

234   experimentally (Tables 1 and 2). All eluted tigecycline samples showed activity comparable

235   with the standard solution and established breakpoints[21,22] for all organisms tested (Table 1).

236   The linezolid samples eluted up to 72 h all showed activity comparable to determined MICs

237   and breakpoints against the Gram positive organisms.[21] The linezolid samples eluted over 1

238   week (168 h) showed higher MICs compared to the other samples and the Gram negative *E.*

239   *coli* was not inhibited by any of the linezolid samples, as expected (Table 2).

240   **Cytotoxicity of antibiotics towards MG63 cells**

241   The cytotoxic effects of standard solutions of linezolid and tigecycline against MG63 cells

242   were determined using the MTT assay. The addition of increasing concentrations of

243   tigecycline resulted in a marked reduction in cell activity with an IC$_{50}$ between 5 – 10 mg/L.

244   The addition of linezolid showed a small reduction in activity that was not statistically

245   significant (P > 0.05). Up to 300 mg/L of linezolid resulted in < 50% reduction in cell activity

246   and so an IC_{50} for linezolid could not be determined (Supplementary material Fig S1).

247   Comparing these results to the concentrations achieved in the elution experiments (Figures 1-

248   4), it is possible that cellular toxicity of tigecycline may be an issue if the *in vivo* eluted

249   concentrations are comparable to those in this laboratory system, whereas linezolid did not

250   show toxicity to mammalian cells, even at substantially higher concentrations than those

251   achieved in the elution experiments.

252   **Impact testing to assess physical strength of bone cements samples**

253   A Charpy type impact test machine was used to evaluate the impact strength of the antibiotic

254   loaded bone cement. Separate bone cement samples loaded either with tigecycline or

255   linezolid at 3 % and 10 % wt/wt were tested, and the results compared to both bone cement

256   without antibiotic and a commercially prepared gentamicin-loaded bone cement, Refobacin[®]

257   Bone Cement R (Table 3). There was no significant difference in the impact strength of the

258   tigecycline-loaded cement samples at either concentration, compared to the control without

259   antibiotic. The 10% (wt/wt) tigecycline-loaded cement was the only cement that had an

260   impact strength that appeared slightly lower than the bone cement without antibiotic,

261   however that difference was not statistically significant. Further, there was no significant

262   difference between the linezolid-loaded samples at either concentration and the Refobacin[®]

263   Bone cement R samples (P > 0.05). The impact strength of both the 3% and 10% (wt/wt)

264   tigecycline cement samples were significantly less (P < 0.05) than, though still comparable

265   to, the commercially available Refobacin[®] Bone Cement R.

266

267

Discussion

The results presented here indicate that tigecycline and linezolid can be included within bone cement and that the elevated temperatures that occur during the curing stage do not compromise their antimicrobial and biocompatibility properties. Both antibiotics elute to clinically relevant concentrations within the first hour in our laboratory elution system (Fig 1 and 2) and retain antimicrobial activity up to one week later. The concentrations of eluted tigecycline peaked around 1 h (Fig 2) and then declined, presumably due to decomposition of the antibiotic. The MICs for eluted tigecycline based upon the concentrations measured by LC-MS showed results comparable with those determined using standard antibiotic solutions (Table 1; Supplementary material Table S1). The MICs of eluted linezolid, the concentration of which increased progressively throughout the experiment (Fig 1), were comparable with those determined using standard antibiotic solutions over the first 72 h. After 1 week, eluted linezolid showed approximately 5-20-fold higher MICs than the standard linezolid (Table 2; Supplementary material Table S1) , which may indicate slow decomposition of the eluted antibiotic that was not revealed by LC-MS. Previously, Anagnostakos *et al.* reported elution of 1% of total linezolid from bone cement, compared to 3% for gentamicin loaded cement over 8 days and Jackson *et al.* reported up to 3% elution over a 4 week period.[14,15] Cement containing linezolid and gentamicin has shown inhibited growth of methicillin-resistant *S.aureus* for up to 8 days.[14] However as this previous study is in conjunction with gentamicin it does not necessarily confirm the activity of the linezolid on its own.

The effect of wear on the tigecycline-loaded bone cement samples significantly reduces the elution of tigecycline. After 1 hour there was > 4-fold reduction in the elution rate from the worn sample, compared to the unworn control (Fig 3). Conversely, wear has very little effect

293   on the elution of linezolid from the bone with similar elution rates and profiles for both worn

294   and unworn samples (Fig 4). This may be relevant in the clinical application of these systems

295   where the cement surface experiences wear. Previously we have reported similarly

296   contrasting results with gentamicin and daptomycin–loaded bone cements where elution of

297   gentamicin was significantly reduced by wear, yet elution of daptomycin was not affected.[16]

298   In this study it was suggested that crystal size and distribution were the two main factors

299   influencing this difference in elution characteristics between the two antibiotics. It was

300   observed that the larger crystals of gentamicin within the orthopaedic cement created voids

301   on the surface upon contact with the aqueous solution, thus allowing greater deformation of

302   the bone cement surface due to wear. It was further proposed that this deformation prevented

303   the solution from penetrating deep into the bone cement, thereby limiting the amount of

304   antibiotic that can be eluted. In the current study we have shown that the crystals of

305   tigecycline are smaller than the linezolid crystals and so crystal size appears not to be the

306   main factor determining the reduced elution from worn bone cement samples here

307   (Supplementary material Fig S1). However there is a much greater tendency for the

308   tigecycline crystals to aggregate within the cement compared to the linezolid. The surface of

309   the tigecycline loaded cement showed areas of aggregated tigecycline crystals, which may

310   also produce voids upon contact with the aqueous solution and so increase the deformation of

311   the bone cement surface (Supplementary material Fig S2, S3).

312

313   The impact strength of the linezolid and tigecycline loaded cements produced results

314   comparable to those commercially available bone cements. The lowest impact strength was

315   seen in the 10% tigecycline containing cement suggesting that tigecycline may have some

316   effect on the mechanical strength of the cement. A previous study by Kries et al showed the

317   addition of tigecycline had a detrimental effect on compressive and bending strength of

318   tigecycline-loaded bone cement.[23] Kries et al. also mentioned a 3.8-fold increase in curing

319   time compared to cement only. Curing time was not specifically investigated during the

320   current study, but all cement samples were fully cured within < 1 h.

321

322   The MTT assay showed that linezolid had low cytotoxicity towards MG63 cells. Up to 300

323   mg/L linezolid concentration resulted in <50% loss of cell activity and so an IC_{50} was not

324   determined. Tigecycline showed greater cytotoxicity with an IC_{50} of 5 - 10 mg/L. This result

325   is consistent with the findings of Pina et al.,[24] who also found that tigecycline concentrations

326   >10 mg/L severely affected the cell growth of osteoblastic cells.

327

328   **<u>Conclusions</u>**

329

330   The antimicrobial activity of linezolid and tigecycline eluted from within bone cement,

331   reaches therapeutically relevant concentrations within the critical perioperative period (based

332   on a typical arthroplasty operation of 1-2 h). Antimicrobial activity is observed up to 1 week

333   later. However, the concentration of tigecycline added to cement may need to be controlled

334   due to the possible cytotoxicity of the eluted antibiotic towards osteoblast cells. The effect of

335   wear in reducing elution of tigecycline in the laboratory reported here is also a factor to be

336   borne in mind if this antibiotic is used in revision surgery. Owing to ongoing antibiotic

337   resistance problems, there is a need to use antibiotics such as linezolid and tigecycline both

338   alone and in conjunction with other antibiotics (such as gentamicin which is included in

339   commercial bone cement preparations currently widely used in arthroplasty surgery). The

340   current study is an *in vitro* assessment of the performance and do not model the conditions *in*

341   *vivo*. Upon implantation the prosthetic comes into contact with extracellular fluid, bone and

342   muscle tissue, all of which will affect elution and the local accumulation of antibiotic. Further

343   work assessing the *in vivo* performance of these cements as well as more mechanical testing

344   needs to be carried out to fully evaluate these antibiotic loaded cements. However, based on

345   the results presented above we propose that linezolid and tigecycline are encouraging

346   candidates for local delivery via antibiotic loaded bone cement, in the treatment and

347   prevention of prosthetic joint infection.

348

355

356   **Transparency Declarations**

357   None.

358

359

360   **<u>References</u>**

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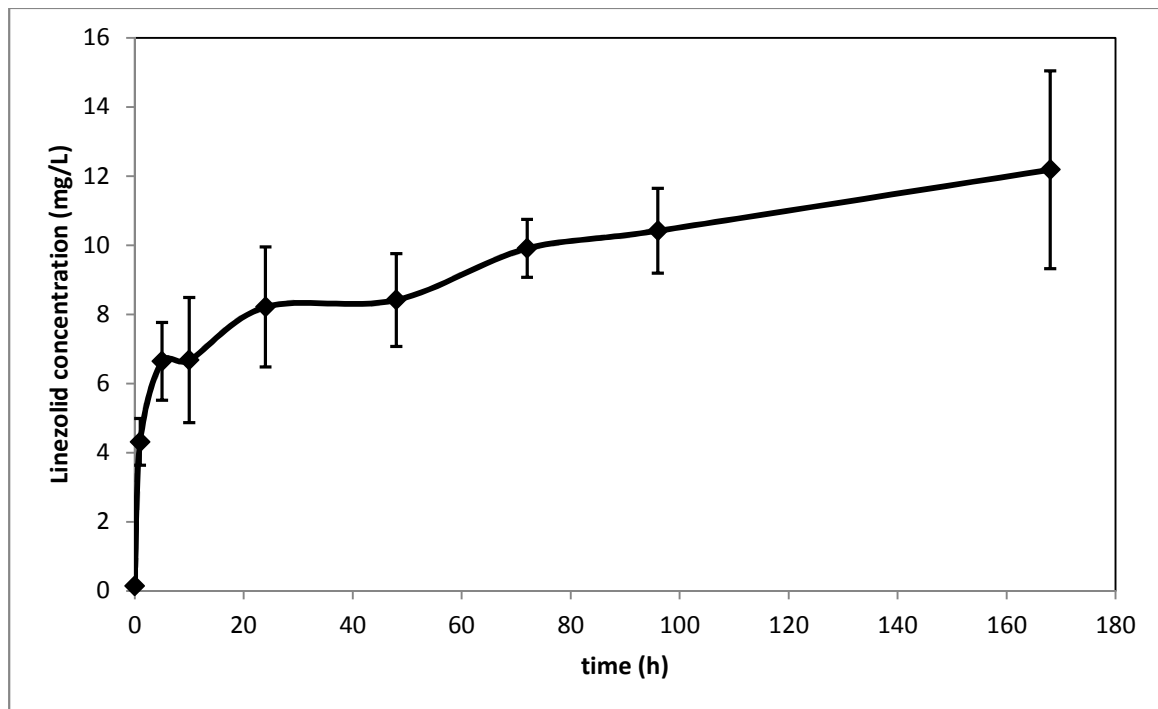
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420 **Figures and tables**

Fig 1: Concentration of linezolid eluted from bone cement over a 1-week period. Results are shown as the mean of three separate experiments ± standard deviation and have been normalised to 1 g bone cement in 5 mL of buffer.
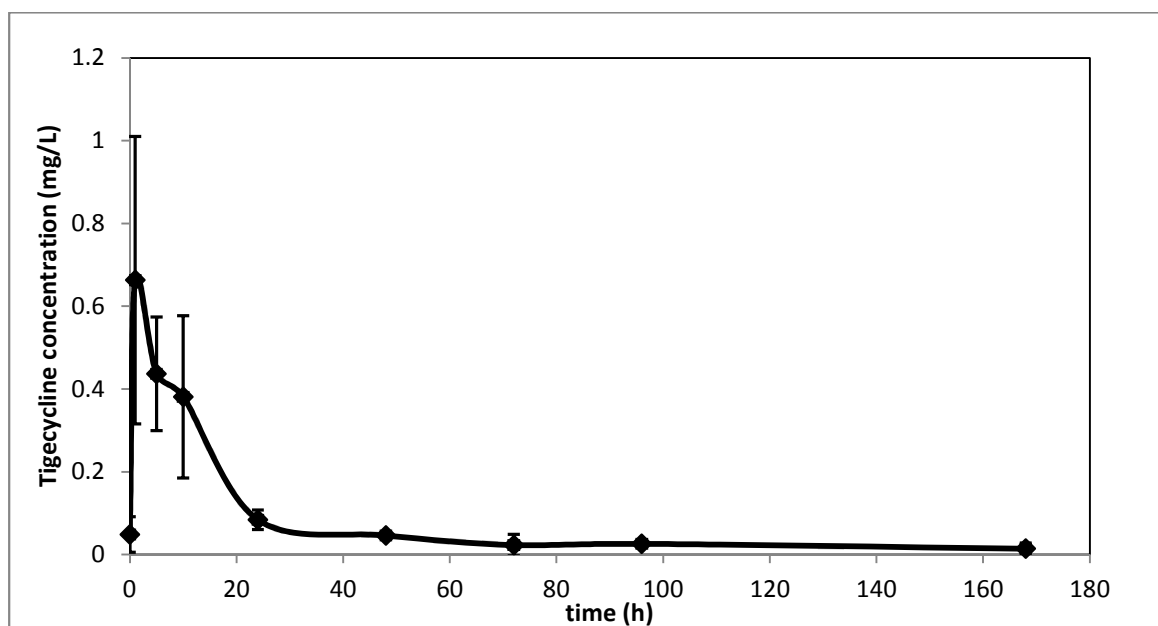

Fig 2: Concentration of tigecycline eluted from bone cement over a 1 week period. Results are shown as the mean of three separate experiments ± standard deviation and have been normalised to 1 g bone cement in 5 mL of buffer.
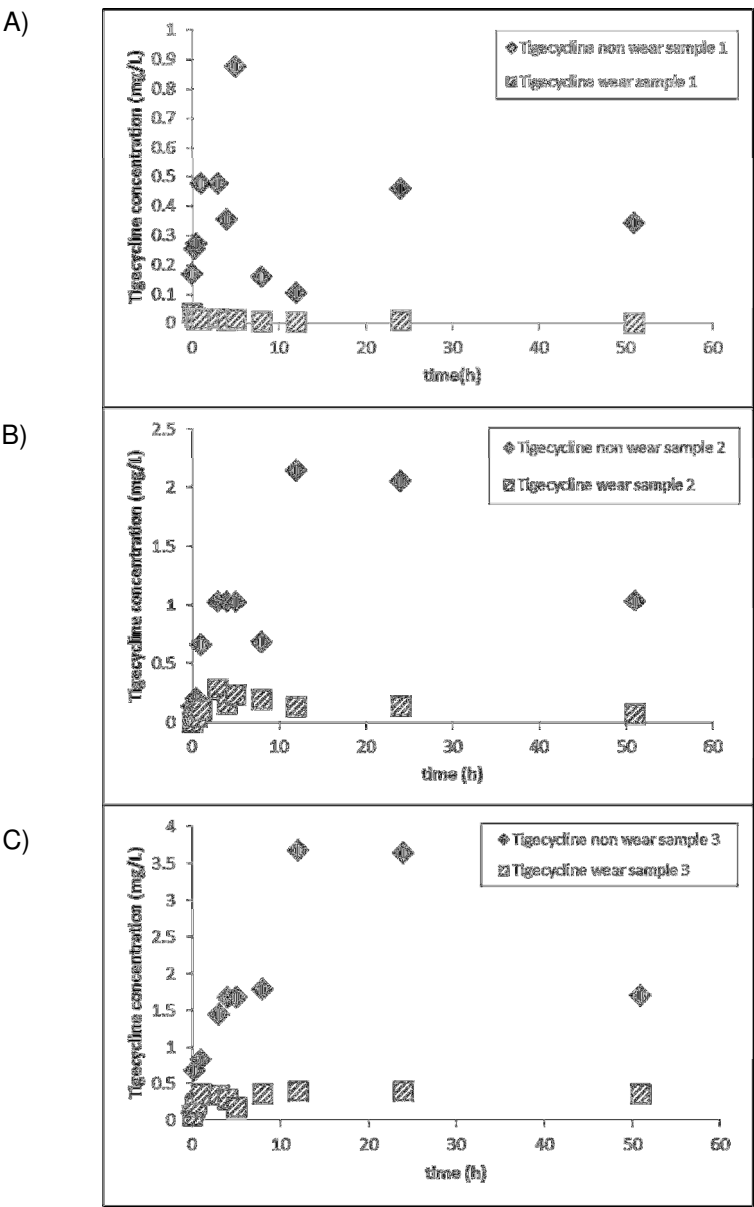
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A)

B)

C)

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Fig 3) Results from three separate experiments (A, B and C) comparing elution of tigecycline

429 from worn and unworn tigecycline-loaded bone cement. Concentration of antibiotic was
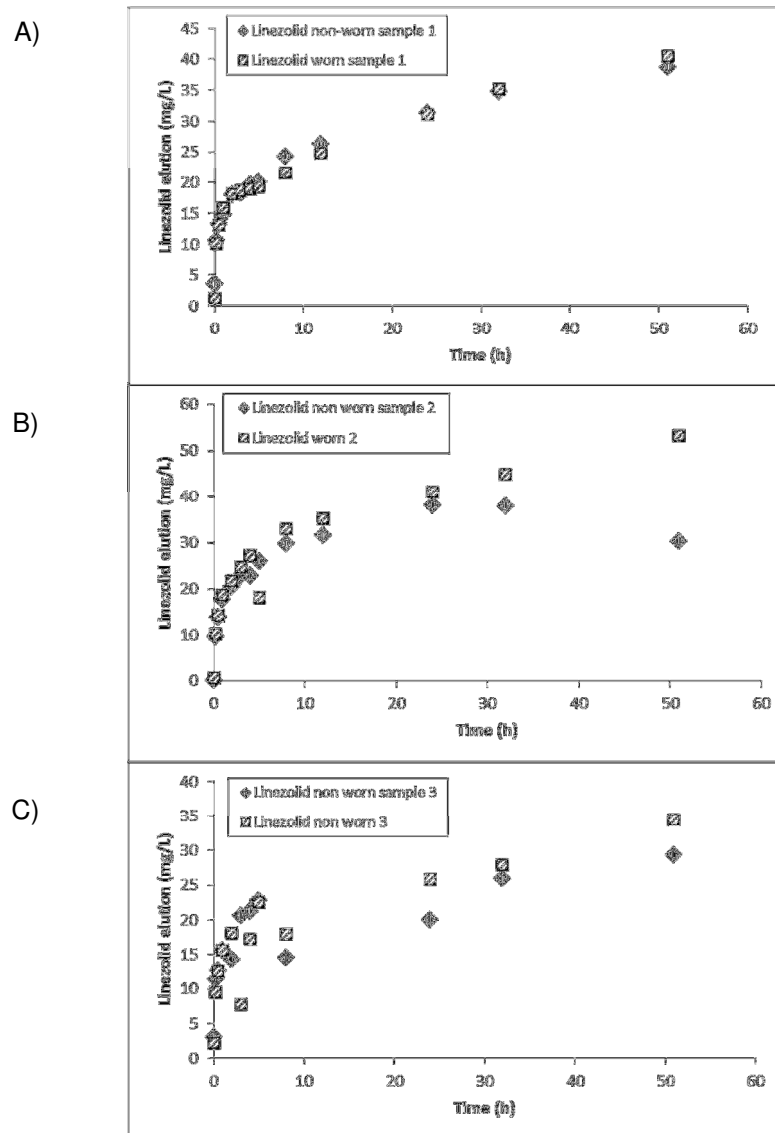
quantified by LCMS.

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433   Fig 4) Results from three separate experiments (A, B and C) comparing elution of linezolid

434   from worn and unworn linezolid-loaded bone cement. Concentration of antibiotic was

       quantified by LCMS.

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Organism	1 h eluate (mg/L)	24 h eluate (mg/L)	48 h eluate (mg/L)	72 h eluate (mg/L)	168 h eluate (mg/L)
S.aureus SH1000	0.2	0.1	0.059	0.088	0.044
Methicillin-resistant *S.aureus (clinical isolate)*	<0.10	0.056	0.059	0.088	0.044
S.epidermidis (clinical isolate)	0.41	0.225	0.12	0.18	>0.18
S.epidermidis (DSM 3269)	0.41	0.28	0.12	0.088	0.052
E.coli (clinical isolate)	0.41	0.7	0.24	0.35	>0.18

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439 Table 1:MICs of tigecycline eluted from bone cement, determined by the broth microdilution method.
440 Experiments were carried out in triplicate.
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Organism	1h eluate (mg/L)	24 h eluate (mg/L)	48 h eluate (mg/L)	72 h eluate (mg/L)	168 h eluate (mg/L)
S.aureus SH1000	1.9	0.89	0.93	1.06	9.75
Methicillin-resistant *S.aureus (clinical isolate)*	1.9	0.89	0.93	1.06	9.75
S.epidermidis (clinical isolate)	0.95	0.89	0.93/1.88	0.53	9.75
S.epidermidis (DSM 3269)	0.95	0.89	0.93	0.53	9.75
E.coli (clinical isolate)	>15.27	>28.50	>30.00	>34.00	>9.75

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452 Table 2: MICs of linezolid eluted from bone cement, determined by the broth microdilution method.

453 Experiments were carried out in triplicate..

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Bone cement	1. Impact strength $(kJ.m^2)$
Cement only	0.259 ± 0.0444
3% tigecycline	0.2649 ± 0.0299
10% tigecycline	0.2271 ± 0.0217
3% linezolid	0.3175 ± 0.0422
10% linezolid	0.3187 ± 0.0493
3% gentamicin	0.3205 ± 0.05
10% gentamicin	0.3673 ± 0.0133
Refobacin® Bone Cement R (1.25 % gentamicin)	0.3343 ± 0.0212

456   Table 3: Impact strength of antibiotic loaded bone cements determined using a Charpy-type testing apparatus.

457   Results are shown as a mean of five separate experiments ± standard deviation. Biomet Bone Cement® was used

458   for all preparations unless stated otherwise.

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