

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

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2 prophylaxis via antibiotic-loaded bone cement

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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 **Introduction**

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 glycylycycline 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 10⁵ 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 % CO₂) 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 μ L of EMEM containing the
109 appropriate concentration of antibiotic. Cells were incubated at 37°C (5 % CO₂) 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 μ L added to each well before incubating at 37°C (5 % CO₂) for 4
112 h. An SDS-HCl (100 mg/mL, 0.01M HCl) stock solution was prepared and 100 μ L 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 μ L 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 C₁₈ 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 specifications²⁰ 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 **Results**

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 $0.014 \text{ mg/L} \pm 0.013$ after 168 h (Fig 2). The initial elution rate of tigecycline
200 from bone cement was calculated as 32.8 ± 17.2 $\mu\text{g}/\text{hour}/\text{g}$ 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 ± 2.6 $\mu\text{g}/\text{hour}/\text{cm}^3$
213 surface and the rate of elution from the worn samples was 2.3 ± 2.5 $\mu\text{g}/\text{hour}/\text{cm}^3$ 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 $232.5 \pm 22.4 \mu\text{g}/\text{hour}/\text{cm}^3$ surface and the rate of elution from
223 the worn linezolid samples was $242.4 \pm 24.3\mu\text{g}/\text{hour}/\text{cm}^3$ 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 IC₅₀ 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

268 **Discussion**

269

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

289

290 The effect of wear on the tigecycline-loaded bone cement samples significantly reduces the
291 elution of tigecycline. After 1 hour there was > 4-fold reduction in the elution rate from the
292 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 tigeacycline-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 IC₅₀ was not
324 determined. Tigecycline showed greater cytotoxicity with an IC₅₀ of 5 - 10 mg/L. This result
325 is consistent with the findings of Pina et al.,²⁴ who also found that tigeacycline concentrations
326 >10 mg/L severely affected the cell growth of osteoblastic cells.

327

328 **Conclusions**

329

330 The antimicrobial activity of linezolid and tigeacycline 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 tigeacycline 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 tigeacycline 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 tigeacycline 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

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351

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355

356 **Transparency Declarations**

357 None.

358

359

360 **References**

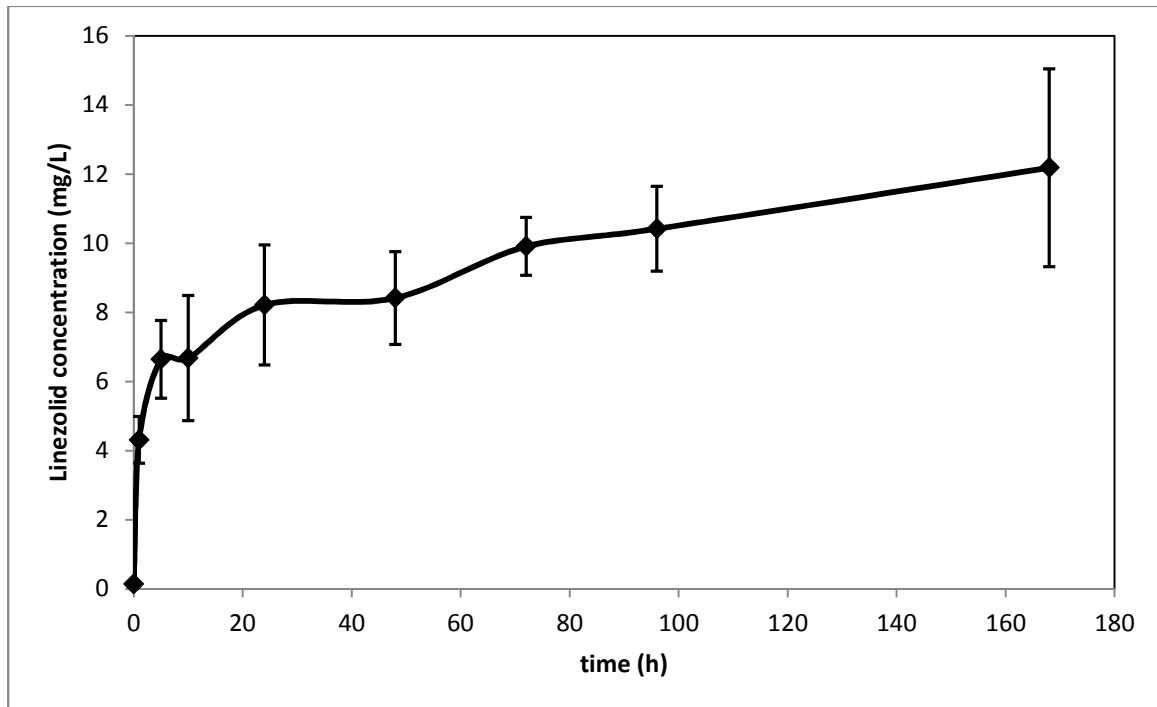
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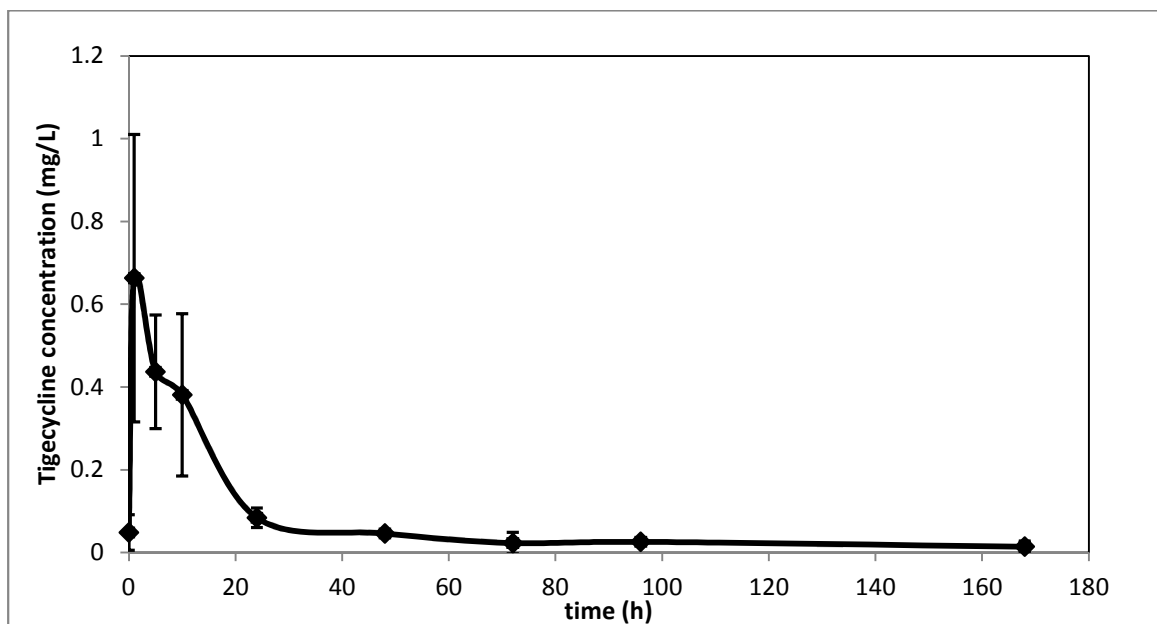


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Fig 1: Concentration of linezolid eluted from bone cement over a 1-week period. Results are shown as the mean of three separate experiments \pm standard deviation and have been normalised to 1 g bone cement in 5 mL of buffer.

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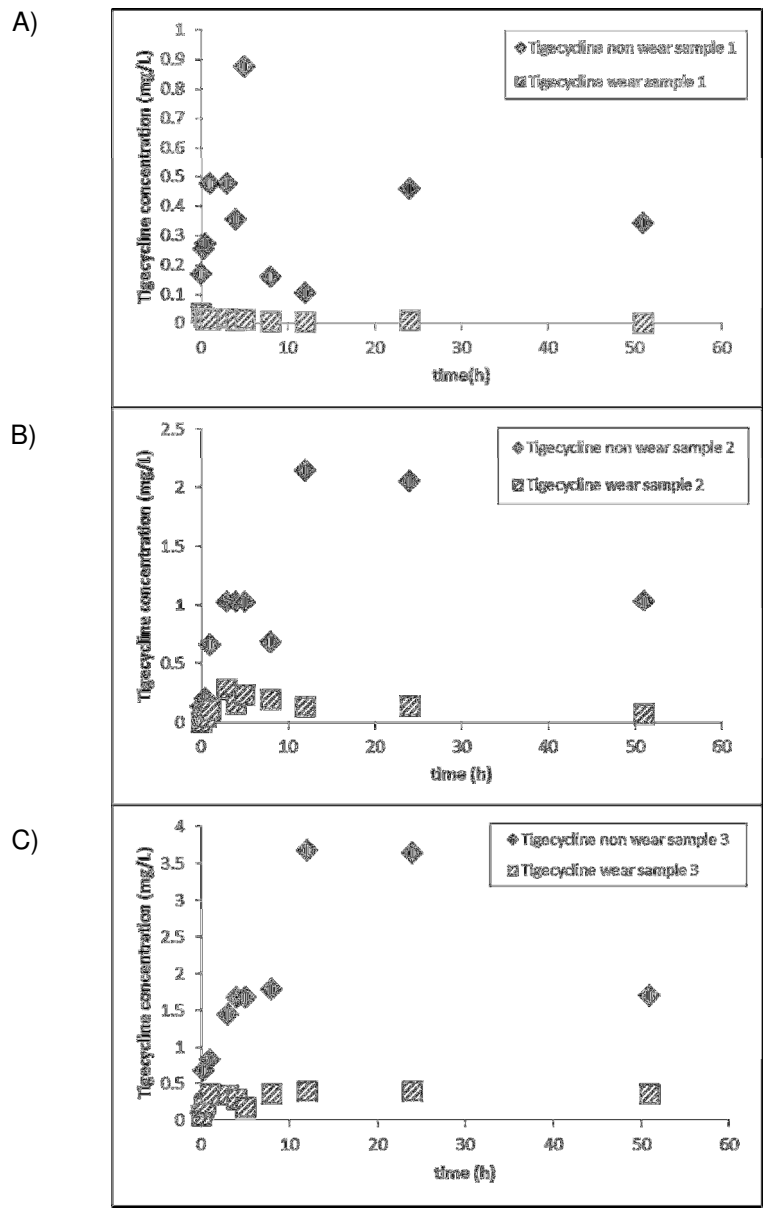
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Fig 2: Concentration of tigecycline eluted from bone cement over a 1 week period. Results are shown as the mean of three separate experiments \pm standard deviation and have been normalised to 1 g bone cement in 5 mL of buffer.

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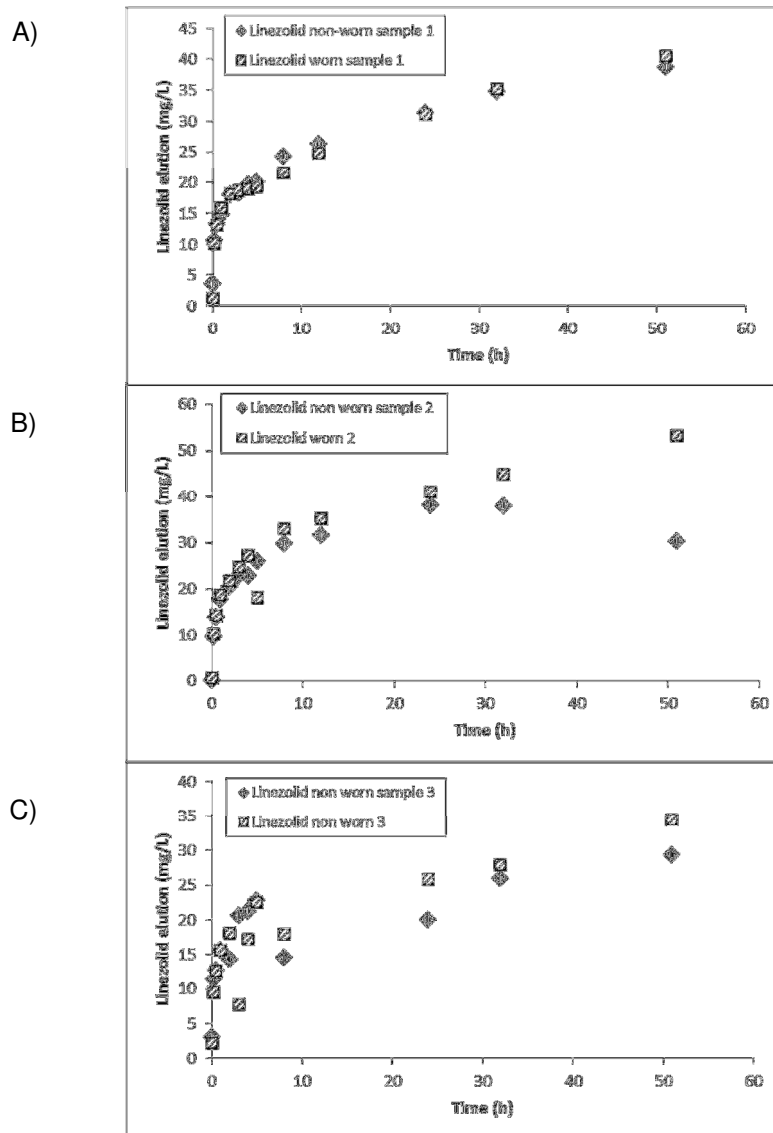
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Fig 3) Results from three separate experiments (A, B and C) comparing elution of tigecycline from worn and unworn tigecycline-loaded bone cement. Concentration of antibiotic was quantified by LCMS.

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

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

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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)
<i>S.aureus</i> SH1000	0.2	0.1	0.059	0.088	0.044
Methicillin-resistant <i>S.aureus</i> (clinical isolate)	<0.10	0.056	0.059	0.088	0.044
<i>S.epidermidis</i> (clinical isolate)	0.41	0.225	0.12	0.18	>0.18
<i>S.epidermidis</i> (DSM 3269)	0.41	0.28	0.12	0.088	0.052
<i>E.coli</i> (clinical isolate)	0.41	0.7	0.24	0.35	>0.18

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Table 1: MICs of tigecycline eluted from bone cement, determined by the broth microdilution method.

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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)
<i>S.aureus</i> SH1000	1.9	0.89	0.93	1.06	9.75
Methicillin-resistant <i>S.aureus</i> (clinical isolate)	1.9	0.89	0.93	1.06	9.75
<i>S.epidermidis</i> (clinical isolate)	0.95	0.89	0.93/1.88	0.53	9.75
<i>S.epidermidis</i> (DSM 3269)	0.95	0.89	0.93	0.53	9.75
<i>E.coli</i> (clinical isolate)	>15.27	>28.50	>30.00	>34.00	>9.75

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

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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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