Tobramycin and Gentamycin Elution Analysis between Two in Situ Polymerizable Orthopedic Composites

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Abstract: This research analyzed Tobramycin and Gentamycin elution characteristics for two antibiotic-impregnated bone composites: PMMA-based Simplex P® and the novel, hybrid, bioactive, CORTOSS™. Experimental results were correlated with composite hydrophilicity and antibiotic phase partitioning behaviors. The phase partitioning experiment was conducted to understand antibiotic solubility in aqueous environments. By comparing experimental results with calculated data, antibiotic release behavior was predicted. Total Tobramycin elution percentages from CORTOSS and Simplex P were 12.5 and 6.4%, respectively. Total Gentamycin elution percentages from CORTOSS and Simplex P were 6.95 and 10.17%, respectively. Phase partitioning data indicate 100% of Tobramycin remains in aqueous phases, being extremely hydrophilic. This is supported by its calculated theoretical value ($\log P = -7.32$). Results suggest that Tobramycin elution can be attributed to composite hydrophilicity as well as its high degree of hydrophilicity. Fifteen percent of Gentamycin distributes in hydrophobic phases ($\log P = -4.22$). Despite a lower Gentamycin hydrophilicity, its release was affected by its complexation with polar salts in the leaching buffer, thereby increasing its elution potential, making it appreciably water soluble. CORTOSS is more hydrophilic; therefore the migration of aqueous liquids into the polymer network of CORTOSS facilitates greater antibiotic elution compared with hydrophobic Simplex P. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 65B: 137–149, 2003

Keywords: bone cement; tobramycin; gentamycin; antibiotic elution; fluorescence

INTRODUCTION

Polymethylmethacrylate (PMMA) -based polymerizable bone cements have found wide use in orthopedic prosthetic implant surgery. These cements are also used in several dental procedures. However, during and after implant fixation, there is the risk for osteomyelitis, deep sepsis, and other bacterial infections. Osteomyelitis was traditionally treated by systemic administration of antibiotics and continuous irrigation of the lesion. Bone grafts were performed secondarily to treat any bone defects. These treatments, however, are associated with major invasion of pathogens and also have effects on other organs.[1] The incorporation of antibiotics especially into PMMA composites during the last two decades has become an accepted clinical practice and has had the advantage of preventing and treating orthopedic infections that result from implantation. Almost 90% of all orthopedic surgeons in the U.S. use antibiotic-impregnated composites as well as for temporary beads and spacers during revision surgery. The focal application of antibiotics in surgical procedures offers an additional therapeutic approach to the treatment of infections and the possibility of minimizing systemic use of antibiotics.[2–5] Antibiotic-impregnated bone cements facilitate the localized delivery of the drug where it is most crucially required from the cement–bone interface to prevent infections. Over an extended time period, the antibiotic is released at high concentrations to the bone and/or tissue area to prevent or treat an existing infection while encouraging new bone formation. This highly localized delivery of the antibiotics is advantageous and more effective than traditional systemic routes where antibiotics are vulnerable to metabolism and devascularization of bone after a surgical procedure. In addition, high local levels of antibiotics would require high serum concentrations, which may also introduce risk for systemic toxicity.[6–8]

Antibiotics in bone-void fillers must be able to elute in therapeutic concentrations and possess excellent aqueous solubility. They must also possess a wide spectrum of antimicrobial activity against a vast array of clinically relevant pathogens. More importantly, they must be stable at the temperatures reached during the polymerization reaction of

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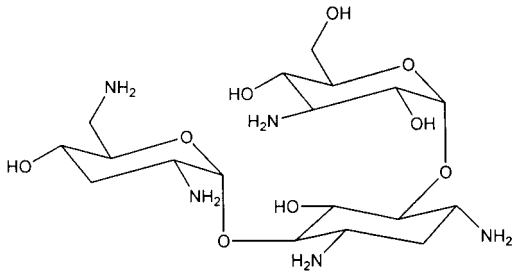
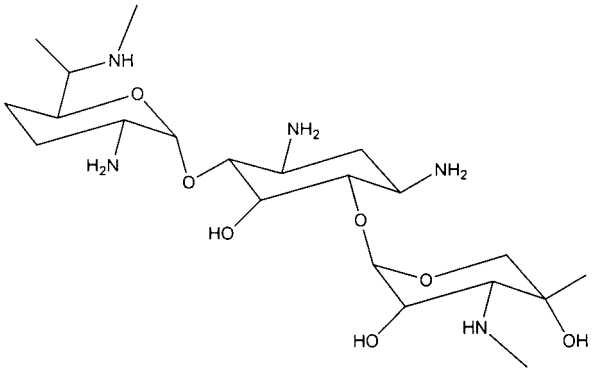


4-Amino-2-[4,6-diamino-3-(3-amino-6-aminomethyl-5-hydroxy-tetrahydro-pyran-2-yloxy)-2-hydroxy-cycloh
exyloxy]-6-hydroxymethyl-tetrahydro-pyran-3,5-diol



2-{4,6-Diamino-3-[3-amino-6-(1-methylamino-ethyl)-tetrahydro-pyran-2-yloxy]-2-hydroxy-cyclohexyl
oxy}
-5-methyl-4-methylamino-tetrahydro-pyran-3,5-diol

Figure 1. Chemical structures of Tobramycin and Gentamycin.

the composite. Aminoglycosides are a diverse class of antibiotics that possess these attributes. Two of the most common aminoglycoside-type antibiotics, Tobramycin and Gentamycin, are the most widely used in orthopedic applications. These aminoglycoside antibiotics have also been shown to possess superior elution properties in Palacos R® and CMW® orthopedic composites when compared to other nonaminoglycosidic antibiotics such as the peptide-based Vancomycin.[9–11] Tobramycin and Gentamycin have been shown to be effective against Gram-positive and Gram-negative aerobic organisms, anaerobes, and mycobacteria. Tobramycin has superior activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and Gentamycin is most active against *E. coli*, *Proteus*, and *Citrobacter* species. Some studies even suggest that Tobramycin is less nephrotoxic than Gentamycin. Tobramycin and Gentamycin bind irreversibly to one of two aminoglycoside receptor sites on the 30S ribosomal subunit, which, in turn, inhibits bacterial protein synthesis.[12] Tobramycin possesses five primary amine functionalities ($R\text{-}NH_2$), and Gentamycin possesses three primary and two secondary amine functionalities ($R_2\text{-}NH$). The chemical structures and chemical designations of Tobramycin and Gentamycin are illustrated in Figure 1. The melting-decomposition temperatures for Tobramycin and Gentamycin (287.2 °C and 218–237 °C, respectively) have also been reported and are sufficiently high in preventing degradation during composite polymerization.[13] These antibiotics can be loaded evenly and thoroughly into bone cements typically by a simple hand-mixing procedure that surgeons employ. Both antibiotics are adequately polar, which should indicate a good leaching potential from orthopedic composites into an aqueous media.

Antibiotic release from PMMA composites is determined by the extent of polar dissolution fluids beyond the surface and into the pores of the composite.[14,15] The extent of fluid penetration also depends on the porosity and the wettability of the composites.[6] Porosity of the composite depends on air entrapment during the wetting and mixing of the cement powder during transfer to the cement gun and on the effects of monomer boiling upon curing.[16] Wettability reflects the surface roughness and the degree of hydrophilicity that a composite possesses. The greater hydrophilicity a composite possesses, the more likely body fluid contact will occur. Release of antibiotics can then be viewed as a combined effect resulting from composite porosity, surface characteristics, hydrophilicity, and antibiotic solubility.[6] However, much debate has arisen concerning the poorly understood antibiotic release mechanisms.[17] Another ramification of greater porosity is that it points to the potential for a decrease in mechanical properties because of the increase in stress and

crack initiation sites due to the presences of pores. It has been suggested, however, that slight wear, in the form of fissures, air pockets, surface roughness, etc. in the cement are needed to permit antibiotic release.[18] Efforts can be made to substantially reduce the number and size of pores during preparation and curing of composites while upholding favorable mechanical and release properties. Conversely, composites that have high mechanical strength as a direct result from a higher degree of curing and cross-linking will essentially have smaller and fewer pores, which may inhibit the release of the antibiotic by making it more difficult for fluids to penetrate the matrix. There are growing concerns on whether body fluids will effectively penetrate antibiotic-loaded bone cements that are prepared by vacuum mixing to reduce void spaces, which is performed on an ever-increasing scale to increase mechanical strength. A combination of these criteria gives rise to specific elution characteristics of the antibiotics.

Despite the widespread use for the positive antimicrobial effects afforded by antibiotic-impregnated composites, there are some negative consequences that compromise their clinical efficacy. Because the primary function of bone cement is to be load bearing, impregnating composites with excess amounts of antibiotics will significantly affect degree of polymerization, and, in turn, decrease its mechanical strength. It has been shown that compressive strength is compromised with increased loading amounts of antibiotics.[19] Klekamp et al. has demonstrated that Tobramycin altered the compressive strength, which Vancomycin decreased the fatigue life of PMMA composites.[11] Therefore, minimal amounts of antibiotics are loaded in an effort to uphold favorable mechanical properties afforded by generally low antibiotic release amounts at subinhibitory concentrations over extended time periods.[17]

Numerous analyses of Tobramycin- and Gentamycin-impregnated PMMA composites and other types of cements have been reported in the literature. Most of the studies compare the elution characteristics of various antibiotics with the different composites, whereas others are concerned with the treatment of clinical infections. Ragel et al., Weisman and his co-workers, and many others all reported that there is a fast initial release of antibiotics from PMMA-type cements during the first 10 h of soaking, which decreases substantially within the first 24 h, followed by sustained release.[20] In addition, antibiotic levels remained near minimum inhibitory concentration (MIC), inhibiting bacteria growth for 7–10 days.[10,17,19,20] Other authors have reported more profound results, and that antibiotics were detected even long after implantation: Sasaki et al. demonstrated that there was sustained release of Gentamycin in vitro and in vivo from calcium-phosphate cement at effective levels for over 2 months.[1] Kanellakopoulou revealed that a biodegradable quinoline-polyacetate system had antibiotic concentrations 100–1000 times above MIC for methicillin-resistant strains.[21] Another study concluded that Gentamycin is released when cements are fractured during revisions taking place 10 years after implantation.[22] Finally, there are studies under way that are investigating the potential use of fusidic acid in delivery systems.[23] Nevertheless, most reports in the literature generally have similar findings that establish defined characteristics for antibiotic/composite systems and contribute considerable insight pertaining to research in this field.

The objective of this in vitro dynamic analysis is to investigate and compare the local elution of Tobramycin and Gentamycin as a function of time for Simplex P® and from a novel, bioactive orthopedic composite, CORTOSS™. CORTOSS is currently marketed in Europe and may have the potential to serve as a new drug-delivery system. In addition, a phase partitioning analysis was conducted in order to reinforce the notion that antibiotic release is directly correlated to antibiotic and composite hydrophilicity.

EXPERIMENTAL DESIGN

Materials

CORTOSS™ was obtained from Orthovita® (Malvern, PA) and Simplex P was obtained from Stryker-Howmedica-Osteonics, Inc. (East Rutherford, NJ). Tobramycin was purchased from Professional Compounding Centers of America, Inc. (Houston, TX), and Gentamycin was purchased from Medisca Inc. (Plattsburgh, NY). CBQCA© fluorescent dye was ordered from Molecular Probes (Eugene, OR). Phosphate-buffered saline (PBS) was purchased from Fischer Scientific (Pittsburgh, PA), and octanol, which was used in the phase partitioning analysis, was ordered from Aldrich Co. (Milwaukee, WI). All assays were analyzed with the use of a standard spectrofluorometer (FP-750 Jasco, Japan). All test samples were prepared according to the manufacturer's supplied instructions.

METHODS

Methods Validation

Fluorescent intensities increased substantially after the addition of several milligrams of Tobramycin and Gentamycin to the assay, proving that at least a sixfold molar excess of CBQCA [10 millimol/L (mM)] with a fivefold molar excess of potassium cyanide (KCN), a derivatization reagent (10 mM) is sufficient to derivatize all antibiotic present in the leachate assays. Standard solutions (1.15×10^{-6} to 5.9×10^{-7}M) were prepared from a stock solution of 10^{-4} M Tobramycin and Gentamycin in PBS, which generated reliable standard curves (refer to Figure 2). A 2-h incubation period was sufficient for complete derivatization. Blanks from the PBS and CBQCA mixture were evaluated and subtracted from overall intensity readings. The limits of detection (LOD) and limits of quantification (LOQ) values have been established, and are 14.6 and 73.1 parts per billion (ppb), respectively.

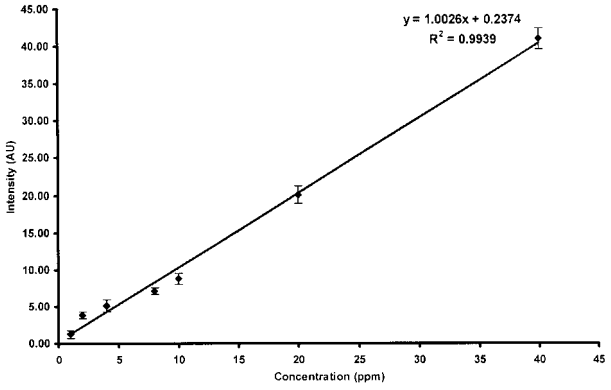


Figure 2. Linear fluorescence standard curve used to determine antibiotic elution concentration (AU; arbitrary units).

Material Mixing and Derivatization

CORTOSS™ is formulated with approximately 30% high-molecular-weight monomers *Bis*-EMA (2,2-*bis*[4-(2-methacryloxy-ethoxy)]phenylpropane), *Bis*-GMA (2,2-*bis*[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane), and the reactive diluent TEGDMA (triethylene glycol dimethacrylate) as its copolymerizing organic components. CORTOSS™ also utilizes a tertiary amine accelerator, DHEPT (dihydroxyethyl-*p*-toluidine), and an initiator, BPO (benzoyl peroxide), to initiate the polymerization reaction. Glass-ceramic fillers comprise approximately 70% of the composite. The density of CORTOSS is approximately 1.90 g/cm^3. Simplex P is one of the most widely used PMMA-based orthopedic materials having an approximate density of 1.10 g/cm^3. A complete summary of the various properties of CORTOSS™ and Simplex P are reported in Table I. The chemical structures of all monomers and other components that comprise the composition of the composites are shown in Figure 3. Because Tobramycin and Gentamycin are the only molecules possessing primary amine functionalities to be found in the leachate bath, separation is unnecessary and instead, derivatized with a fluoroprobe. Fluorometry uses absolute intensity measurements rather than separation/detection. One method proposed for antibiotic detection by Ragel et al. is the *o*-phthaldialdehyde method by UV-VIS spectroscopy (λ_{max} = 331 nm) to form an aminoglycoside-*o*-phthaldialdehyde complex.[20]

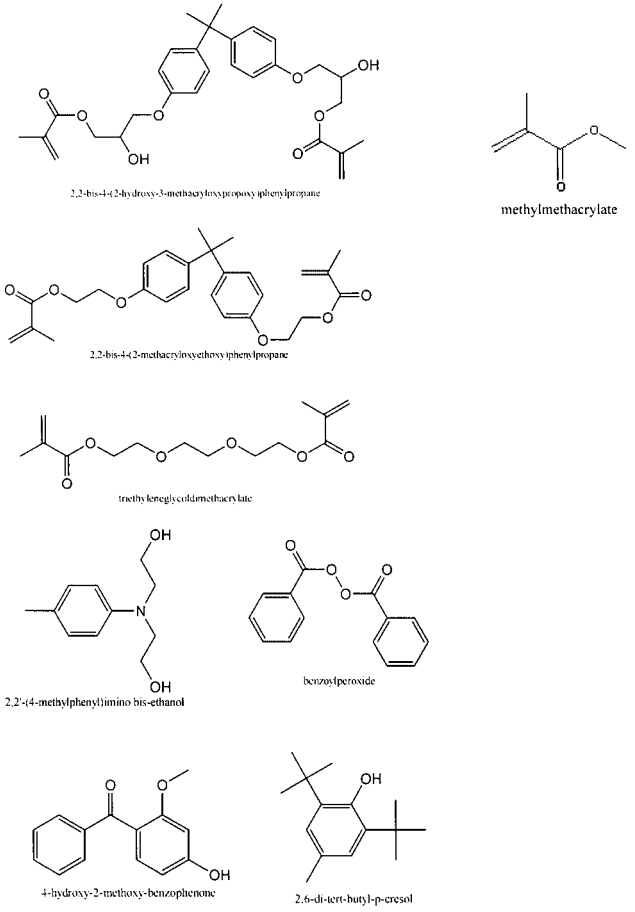


Figure 3. Chemical structures of monomers/compounds comprising CORTOSS and Simplex P.

TABLE I. Relevant Properties and Data Summary of CORTOSS, Simplex P, and PMMA Literature

	CORTOSS	Simplex P	PMMA Literature
Polymer Type	Thermoset	Thermoplastic	Thermoplastic
Polymer Structure	Cross linking	Linear	Linear
Polymer Miscibility	Hydrophillic	Hydrophobic	Hydrophobic
Composition (Monomer)	Bis-GMA, Bis-EMA, TEGDMA (bifunctional)	MMA (monofunctional)	MMA (monofunctional)
Initiator	BPO/DHEPT	BPO/DMEPT	BPO/DMEPT
Filler	68% Combeite/BBAS/SiO_2	10% $BaSO_4$	10% $BaSO_4$
Leachate	DHEPT/TEGDMA	N/A	DMEPT/MMA
Porosity	4.5 ± 2.5%	9.4 ± 1.5%	4–15%
Bioactivity	Apatite layer	None	Various
Water Sorption	4.5%	N/A	3.8%
Set-Time	4.77 ± 0.32 min	10.53 ± 0.42 min	N/A
Polymerization Exotherm	54.4 ± 5.1°C	93.1 ± 1.0°C	N/A
Density	1.90 g/cm^3	1.10 g/cm^3	N/A

(Abbreviations: MMA: Methylmethacrylate, DMEPT: Dimethoxyethyl-p-toluidine, BBAS: Bario-boro-alumina Silicate Glass, and $BaSO_4$; Barium Sulfate)

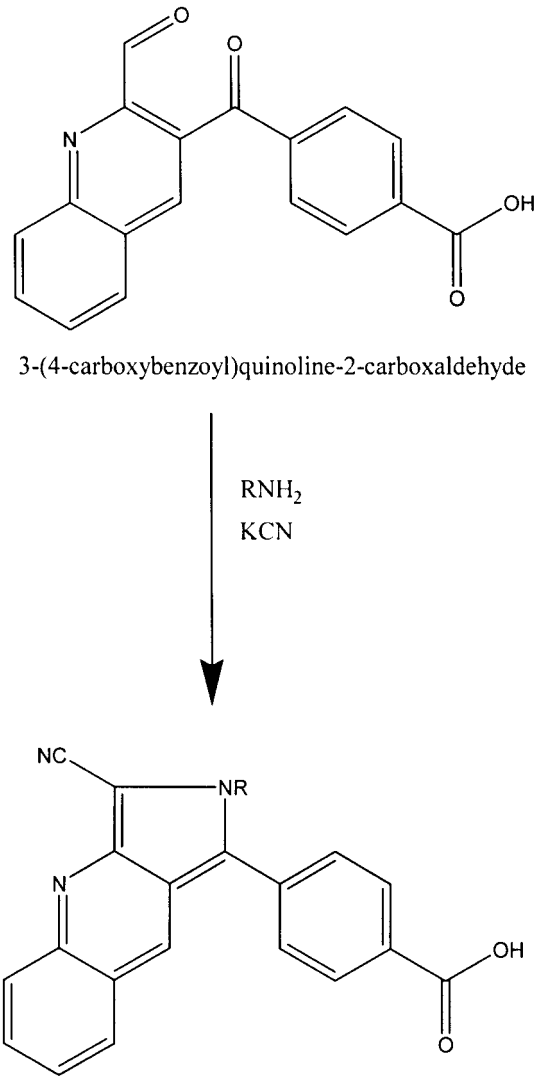


Figure 4. Chemical structure of CBQCA including reaction scheme with aminoglycoside antibiotics.

However, most studies in the literature chose various fluorometric techniques due to their high sensitivity and specificity. The fluoroprobe used in this study is CBQCA (3-(4- carboxybenzoyl)quinoline-2-carboxyaldehyde), which reacts exclusively with the primary amine functionalities present in aminoglycosidic antibiotics.[24] The reaction scheme of the derivatization between CBQCA and aminoglycoside antibiotics is illustrated in Figure 4. The CBQCA-antibiotic conjugate excites and emits at 460 and 550 nm, respectively, which is evident in the peaks of the fluorometric spectra contained in Figure 5.

Both CORTOSS™ and Simplex P composites were impregnated with either Tobramcyin or Gentamycin via a vigorous mixing technique, which consisted of approximately 40 g of composite impregnated with approximately 1 g [2.5 weight/weight percent (w/w%)] of the antibiotics. Five cubic centimeters (cc) of the impregnated composites (≈5.5–8.5 g) were then loaded into a syringe and extruded into 45 mL of PBS at pH 7.0 that was previously heated to 37 °C in a light-impervious container. 25 μL samples of the eluent were extracted daily for derivatization and analysis, with the PBS refreshed daily. All leachates were stored in a dark oven programmed at 37 °C. At the same time, 25 μL of a 10^{-4} M Tobramycin and Gentamycin standard solutions were prepared on the same day that the composites were impregnated to generate the standard curves. 50 μL of CBQCA and 100-μl KCN solution were then added to the samples and standard solution to yield a total reaction volume of 175 μl. These solutions were incubated for 2 h in dark conditions with the use of a magnetic spin vane in a microvial set atop a magnetic stirrer plate to allow for complete derivatization of the antibiotics. The derivatized reaction mixtures were then diluted with 2 mL of PBS to cease any further derivatization and to bring the concentration within the range of medium to low-intensity sensitivity on the fluorometer. This step was also required to fill the standard spectrometer cuvette (max. volume of 4 mL) with a volume greater than 2 mL, the volume required for detection. Standard solutions were made with the use of the standard addition method by adding subsequent 0.5-mL amounts of PBS to the cuvette to generate five data points for the standard curve. Depending on the concentration of the antibiotics, eluent samples were further diluted until the antibiotics were within the detectable range of the standard curve. Each of the eluent samples, standards, and blanks were scanned five (n = 5) times. The unknown antibiotic concentrations were determined by comparing fluorescent values to the standards of known concentration. Reported values were given as a mean from the five scans with their standard deviations.

Preparations for Phase Partitioning Evaluation

Preparation for the phase partitioning study employed an equal volume of octanol in which 10^{-4} M of Tobramycin and Gentamycin was dissolved in the aqueous phase (2.0 mL).

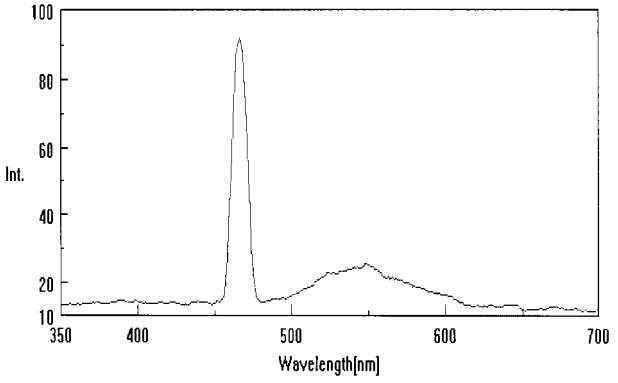


Figure 5. Fluorometric spectra of a derivatized CBQCA-antibiotic conjugate with characteristic excitation and emission peaks (460 and 550 nm, respectively).

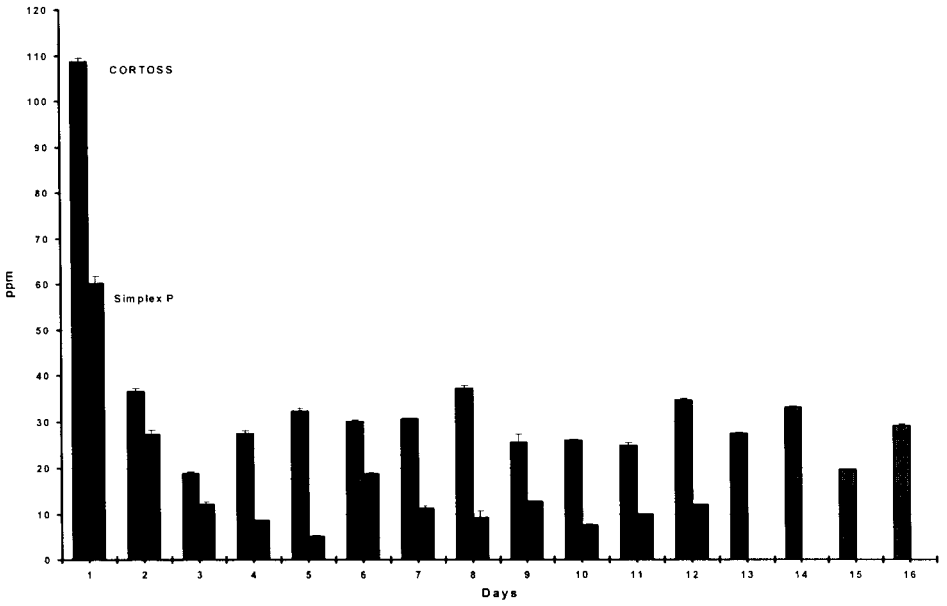


Figure 6. Daily elution profile of Tobramycin from CORTOSS™ and Simplex P® composites, Study 1.

Samples were gently inverted approximately 30 times in order to prevent formation of an inseparable emulsion, and then were allowed to settle for partitioning. The control consisted of only an aqueous phase with equal concentration of the antibiotics. Extraction from both the controls and aqueous/organic phases were derivatized with CBQCA and then evaluated in the Jasco spectrofluorometer ($n = 5$ times) in a similar manner as stated above. The intensities from the partitioned phase were then compared to the control intensities as a ratio in order to determine if any phase partitioning occurred. Log *P* [octanol]/[water] partitioning values for Tobramycin and Gentamycin were calculated with the use of Chem Draw© software package in order to compare the experimental results to the calculated values. The algorithm used to determine log *P* is a method based on 120 atomic contributions evaluated from 893 molecules by least-square analysis, with the use of a standard deviation of 0.50 log *P* units, and handling molecules containing hydrogen, oxygen, nitrogen, sulfur, phosphorus, and halogens.[25]

Determination of Composite Porosity

Porosity measurements performed on CORTOSS and Simplex P were conducted on a Faxitron™ X-ray imaging scan-

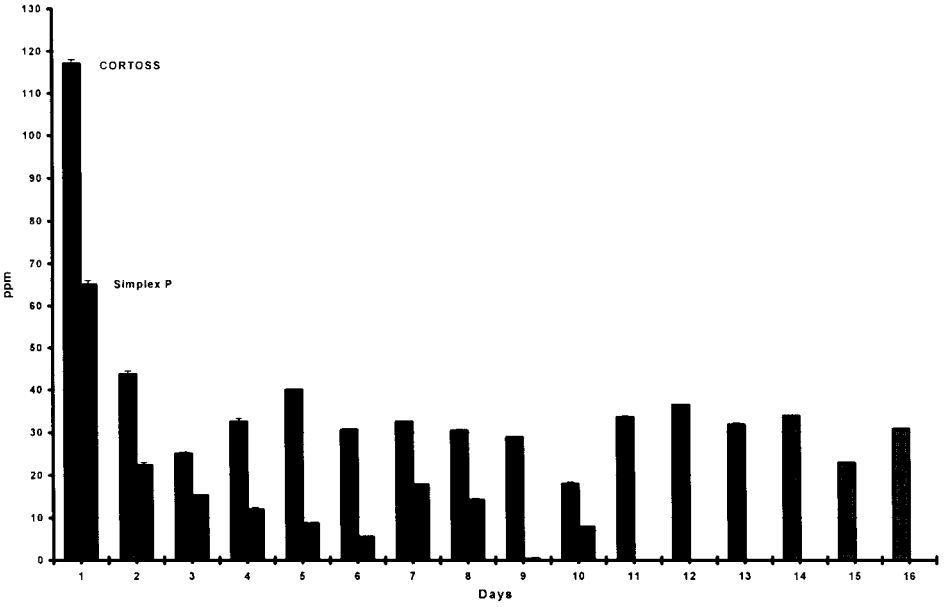


Figure 7. Daily elution profile of Tobramycin from CORTOSS™ and Simplex P® composites, Study 2.

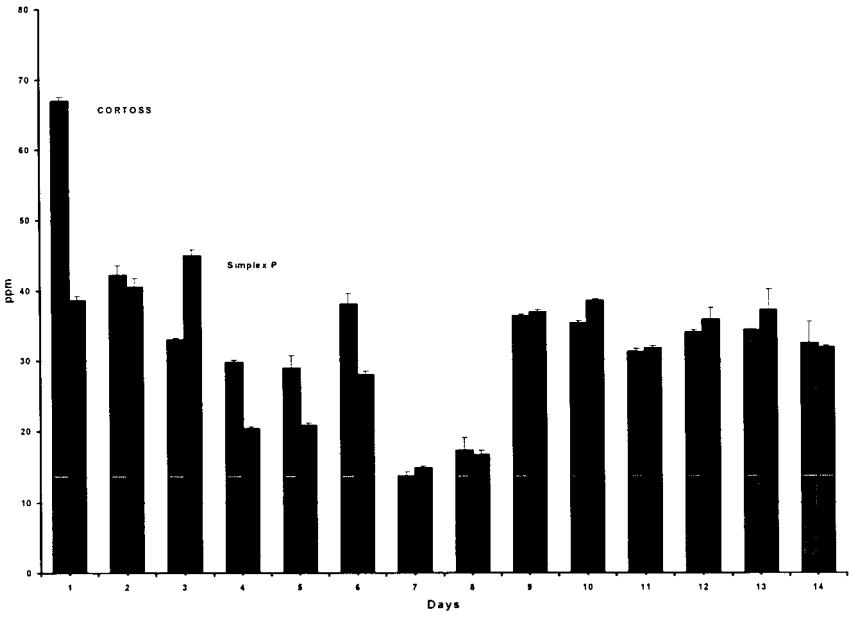


Figure 8. Daily elution profile of Gentamycin from CORTOSS™ and Simplex P®.

ner and were provided as supplement courtesy of Orthovita, Inc.[26] A total of 123 samples (n = 123) of polymerized CORTOSS and 26 samples (n = 26) of Simplex P were evaluated for a direct comparison of porosity. Composites were filled retrograde into a modified 5-cc syringe until material was overfilled. Prior to porosity testing, any samples with visibly noticeable large air bubbles were discarded. The cured cylindrical specimens were removed from the syringes and were serially sectioned in 5-mm-thick slices with a diamond wafer blade. The slices were then X-rayed with adequate exposure time and energy (60 s and 90 kVp, respectively). Air entrapment was measured from the cross-sectional area of each slide. The porosity values are determined from an area of interest, not a volume; therefore, the value is calculated as a fractional area. The film was then developed and the X-rays were digitized.

RESULTS

The daily elution profiles of Tobramycin from CORTOSS™ and Simplex P, illustrated in Figure 6, clearly indicate that Tobramycin elution amounts reach a steady state within the second week after the concentrated initial elution from the composite network in the first few days. Tobramycin elution from CORTOSS™ was not detectable after Day 16, and Tobramycin elution was not detectable from Simplex P after Day 12.

Tobramycin elution was replicated in a congruent second study of CORTOSS™ and Simplex P, illustrated in Figure 7. Tobramycin, again from CORTOSS™, was not detected after Day 16. Tobramycin elution from Simplex P, however, was not detectable after Day 10.

The daily elution profiles of Gentamycin from CORTOSS™ and Simplex P are illustrated in Figure 8. It is apparent that the elution characteristics of Gentamycin follow a less uniform trend compared to theoretical elution behavior. The elution of Gentamycin from CORTOSS™ decreases in the first 7 days whereas Gentamycin elution appeared to slightly increase in the first 3 days from Simplex P.

The cumulative elution profile of loaded Tobramycin from both the CORTOSS™ and Simplex P studies is depicted in Figure 9. A comparison of CORTOSS™ with Simplex P indicates that their elution characteristics are different, and comparisons of data obtained from replicating the initial study indicates that the data are consistent and reproducible. Percent elution amounts were calculated from a ratio of the sum of the concentrations of antibiotics that were eluted daily over the total amount of antibiotic loaded into the composites. The percent elution characteristics of both CORTOSS™ and

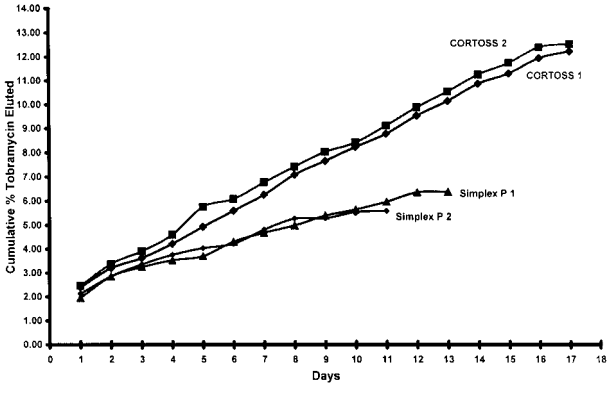


Figure 9. Cumulative elution profiles of Tobramycin from CORTOSS Studies 1 and 2 and Tobramycin from Simplex P® Studies 1 and 2.

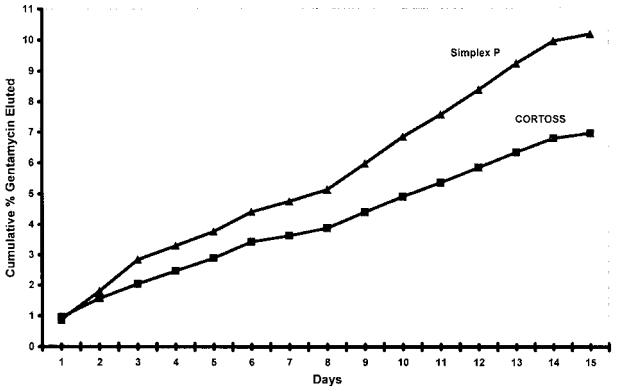


Figure 10. Cumulative elution profiles of Gentamycin from CORTOSS™ and Simplex P®.

Simplex P behave linearly indicating that Tobramycin elution occurs in a slow and steady fashion. Figure 9 also reveals that there is greater overall elution of Tobramycin from CORTOSS™ than from Simplex P in the time period studied. Elution of Tobramycin was not detectable after Day 16 and Days 10–12 for CORTOSS™ and Simplex P, respectively.

The cumulative elution profile as a function of percent elution of loaded Gentamycin from CORTOSS™ and Simplex P is illustrated in Figure 10. These data indicate that Gentamycin elution characteristics differ significantly from that of Tobramycin elution characteristics. For Gentamycin, greater elution occurs from Simplex P as compared to CORTOSS™ in the time period studied. The rate at which Gentamycin elutes from Simplex P is increasing when compared to CORTOSS™. The equilibrations of both CORTOSS™ and Simplex P data points at the end of both antibiotic analyses in Figures 9 and 10 were a result of the sample intensities being approximately equal to background intensities. This behavior indicates that antibiotic elution was not detectable after this time period for both analyses from both composites. After these data points, control blank intensities were approximately equal to sample intensities. Subtraction of blanks from sample intensities resulted in near-zero concentration of antibiotics. Therefore, the flattening out of the curves at the end of the elution periods for both composites point to the fact that elution has vastly diminished and reaches a steady state.

Figures 11(a) and 11(b) illustrate porosity through cross-sectional-area visual representations of slices from both CORTOSS™ and Simplex P. Figure 12 graphically illustrates the mean porosity with standard deviation for total combined CORTOSS™ and Simplex P samples. It is apparent that Simplex P displays an approximately twofold greater air void volume compared to CORTOSS, which is clearly evident from the images. Statistical results summarized from the figures indicate that CORTOSS™ is significantly less porous than Simplex P ($p < 0.01$) (Student's t-test: two-samples, assuming equal variance). The porosity value for CORTOSS™ was 26% less than the Simplex P samples (95% confidence limit). Simplex P data fall within the results of previous reported porosity measurement data for PMMA composites.[27] This data points toward the fact that the CORTOSS™ composite is considerably less porous than PMMA-based composites under the same test conditions.

Table II summarizes the relevant data of the Tobramycin and Gentamycin elution studies with total percent elution, total elution amounts in parts per million (ppm; μg/mL), total amounts of loaded antibiotics that were present in $\approx$5 cc of

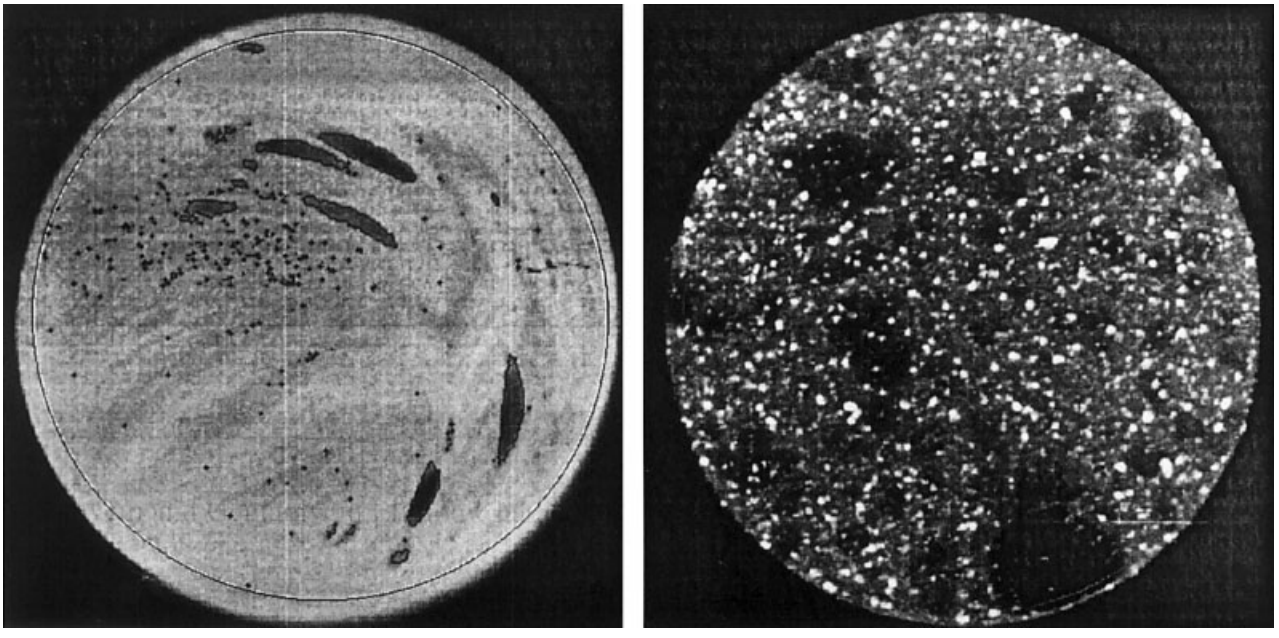


Figure 11. (a) Representative sample of polymerized CORTOSS™ injectable with a porosity measurement of 4.5%, and (b) representative sample of Simplex P® with a porosity measurement of 9.4%.

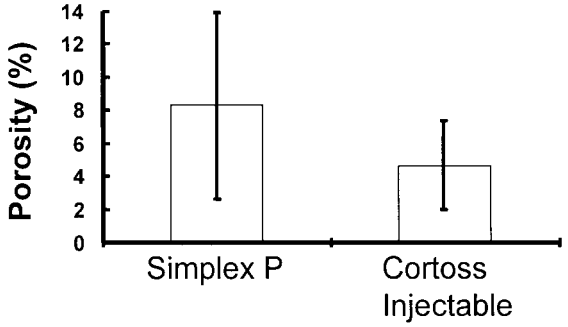


Figure 12. Porosity percentage for CORTOSS™ and Simplex P®.

composites, and the amount of composites that was delivered into PBS. Total elution percents were determined by dividing total amounts of antibiotic in parts per million by the amount of antibiotic loaded in grams converted into parts per million multiplied by 45-mL volume of PBS. Loading amounts, although, were different but represent approximately 2.5 w/w% loaded Tobramycin. Empirically, Gentamycin was loaded at 3.7 w/w%, ensuring that both antibiotics had equivalent activities. The initial Tobramycin analysis yielded a slightly greater elution amount than the replicate study, due to a slightly greater volume of antibiotic-impregnated composite that was delivered into the PBS. The same relationship is observed for the Simplex P analyses. Tobramycin elution concentrations were proportional to the amounts of antibiotics that were impregnated in the composites at delivery. Overall, the initial loading mass of Simplex P is lower, due to the fact that Simplex P is approximately one-third less dense as CORTOSS™, despite being delivered in approximately equal volumes. As a result, a lesser amount of antibiotics were loaded into Simplex P compared to CORTOSS™. Almost equivalent total Gentamycin elution amounts were detected for both composites, but total elution percent is greater in Simplex P due to a lesser Gentamycin delivery amount as a result of the lower density of Simplex P compared to CORTOSS™. Even though elution/loading proportionality factors were not a primary concern of this work, the results of this study support the findings of previous studies described in the literature, which were primarily focused on the relation between elution and initial antibiotic concentrations. The literature indicates that antibiotic release rates increased with increasing antibiotic amount concentration, and that varying the amount of antibiotic in the composite can control release rates.[7,28]

DISCUSSION

Antibiotic release from composites is necessarily a surface area effect. In addition, porosity and surface area wettability[6] also play major roles in the antibiotic release from composites. The amount of dissolution fluid that enters the polymer matrix is governed by how well it contacts the composite surface. Porosity is directly related to surface area, and the degree of porosity will determine the surface roughness of the composites, and, in turn, the amount of body fluid that will contact the surface to facilitate antibiotic release. In other words, greater porosity points to greater surface area, thus allowing for more body fluid to contact the composite enhancing antibiotic release. From this theory, many researchers agree that antibiotic release from composites is essentially a surface chemistry phenomenon.[14,15] For CORTOSS™ and Simplex P, antibiotic release is aided by the penetration of bodily fluids beyond the surface interface and into the pores of the polymer network of these composites. Only antibiotic molecules located in the superficial layers of these composites where the solution can penetrate to dissolve them through the pores of the matrix, will be released. When antibiotics are dissolved, the antibiotic liberation from the composites will cease because the polymeric matrix will cover those antibiotic molecules located inside the matrix and isolate them from the solution, so they cannot be released to the medium.[29] From a materials perspective, it is evident from the porosity data that Simplex P possesses greater porosity when compared to CORTOSS™. The porosity data also suggest that CORTOSS™ has a higher resistance to crack initiation, as it is 70% ceramic-filled terpolymer composite. The implications from these porosity data may yield insight into the antibiotic elution characteristics as well. Neglecting the fact that Simplex P is less hydrophilic than CORTOSS™, it would be anticipated that there would be a free-flow release of antibiotics from Simplex P because of greater porosity. However, porosity is not solely responsible in governing antibiotic release, as in the case of the greater elution amounts

TABLE II. Summary and Data of Complete Elution Studies Conducted with Tobramycin/Gentamycin Loaded into CORTOSS/Simplex P

	Total Antibiotic Elution (%)	Total Antibiotic Elution (ppm)	Loaded Antibiotic (g)	Composite Delivery (g)	Composite Delivery (cc)	Antibiotic: Composite (w/w%)
CORTOSS/Tobramycin 1:	12.19	553.01	0.2041	8.193	4.97	2.49
CORTOSS/Tobramycin 2:	12.49	593.02	0.2137	8.538	5.17	2.50
Simplex P/Tobramycin 1:	6.37	195.60	0.1381	5.490	4.99	2.52
Simplex P/Tobramycin 2:	5.58	170.25	0.1373	5.450	4.95	2.52
CORTOSS/Gentamycin:	6.95	483.74	0.3130	8.493	5.15	3.69
Simplex P/Gentamycin:	10.17	445.15	0.1970	5.318	4.83	3.70

of Gentamycin detected from Simplex P when compared to CORTOSS™. Other factors aid in determining the extent of antibiotic release. In fact, Gentamycin elution from Simplex P exhibits a slight increase during the first 3 days, followed by a slow average decrease during the second week, as opposed to CORTOSS™, where there is an overall decrease in elution concentration after the initial release maximum. A plausible explanation for this behavior is that water penetration is occurring randomly through voids, cracks, and imperfections in the Simplex P matrix, thus allowing the Gentamycin to diffuse randomly. In addition for Gentamycin, release is enhanced by its complexation with five highly polar sulfate salts (SO_4^{2-}), complemented by the greater porosity of Simplex P. At the same time, the incorporation of a salt in order to increase the polarity of Gentamycin may significantly interact with the solution chemistry and potentially cause less uniform elution characteristics, as evident in the elution profile of Figure 8. The salt may simultaneously assist and inhibit the Gentamycin dissolution, competing with various ions for complexation. Another possibility is that the composites may have been extruded unevenly, which in turn produced peculiar porosity and surface area characteristics responsible for the less-uniform elution behavior. This interaction of an antibiotic with similar miscible specie, however, is favored, because the integration of two or more antibiotics into a composite greatly enhances overall elution.[10] Nevertheless, the presence of a salt is responsible for the greater observed elution amounts of the less hydrophilic Gentamycin from a more hydrophobic Simplex P when compared to CORTOSS™. Based on the data, this may be viewed as an overall minimal contribution, because total elution percentage values of Gentamycin from CORTOSS™ and Simplex P are relatively similar (7 and 10%, respectively). Gentamycin release is virtually completed within 13–15 days after loading from both composites. In support of this finding, there exists pharmacokinetic data on Gentamycin and Vancomycin/PMMA systems indicating that Gentamycin is undetectable in the urine after the tenth day of fixation.[30]

Concern arises when impregnating any composite system with significant amounts of materials other than the initial components that confer the inherent structural morphology of a system through polymerization reaction. These materials, such as antibiotics, can be suspect in compromising the mechanical properties of any given composite system. To ensure whether the mechanical strength of CORTOSS™ was affected by antibiotic impregnation, an adjunct EPR analysis was conducted previously on antibiotic-impregnated CORTOSS™ in order to investigate whether the addition of antibiotics altered its polymerization profiles. Results from the EPR analysis indicate that the radical populations are slightly lower for 2.5 w/w% antibiotic-impregnated CORTOSS™ when compared to nonimpregnated CORTOSS™ and 1.0 w/w% antibiotic-impregnated CORTOSS™. Moreover, the incorporation of antibiotics at 1.0 w/w% yielded a small increase in the EPR signal, therefore slightly stabilizing the radicals. The origin of the slight decrease observed in radical populations for 2.5 w/w% antibiotic-impregnated CORTOSS™ is due to free radicals being suppressed in the initiation step of polymerization. Radical populations are indicative of the extent of polymerization occurring as a result of the double-bond conversion rate of the monomers through a radical propagation mechanism. However, this also suggests that the polymerization reaction for antibiotic-impregnated CORTOSS™ goes to completion efficiently in the same time (set time) compared to the nonimpregnated CORTOSS™ samples. The same EPR data from antibiotic-impregnated Simplex P were not obtainable because Simplex P contains pre-polymerized MMA powder, which prohibited acquiring a signal for the propagation mechanism during polymerization. It was also concluded from differential scanning calorimetry (DSC) analysis that the incorporation of antibiotics at 2.5 w/w% slightly lowered the degree of polymerization of CORTOSS™ from 82.1 to 78.5% while not affecting the set time of the composite. The decrease in degree of polymerization reinforces the decrease in radical populations observed from the EPR analysis. A 3.6% decrease in degree of polymerization, however, does not suggest a compromise in the mechanical properties of CORTOSS™, because the degree of polymerization of CORTOSS™ exceeds the typical degree of polymerization values for other PMMA composites, ranging from 55 to 75%.[31,32] DSC analysis of antibiotic-impregnated Simplex P is currently being conducted and thus has not yet been reported.

Tobramycin exhibited greater elution by approximately twofold from CORTOSS™ when compared to Simplex P. This outcome was anticipated, as previous studies demonstrated that moderately polar composites such as Palacos-R® generally exhibited greater antibiotic elution when compared to Simplex P.[10] These results are attributed to the fact that CORTOSS™ possesses a greater degree of hydrophilicity afforded by its polar bifunctional monomers such as *Bis*-EMA, *Bis*-GMA, and TEGDMA, giving CORTOSS™ a high affinity for PBS. Consequentially, water can migrate more easily into the polymer matrix network of CORTOSS™ and solvate the Tobramycin. Therefore, the hydrophilicity of the polymer matrix will indicate the extent of penetration of polar extracellular dissolution fluid that enters the composite and solvates the antibiotic. Conversely, Simplex P is a highly hydrophobic/lipophilic composite because of its prepolymerized monofunctional methacrylates, and thus it is more impervious to antibiotic diffusion. Phase boundaries are more easily established in Simplex P when compared to CORTOSS™. Consequentially, a lesser amount of Tobramycin elution is exhibited, as Simplex P will have a lesser affinity for polar fluids, therefore making it highly unfavorable for PBS to enter into its hydrophobic polymer network. Nevertheless, the complete release of Tobramycin in these studies generally occurred within 12 and 16 days after loading for Simplex P and CORTOSS™, respectively.

The progressive formation of an apatite-like layer on the surface of bioactive CORTOSS™ can also affect antibiotic elution behavior in that it may inhibit the release of the loaded antibiotic in the latter stages of the analysis. The initial concentrated release of Tobramycin and Gentamycin from

TABLE III. Tobramycin and Gentamycin Phase-Partitioning Analyses, Experimental and Theoretical Results

	Tobramycin	Gentamycin
Initial Concentration (M)	0.0001	0.0001
Control Concentration (M)	0.0001	0.0001
Partitioned Intensity (AU)	94.20 ± 1.21	81.96 ± 0.46
Control Intensity (AU)	94.13 ± 1.31	97.23 ± 1.19
Δ Intensity (%)	~0.0	15.71
Concentration Remaining in Aqueous Polar Phase (M)	~0.0001	8.43×10^{-5}
Concentration Partitioned in Non-polar Phase (M)	~0.0	1.57×10^{-5}
% Remaining in Aqueous Polar Phase	~100.0	84.29
% Partitioned in Non-polar Phase	~0.0	15.7
Log P (Partitioning Coefficient)	−7.32	−4.22
K (Equilibrium Constant)	4.79×10^{-8}	6.03×10^{-5}

CORTOSS™ is in agreement with the Ca^{2+}-H_3O^+ ions exchanges between the glass fillers of the composite and the PBS. This exchange is catalyzed by the inherent alkalinity of the inorganic fillers in CORTOSS. The primary release of the antibiotics thus occurs prior to the initial formation of the bioactive apatite layer. Confirmation of the formation of a bioactive apatite layer has been verified previously by utilizing Fourier-transform infrared spectroscopy (FTIR) approximately 7–9 days after soaking CORTOSS™ in PBS. The mechanism for the release of the antibiotics from bioactive composites is not completely diffusion controlled; rather it is enhanced by the ionic exchanges with the surrounding environment.[20]

The elution behavior indicates that Tobramycin and Gentamycin are released reaching a maximum initially during the first 24–36 hours followed by slow, steady elution. The initial maximum may be attributed to the dissolution of antibiotic molecules adsorbed to the cement surface or to the diffusion of antibiotic molecules close to the surface.[29] This elution behavior is independent of composite type and has been reported previously.[6,7,19,20] This behavior suggests that the elution of antibiotics from these composites may follow first-order rate kinetics in which the rate of elution is directly dependent on the concentration of antibiotics present.[33] Elution levels will decrease as a direct result of fewer antibiotic molecules present in the composites. Other prevailing factors such as fluid saturation may play an additional role in the elution characteristics. The continued steady elution of Tobramycin and Gentamycin at or near equivalent levels up to the second week after impregnation demonstrates that specific solution chemistry phenomena may play a role in the observed elution characteristics. A plausible explanation for this is that the concentrations of antibiotics have achieved a state of hydrostatic equilibrium that results from constant refreshing of a fixed amount of PBS at equally spaced intervals in which the rate of antibiotic solvation is equivalent to the rate of antibiotic recrystallization. This occurs as a result of water pressure (PBS) within the composite network directly solvating antibiotic molecules that are embedded in the network and antibiotic molecules reversibly precipitating into the network. It is uncertain at this point whether greater amounts of PBS or more frequent refreshing would increase pressure at the composite/liquid interface and achieve a state of hydrostatic equilibrium in a shorter time, facilitating greater release of the antibiotics. This precipitation/recrystallization effect is expected to be more prominent in a semi-static in vitro analysis where equilibrium is more likely to be reached. However, in a dynamic in vivo analysis where bodily fluids are continuously refreshed and circulating, the process is pushed further in the direction of solvation, making it more unlikely to establish hydrostatic equilibrium. In in vitro analyses, this behavior is predicted to gradually diminish over the next several days, thus facilitating optimal release of the antibiotics. Consequentially, the sample eluent was not further refreshed after Day 18 and the analyses were terminated, suggesting that the elution of the antibiotics approaches zero concentration. Here, at lower detectable levels of antibiotic elution, concentrations approached the limit of quantification.

Experimental evidence from the phase partitioning study reinforces the effect of antibiotic water solubility in which overall results are compiled into Table III. The calculated log *P* values for Tobramycin and Gentamycin were − 7.32 and −4.22, respectively. The greater magnitude of the log *P* and smaller *K* value for Tobramycin compared to Gentamycin reflects its higher degree of hydrophilicity; it is capable of more easily distributing into the aqueous phase. The ratios of intensities for partitioned and control Tobramycin and Gentamycin demonstrate that essentially 100% of Tobramycin present remains in the aqueous phase and a vast percentage (85%) of Gentamycin remains in the aqueous phase. Tobramycin is by far mostly water soluble, and trace amounts of Tobramycin molecules may partition in the octanol nonpolar phase. Hence, Tobramycin is highly hydrophilic, giving it the capability to simultaneously disperse into PBS and the CORTOSS™ network, facilitating its solvation and release. Gentamycin was found to have three orders of magnitude greater possibility of distributing in the organic phase as compared to Tobramycin, where approximately 15% distributes into nonpolar phases. Log *P* calculations for Gentamycin were determined for free-base forms without any complexation.

The initial release maximum observed for both antibiotics from CORTOSS™ and Simplex P suggests that optimal antimicrobial activity is most profound during the early

stages of the elution periods. Antibiotic elution concentrations are above the MIC in the early stages after implantation, thereby providing a high level of local antiseptic effects around the bone-composite surface interface. For these systems, the total amounts of Tobramycin and Gentamycin that elute are greater than conventional systemic treatments. In addition, these antibiotics are also delivered for longer periods of time compared to systemic routes. Both antibiotics can be delivered at concentrations necessary for microbial inhibition. This is most profound in CORTOSS™, where Tobramycin release amounts are the greatest for up to 16 days. Compared to Simplex P, there is a higher and longer release dosage of Tobramycin from CORTOSS™. In fact, the serum level/day concentration for Tobramycin required to provide microbial inhibition is between 2 and 30 ppm.[12] A direct delivery system like the CORTOSS™/Tobramycin system therefore delivers Tobramycin levels appropriate for microbial growth inhibition. This combination of a highly hydrophilic CORTOSS™ and Tobramycin allows for an optimal drug delivery system. CORTOSS™ also provides a fairly significant heat tolerance for antibiotic stability compared to the higher polymerization exotherm of Simplex P. Because both Tobramycin and Gentamycin are sufficiently hydrophilic with substantial elution potentials, a mixed Tobramycin–Gentamycin delivery system with CORTOSS™ would be desirable due to their favorable water-solubility properties. Because hydrophilic antibiotic elution characteristics from CORTOSS™ have been assessed, a drug delivery system like CORTOSS™ can be exploited to include other hydrophilic antibiotics. Moreover, a combination of Tobramycin with a less hydrophilic antibiotic such as the peptide-based Vancomycin can be utilized to increase its elution potential and widen the spectrum for antimicrobial inhibition. Therefore, CORTOSS™ is an excellent candidate for antibiotic delivery and should compete well with more established composite systems such as Simplex P.

CONCLUSION

Simplex P was more porous when compared to CORTOSS™, which may be why a greater Gentamycin elution amount resulted from a more hydrophobic Simplex P. However, the complexation of Gentamycin to highly polar salts is another prominent factor responsible for this observed elution characteristic, enhancing its release potential from a hydrophobic environment. Higher porosity for Simplex P suggests that it may be mechanically weaker when compared to CORTOSS™. The lower porosity of CORTOSS afforded by its high degree of monomer cross linking compared to the linear polymerization of Simplex P also suggests that CORTOSS™ is more robust in regards to load bearing. In addition, through utilization of EPR and DSC analyses, it has been shown that the incorporation of antibiotics into CORTOSS™ slightly lowers radical populations and hence, degree of polymerization, while polymerizing efficiently within the same time when compared to nonimpregnated CORTOSS™. Nevertheless, the mechanical properties of CORTOSS™ have not been compromised. Tobramycin and Gentamycin elution characteristics indicate that there is an initial release maximum during the first 24 h, which levels off in the second week after impregnation from both CORTOSS™ and Simplex P. Generally, total elution percentage amounts given in Table II reflect the amounts of aminoglycoside antibiotics typically retrieved (3–8%)[8] from the original incorporation into bone cements, with antibiotic elution from CORTOSS™ being slightly greater due to its greater hydrophilicity when compared to Simplex P. Gentamycin elution characteristics behave similarly to other antibiotics, which are released at high concentrations initially and then decrease steadily over time.[6] Because CORTOSS™ is a bioactive cement that eventually forms apatite-like layers on its surface, the initial concentrated release of Tobramycin and Gentamycin, enhanced by ionic exchanges between the glass and environment, occurs prior to the initial formation of the bioactive layer. Approximately twofold greater elution amounts of Tobramycin were detected from CORTOSS™ when compared to Simplex P. This arose from the fact that the monomers that comprise CORTOSS™ are significantly hydrophilic when compared to the MMA in Simplex P, thus making it favorable for antibiotic dissolution. Based on this study, it has been shown conclusively that composite hydrophilicity determines the extent that body fluids may enter into the composites and promote release for these appreciably polar antibiotics. Tobramycin elution was also shown to be aided by its high degree of hydrophilicity. Phase partitioning studies yielded experimental results that were in direct agreement with calculated values demonstrating the high hydrophilicity of Tobramycin and that a small percentage of Gentamycin partitions in the nonaqueous phase, such as the monomers of Simplex P. Therefore, the incorporation of adequately polar antibiotics such as Tobramycin and Gentamycin into a hydrophilic composite such as CORTOSS™ affords optimal release of the antibiotics, thereby providing high local levels at the bone–composite interface where it is needed the most. Based on the data from this research, CORTOSS™ would therefore be an excellent candidate for an antibiotic delivery system, providing antibiotic concentrations high enough and at a duration long enough to be effective against the numerous microbes that are encountered in the operating theatre.

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