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Effects of water-soluble component content on cephalexin release from bioactive bone cement consisting of *bis*-GMA/TEGDMA resin and bioactive glass ceramics

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The effect of the amount of a water-soluble, lactose, on cephalexin (CEX) release from bioactive bone cement consisting of bisphenol-α-glycidyl methacrylate (*bis*-GMA), triethylene-glycol dimethacrylate (TEGDMA) resin and apatite- and wollastonite-containing glass-ceramic (A–W GC) powder was investigated. A–W GC powder containing 5% CEX and lactose powders hardened within 5 min after mixing with *bis*-GMA/TEGDMA resin, and furthermore its compressive strength was expected to be higher than that of polymethylmethacrylate cement. *In vitro* CEX release from bioactive bone cement pellets in a simulated body fluid at pH 7.25 and 37 °C continued for more than 2 wk. The drug-release rate increased with increasing amount of lactose powder in the mixture. CEX release profiles followed the Higuchi equation in the initial stage, but not in later stages. As hydroxyapatite was precipitated out on the cement surface, the CEX release rate decreased. The micropore distribution of the cements measured by mercury porosimetry also supported the variation in drug release due to cement porosity being mainly a result of the dissolution of lactose in the cements. These results suggest that the rate of CEX release from bioactive bone cement could be controlled by varying the amount of lactose in the cement system. © *1999 Kluwer Academic Publishers*

1. Introduction

Polymeric biomaterials have been used with clinical success as artificial bone-filler cements for the stabilization of prostheses. Bisphenol-α-glycidyl methacrylate (*bis*-GMA) is suitable for use as a binder for reinforcing fillers in commercial dental composites, because it is non-volatile and hardens rapidly under oral conditions when suitably formulated with an appropriate initiator system [1]. Polymethylmethacrylate (PMMA) is used in a wide variety of medical applications and has exhibited excellent biocompatibility when in its bulk, polymerized form [2–6]. Salvati *et al.* [7] developed an antibiotic-loaded bone cement based on PMMA which is currently in use clinically as a combination bone filler and drug delivery system for fixing non-bioactive prostheses to surrounding bone. However, the polymeric cement has several clinical problems for long-term application, the most serious of which are non-adhesiveness with bone surfaces and polymerization shrinkage. In addition, the polymer has considerably weaker mechanical properties than bone cortex [8].

Inorganic biomaterials, such as self-setting apatitic cements [9, 10] and bioactive glass ceramic bone cements [11, 12], etc., have been transformed into hydroxyapatite, which has the same elementary chemical composition as natural bone and teeth, and a high affinity with hard tissue, and was demonstrated to form a chemical bond with living bone through an apatite layer. Self-setting apatitic cements, therefore, have been applied in several drug-delivery systems [13–16]. However, inorganic biomaterials are brittle and have poor strength characteristics, limiting their use as an implant in loaded areas [17].

To improve the biological affinity and the mechanical properties of polymeric and/or inorganic cements [18], Kawanabe *et al.* [19] have developed a new bioactive bone cement consisting of *bis*-GMA/triethylene-glycol dimethacrylate (TEGDMA) (1 : 1) resin and apatite- and wollastonite-containing glass-ceramic

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(A–W GC) powder, which forms chemical bonds with living bones and has sufficient mechanical strength. We used this bioactive bone cement as the basis of a drug-delivery system, and examined *in vitro* antibiotic, cephalexin (CEX) release rates [20]. The results implied that the CEX release from bioactive bone cement could be controlled by varying the drug concentration in the bioactive bone cement system. In the present study, to control further the drug release through additional pharmaceutical factors, the effects of the amount of a water-soluble lactose on the *in vitro* CEX release from the cement matrix, were investigated.

2. Experimental procedure

2.1. Materials

Bioactive bone cement consisting of *bis*-GMA/TEGDMA resin and A–W GC powder was prepared as described by Kokubo *et al.* [11]. Crystalline α-lactose monohydrate (200 mesh pass, DeMelkindustrie Veghel Co., The Netherlands) was used as a water-soluble additive.

Modified A–W GC bulk powder: particles of A–W GC were provided by Nippon Electric Glass Co., Ltd, Japan. The material contained 38% oxyfluoroapatite ($Ca_{10}(PO_4)_6(O, F_2)$), 34% β-wollastonite ($CaO \cdot SiO_2$), and 28% MgO–CaO–SiO_2 glass matrix. The average diameter of the particles was 5 μm. This powder was sifted into a 1.0% aqueous solution of γ-methacryloxy propyl trimethoxy silane (Chisso Co., Tokyo, Japan) and mixed with a magnetic stirrer on a hot plate for 1 h. The slurry was dried at about 120 °C, and then benzoyl peroxide, 0.4% per unit weight of the treated powder, was added.

Bis-GMA/TEGDMA resin: the resin was prepared from equal weights of *bis*-GMA and TEGDMA (Shin-Nakamura Chemical Industry, Wakayama, Japan). *N*,*N*-dimethyl-*p*-toluidine, 0.2% per unit weight of resin, was dissolved into the mixture.

Bulk CEX powder (lot no. Y172) was obtained from Yamaguchi Pharmaceutical Co., Japan. All other chemicals were of analytical grade.

2.2. Formation of bioactive bone cement

The cement powder consisted of 5% CEX powder, the A–W GC powder, *bis*-GMA/TEGDMA resin and lactose powder as summarized in Table I. Lactose and CEX powders were carefully mixed with A–W GC powder, then the *bis*-GMA/TEGDMA resin was added and stirred for 1 min. The unpolymerized composite was placed in a Teflon mould (15 mm diameter, 2 mm thick), and stored at room temperature for 5 min, then the cement was removed from the mould. The total weight of the cement pellets was 480 ± 5 mg. The hardened cement pellet was coated with silicon rubber so that only one face of the pellet surface was exposed (1.77 cm^2).

TABLE I Formulation of bioactive bone cements

Sample	CEX (%)	Resin (%)	Ceramics (%)	Lactose (%)
Cement A	5.0	66.5	28.5	0.0
Cement B	5.0	65.1	27.9	2.0
Cement C	5.0	59.5	25.5	10.0

2.3. Mechanical strength of the cement

A–W GC powders with or without drug and lactose, were mixed with the resin as described above. The mixed paste was placed in a mould (6 mm diameter, 12 mm long), and stored at room temperature for 5 min, then, the cement was removed from the mould. The compression strength of the hardened cement was measured using an accurate compression/tension testing machine (Autograph model IS-5000, Shimadzu Co.) at a compression speed of 0.5 mm min^{-1} after storage at room temperature for 24 h.

2.4. *In vitro* drug-release test

The drug-release rates from all bioactive bone cement pellets containing CEX (15 mm diameter) were measured after storage at room temperature for 24 h as follows: a sample of cement was placed in 15 ml simulated body fluid (SBF) [11] comprised of 142 mmol Na^+, 5.0 mmol K^+, 1.5 mmol Mg^{2+}, 147.8 mmol Cl^-, 2.5 mmol Ca^{2+}, 4.2 mmol HCO_3^-, 0.5 mmol SO_4^{2-}, 1.0 mmol HPO_4^{2+} (pH 7.25) in a 50 ml capped test tube at 37.0 ± 0.1 °C. During the release test, the entire dissolution medium was replaced with fresh buffer at various intervals. Because the amount of unpolymerized *bis*-GMA and TEGDMA monomers released from the cement was negligible compared with the concentration of CEX in SBF in the previous study, the concentrations of CEX were measured spectrophotometrically (W 160A, Shimadzu Co., Kyoto, Japan) at 245 nm. Each value shown represents an average of three runs.

2.5. X-ray diffraction measurement

The powder samples were obtained by grinding hardened bioactive cement in an agate mortar. The X-ray powder diffraction profiles of the cement and drug-loaded cement samples were measured by powder X-ray diffraction analysis (XD-3A, Shimadzu Co., Japan, copper radiation, 15 mA, 35 kV).

2.6. Fourier transform–infrared (FT–IR) measurement

Reflectance FT–IR spectra of the sample pellet surfaces were obtained by spectral reflectance on an FT–IR spectrophotometer (type FT–IR 1600, Perkin–Elmer Co., Yokohama, Japan) and corrected using the Kramers–Krig equation.

2.7. Scanning electron microscopy (SEM)

The samples were coated with gold in an ion-sputter JFC-1100 (Jeol Datum Co., Tokyo, Japan). Scanning

electron micrographs of the samples were taken with a model JSM-5200LV (Joel Datum Co., Tokyo, Japan) at magnifications of $\times 5000$ and $\times 10\,000$.

2.8. Micropore distribution measurement

Micropore distribution of the cement was measured by means of mercury porosimetry (type 2000, Carlo Erba Strumentazione, Italy). The contact angle and surface tension of mercury were 141.3° and 480 dyn cm^{-1}, respectively. The pore radius ranged from 300–6×10^{-3} μm.

3. Results

3.1. CEX release from bioactive bone cements containing lactose

Fig. 1 shows the effect of lactose content on CEX release profiles from 5% drug-loaded bioactive bone cement systems in SBF at pH 7.25 and 37 °C. The CEX release rate from the cement was initially very rapid, but after 50 h slowed markedly. The concentration of CEX released increased with increasing amounts of lactose in the cement, suggesting that the initial rates were dependent on the amount of lactose loaded. After 2 wk, total drug release from the 5% CEX loaded cement system containing 0%, 2% and 5% lactose was $5.41 \pm 0.65\%$, $6.26 \pm 1.04\%$ and $8.78 \pm 0.89\%$, respectively.

Fig. 2 shows the effect of lactose content on the plots of *in vitro* CEX release from the cement against the square root of time. *In vitro* CEX release profiles of all cements were linear at the initial drug-release stage on the plot. However, drug-release rates were reduced after 4–6 d. The initial and later CEX release rates on Higuchi plots were estimated by the least-squares method and are shown in Fig. 3. The initial release rate from the cement increased with increasing amount of lactose. The value for the effective diffusivity of CEX in the pores of the matrix were calculated from the Higuchi constant and are summarized in Table II.

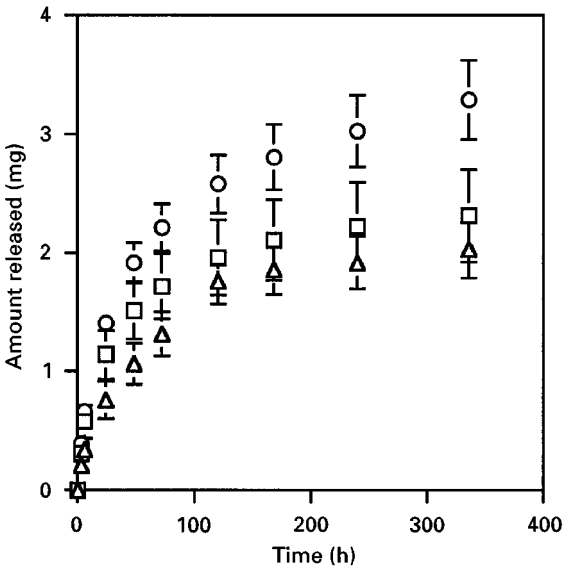

Figure 1 Effect of lactose content on the *in vitro* CEX release profiles from 5% CEX-loaded bioactive bone cement systems in SBF at pH 7.25 and 37 °C. Lactose content: (△) 0%, (□) 2%, (○) 10%. All data are the mean of three different experiments ± standard deviation.

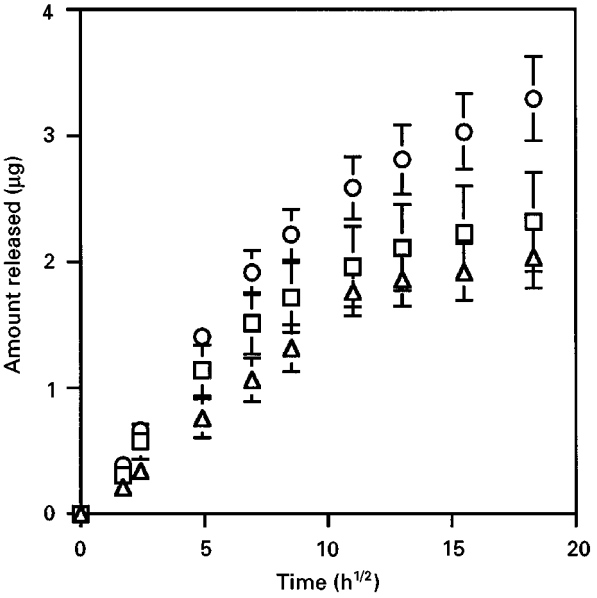

Figure 2 Effect of lactose content on the plots of *in vitro* CEX release from 5% CEX-loaded bioactive bone cement systems in SBF against square root of time. Lactose content: (△) 0%, (□) 2%, (○) 10%. All data are the mean of three different experiments ± standard deviation.

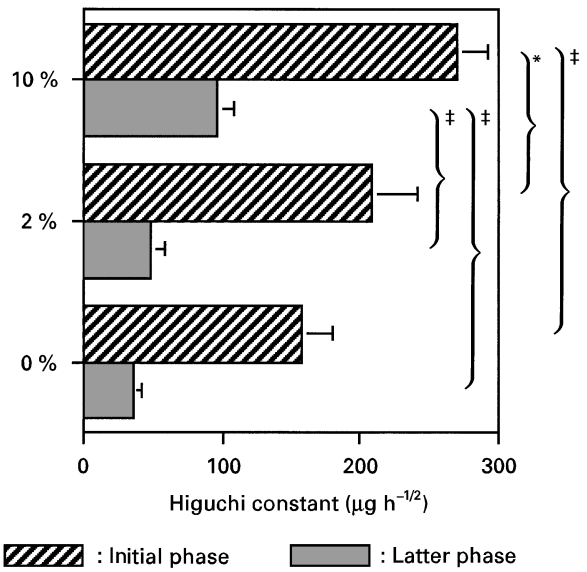

Figure 3 Effect of lactose content on initial and later CEX release-rate constants of bioactive bone cements. All data are the mean of three different experiments ± standard deviation. Statistical analysis was realized with an analysis of variance (ANOVA), $^{*}P < 0.05$ and $^{\ddagger}P < 0.01$.

3.2. Characterization of bioactive bone cements

The X-ray diffraction profiles of drug-loaded bioactive bone cement systems before and after the drug-release test suggested that the hardened cement exhibited characteristic peaks at 25.8°, 30.5°, 32.1° and 33.2°,

TABLE II Effect of lactose content on effective diffusivity of CEX-loaded bone cements

Lactose (%)	D_{eff} at initial stage ($cm^2 h^{-1}$)		D_{eff} at later stage ($cm^2 h^{-1}$)	
0.0	$99.5 \times 10^{-8} \pm 28.3 \times 10^{-8}$	NS ‡	$5.31 \times 10^{-8} \pm 1.85 \times 10^{-8}$	NS *
2.0	$176 \times 10^{-8} \pm 54 \times 10^{-8}$		$9.26 \times 10^{-8} \pm 3.92 \times 10^{-8}$	
10.0	$290 \times 10^{-8} \pm 51 \times 10^{-8}$ *		$37.1 \times 10^{-8} \pm 8.99 \times 10^{-8}$ *	

Note: All data are mean of three different experiments ± standard deviation. Statistical analysis was realized with an analysis of variance (ANOVA), $*P < 0.05$ and $‡P < 0.01$. NS = not significant.

that were attributable to A–W GC. However, the result of the reflection FT–IR spectra of the fixed cements containing 5% CEX before and after drug-release tests, suggested the formation of hydroxyapatite on the surface.

Fig. 4 shows scanning electron micrographs of 5% CEX-loaded bioactive bone cement containing 10% lactose after drug release for 2 wk. The fresh bioactive bone cement had a smooth surface. However, after drug release, the cement had many aggregated particles on its surface.

Fig. 5 shows the effect of lactose content on the micropore distribution of the cements before and after drug release. Most of the micropores of cement A (without lactose) were less than 0.01 μm in radius, but increased to 0.1–1 μm after drug release. In cement C (10% lactose), pore radius was increased to 0.07–1 μm, and pore volume to around to 25 $mm^3 g^{-1}$. The total pore volume of the cement systems increased with increasing amount of lactose, suggesting that the pores 0.1–1 μm in radius were formed by dissolution of lactose after drug release.

3.3. Effect of lactose on mechanical strength of bioactive bone cements

Table III shows the compressive strength of bioactive bone cements. The compressive strength of the cement was decreased by adding CEX and lactose, but that of the cement containing 10% lactose was 102 MPa, about 1.5 that of PMMA cement.

4. Discussion

4.1. Effect of lactose content on CEX release from bioactive bone cements

In general, diffusion-controlled drug release from matrix-type drug-delivery systems has a linear relationship with the square root of time [21]. Drug release from the matrix tablets is given by

$$M_r = A\left[\frac{D_i \varepsilon C_s (2C_d - \varepsilon C_s) t}{\tau}\right]^{1/2} \quad (1)$$

$$D_{eff} = \frac{D_i \varepsilon}{\tau} \quad (2)$$

where M_t is the amount of drug released from the cement at time t, A is the surface area of the tablet, D_i is the diffusivity of the drug, D_{eff} is the effective diffusivity of the drug in the pores of in the matrix, C_s is the solubility, C_d is the concentration of the drug, τ is the tortuosity and ε is the porosity.

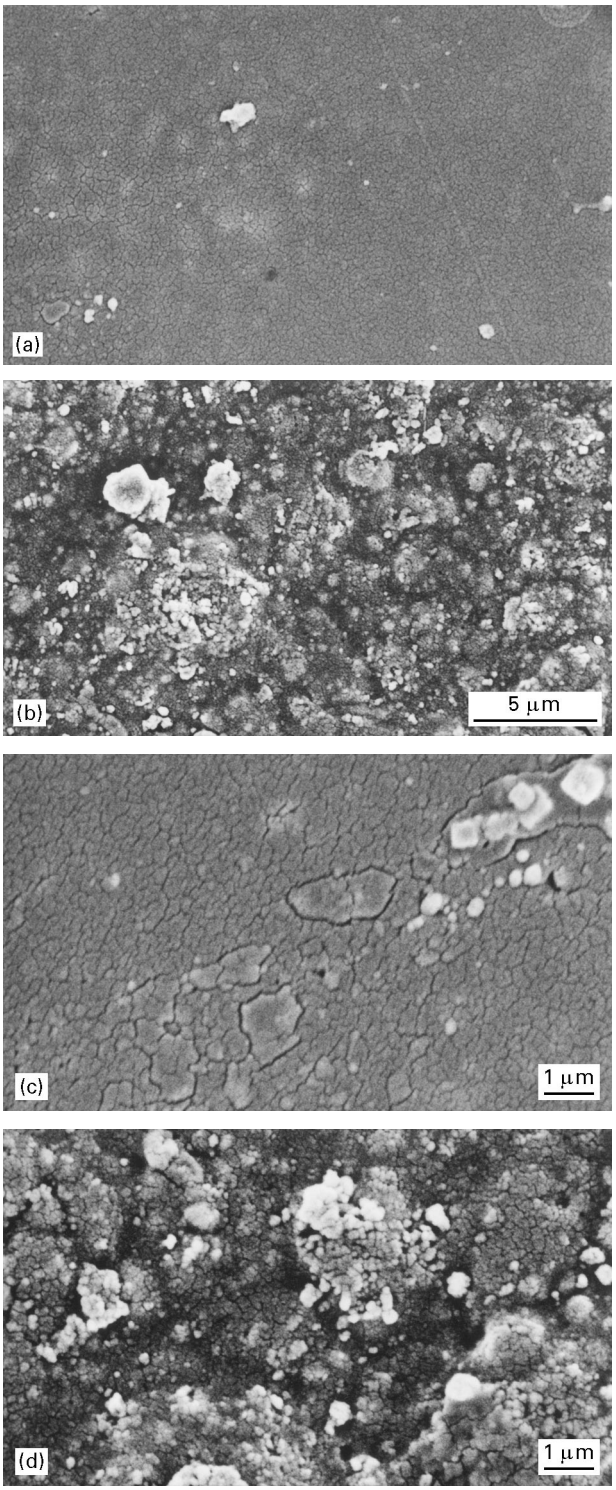

Figure 4 Scanning electron micrographs showing the effect of lactose content on CEX-loaded bioactive bone cements before and after drug-release tests: (a) the cement without lactose before drug release, ×5000, (b) after drug release, (c) the cement with 10% lactose before drug release, (d) after drug release.

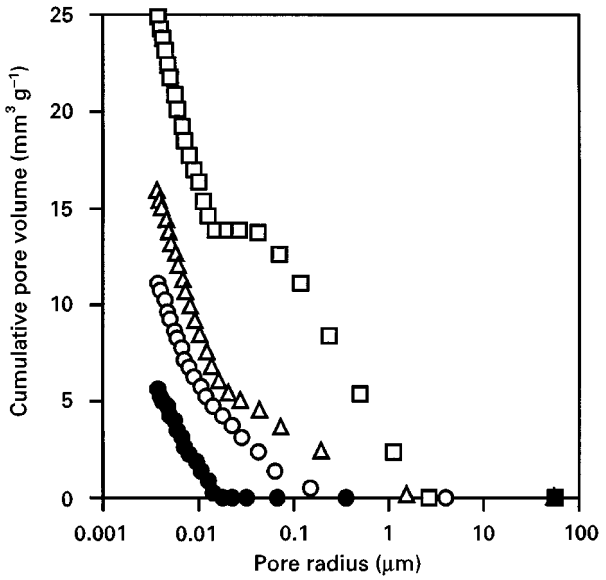

Figure 5 Effect of lactose content on micropore distribution of CEX-loaded bioactive bone cements before and after drug release tests. (●) CEX loaded cement without lactose before drug release, (○) CEX loaded cement without lactose after drug release, (△) the cement with 2% lactose after drug release, (□) the cement with 2% lactose after drug release.

TABLE III Effect of lactose on compressive strength of bioactive bone cements

CEX (%)	Compressive strength (MPa)
Cement without drug and lactose	180 ± 10
Cement A	127 ± 6
Cement C	102 ± 3
PMMA cement	68 ± 1

Note: all data are the mean of three different experiments ± standard deviation. Statistical analysis was realized with an analysis of variance (ANOVA). *$P < 0.01$.

In the previous study, *in vitro* CEX release from 5% CEX-loaded cement consisting of *bis*-GMA/TEGDMA resin and A–W GC as a plot of drug release against square root of time, was linear at the initial stage, and the initial release rate could be controlled by varying the drug concentration in the bioactive bone cement system. However, it showed a reduction at the later stages of the release test, and did not follow the Higuchi equation. The IR spectrum of the cement after release suggested that the cement surface was covered by hydroxyapatite generated for A–W GC powder, and it seemed that the bioactive bone cement chemically bonded with hydroxyapatite in the hard tissue. Therefore, these results indicated that change in the geometrical structure of the pores in the cement is dependent on the calcium concentration in SBF. This calcium is a result of the precipitation of hydroxyapatite on the cement surface during the drug-release test due to the high degree of supersaturation of SBF with respect to the solubility of hydroxyapatite.

In the present study, the rate of CEX release from the cements containing lactose increased with increasing amount of lactose, but it also showed a reduction at the later stages of the release test. Thus CEX release from the cement matrix at the later stages reflected the rate of decrease in the effective diffusivity of drug in the pores, and did not follow the Higuchi equation. However, when drug release is evaluated based on the equation, the parameters indicate a change in the geometrical structure of the pores in the cement matrix during the drug-release test. Therefore, we assumed that the drug release followed the Higuchi equation and evaluated the initial and later drug release rates based on the equation. Initially, the rate of *in vitro* CEX release significantly increased with increasing lactose content in the cement, because drug release was accelerated by increasing porosity due to the addition of lactose.

The micropore distribution of the cements measured by mercury porosimetry also supported the variation in drug release due to cement porosity being mainly a result of the dissolution of lactose in the cements. SEM results before CEX release significantly differed from those after. Surface morphological differences in the cements were evident before and after the drug-release test. These results suggested that a portion of the A–W GC in the bioactive bone cement was converted into fine hydroxyapatite crystals on the surface of the cement during drug release. The results indicated that it was possible to control CEX release from the bioactive bone cement by adding lactose.

The compressive strength of the cement decreased with increasing CEX concentration, and for 5% CEX loaded cement with (10%) and without lactose was 102 and 127 MPa. It appears that the decrease was caused by CEX and lactose adsorption on to the interface between the particles and/or increasing porosity. The compressive strength of the cements was significantly lower by about 1.5 times than that of PMMA cement. However, the mechanical strength of the present cement system was considered to be sufficient for clinical use in hard tissues.

5. Conclusion

These results imply that the rate of CEX release from bioactive bone cement can be controlled by varying the lactose concentration in the cement system. Therefore, this cement can be used as an effective delivery system for the local application of antibiotics in bone.

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