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Vancomycin release from bioresorbable calcium phosphate–polymer composites with high ceramic volume fractions

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Abstract Bioresorbable calcium phosphate–polymer composite implants are a desirable alternative to the traditional metal bone-healing devices. Incorporation of antimicrobial drugs into the composite material and their sustained delivery may dramatically reduce the risk of implant infections. The paper reports the fabrication of drug-incorporated bioresorbable CaP–polymer nanocomposites that can be used for fracture fixation devices and at the same time function as local delivery systems. Vancomycin was incorporated into β-tricalcium phosphate (β-TCP)- and biphasic CaP (BCP)-based composites containing $\leq$30 vol.% polycaprolactone (PCL) or polylactic acid (PLA), during their high pressure consolidation at 2.5 GPa and room temperature. The antibiotic release was studied in Tris buffer solution at 37 °C. Up to 5 wt% vancomycin could be included without compromising material's integrity upon immersion into Tris solution. Vancomycin release profile was found to depend on the specific surface area of the test specimens and on the composite porosity. β-TCP–30 vol.% PLA composites were found to have the best combination of compression strength and drug release pattern. Complete drug release was accompanied by only negligible material dissolution suggesting a diffusion mechanism of release. In the context of bone-healing applications, such a release-dissolution pattern will allow local prophylaxis against implant-related infection at the early stages after implantation followed by a much more slow dissolution of the load-carrying device.

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Introduction

Bone-fixation devices such as intramedullary nails, plates, and screws are customarily used to assist in the healing of complex fractures and in bone regeneration [1, 2]. Bioresorbable implants are immerging as an attractive alternative to the traditional metal bone-healing devices because they do not require a second surgery to remove the implant. To be effective for clinical therapy, such bioresorbable fixation devices should be sufficiently strong initially as well as retain their strength during the bone-healing process [3]. Polymer–ceramic composites are increasingly considered as materials for bioresorbable devices with poly(α-hydroxyester)s (polylactic and polyglycolic acids (PLA and PGA), their copolymers and polycaprolactone (PCL)) used as the polymer component, and calcium phosphates, CaP (hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and ratios thereof—biphasic CaP (BCP)) used as the ceramic component. Such composites are believed to be more biocompatible than their pure polymeric counterparts since the alkaline resorption products of calcium phosphates can buffer the acidic products of hydrolytic degradation responsible for late inflammation around α-hydroxyester implants [4].

A common feature of most proposed materials is the low volume fraction of CaP particles dispersed in a continuous polymer matrix [2, 5–9]. In such a design, the ceramic particles increase stiffness and improve biocompatibility; the strength, however, is not increased and may actually decrease [2, 6]. Therefore, polymer–CaP composites with low ceramic fractions cannot be used in load-bearing body locations. We've previously reported that the inverse approach where small volume fractions of the polymer (PCL, PLA) are added to the largely ceramic material can yield strong CaP–polymer composites [10, 11].

Orthopedic implant procedures carry a high risk of infection with about 5% of inserted internal fixation devices becoming infected [12]. Implant infections are extremely resistant to antibiotic therapy and host defenses, and frequently persist until the implant is removed. Local antibiotic delivery systems are considered advantageous for treatment and prevention of implant-related infections because they may deliver high drug concentrations to the infected bone while avoiding systemic side-effects [13–16]. Antibiotic-loaded polymethylmethacrylate (PMMA) cement beads are often used for local drug delivery; however, PMMA beads are nonbiodegradable and must be removed by an additional surgical procedure [17]. Therefore, much attention has been focused on the development of bioresorbable materials as antibiotic carriers for local delivery, including for the prevention and treatment of infections in fixation of bone fractures [18]. Incorporation of antimicrobial drugs into high strength bioresorbable biomaterials seems especially attractive as it can produce mechanically reliable multifunctional implants that not only reduce the risk of bone infection but also provide secure fracture fixation [19]. Promising results were obtained for bioresorbable ciprofloxacin-releasing osteofixation screws for nonload-bearing applications made of pure polymer and bioglass-reinforced PLGA and PLDLA [19, 20], however, incorporation of drugs into dense bioresorbable CaP–polymer composite implants has not been reported. In principle, active ingredients may be incorporated into the composite powder prior to densification provided the biological activity is not lost during processing. Unfortunately, most processing methods of dense calcium phosphate–bioresorbable polymer composites employ relatively high temperatures that may compromise the bioactivity of incorporated drugs [5–9, 21–23]. Using higher pressures could allow one to achieve high density at low temperatures that are less likely to be harmful for biomolecules.

This work reports high pressure consolidation at room temperature of CaP (≥70 vol.%)–polymer nanocomposites with incorporated antibiotic designed for the fabrication of bioresorbable implants that are sufficiently strong to provide secure fixation of bone fractures and at the same time function as local delivery systems. The antibiotic investigated was vancomycin, a highly water soluble drug effective in treating osteomyelitis and preventing osseous staphylococcal infections [24, 25].

Experimental

Materials

The starting materials for the synthesis of β-TCP and BCP ceramic powders were dried calcium acetate, $(CH_3COO)_2Ca$ (anhydrous basis 99.0–100.5%, Spectrum Chemical Mfg. Corp., USA) and aqueous o-phosphoric acid, H_3PO_4 (85%, BIO LAB LTD, Jerusalem, Israel). Chloroform and ethanol (AR grade, BIO LAB LTD, Jerusalem, Israel) were used as solvents. Poly(ε-caprolactone) (PCL) pellets, mean $M_w > 100{,}000$, $\rho = 1.1$ g/cm^3, $T_m = 58$–60 °C, were purchased from Solvay Caprolactones; poly(lactic acid) (PLA) pellets, $T_m = 170$ °C, $\rho = 1.21$ g/cm^3, were purchased from Performance Alloys & Materials, Inc. Vancomycin–HCl antibiotic powder was purchased from ACROS Organics. Reagents used for Tris buffer solution preparation were Trizma base (Sigma), HCl 1 M (Carlo Erba) and deionized water.

CaP–polymer composite fabrication

CaP synthesis was based on the procedure reported in [26]. 16 mL of H_3PO_4 was dissolved in 300 mL of ethanol, after which calcium acetate powder was slowly added to the same beaker under forceful stirring. 57.12 and 60.93 g of calcium acetate were used to obtain β-TCP and BCP, respectively. The beaker was sealed to prevent ethanol evaporation and left for 72 h at room temperature under stirring. After 72 h the beaker was opened to evaporate ethanol and its content dried in an oven at 100 °C overnight. The powder received was crushed, calcined in open air at 650 °C for 1 day and characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

To obtain a calcium phosphate–polymer composite powder, PCL or PLA was dissolved in 60 mL chloroform after which 8 g of CaP (β-TCP or BCP) powder was added under vigorous stirring. 0.5058 g PCL was used to produce a CaP–15 vol.% PCL composite; 0.5564 and 1.3513 g PLA were used to produce CaP–15 vol.% PLA and CaP–30 vol.% PLA composites, respectively. After 1 h stirring, the substance was poured into a beaker containing 60 mL of ethanol, sealed and left under continuous stirring for 30 min. The suspension was then poured into a mortar, stirred, and crushed until all the chloroform and ethanol evaporated and dried in vacuum for 48 h. The polymer content of the composite powders was measured by thermo-gravimetric analysis (TGA).

0.1–0.3 g vancomycin was dissolved in 5 mL deionized water and mixed with 1 g of dry CaP–PLA and CaP–PCL composite powders to yield materials containing 3–10 wt% antibiotic. The vancomycin-containing powders were dried in vacuum.

Composite powders obtained were high pressure consolidated [27] at room temperature and 2.5 GPa pressure in a Toni Technik Press. Three types of specimens were prepared. Disks 6.5 mm in diameter and 3 mm high were used for density measurements and mechanical testing. Disks 10.7 mm in diameter and ~1.1 mm high were used

for immersion tests. Disks 10.7 mm in diameter and ~0.9 mm high were crushed into 5–6 small fragments (crumbs) and also used for immersion tests.

Vancomycin release

Vancomycin release was studied by immersing composite samples in a 50 mM Tris buffer solution (pH 7.4) at 37 °C. To prevent saturation, a 1 mg material-to-1 mL solution ratio was used. At pre-determined intervals, 10-mL aliquots were collected and replaced with fresh Tris solution to maintain the same total volume throughout the study. The vancomycin concentration was evaluated using a UV spectrophotometer at 280 nm. Release studies were performed in triplicate for disk specimens and in duplicate for crumb specimens.

Results and discussion

In Fig. 1a, XRD patterns of the two types of synthesized CaP powders are presented. The upper pattern corresponds to the pure β-TCP with no visible peaks of other CaP phases. The lower pattern shows that the synthesized BCP powder consists of ~40% β-TCP and 60% HA (wt%). Both synthesized CaP powders (β-TCP and BCP) consisted of very fine nanoparticles not larger than 200 nm. When PCL or PLA was dissolved in chloroform and added to the CaP powder, it became uniformly distributed between the ceramic nanoparticles forming tiny polymer fibers, Fig. 1b. The results of TGA analysis confirmed that polymer contents of the CaP–PLA and CaP–PCL composite powders roughly corresponded to the nominal composition (15 or 30 vol.%). This means that the polymer dissolved in chloroform was fully incorporated in the composite powders.

High pressure consolidation of the composite powders at room temperature yielded relatively dense and strong ceramic–polymer composites, Table 1. The best mechanical properties so far were obtained for the β-TCP–30 vol.% PLA composition. The compression strength of this material (197 MPa) is significantly higher than the highest literature value of 115.3 MPa reported for the PLA–HA composite with low (32 vol.%) ceramic fraction forged at 103 °C [2].

Table 1 Density and compression strength of β-TCP–polymer composites

Material	Density (% TD)	Porosity (%)	Compression strength (MPa)
β-TCP–15% PCL	89 ± 2	11 ± 2	137 ± 13
β-TCP–15% PLA	87 ± 2	13 ± 2	118 ± 21
β-TCP–30% PLA	94 ± 1	6 ± 1	197 ± 12

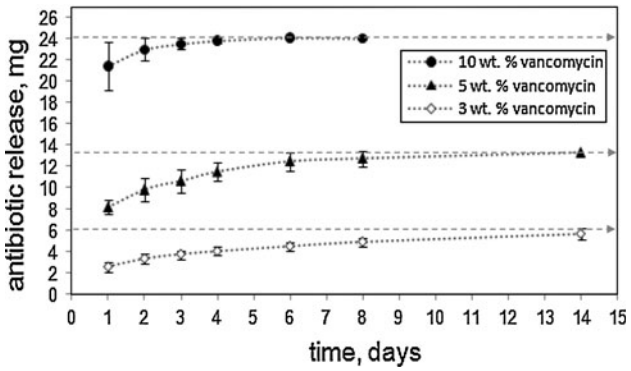

Fig. 2 Cumulative antibiotic release from β-TCP–15% PCL disks containing different amounts (3, 5, and 10 wt%) of vancomycin as a function of immersion time in Tris buffer solution at 37 °C. *Arrows* indicate 100% initial vancomycin load

Figure 2 shows drug release from 10.7 mm diameter β-TCP–15% PCL disks containing different amounts of vancomycin. Specimens with 10 wt% vancomycin (~24 mg) released the drug very rapidly and disintegrated completely after 2 days immersion. In contrast to this, disks with 3 and 5 wt% vancomycin (6 and 13 mg, respectively) released much more slowly and remained intact after 14 days immersion. 5 wt% vancomycin was

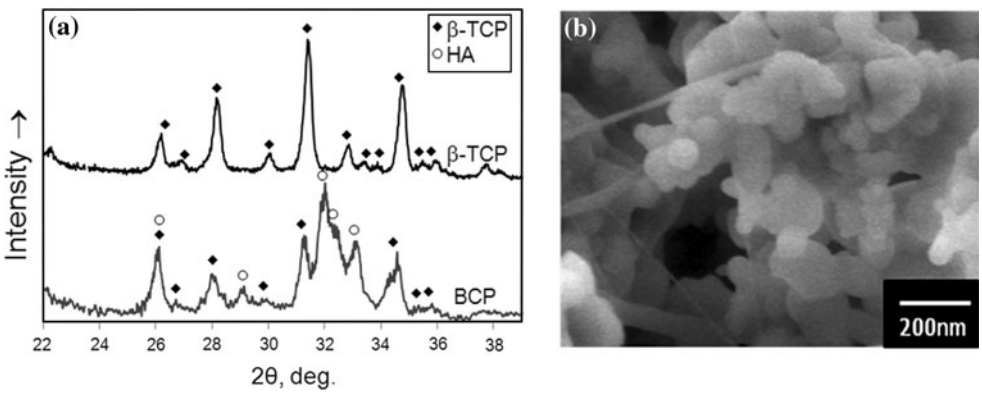

Fig. 1 **a** XRD pattern of the synthesized β-TCP and BCP powders; **b** SEM micrograph of β-TCP–15% PCL composite powder

chosen for further experiments as the largest antibiotic load that does not cause material disintegration upon immersion. As can be seen in Fig. 2, 1-mm-thick β-TCP–15% PCL disks containing 5 wt% vancomycin released $\sim$30% of the total drug load in an initial burst, and the remaining 70%—over the period of $\sim$10–12 days. The weight loss of the β-TCP–15% PCL disks measured after 14 days immersion, constituted only 3% of the initial weight. This suggests that the drug is released from the β-TCP–15% PCL composite by a diffusion mechanism rather than as the result of material dissolution. In the context of bone-healing applications, such a release-dissolution pattern will allow local prophylaxis against implant-related infection at the early stages after implantation followed by a much more slow dissolution of the load-carrying device.

As shown in Table 2, the incorporation of 5 wt% vancomycin did not strongly affect the compressive strength of β-TCP–15% PCL. Moreover, no significant strength deterioration was measured following 2 weeks immersion of the vancomycin-containing as well as vancomycin-free material. This must be due to the negligible material dissolution of 3%, see above. Although dissolution pits are clearly visible on the material surface after 2 weeks immersion, Fig. 3, these must be confined to the thin surface layer and do not significantly affect the bulk properties.

Figure 4 shows plots of vancomycin release from small crumbs and larger disks made of β-TCP–15% PCL and BCP–15% PCL ($\pm$5 wt% vancomycin) composites. The release from the crumbs is much faster than from the disks

Table 2 Compression strength of β-TCP–15 vol.% PCL composites before and after 2 weeks immersion in Tris buffer solution at 37 °C

Vancomycin content in β-TCP–15% PCL	No vancomycin	5 wt%
Compression strength (MPa)		
Before immersion	137 $\pm$ 13	120 $\pm$ 30
After 2 weeks immersion in Tris	110 $\pm$ 26	123 $\pm$ 24

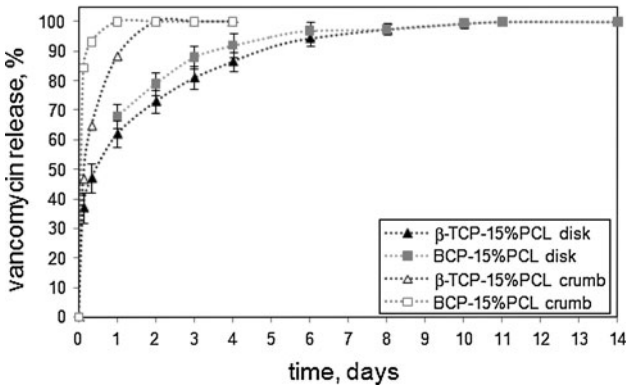

Fig. 4 Cumulative drug release (% of initial load) from β-TCP–15% PCL and BCP–15% PCL composite disks and crumbs containing 5 wt% vancomycin as a function of immersion time in Tris buffer solution at 37 °C

suggesting a strong effect of the specimen surface-to-volume (S/V) ratio. In the context of fracture fixation plates, this means that thicker plates will release antibiotics more slowly than thinner ones, at the same drug concentration. This also suggests that drug release profile from CaP–PCL composites can be tailored to a specific application by creating drug concentration gradient across the plate, e.g., by stacking composite powder layers with different drug contents prior to consolidation.

For the large disk specimens, no significant effect of CaP type (BCP or β-TCP) on vancomycin release was observed. For the crumb specimens, though, a much larger burst release was observed for BCP–PCL compared to β-TCP–PCL composite. Even so, the geometry (and the corresponding S/V ratio) of the small crumbs is not well defined which precludes a meaningful comparison of BCP- versus β-TCP-based material.

Figure 5 compares drug release profiles from β-TCP–polymer composites with 5 wt% vancomycin for different types (PCL and PLA) and fractions (15 and 30 vol.%) of the polymer. It can be seen that for the same polymer (PLA), drug release is slower from the composite with the higher (30 vol.%) polymer fraction. This must be due to

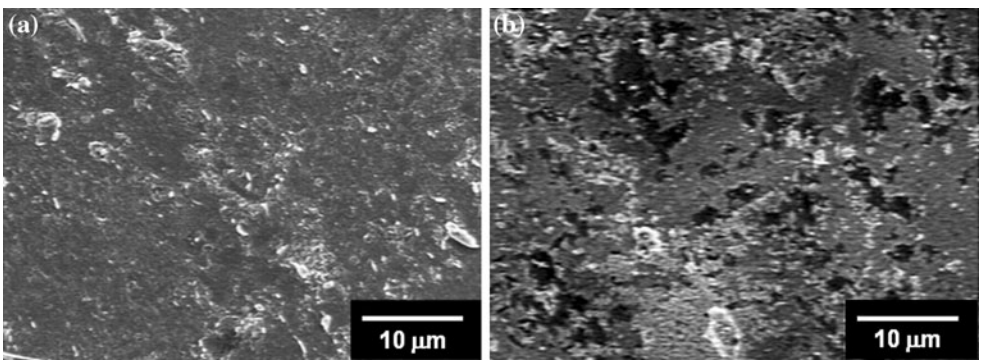

Fig. 3 Surface of (β-TCP–15 vol.% PCL)–5 wt% vancomycin disk before (**a**) and after (**b**) 2 weeks immersion in Tris buffer solution, SEM

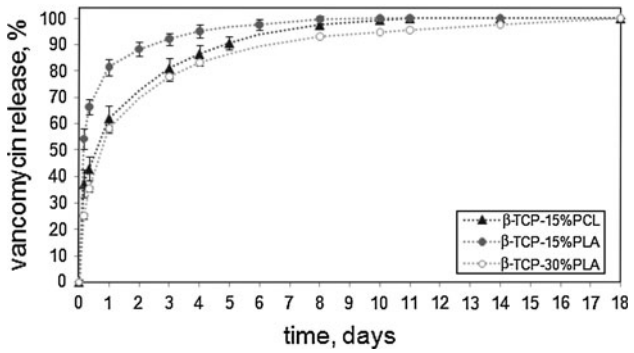

Fig. 5 Cumulative drug release from β-TCP-based composite disks with different polymer contents containing 5 wt% vancomycin as a function of immersion time in Tris buffer solution at 37 °C

the significantly lower porosity of the latter material (6 vs. 13%, Table 1). Given our earlier assumption that vancomycin is released via a diffusion mechanism, higher porosity should favor faster drug release. Among the two composites with the same polymer volume fraction (15%), the material with PLA as the polymer phase releases vancomycin faster than the one with PCL. The small difference in the general porosity of these two composites (11 vs. 13%, Table 1) can hardly have such a pronounced effect on drug release. The slower vancomycin release from the PCL- versus PLA-containing material could be due to the smaller water uptake of the more hydrophobic PCL polymer which makes diffusion of the drug more difficult. So far, a β-TCP–30 vol.% PLA composite has exhibited the most attractive gently sloping drug release profile and the highest compression strength ($\sim$200 MPa, Table 1). Therefore, we are now concentrating on investigation of vancomycin release, dissolution behavior and its effect on the mechanical properties of β-TCP–30 vol.% PLA and BCP–30 vol.% PLA composite specimens with different specific surface area.

Conclusions

It has been shown that an antibiotic drug (vancomycin) can be incorporated into strong bioresorbable calcium phosphate (CaP)–polymer composites, during their high pressure consolidation at the low (room) temperature that is not harmful for biomolecules. Up to 5 wt% vancomycin could be incorporated into β-TCP- and BCP-based composites containing $\leq$30 vol.% PCL and PLA as the polymer phase without compromising material's integrity upon immersion into Tris buffer solution. Vancomycin release profile was found to depend on the specific surface area of the test specimens and on the composite porosity. β-TCP–30 vol.% PLA composites were found to have the best combination of compression strength ($\sim$200 MPa) and drug release pattern (complete release of a 5 wt% vancomycin load from $\sim$1-mm-thick disks after 18 days immersion). Complete drug release was accompanied by only negligible material dissolution suggesting a diffusion mechanism of release. The drug release and strength retention properties of high pressure consolidated vancomycin-loaded β-TCP–polymer composites make them an attractive candidate material for bioresorbable bone-fixation devices combining tissue supporting function with bone infection prophylaxis.

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