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Effects of the addition of vancomycin on the physical and handling properties of calcium sulfate bone cement

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ABSTRACT

Intra-operative applications of bone graft substitutes into bone voids support mechanical stability, and accelerate fracture healing. Calcium sulfate bone cement, an injectable substitute, is used widely in non-loading bones with favorable results. In addition, calcium sulfate also serves as a vehicle for antibiotics that treat osteomyelitis or prevent contaminations. However, the effects of the addition of antibiotics on the physical properties of calcium sulfate are rarely addressed. In this study, calcium sulfates mixed with vancomycin at different weight ratios (4:0, 4:0.025, 4:0.05, 4:0.075, and 4:0.1) were evaluated *in vitro*. No obvious temperature increase or pH change was observed during setting and immersion in the simulated body fluid. The added vancomycin did not influence the mechanical strength, crystalline phase, or microstructure of the calcium sulfate cement. However, the addition of vancomycin extended the initial and final setting time (4:0.075, and 4:0.1). A higher amount of vancomycin resulted in a higher initial boosting release, but did not lead to faster degradation. The vancomycin-impregnated cement exhibited inhibitory effects against *Staphylococcus aureus*. These data indicate that the extended initial and final setting time of the calcium sulfate bone cement with the addition of vancomycin should be considered during operation.

1. Introduction

Bone grafts are used frequently in orthopedic surgery [1,2]. Besides the use of different kinds of implants to hold the reduction of displaced fracture fragments during surgeries, intra-operative applications of bone grafts into bone voids are considered to support mechanical stability, or to provide structural replacement to accelerate fracture healing and promote early rehabilitation [3]. In addition to supporting mechanical stability to improve bone defect repair, bone graft substitutes also serve as a controlled drug delivery system for various purposes [4–7]. A number of studies have reported using bone substitutes to deliver antibiotics locally for open fractures, infection prevention, and osteomyelitis [8].

In terms of minimally invasive surgery, developments in injectable bone graft substitutes, which can be injected into irregular bone defects and integrated into bone completely, do benefit fracture treatments [9]. Calcium sulfate, an injectable bone graft substitute with the longest proven clinical history, is used widely in treating non-loading bone injuries such as distal radial fractures [10–12]. In addition, calcium sulfate cement can also be used as a vehicle to deliver antibiotics. Thomas and Puleo added tobramycin to calcium sulfate to prevent infection in contaminated wounds [13]. Tsai et al. treated an infected tibial nonunion using a plate fixation with tobramycin-impregnated calcium sulfate [14]. Helgeson used antibiotic-impregnated calcium sulfate in open fractures [15]. Cephalexin-loaded calcium sulphate-based cements have also been

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Table 1
The compositions, initial and final setting time of calcium sulfate/vancomycin cement samples.

	Percentage of calcium sulfate content (%)	Percentage of vancomycin content (%)	Initial setting time (min)	Final setting time (min)
4:0	100	0	16.50 ± 1.64	18.50 ± 1.38
4:0.025	99.38	0.62	16.83 ± 1.17	19.00 ± 1.10
4:0.05	98.76	1.23	19.00 ± 1.41	21.33 ± 1.63
4:0.075	98.14	1.84	18.17 ± 2.23	20.17 ± 1.94
4:0.1	97.52	2.44	20.50 ± 1.38	24.00 ± 0.89

The weight of prepared calcium sulfate/vancomycin sample was about 0.48 g.

proposed [16]. In the previous studies [13–16], various antibiotics were added into calcium sulfate cements. However, the change of physical properties of calcium sulfate cements caused by antibiotic did not mention and discuss before.

Accordingly, we wanted to analyze the influences of antibiotics on calcium sulfate cement in this study. Vancomycin was chosen to be the studied antibiotic, and was added to calcium sulfate cement in different weight ratios. The mechanical strength, setting time and temperature, microstructures, degradation, and vancomycin-releasing pattern of antibiotic-loaded calcium sulfate were demonstrated *in vitro*. The purpose of this study was to demonstrate the effects of the addition of vancomycin on the physical properties of calcium sulfate cement.

2. Materials and methods

2.1. Preparation of calcium sulfate/vancomycin samples

The calcium sulfate powders (calcium sulfate hemihydrate, 237132, Sigma–Aldrich, USA) and vancomycin (vancomycin hydrochloride, pure form without excipient, NDC 0409-4332-01, Hospira, IL, USA) were mixed well in a series of weight ratios: 4:0, 4:0.025, 4:0.05, 4:0.075, and 4:0.1. The maximal weight ratio for antibiotic addition was decided upon our clinical experiences, and the vancomycin contents of samples were summarized in Table 1. Saline was added to the mixed powders to form paste (power to saline ratio: 1 g/0.25 mL), and the paste was put into a plastic mold manually in order to obtain uniform cylinders (5 mm in diameter and 10 mm in length). The dried weight of the cylinders was about 0.48 ± 0.03 g.

2.2. Setting time and setting temperature

The initial and final setting time was measured using a Vicat needle as previously described [17]. The setting temperature was recorded for 20 min using an infrared thermometer. The calcium sulfate/vancomycin cylinders were stored at room temperature for 24 h for complete hydration. Six samples for each composition were recorded for the setting time/temperature.

2.3. Vancomycin-releasing pattern

Simulated body fluid (SBF, which containing Na^+, K^+, Mg^+, Ca^+, Cl^-, HCO_3^-, HPO_4^{2-}, and SO_4^{2-} ions [18]) was prepared to test the releasing pattern of vancomycin. A calcium sulfate/vancomycin cylinder was located in the center of a 6-cm glass dish with 10 mL of SBF, and the dish with the sample was placed in an incubator set at 37 °C. Under static condition, the SBF was sampled on days 0, 1, 3, and 7 of the first week, and twice a week thereafter for a total of 4 weeks. Four samples of each composition were tested in this study. The concentration of vancomycin in the SBF was quantified. Four independent samples were tested for each condition.

2.4. Degradation of calcium sulfate/vancomycin samples and pH value variation of simulated body fluid

The degradation of calcium sulfate/vancomycin cylinders was determined based on weight loss. Samples were immersed in the SBF under static condition at 37 °C for 7, 14, 21, 28 days. At pre-determined intervals, samples were removed from SBF and weighed after drying in an oven at 65 °C overnight. Six samples were tested for each composition. The pH value of the SBF was also recorded to identify the pH variation.

2.5. Test of mechanical strength

The compressive strength of the vancomycin-impregnated calcium sulfate was tested. The calcium sulfate/vancomycin cylinders were removed from the SBF and dried in an oven at 65 °C overnight, and samples were tested using a tensile test machine (Texture Exponent 32, TA.XTplus, Surrey, UK) under compression at a crosshead speed of 0.5 mm/s at predetermined intervals. The mechanical strength of wet samples was also determined. Six samples of each composition were tested.

2.6. Microstructure observation

A scanning electron microscope was used to observe the microstructure and crystal morphology of calcium sulfate/vancomycin cylinders. Samples hydrated at room temperature overnight, and samples immersed in SBF for 2 and 4 weeks. The cylinders were dried for 3 days and then broken into small pieces. Central parts of broken samples were chosen for critical point drying (HCP-2, Hitachi, Tokyo, Japan). The samples were subsequently coated with gold ions using an ion sputter (E101, Hitachi, Tokyo, Japan) and then imaged by field emission scanning electron microscope (S-4800, Hitachi, Tokyo, Japan).

In order to demonstrate the influence of vancomycin on the crystalline phases of calcium sulfate, the calcium sulfate and calcium sulfate/vancomycin samples were identified by X-ray diffraction (XRD, Rigaku Denki, Japan) after being hydrated at room temperature overnight. The crystalline phases of the calcium sulfate samples with/without vancomycin were also identified by XRD after being immersed in SBF for 4 weeks.

2.7. Antibacterial activity assessment

The *in vitro* antibacterial activities of calcium sulfate/vancomycin samples were assessed by determining the zone of inhibition. The Gram-positive bacterial *Staphylococcus aureus* (*S. aureus*, BCRC 10780, Bioresources Collection and Research Center, Taiwan) and the agar gel diffusion method were conducted. 0.4 ml of diluted inoculum (4×10^5 CFU/mL) of test organism was spread on Muller–Hinton agar plates and incubated at 37 °C for 24 h. Three circular holes (5 mm in diameter) were punched in the peripheral area of one agar plate, and a calcium sulfate/vancomycin sample was placed in each hole. Ampicillin, which loaded in the central area of agar plate, was used as a reference material in the antibacterial

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