

Setting Time Comparison of Four Antimicrobial Laden Calcium Sulfate Plasters

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ABSTRACT

Background: The surgeon may implant calcium sulfate pellets (aka gypsum) as a resorbable antimicrobial vehicle at the surgical site in severe cases of osteomyelitis. Gypsum setting times with or without antibiotic additives are found scattered throughout the literature, but often factors known to alter setting time are either not disclosed or not held constant between experiments. To our knowledge, no prior study compares the setting time of calcium sulfate plaster mixed with the four commonly used antibiotics under constant conditions as presented here.

Purpose: To compare the setting times of calcium sulfate hemihydrate mixtures containing vancomycin, cefazolin, tobramycin, or amphotericin B.

Materials and methods: Groups consisted of samples comprised of 6.3 gm calcium sulfate hemihydrate (CSH) mixed with approximately 1/4th a vial of lyophilized antimicrobial (vancomycin, cefazolin, tobramycin or amphotericin B) with CSH powder to normal saline ratio of 1.7 gm/ml and mixed for 30 seconds at controlled speed and humidity. Each sample initial setting time (Ti) and final setting time (Tf) were established by Gillmore needles method according to ASTM standard C266-08 apparatus specifications.

Results: Kruskal-Wallis one-way analysis of variance by ranks revealed that antibiotic type affected the initial and final setting times of gypsum ($p < 0.05$). Post hoc analysis using Dunn's multiple comparisons indicated that there was no difference between control Ti (7.2 ± 1.1 min) and that of vancomycin or cefazolin group (9.8 ± 1.7 or 14.2 ± 1.3 min, respectively, $p > 0.05$), but the Ti of the tobramycin and amphotericin B groups (31.8 ± 5.7 and 140.4 ± 18.0 min) differed from the control Ti ($p < 0.05$). Likewise, there was no difference of control Tf ($p > 0.05$, 12.2 ± 1.1 min) when compared to vancomycin or cefazolin groups (22.2 ± 6.9 or 25.7 ± 4.1 min), but that the Tf of tobramycin and amphotericin B groups (76.3 ± 5.9 and 200.0 ± 21.1 min) each differed from the control group ($p < 0.05$).

Conclusion: This experiment is aimed to help surgeons plan when they should begin preparing their calcium sulfate antibiotic beads during surgery. As a general guideline, allow 15 minutes to set when adding a 1 gm vial of vancomycin or cefazolin, 30 minutes for adding a 1.2 gm vial tobramycin, and 2.5 hours for adding a 50 mg vial of amphotericin B.

Keywords: Calcium sulfate plaster, Antibiotic cement, Setting time.

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INTRODUCTION

Microbial infections are of increasing concern in the clinical setting.^{1,2} Deep seated bacterial infection after hip and knee

replacement are recognized as a serious threat to patients.³ Fungal infections, such as candidemia are also associated with economic burden caused by increased length of hospital stay (average of approximately 11 days more per case) and three-fold increased mortality rate.^{4,5} Select cases of osteomyelitis require aggressive surgical intervention due to their severity, leaving a void in which scar tissue may invade and prevent bony regeneration.⁶ Calcium sulfate bead implants at the surgical site show promise in the treatment and prevention of infection as a bone void filler and antimicrobial vehicle.⁷⁻¹³

Calcium sulfate hemihydrate (also called Plaster of Paris while unset, and gypsum when set) is commercially available as Osteoset (Wright Medical Technology, Inc, Arlington, TN, USA) and is historically known as a bone void filler with favorable scaffolding properties, safety, and *in vivo* resorption.^{7,14-16} Calcium sulfate is mainly used by the orthopaedic surgeon as antibiotic impregnated beads, where the rapid absorption rate of calcium sulfate may circumvent the need for removal that is seen when implanting other nonbioactive cements, such as polymethyl-methacrylate (PMMA).¹⁷ The literature lacks a single resource for surgeons to reference when attempting to mix their own antimicrobial laden calcium sulfate. The awareness of cement setting time is important when planning a timeline during surgery and predicting workability of the material.¹⁸ Gypsum setting times can be seen scattered throughout the literature, and it is known that antibiotic additives will affect gypsum setting and mechanical properties.¹⁸⁻²⁰ Method of setting evaluation, cement mixing and ambient temperature, and humidity are several key variables that affect setting time. However, they are not necessarily kept constant among calcium-based cement setting experiments.^{21,22} Here we provide the setting times of 98% pure calcium sulfate hemihydrate into calcium sulfate dihydrate when mixed with four commonly used antimicrobial agents: Vancomycin, cefazolin, tobramycin, and amphotericin B. In this study, we are trying to mimic what occurs in the clinical setting by mixing calcium sulfate in a ratio of one vial antibiotic to 25 gm calcium sulfate hemihydrate.

MATERIALS AND METHODS

Antimicrobial Sample Preparation

Vials of 1 gm vancomycin hydrochloride (Hospira, Inc, Lake Forest, IL USA), 1 gm cefazolin sodium (Sagent Pharmaceuticals, Schaumburg IL, USA), 1.2 gm tobramycin

sulfate (X-gen Pharmaceuticals, Inc, Big Flats, NY, USA), and 50 mg amphotericin B deoxycholate (Fungizone[®], X-gen Pharmaceuticals, Inc, Big Flats, NY, USA) were used. Calcium sulfate hemihydrate (CSH), GFS Chemicals, Inc, Columbus, Ohio, USA) was combined with lyophilized antibiotic powder in each sample per experimental group.

All samples contained 6.3 gm powdered CSH. Antimicrobial powders were weighed and the approximate amount added to each respective sample per group designated as follows (weights added reflect a 1 vial to 25 gm CSH ratio, with 25 gm CSH being obtained from common Commercial bead kits such as Osteoset[®]): Control group—no antimicrobial added; vancomycin group—0.25 gm vancomycin hydrochloride; cefazolin group—0.26 gm cefazolin sodium; tobramycin group—0.42 gm tobramycin sulfate; amphotericin B group—0.025 gm amphotericin B deoxycholate. Weighed CSH and antimicrobial powder was placed into 50 ml conical polypropylene tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA).

Mixing

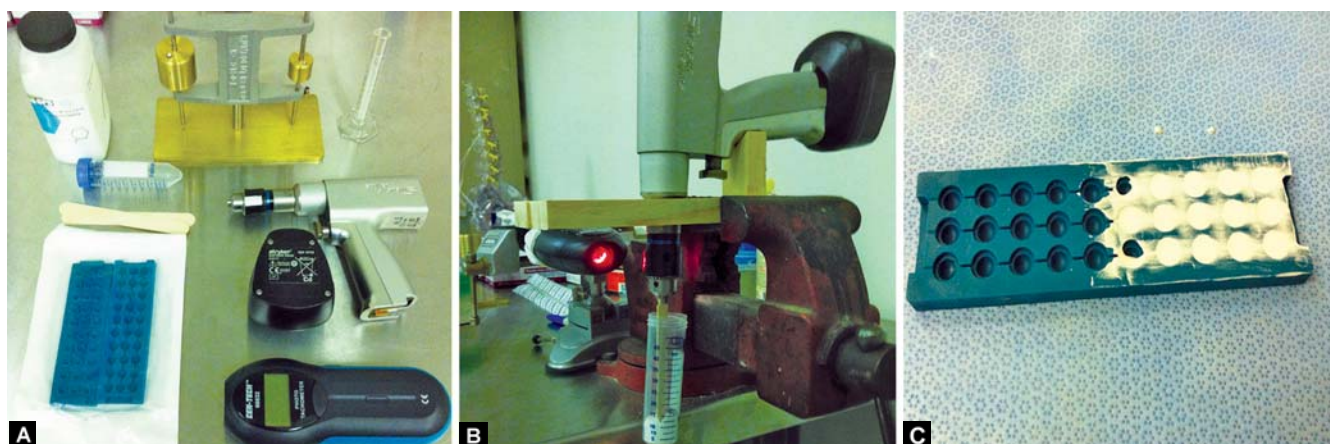
A surgical rotary drill (Stryker Orthopaedics, Kalamazoo, MI, USA) was mounted on a custom-made holster to stabilize the drill during mixing. A noncontact phototachometer (Harbor Freight Tools, Camarillo, CA, USA) was used to help maintain the drill speed between 90 and 100 rpm. The room was maintained at 24 to 26% humidity, with a temperature range of 17.8 to 20.0°C. A new wooden tongue depressor was used as the mixing blade and spatula for each cement sample. 3.7 ml of saline (sterile 0.9% sodium chloride solution, B Braun Medical Inc, Irvine, CA, USA) was added to each tube and a timer was set (CSH powder/normal saline ratio, P/L= 1.7). The solution was allowed to stand for 1 minute and then mixed for 30 seconds. The paste was applied evenly to a clean silicon bead mold (Wright Medical, Arlington, TN, USA) to produce 7 mm diameter hemispheres (Figs 1A to C).

Setting Assessment

Recording elapsed time began at the instant and saline was added to each respective sample. Setting times were assessed using Gillmore needles (Humboldt Mfg Co, Schiller Park, IL, USA) following ASTM standard C266-08 apparatus specifications. Gillmore needle initial setting time (Ti) was determined when the larger needle (2.12 mm diameter) with smaller 113.4 ± 0.5 gm weight attached rested on the cement for 5 seconds and made no discernable imprint on the pat in the bead mold when visually inspected by both investigators. After initial time was recorded for the sample, final setting time (Tf) was determined when the smaller, heavier needle (1.06 mm and 453.6 ± 0.5 gm) no longer made a complete circular impression after resting on the pat for 5 seconds. The first measurement of Ti was taken 5 minutes after saline was first added to the sample. Each subsequent measurement was taken at 2.5 minutes intervals until Ti and Tf had been reached. An illustration of materials used, drill holster, and CSH hemisphere pat can be seen in Figures 1A to C.

RESULTS

Setting time Ti and Tf means and standard deviations are reported in Table 1. Statistical analysis was performed using GraphPad Prism[®] software suite (GraphPad Software Inc, La Jolla, CA, USA). Kruskal-Wallis one-way analysis of variance by ranks revealed that antibiotic type affected the initial and final setting times of gypsum ($p < 0.05$, Fig. 2). Post hoc analysis using Dunn's multiple comparisons indicated that there was no difference between control Ti (7.2 ± 1.1 min) and that of vancomycin or cefazolin group (9.8 ± 1.7 or 14.2 ± 1.3 min respectively, $p < 0.05$), but the Ti of the tobramycin and amphotericin B groups (31.8 ± 5.7 and 140.4 ± 18.0 min) differed from the control Ti ($p < 0.05$). Likewise, there was no difference of control Tf ($p < 0.05$, 12.2 ± 1.1 min) when compared to vancomycin or cefazolin groups (22.2 ± 6.9 or 25.7 ± 4.1 min), but that the Tf of tobramycin and amphotericin B



Figs 1A to C: Materials and Gillmore apparatus (A), mixing setup (B) and calcium sulfate plaster pat in bead mold (C)

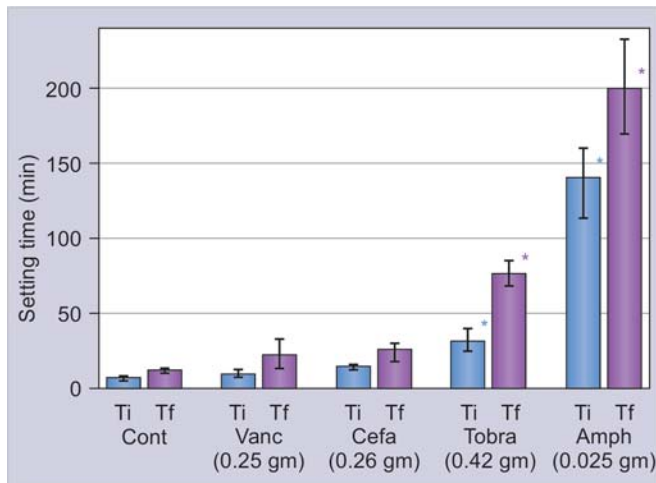


Fig. 2: Initial (Ti) and final (Tf) setting times of four calcium sulfate hemihydrate mixtures. Setting times (Ti) shown of cements mixed with vancomycin hydrochloride (n = 7), cefazolin sodium (n = 8), tobramycin sulfate (n = 7), or amphotericin B deoxycholate (n = 7) as determined by Gillmore needles method. Kruskal-Wallis analysis of variance by ranks determined antimicrobial type had an effect on gypsum setting time (Ti, p < 0.05; Tf, p < 0.05). Error bars display range. *Dunn’s multiple comparisons (p < 0.05)

groups (76.3 ± 5.9 and 200.0 ± 21.1 min) each differed from the control group (p < 0.05).

DISCUSSION

This report describes the setting times of four commonly used antibiotic laden plasters as determined by Gillmore needles method. Vancomycin hydrochloride, cefazolin sodium, tobramycin sulfate, and amphotericin B deoxycholate were mixed with calcium sulfate hemihydrate at the approximate ratio of 1 vial lyophilized antimicrobial per 25 gm calcium sulfate hemihydrate (CSH powder/saline ratio: 1.7 gm/ml). Antibiotic and CSH values were chosen to represent product weights commonly prescribed in the clinical setting to form 7 mm spherical beads to be implanted as a local antimicrobial vehicle, with setting times reported in Table 1.

Setting comparisons of antimicrobial laden gypsum under constant reaction conditions are scarce in the literature. This lack of consistency among reports makes it difficult for the surgeon to predict when he or she should begin preparing antibiotic beads. Failure to maintain controlled mixing or powder to liquid ratios may lead to unpredictable setting and resultant mechanical characteristics.²³

Amphotericin B and tobramycin groups showed statistical difference in setting times in comparison with controls. It has been shown anecdotally that tobramycin sulfate acts as a mild retarder of gypsum setting,¹² and our results are consistent with the literature.

Amphotericin B cements showed a markedly prolonged setting time not just over the control group, but also over all other antibiotic cement formulations. Sodium phosphate found in the amphotericin B formulation used is known as a setting retarder of gypsum and is found in phosphate-based commercial retarders. The mechanism of gypsum retardation by polyphosphate has recently been investigated by Nilles et al.²⁴ Amphotericin B is practically insoluble in water in the pH range of 6 to 8, and as such, pharmaceutical formulations of the drug invariably contain surfactants such as cholesteryl sulfate (Amphotec[®]), deoxycholate (Fungizone[®]) and various phospholipids (Abelcet[®], Ambisome[®]) in order to effectively solubilize the drug. The setting of calcium sulfate cements is predicated on the nucleation of calcium sulfate dihydrate crystals to form an interlocking rod-like structure, and this crystallization process is greatly affected by the presence of surfactants,²⁵ which not only substantially retard the kinetics of crystal formation and growth, but also modify both the morphology and size distribution of crystals, via adsorption of surfactant molecules onto crystal faces.

The results of our study could be used by the clinician to predict setting times of calcium sulfate and allow the clinician to appropriately time when the calcium sulfate and antibiotics should be mixed intraoperatively. Commercial Osteoset[®] formulations commonly use a proprietary mixture of 25 gm calcium sulfate with 7.8 ml saline (3.2 gm/ml powder/liquid ratio). It is known that increasing the P/L ratio decreases time required to reach a clinically relevant setting time. With this understanding, we predict that commercial mixtures of CSH with accelerators or high P/L ratios will require less time in setting on average than the Ti values (Table 1). In the case that a paste cannot be formed after adequate mixing, small volumes of saline may be added until a free-mixing paste is acquired.

LIMITATIONS

The Gillmore needles method of setting time evaluation uses a subjective visual inspection to determine setting endpoints.

Table 1: Initial (Ti) and final (Tf) setting times of antibiotic laden gypsum

Groups	Ti (minutes)			Tf (minutes)		
	Mean	SD	n	Mean	SD	n
Control	7.2	1.1	6	12.2	1.1	6
Vancomycin	9.8	1.7	7	22.2	6.9	7
Cefazolin	14.2	1.3	8	25.7	4.1	8
Tobramycin	31.9	5.7	7	76.3	5.9	7
Amphotericin B	140.4	18.0	7	200.0	21.1	7

This subjectivity has led to additional criticism of the comparisons between setting experiments by those in favor of quantifiable methods.^{22, 26, 27} We believe that setting times by Gillmore needles are still relevant to clinical practice despite this subjectivity,²⁸ with our antibiotic hemispheres appearing stable enough for implantation at Ti and later. We are concerned with implantation of CSH before adequate curing, as mechanical properties and altered hygroscopic setting expansion^{29, 30} may contraindicate the implantation of uncured antibiotic-impregnated beads in a confined space.

Amphotericin B samples showed marked setting contraction, most likely due to surfactant-induced modification of crystalline morphology and size distribution, and this may have contributed to increased variability in setting time. It became difficult to find flat surfaces for needle placement on the hemisphere pats, and subsequently made it slightly more challenging to confirm an incomplete circular impression at Tf.

CONCLUSION

This experiment is aimed to help surgeons plan when they should begin preparing their calcium sulfate antibiotic beads during surgery. As a general guideline, allow 15 minutes to set when adding a 1 gm vial of vancomycin or cefazolin, 30 minutes for adding a 1.2 gm vial tobramycin, and 2.5 hours for adding a 50 mg vial of amphotericin B.

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