Effects of Composition and Setting Environment on Mechanical Properties of a Composite Bone Filler

M.E. Brown, Y. Zou, D.A. Puleo
Center for Biomedical Engineering
University of Kentucky
Lexington, KY, USA

Introduction

Calcium sulfate (CS) is a commonly used bone grafting material due to its good biocompatibility and long history of clinical use as a biomaterial. High energy traumas often involve significant damage to both the soft and hard tissue of extremities and the head region. These defects are irregular in shape and are usually accompanied by infection which inhibits the healing process. Addition of viscous biopolymers to CS can create a bone filler that is capable of filling irregular voids in hard tissue. To further enhance CS's osteoconductive properties, bioactive molecules can be incorporated into the material to promote bone growth and prevent infection.

The goal of this work was to create an implantable, moldable CS bone filler loaded with bioactive molecules to enhance bone formation in irregular defects.

Methods

Gelatin microspheres were prepared by a single emulsion procedure and then crosslinked with 5mM glutaraldehyde for 12 hours at 4°C. The crosslinked microspheres were collected and immersed in a 50mM glycine solution to block residual aldehyde groups. Cellulose acetate phthalate/Pluronic F-127 (CAPP) microspheres were prepared using a double emulsion process. Both types of microspheres were lyophilized for 24 hours prior to use.

Samples were made with varying amounts of CS, CMC, gelatin microspheres, and CAP microspheres. Expressed in wt. %, the primary ratios studied were 80/10/5/5, 85/5/5/5, and 90/5/2.5/2.5 (%CS/%CMC/% Gelatin/%CAPP). Components were dry mixed before the addition of 700, 650, or 600 µl/g deionized water, respectively. After mixing, the resulting material was packed into cylindrical Delrin molds. The composite remained in the molds until the cylindrical samples, 3mm in diameter and 6.15mm tall, could be pushed out without deformation, between 10-20 minutes. Samples were then placed in one of three environments to set: 1) air dry at room temperature, 2) a fully humidified cell culture incubator at 37°C, or 3) immersion in 5ml phosphate-buffered saline, pH 7.4 (PBS) 37°C.

Compression testing was performed using a Bose ELF 3300 mechanical testing system on samples at 1, 2, 4, 7, and 14 days of setting. Samples were loaded at 5N/sec until failure, and the elastic modulus (E) and ultimate compressive strength (UTS) were calculated.

Results and Discussion

Because the ultimate goal of this project is to develop a moldable bone filler material that can set *in vivo*, the environment in which samples are dried is of great importance. Three different scenarios were examined to simulate the warm and moist environment that an implanted material would be exposed to (Figure 1). A drastic difference in the resulting moduli, ranging from 0 to 500 MPa, was seen depending on what environment the filler was cured in. When CS was placed in a wet or humidified environment before it had completely dried, it remained saturated with water and was unable to set completely. It is likely that the bone filler will be curing in an environment somewhere between the completely wet PBS and the fully humidified incubator, and care will have to be taken to ensure that the material maintains its mechanical integrity while setting *in vivo*.

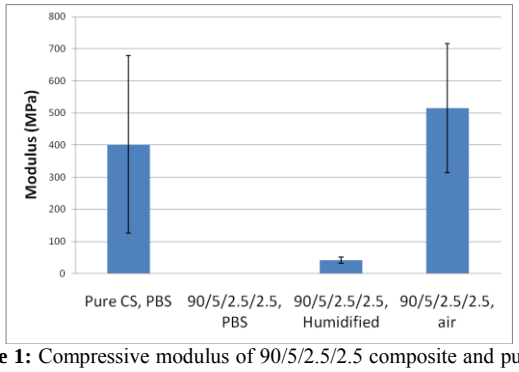

Figure 1: Compressive modulus of 90/5/2.5/2.5 composite and pure CS in different drying environments for 1 day. (Data are mean ± SD, n=4)

To better understand how the different components of the filler material affected the overall mechanical and drying properties, samples were mechanically tested at five time points as they dried in a fully humidified chamber (Figure 2). Addition of more microspheres and CMC to the CS caused a drop in the peak modulus and a slower setting time, likely due to CMC and gelatin microspheres retaining water.

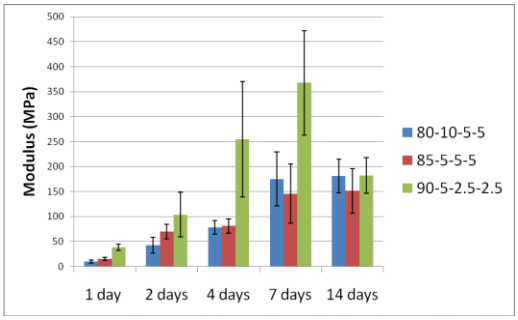

Figure 2: Compressive modulus of moldable bone filler dried in a humidified environment. (Data are mean ± SD, n=4)

Conclusion

To create a moldable bone filler material that is also biodegradable and capable of promoting bone growth while also treating infection, a balance will have to be found between the mechanical properties, setting times, and drug release characteristics of the bone filler material.

Acknowledgement

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