INTRODUCTION:
The composite of dimineralized bone matrix (DBM) has been used to induce osseous integration including bone defect repairs as well as spinal fusion due to its osteoconductive and osteoinductive properties.[1] However, DBM, in the form of powder, gel or putty, lacks mechanical properties when used at loading sites. A novel gelatin based material crosslinked with silane and fructose (MGSF) was developed and exhibits superior properties over gelatin carrier including improved tensile strength, stable thermodynamic property and slow degradation by proteolytic enzymes as well as retaining biocompatibility.[2] In this study, DBM and MGSF were composed as a bone graft and its mechanical and biological properties were characterized in vitro and in vivo rat fibular defects model.

METHODS:
Preparation of DBM/MGSF: Modified gelatin was prepared with silane, z-6040 and z-6011 (Dow-Corning) and (D)-fructose (MGSF). Briefly, in 100ml of 5% w/v gelatin solution (pH 5.0), fructose (Calbiochem, CA) and silanes were added to make 0.5% (w/v) fructose and 3% z-6040 and 2% v/v z-6011. Solution was heated over 90°C for 10 min and then cooled down to 37°C. MGSF was blended with rat DBM (particle size 210-800 µm) at 1:1 volume ratio. The mixture was molded and lyophilized. DBM activity was previously tested in vivo and inactive DBM was quenched by guadinine-HCl extraction for 48 hours from lyophilized. DBM activity was previously tested in vivo and inactive DBM showed 90.88% release of final growth factor in 2 days. Afterward, it released a negligible amount of growth factor. In contrast, DBM/MGSF released continuously growth factor up to 96 hours.

Cell attachment and growth on DBM/MGSF block: Freezer-dried DBM/MGSF composites (6mm diameter and 2mm thickness) were placed to 24 well-plate. C2C12 (ATCC) with the density of 100k cells/ml in 10% FBS/DMEM were seeded to each MGSF/DBM scaffold and allowed to attach on the surface at 37°C with 5% CO₂ for 6 hours. Then, 1ml of fresh medium was added to cover the disc. After 3 days, cell attachment and growth were visualized under microscope (Olympus).

Accelerated growth factor release assay from DBM/MGSF block: In 200mg of freeze-dried DBM/MGSF composite, 500µl of 20U/ml of collagenase type II solution (Worthington) was added. At each decided time interval, digested solution was collected and replaced with fresh enzyme solution. The extractions were tested with C2C12 cells. Briefly, 12.5x10⁳ cells of C2C12 (ATCC) were plated in each well of 96-well plate in a medium of 10% FBS/DMEM. After 5-hour attachment, the harvest was changed into DBM assay medium containing 1% FBS/DMEM and 30 µl of DBM/MGSF extracts. After 48-hour of incubation, cells were washed with cold PBS three times prior to alkaline phosphatase and total protein assays.[3]

Implantation of fibular critical size defect: Bilateral 6 mm-long diaphyseal defects were created in rat fibula. An 8 mm-long DBM/MGSF rod with drilled orifices on both ends was used to bridge the gap. All animal were used with followed protocols approved by IACUC of the University of Southern California. A total of 4 rats (Fisher 344, 8 weeks old) were used with active DBM/MGSF grafts (n=4) placed at one side and guadinine-HCl inactive DBM/MGSF (n=4) on the other side. Animals were euthanized 3 weeks postsurgery to determine bone repair rates by radiography and histology.

RESULTS

Appearance of DBM/MGSF composite were shown in Fig. 1. MGSF gel adhered DBM particles to form a moldable composite. Lyophilized composite formed 3D porous rigid structure but flexible. When placed in fluid, it maintained its structure over 7 days [Fig 1-(a) and (b)]. Porous structure allowed cell penetration and support cell attachment and growth on DBM surfaces.

DISCUSSION
When DBM were combined with MGSF gel to form a moldable putty, it significantly enhanced the DBM handling and delivery characteristics. After lyophilization, DBM/MGSF composite formed a porous and flexible structure with improved mechanical properties and well developed pores for cell ingrowth and new bone formation. DBM/MGSF released growth factor slower than DBM alone due to slow MGSF degradation rate. In vivo preliminary data showed DBM/MGSF can be an osteoinductivie and osteoconductive bone graft with optimal mechanical properties for orthopedic application.

REFERENCE