Autologous and Allogenic In vivo Engineered Extracellular Matrix as Tissue-Engineered Periosteum for Defect Repair

Chen Jiang[1], Tianfeng Miao[1], Nicholas Lenhard[1], Xinping Zhang[1*]
[1]Center for Musculoskeletal Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY, 14642, USA
[*]xinping_zhang@urmc.rochester.edu

INTRODUCTION: While periosteum tissue engineering has become a mainstay in repair and reconstruction, an emerging surgical technique named Masquelet technique that combines a foreign body reaction-induced vascularized tissue membrane with bone chips/matrix for repair and reconstruction of large bone defect has gained wide attention in regenerative medicine. Combining bone tissue engineering technologies with induced membrane technique provides an exciting new arena for management of bone defect repair. Inspired by Masquelet techniques, we previously reported an approach utilizing foreign-body reaction-induced, *in vivo* engineered decellularized extracellular matrix (dECM) as a periosteum mimetic for repair and reconstruction of segmental bone defects. The approach involves 3D printing, *in vivo* implantation of a polylactic acid (PLA) templates, followed by depolymerization and decellularization to create a dECM matrix with desired pattern and architecture. The advantages of using engineered induced dECM membrane as a functional periosteum mimetic include: 1) ease to control the architectural and overall size of the scaffold through versatile 3D printing technology; 2) creation of a patient-specific, personalized, autologous natural ECM as scaffolding material for regeneration; 3) the reduced immunogenicity of autologous dECM that favors bone regeneration; 4) preservation of natural proteins/proteoglycans, cell adhesion ligands, and biological signals on natural ECM; and 5) an ideal large dECM surface for cell attachment and drug delivery. The goal of our current study is to compare the reparative outcome using allogenic and autologous dECM as tissue-engineered periosteum for allograft bone-mediated large bone defect repair. To enhance the osteo-inductive activity of the engineered matrix, we examined the capacity of the dECM to bind and deliver growth factors to bone healing site. Our study showed that autologous dECM performed better than allogenic dECM, further permitting delivery of low dose BMP2 to enhance bone formation and bone allograft incorporation.

METHODS: *Fabrication of dECM matrix.* Polylactic acid (PLA) templates (4-layers, 10x10 mm) were 3D printed and implanted subcutaneously in B6 mice. The implants were harvested after 4 weeks and processed via a series of steps of depolymerization and decellularization to generate dECM matrix. dECM matrices were placed in 96-well plate containing BMP2 (100 ng/dECM, 50 µl/well) and incubated at 4 °C overnight. *Implantation of dECM for repair of allograft*. A 4mm segmental femoral bone graft transplantation model was used to evaluate the efficacy of tissue-engineered periosteum in reconstruction of long bone defect. Col1(2.3) GFP mice was used to track osteoblasts at the healing site. Allogenic, autologous dECM, and BMP2-dECM scaffolds were wrapped around 4 mm allografts and implanted into the same or a different mouse that generated the dECM matrix. Micro-CT, histomorphometric, and torsional biomechanical analyses were performed at 7-week post-implantation to evaluate healing.

RESULTS: Micro-CT scanning showed that treatment of allogenic, autologous and BMP2 loaded dECM matrix all achieved enhanced callus formation (Fig. 1a). Compared with allograft control, volumetric analysis demonstrated that allogenic dECM, autologous dECM, and BMP2-dECM had 1.36, 1.73, and 2.14-fold larger total new bone callus, respectively (Figure 1c, n=5-11, $p<0.05$). Histologic analyses showed that compared with allograft and allogenic dECM, autologous dECM and BMP2-dECM induced significantly more periosteal bone callus formation across the surface of allograft (Fig. 2a), leading to better osseointegration between host and donor. Fluorescence images further showed newly formed bone tissues with Col1(2.3) GFP^+ osteoblasts on allograft surface in each group (Figure 2b). Quantitative histomorphometric analysis showed that fibrotic tissue formation in autologous dECM and BMP2-dECM treated samples was reduced to 55% and 36% of that of the control allograft. Consequently, bone formation was increased by 2.10 and 2.48-fold, respectively (Figure 2c, n=5-11, $p<0.05$). Finally, the autologous dECM-wrapped allograft significantly improved biomechanics of bone allograft healing, restoring the torsional rigidity and maximum torque to 91% and 55% of those of intact femurs, while allograft alone only had 6% and 13% of those of the intact bone (Fig. 2d and 2e, n=8-16, $p<0.05$).

DISCUSSION: Autologous and allogenic dECM matrices as tissue engineered periosteum were used to improve bone allograft repair. Our data show that autologous matrix induced more periosteal bone callus formation and recruited more osteoblasts to allograft surface than allogenic dECM. This could be attributed to the fact that allogenic scaffolds could generate a stronger immune response than autologous dECM during repair. More analyses are currently underway to determine the immune cells, including macrophage infiltration in allogenic and autologous dECM. In addition, the degradation of the allogenic and autologous dECM will also be examined. BMP2 has been widely used in the Masquelet techniques to repair large segmental defects. However, the success requires a large dose of BMP2, often as high as 12 mg per treatment. Excessive amounts of BMP2 are shown to be associated with increased risk of adverse effects such as infections, osteolysis, heterotopic bone formation, and immune response. Utilizing dECM, we showed that loading ~100ng BMP2 per dECM graft achieved markedly induction of periosteum callus formation, reduction of fibrotic tissue and further improvement of osseointegration. Taken together, our results support that engineered autologous dECM could be directly used in bone regeneration applications or as a vehicle to deliver bioactive molecules to bone healing sites.

SIGNIFICANCE/CLINICAL RELEVANCE: The success of our current study could establish a new line of versatile, patient-specific, and periosteum-like autologous dECM matrices for bone tissue engineering, potentially offering personalized therapeutics to patients with impaired healing for one-stage repair and reconstruction of large weight-bearing defects.

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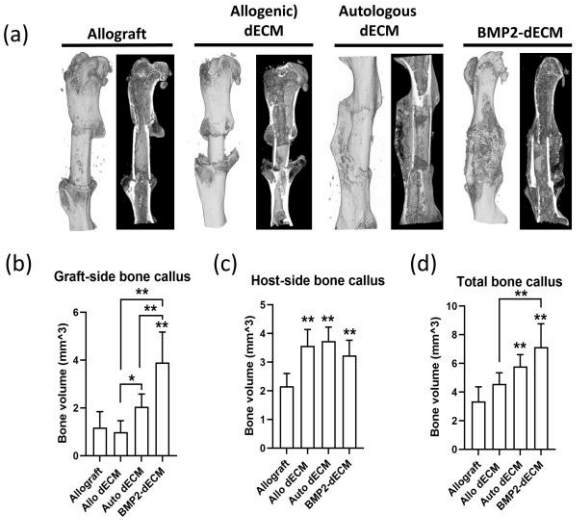

Figure 1. (a) Representative MicroCT 3D images of allograft, allogenic dECM, autologous dECM, and BMP-dECM at week 7 post surgery. Volumetric MicroCT analyses of new bone in graft **(b),** host **(c)** and total callus **(d).** (n=5-11, *p < 0.05 **p < 0.01, one-way ANOVA test, Tukey for multiple comparisons)

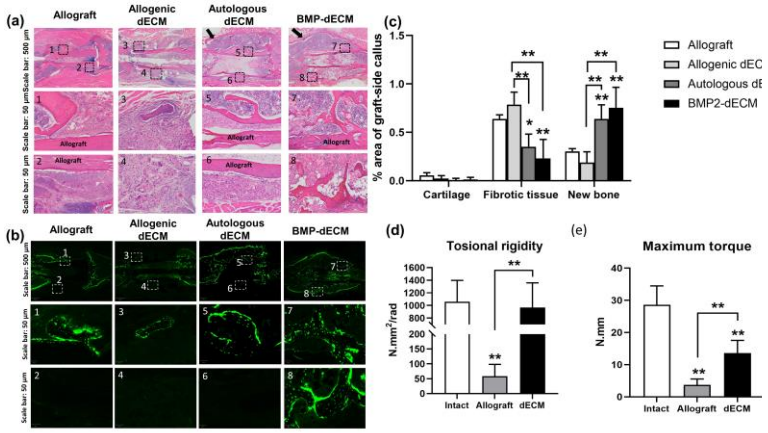

Figure 2. AB/H staining **(a)** and Fluorescence images **(b)** of various types of grafts at injury site. Quantitati histomorphometric analyses show percent of cartilage, fibrotic tissue and new bone in the graft-side periosteal callus **(c)** (n=5, *p<0.05, **p<0.01, one-way ANOVA test, Tukey for multiple comparisons). Torsional rigidi (d) and maximum torque (e) in allograft and autologous dECM groups as opposed to intact femurs from no surgical contralateral femurs of the surgical animals (n=8-16, *p < 0.05 **p < 0.01, one-way ANOVA te Tukey for multiple comparisons).