Microribbon-based Scaffolds Accelerate Bone Regeneration in a Cranial Defect Model

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Introduction: Bone loss affects millions of patients in the United States annually and can be caused by traumatic injury, pathological diseases, congenital malfunction or oncologic resection. Adipose derived stromal cells (ADSCs) represent a promising source of autolgous stem cells cells for bone repair given their relative abundance and potential to differentiate towards bone lineage. For repairing large bony defects, scaffolds with macropores are highly desirable to promote nutrient diffusion, fast blood vessel in growth, and new tissue formation. Various methods have been developed for fabricating macroporous scaffolds including particle-leaching, phase separation, gas foaming and electrospinning. However, these methods require the use of non-physiological conditions and are not cell-friendly. As a result, cells can only be seeded onto the scaffolds post-fabrication, which makes it very difficult to achieve homogeneous cell seeding in scaffolds for repairing large bony defects. To overcome this limitation, we have recently reported development of **microribbon-like, crosslinkable elastomers as scaffold building blocks**, which support direction cell-encapsulation while simultaneously forming macroporous scaffolds [1]. The goal of this study is to evaluate the potential of microribbon (uRB)-based, macroporous scaffolds for repairing bony defects in vivo using a mouse critical size cranial defect model.

Methods: Methacrylated gelatin (GelMA) was prepared by existing protocol. Crosslinkable, gelatin-based uRB were synthesized as reported [1]. Passage 2 mouse ADSCs isolated from GFP-Luciferase positive mice were used for the study. For cell encapsulation, mADSCs were mixed with uRBs or GelMA (10% in PBS) to reach a concentration of 10 M/ml, and photocrosslinked into cell laden cell scaffolds (365nm, 2mW/cm^2, 4 min). Acellular scaffolds were included as controls. Critical size cranial defect (4 mm) was made in athymic mice in the parietal bones as previously reported. Four groups were tested including uRB-based scaffold with mADSCs, HG-based scaffold with mADSCs, and both types of scaffolds without cells. Mice with empty defects were used for negative control. From day 0 to week 6, cell viability and location of implants were monitored weekly via bioluminescence imaging (BLI). At 0, 2, 4, and 6 weeks, the mineralization of the implants was monitor by micro computed tomography (u-CT) scanning. Animals were harvested on day 3 and at week 6 for histology.

Results: Confocal imaging showed extensive cell adhesion and spreading of mADSCs throughout 3D uRB-based scaffolds 24 h post cell-seeding (Fig. 1A). In contrasts, cells in hydrogels remained round morpholgy with minimal cell spreading (**Fig.1B**). By week 6, uRB-based scaffolds also resulted in twice as many ADSCs in comparison with the HG-based implants by week 6 as shown by bioluminescence imaging. Histological staining of CD31 showed higher blood vessel density inside uRB-based scaffolds vs. HG scaffolds. MicroCT imaging results demonstrated that uRB-based scaffolds accelerated bone regeneration with almost complete filling of the defects, yet minimal bone repair was observed in the HG group (Fig. 1C). The acellular control groups showed negligible bone regeneration, with less than 6% of bone volume refilled. Histology also showed the highest level of collagen deposition in the uRB-ADSC group.

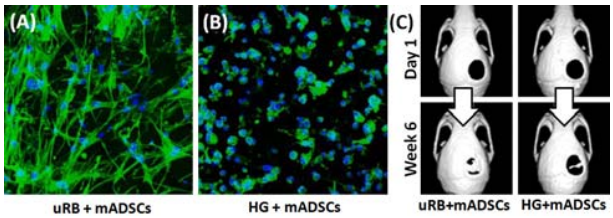

Fig.1. (A-B) uRB-based scaffolds, but not HGs, induced extensive cell spreading 24 h post-encapsulation. (C) MicroCT images showed that uRB-based scaffolds accelerated bone regeneration and almost filled up the defects by wk 6, yet minimal bone formation was observed in HG-based implants.

Conclusions: Here we report microribbon-based scaffolds as a novel platform to accelerate *in vivo* bone repair using in a critical sized, cranial defect model. In comparison with conventional hydrogels, the uRB-based scaffolds supported cell spreading, which may promote osteogenesis via mechanotransduction of cytoskeleton tension. Furthermore, the macroporosity of uRB-based scaffolds promoted cell proliferation, blood vessel ingrowth, and deposition of mineralized bone matrix. The platform reported herein may be particularly useful for repairing large defects of load-bearing tissues such as bone and cartilage.

Reference: [1] L.H. Han, S. Yu, T.Y. Wang, A.W. Behn, F. Yang, *Advanced Functional Materials*, **23**, 346-358, 2013.