Microribbon-based macroporous matrices enhance cartilage repair in a rat osteochondral defect model

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INTRODUCTION.

- Injury of articular cartilage and subsequent joint degeneration represents one of the leading causes of disability with a huge socioeconomic burden. Current surgical treatments such as microfracture result in undesirable formation of fibrocartilage with poor long-term outcomes.
- Hydrogels offer an attractive strategy for treating cartilage defects given its injectability and minimally invasive delivery. However, conventional hydrogels are nanoporous, often slow in supporting neocartilage regeneration, and challenging to maintain structure integrity upon mechanical loading.
- Our group has reported gelatin microribbon (μRB) hydrogels as a novel 3D scaffold that contains macroporosity and support homogeneous cell encapsulation, while demonstrating cartilage-mimicking shock-absorbing capacity [1]. Compared to nanoporous gelatin hydrogels (HG), the macroporous gelatin μRB hydrogels substantially accelerated MSC-based cartilage regeneration in vitro, with over 20-fold increase in Young's modulus in 3 weeks, approaching the range that mimics native cartilage [2]. However, how μRB hydrogels would perform in a cartilage disease model has never been investigated before.
- The goals of this study are to evaluate the biocompatibility and efficacy of gelatin μRB scaffolds for supporting endogenous cartilage regeneration in a rat osteochondral model, and to assess whether in situ delivery of transforming growth factor beta (TGFβ) would further enhance μRB-mediated cartilage regeneration in vivo.

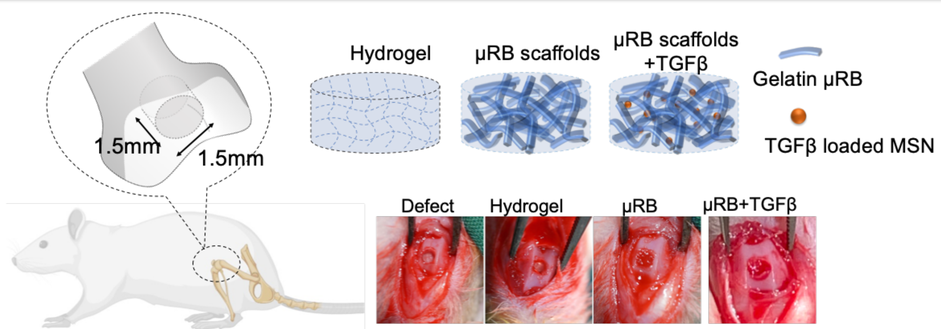


Figure 1. Methods. A rat osteochondral defect model was used as previously reported [3]. The untreated defect was used as a control. Three scaffold implant groups were assessed including nanoporous gelatin **hydrogel**, microporous gelatin μRB hydrogels (**μRB**), and gelatin μRB plus TGFβ. Gelatin μRBs scaffolds were fabricated into sheet with thickness of 1.5 mm. The formed sheets were punched with a biopsy punch (ϕ2.0 mm) to obtain the construct for implantation. The TGF beta was loaded using the dopamine coated mesoporous silica nanoparticles (MSNs), which has been recently reported by our group that supports the prolonged release of growth factors in μRB scaffolds.

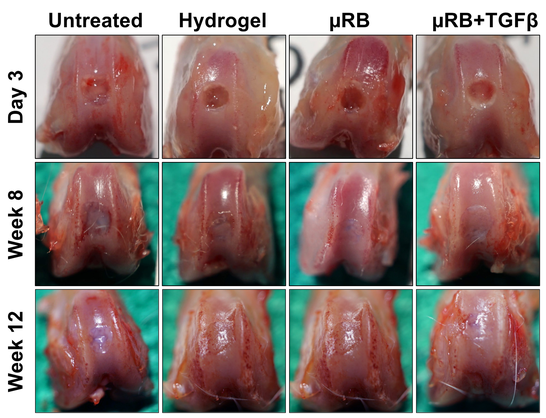
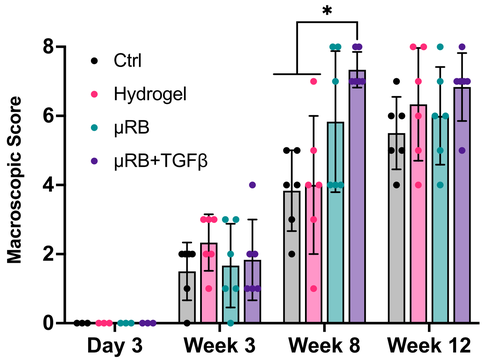


Figure 2. Macroscopic scoring. Morphology of the joint defects was scored using the Niederauer system. By week 8, the μRB scaffolds accelerated cartailge defect healing compared to untreated ctrl or Hydrogel groups. μRB+TGFβ further accelerated defect filling with the highest score. By week 12, the defects were mostly filled in all groups with comparable score.



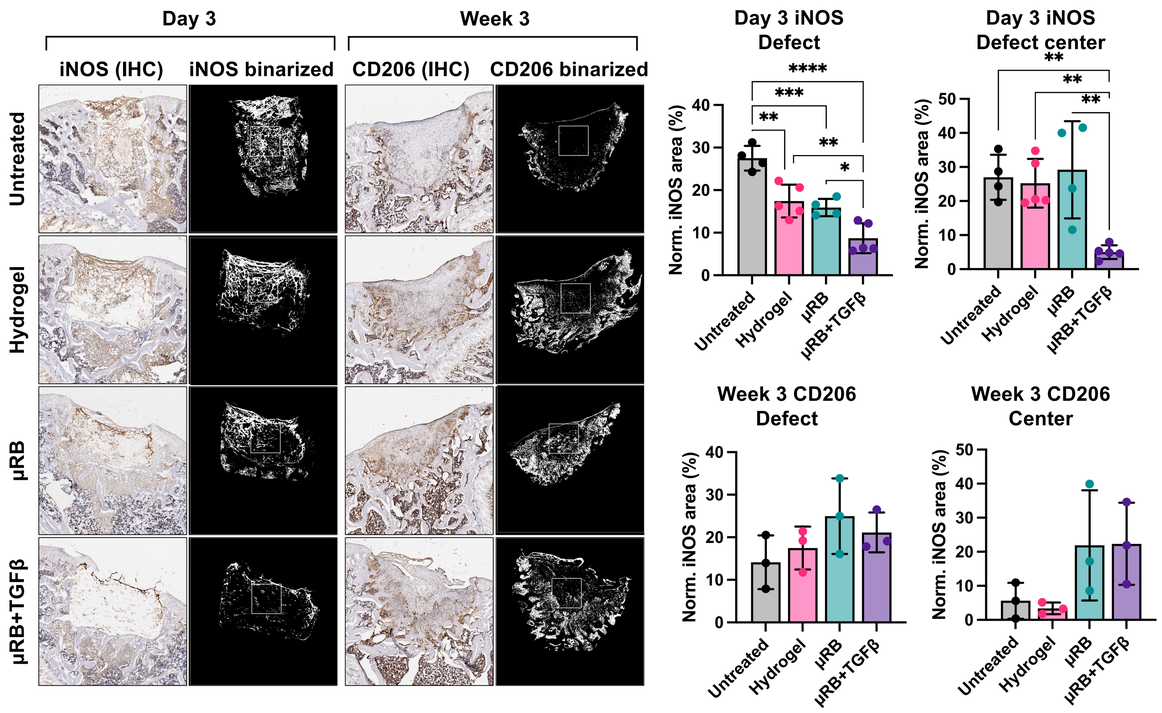


Figure 3. Inflammatory response. By day 3, all implants remained in the defects, while the highest number of pro-inflammatory macrophages (M1) was detected in defect-only control, as shown by immunostaining of iNOS, a marker for pro-inflammatory M1 macrophages. Both HG and μRB groups significantly reduced M1 infiltration, and TGFβ delivery further reduced M1 infiltration . Histological analysis of the patella of the joints revealed minimal inflammatory response in the surrounding tissues for all groups. By week 3, M1 macrophages were rarely seen in all groups and were replaced by pro-regenerative M2 macrophages. All groups treated with scaffold implants recruited more M2 macrophages compared to untreated defects. The quantification is extracted from the coverage of binarized positive staining of the defect area and the center square (0.5 mm x 0.5 mm).

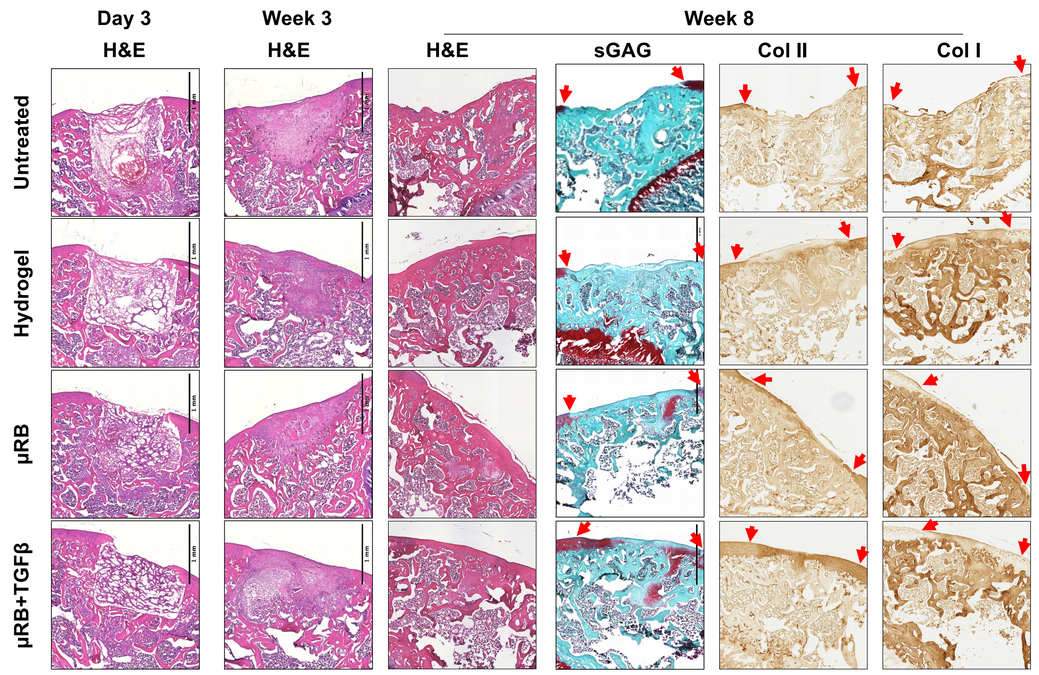


Figure 4. Cartilage defect repair. Harvested joint tissues were assessed by histology and immunostaining. The H&E staining confirmed that the μRB scaffolds were well integrated with the surrounding tissue at early time point (day 3) and were mostly degraded by week 3. By week 3, subchondral bone has started to regenerate, which is required before subsequent cartilage regeneration. By week 8, the μRB and μRB+TGFβ groups showed complete filling of the defects. Positive Saf'O staining of sGAG (cartilage marker) was observed in μRB groups, but not in untreated control or HG. The most robust articular cartilage phenotype was observed in TGFβ+μRB group, as shown by highest Collagen II and less Collagen I staining. In contrast, untreated control and hydrogel groups showed minimal sGAG and collagen II deposition. (↓ indicating defect edges)

Conclusion

- We report the first in vivo evaluation of the biocompatibility and efficacy of macroporous gelatin microribbon hydrogels for supporting endogenous cartilage repair using a disease-relevant model. Compared to the control (similar to microfracture), gelatin μRB significantly reduced the acute pro-inflammatory response by decreasing M1 macrophage recruitment, while supporting the recruitment of regenerative M2 macrophages.
- In situ delivery of TGFβ from microporous gelatin μRB hydrogels further reduced inflammation and accelerated endogenous cartilage regeneration with enhanced hyaline cartilage phenotype. Compared to the current clinical standard (control), adding gelatin μRB with in situ growth factor delivery represent a promising therapy to significantly enhance the efficacy of articular cartilage repair.

REFERENCES: 1. Han, L.H. et. al, *Adva Funcl Mater* 23, 346-358 (**2013**). **2.** Conrad, B., et. al, *Regen Eng Transl Med* 7, 301-311 (**2019**). **3.** Pagnotto, M.R., et al., Gene Ther 14, 804-813 (**2007**).

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