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## Repair and Regeneration of Chondral Defects: An In Vitro Study Demonstrating Feasibility and Mechanism under Low Intensity Ultrasound

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# Repair and Regeneration of Chondral Defects: An In Vitro Study Demonstrating Feasibility and Mechanism under Low Intensity Ultrasound.

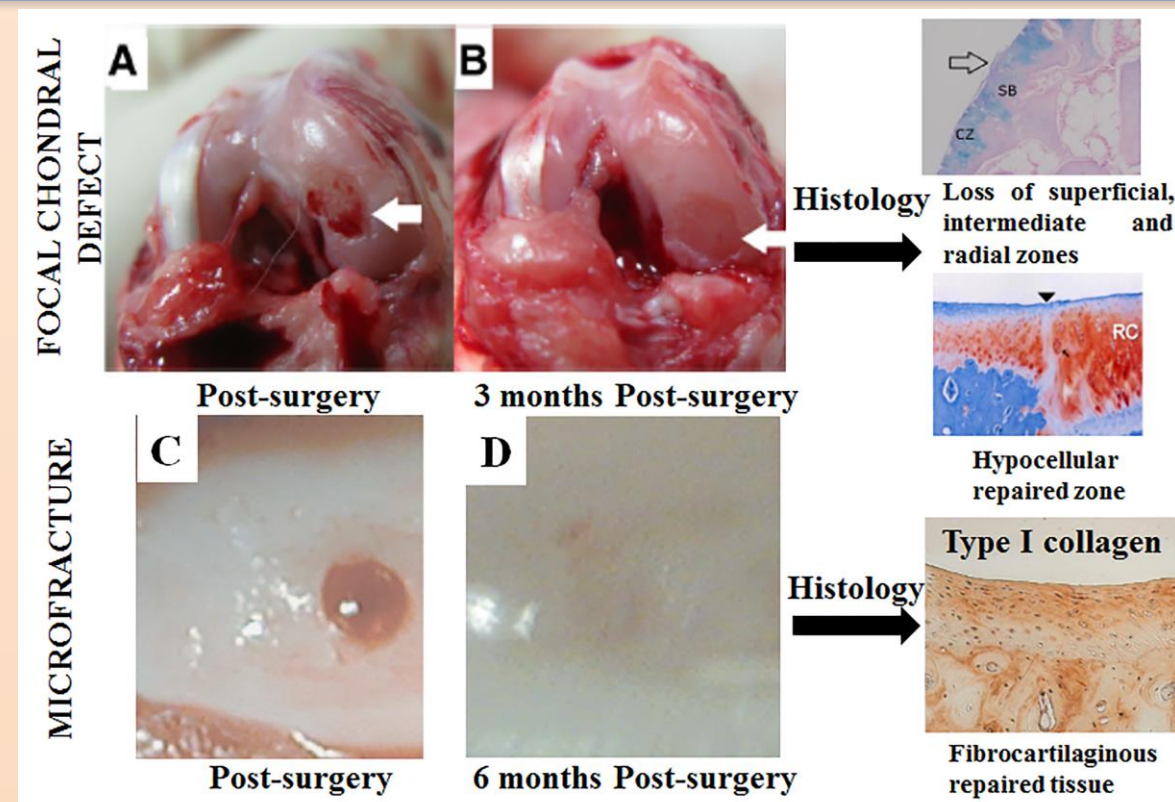
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## ABSTRACT

Holistic repair of damaged cartilage remains an unsolved biomedical problem. Current methods that employ microfracture (MF) or autologous chondrocyte implantation (ACT) or tissue engineered strategies yield inferior repaired cartilage (Fig. 1). Lack of chondroinductive factors at the site of injury, in vivo, has been identified as a factor that limits repair. Clinically amenable strategies that can improve repair are desired. A novel clinically translatable repair strategy based on low-intensity-ultrasound (US) is proposed (Fig.2). Differently from all approaches that use US, our approach employs US at the cell resonant frequency where bioeffects are maximized. We have shown that US impacts the cellular response by promoting proliferation, chondrogenesis and healing at interfaces. In conjunction with the demonstration that US propagates in joint space, this work is impactful in the development of in vivo treatment strategies.

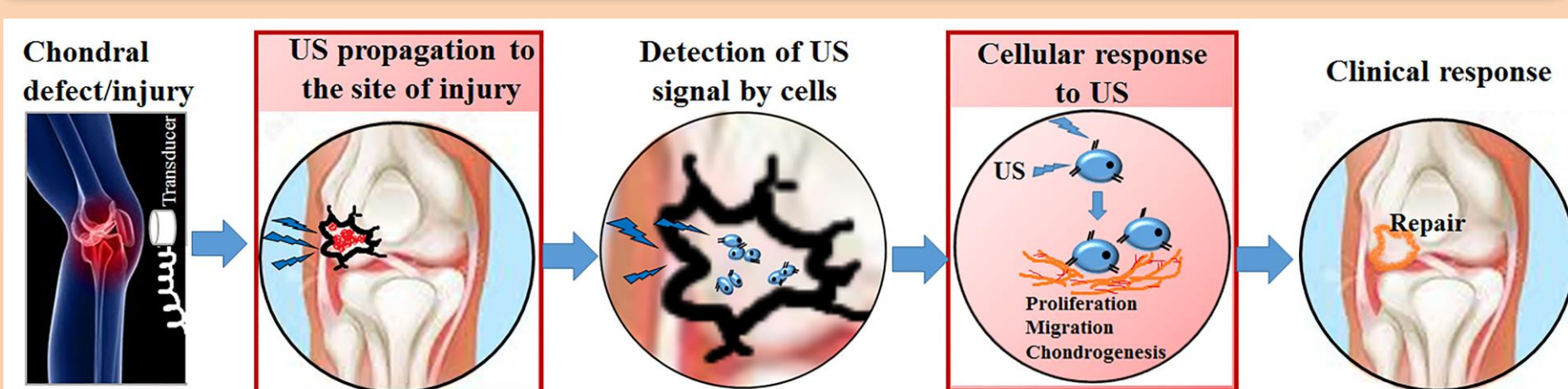
## Current Methods in Cartilage Repair



**Figure 1: Outcomes of surgical methods of cartilage repair.** (A) Focal cartilage defects created surgically in rabbit femoral condyles are harvested after 3 months. (B) Histology shows ineffective restoration of cartilage morphology[2]. (C) 3 mm deep microfracture in rabbit knee are harvested after 6 months (D). Histology reveals fibrocartilage formation due to positive Collagen type I staining[3].

**UNSATISFACTORY REPAIR OF CARTILAGE BY CURRENT SURGICAL METHODS DRIVE THE NEED TO SEEK NEW TREATMENT STRATEGIES.**

## Proposed US-assisted Strategy



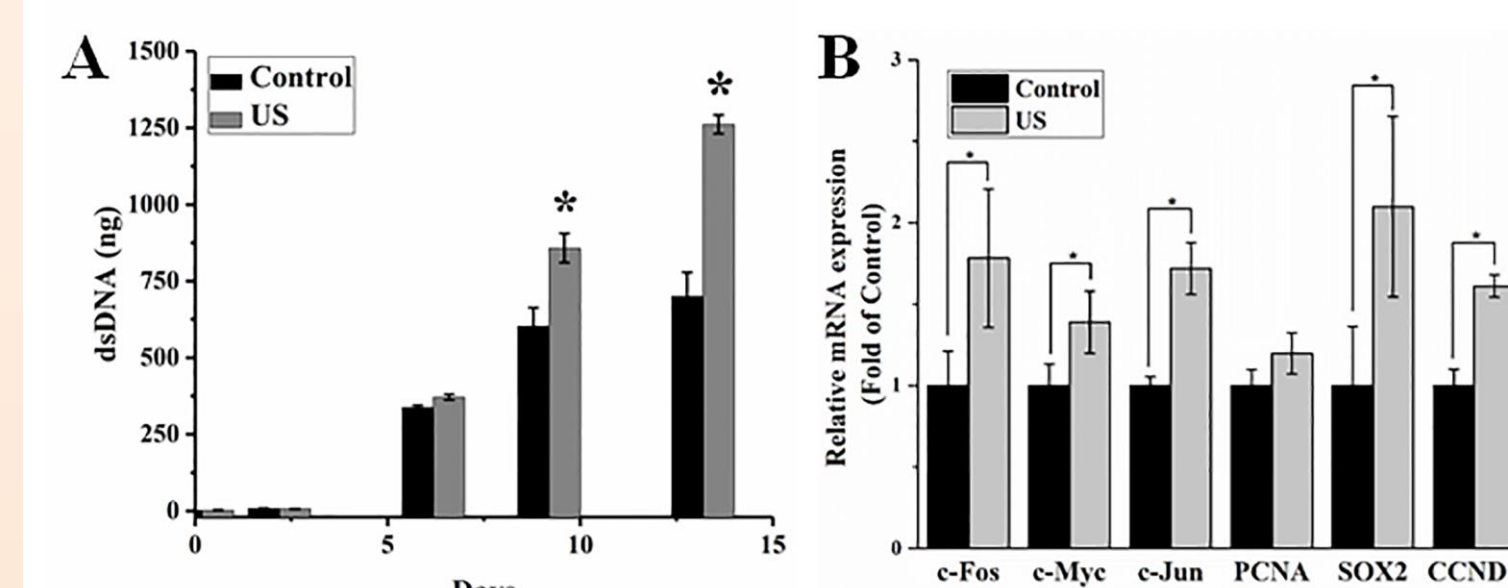
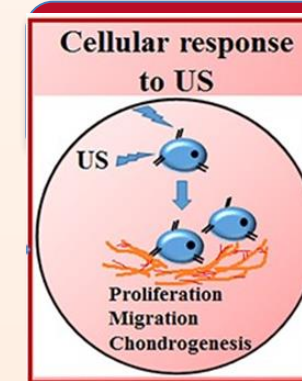
**Figure 2: Proposed US based strategy for cartilage repair.** Illustration representing in vivo translation of bioeffects of US for restorative repair of injured cartilage. An optimal US regimen suited for maximum propagation of signals has to be applied to the knee post trauma for effective cellular response.

## How can US enhance repair?

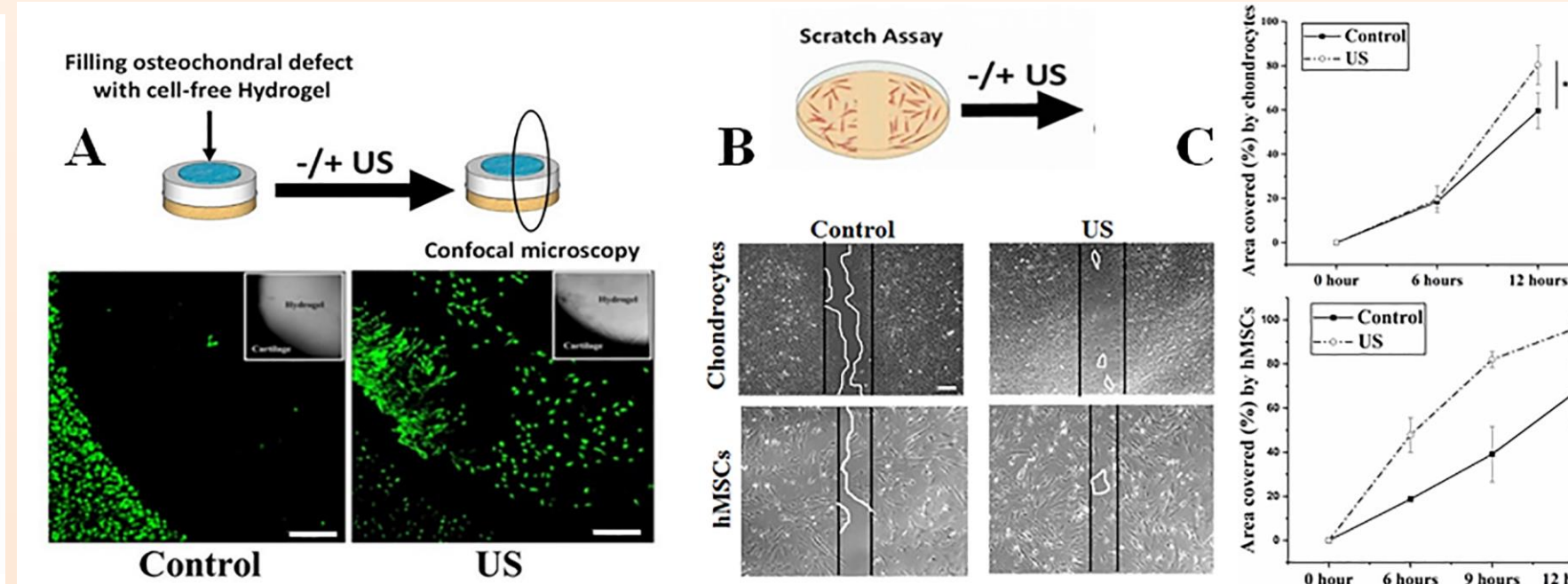
- Promote migration of cells toward the site of defect or injury.
- Promote proliferation of migrated cells.
- Support chondrogenesis of progenitor cells.
- Promote synthesis of functional extracellular matrix (ECM).

## Cellular Response to US

### US promotes cell proliferation and migration

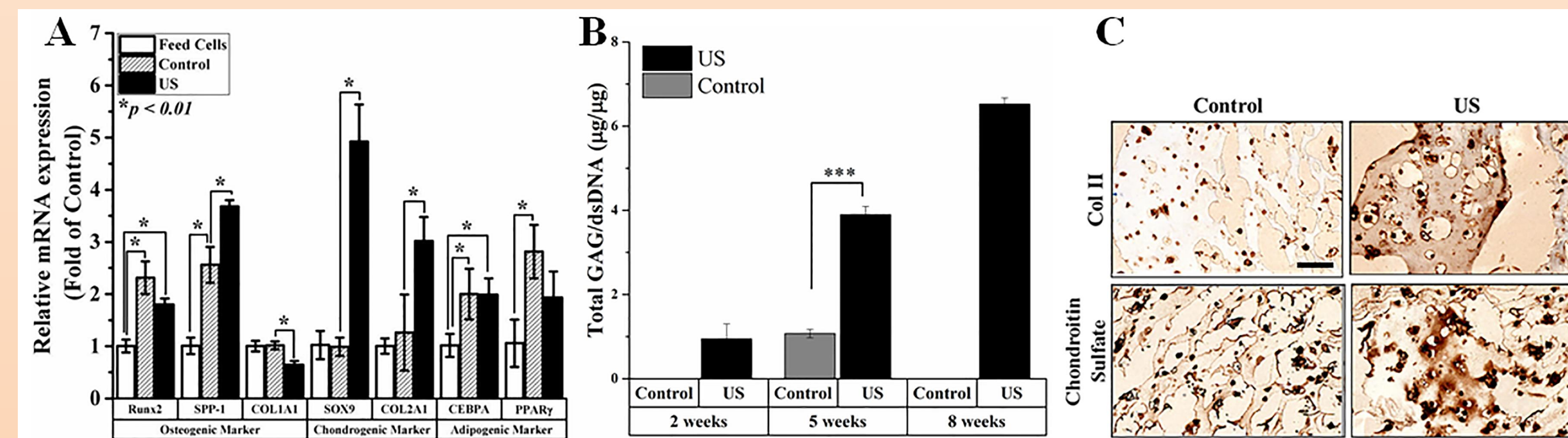


**Figure 3: Proliferation under US.** (A) Elevation of dsDNA content of human mesenchymal stem cells (hMSCs) in culture under US (5 MHz, 2.5 Vpp, 20 minutes/application, 4 applications/day). (B) Increase in the levels of expression of load-inducible genes c-fos, c-jun, c-myc, chondrogenic SOX2 gene and cell proliferation marker CCND1 in hMSCs under 1 application of US. Bars represent mean  $\pm$  standard deviation ( $p < 0.05$ ).



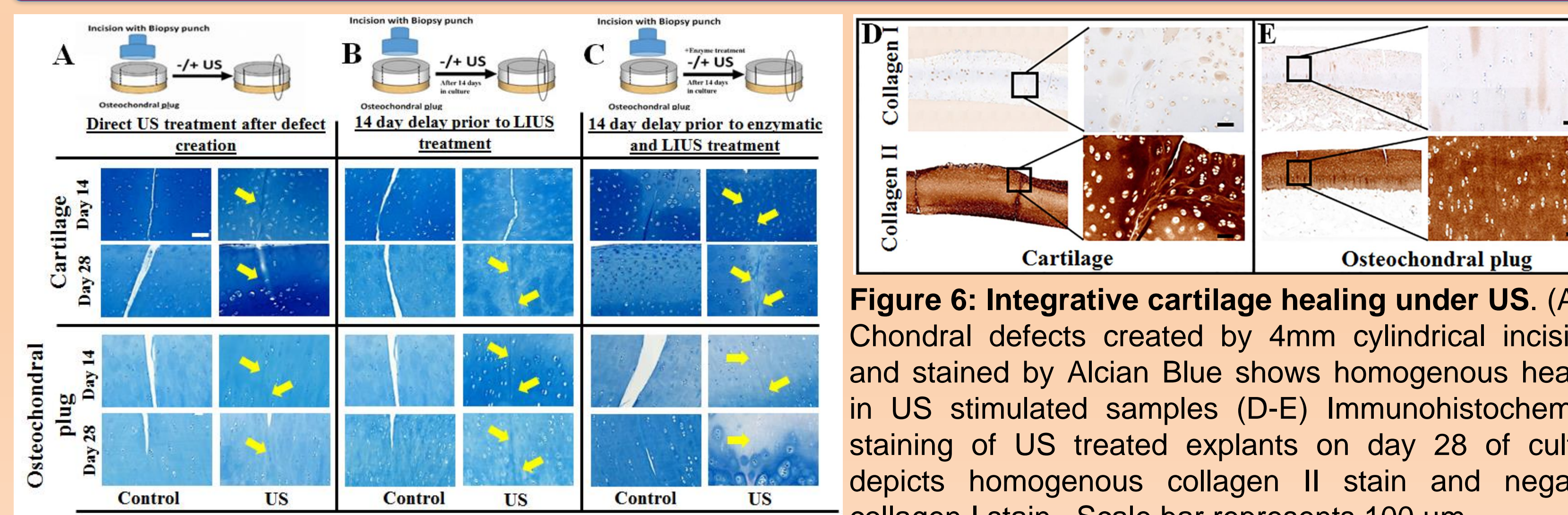
**Figure 4: Migration under US.** (A) Migration of cells (green) from native cartilage to hydrogel filled core (4mm) drilled out of an 8 mm thick cartilage explant is visualized by confocal microscopy. (B) Wound healing by migration of chondrocytes and hMSCs depicted by 2D scratch test. (C) Quantification of wound healing. Graph represents mean  $\pm$  standard deviation ( $p < 0.05$ ).

### US promotes chondrogenesis of hMSCs

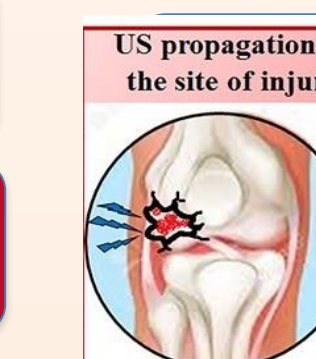


**Figure 5: Chondrogenic differentiation under US.** (A) Gene expression of key chondrogenic marker, SOX9, is substantially increased under US in hMSCs as compared to osteogenic and adipogenic markers. (B) US elevates GAG synthesis in hMSCs undergoing chondrogenic differentiation in 3D biomimetic scaffold. Bars represent mean  $\pm$  standard deviation ( $*p < 0.05$ ,  $***p < 0.01$ ). (C) Immunohistochemical staining of hydrogel constructs laden with hMSCs undergoing chondrogenesis at 4 weeks of culture shows intense collagen II and chondroitin sulfate staining in US samples. Scale bar represents 100  $\mu$ m.

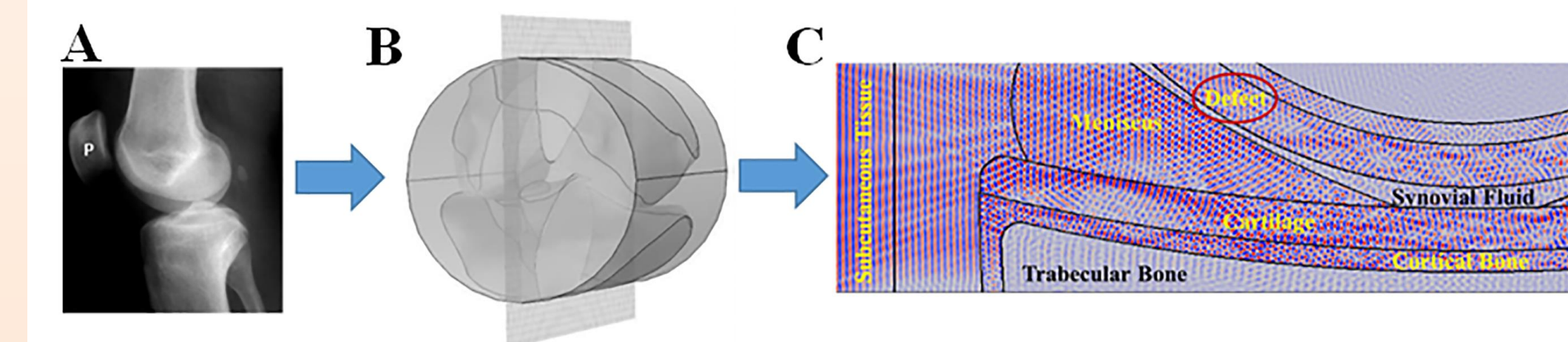
### US promotes integration at the cartilage interface



**Figure 6: Integrative cartilage healing under US.** (A-C) Chondral defects created by 4mm cylindrical incisions and stained by Alcian Blue shows homogenous healing in US stimulated samples (D-E) Immunohistochemical staining of US treated explants on day 28 of culture depicts homogenous collagen II stain and negative collagen I stain. Scale bar represents 100  $\mu$ m.



## US Propagation to the Site of Injury



**Figure 7: Determination of US propagation in joint space.** (A) MRI image of the joint is obtained. (B) MIMICS software is used to convert joint into a 3D model. (C) COMSOL and Biot theory are used to ascertain US propagation as a function of transducer placement. An example with human knee is shown.

## CONCLUSIONS

- US directs positive cellular response
  - Enhances proliferation
  - Enhances chondrogenic differentiation
  - Enhances cartilage-to-cartilage integration
- US propagates in the joint space and therefore, has the ability to reach the site of injury to promote repair.
- Thus, US has the potential to be used as an alternative or in combination with current surgical methods to repair cartilage defects.

## Future Directions

- Translation of in vitro findings to large animal models.
- Optimize transducer placement to effective US application.
- Demonstrate integrative cartilage repair.
- Establish mechanism for in-vivo cartilage repair.

## REFERENCES

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- [2] Khan et al. Cartilage integration: evaluation of the reasons for failure of integration during cartilage repair. A review. Eur Cell Mater. 2008 (3) 26-39.
- [3] Kuo et al, Microfracture and bone morphogenetic protein 7 (BMP-7) synergistically stimulate articular cartilage repair. Osteoarthritis Cartilage, 2006 (11) 1126-35.

## ACKNOWLEDGEMENT

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