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 interior repaired cartilage (Fig. 1). Lack of chondroductive 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).

US PROMOTES CHONDROGENESIS OF hMSCS

Figure 3: Proliferation under US. (A) Elevation of di/dNA 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 application of US . Bars represent mean ± standard deviation (p<0.05).

US PROMOTES INTRODUCTION AT THE CARTILAGE INTERFACE

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 ± 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 µm.

US PROMOTION TO THE SITE OF INJURY

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 ± standard deviation (p<0.05).

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

[1] Sahu, Neety. Doctoral Thesis. UNL-Lincoln @ 2017

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