Early Weight-Bearing Improves Cartilage Repair in an in vitro Model of Microfracture:Comparison of Two Mechanical Loading Regimens on Simulated Microfracture Based onFibrin Gel Scaffolds Encapsulating Bone Marrow Mesenchymal Stem Cells

Tomoya Iseki, MD[1], Benjamin B. Rothrauff, MD PhD[1], Shinsuke Kihara, M.D. PhD.[1], Shinichi Yoshiya, MD[2], Freddie H. Fu, MD[1], Rocky S. Tuan, PhD[1], Riccardo Gottardi, PhD[1]

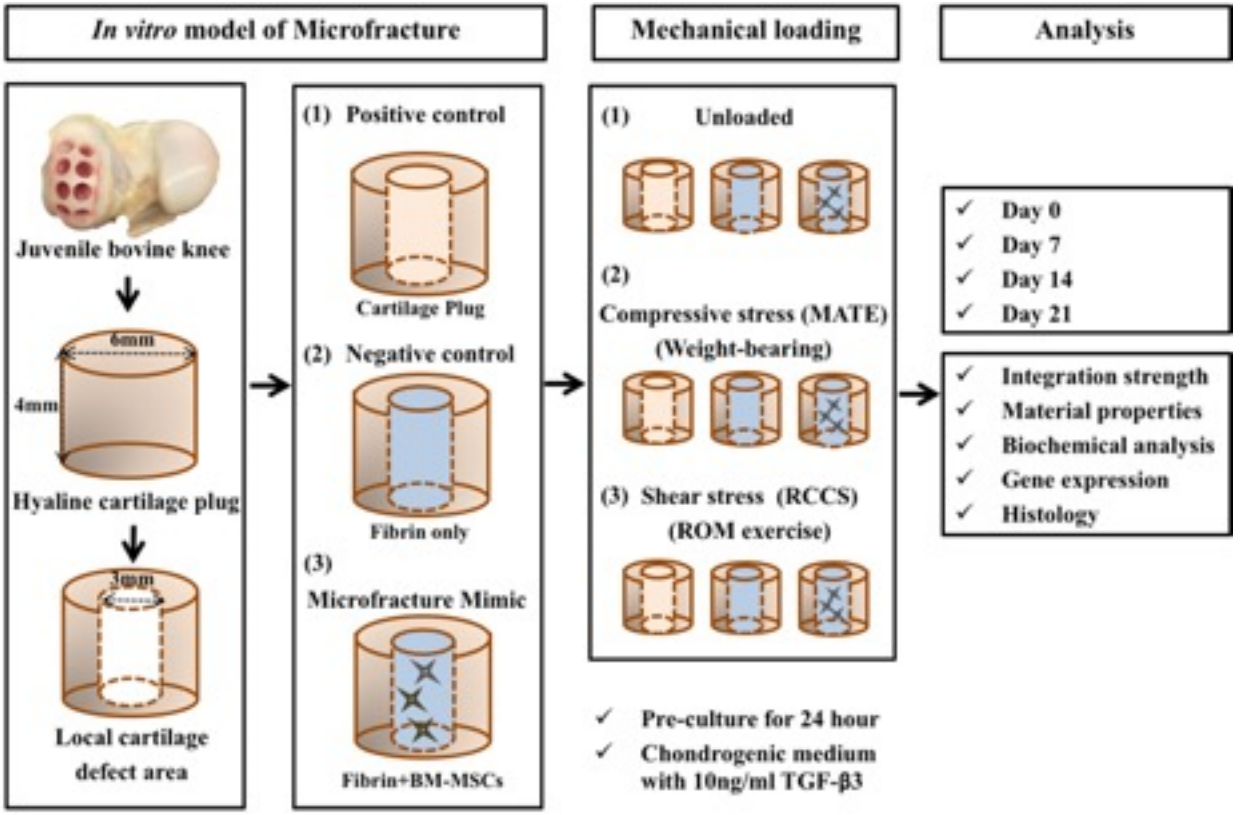
[1]University of Pittsburgh Medical Center, Pittsburgh, PA, USA, [2]Department of Orthopedic Surgery, Nishinomiya Hyogo, Japan

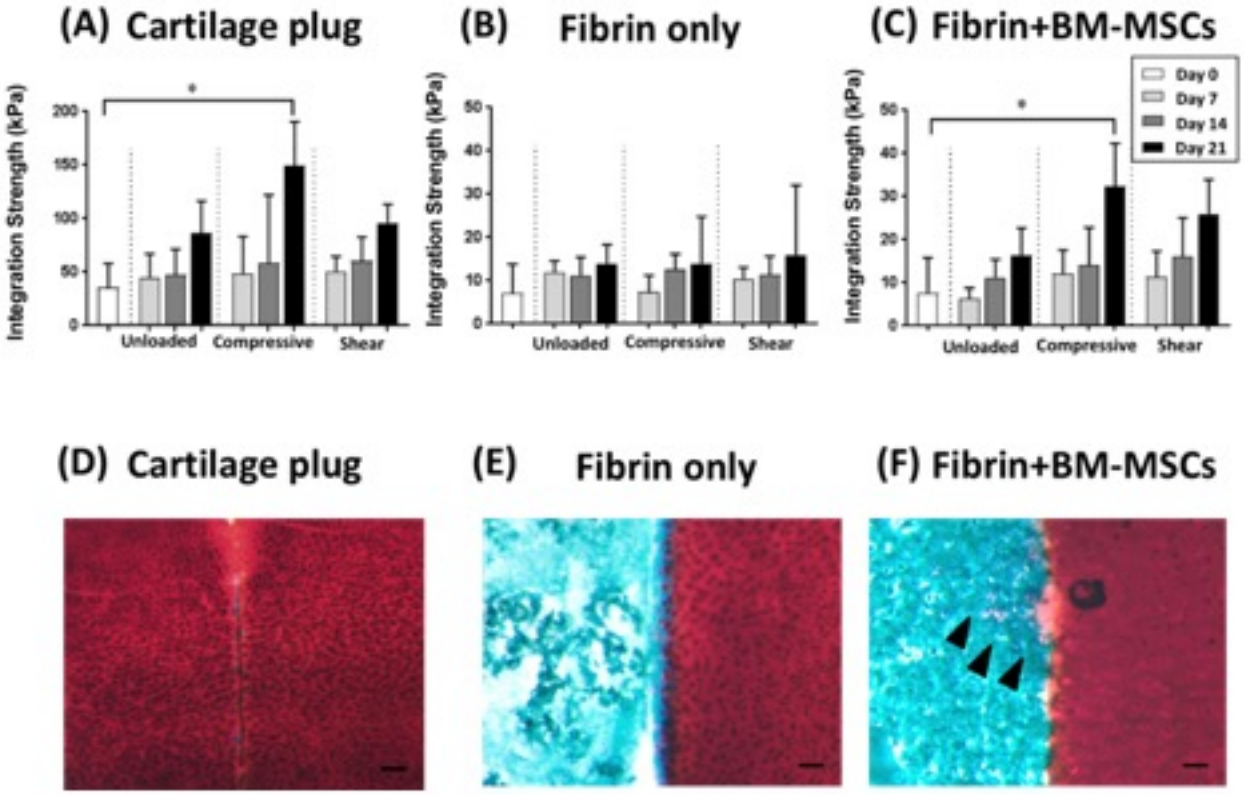
Objectives: Microfracture of focal chondral defects produces fibrocartilage, which inconsistently integrates with the surrounding native tissue and possesses inferior mechanical properties compared to hyaline cartilage. Mechanical loading modulates cartilage during development, but it remains unclear how loads produced in the course of postoperative rehabilitation affect the formation of the new fibrocartilaginous tissue. The purpose of this study was to assess the influence of different mechanical loading regimens simulating weight-bearing or passive motion exercises on an in vitro model of microfracture repair based on fibrin gel scaffolds encapsulating mesenchymal stem cells (MSCs).

Methods: Cylindrical cores were made in bovine hyaline cartilage explants and filled with either: (1) cartilage plug returned to original location (positive control), (2) fibrin gel (negative control), or (3) fibrin gel with encapsulated bone marrow-derived MSCs (BM-MSCs) (microfracture mimic). Constructs were then subjected to one of three loading regimens, including (1) no loading (i.e., unloaded) (2) dynamic compressive loading, or (3) rotational shear loading. On days 0, 7, 14, and 21, the integration strength between the outer chondral ring and the central insert was measured with an electroforce mechanical tester. The central core component, mimicking microfracture neotissue, was also analyzed for gene expression by real-time RT-PCR, glycosaminoglycan and dsDNA contents, and tissue morphology by histology.

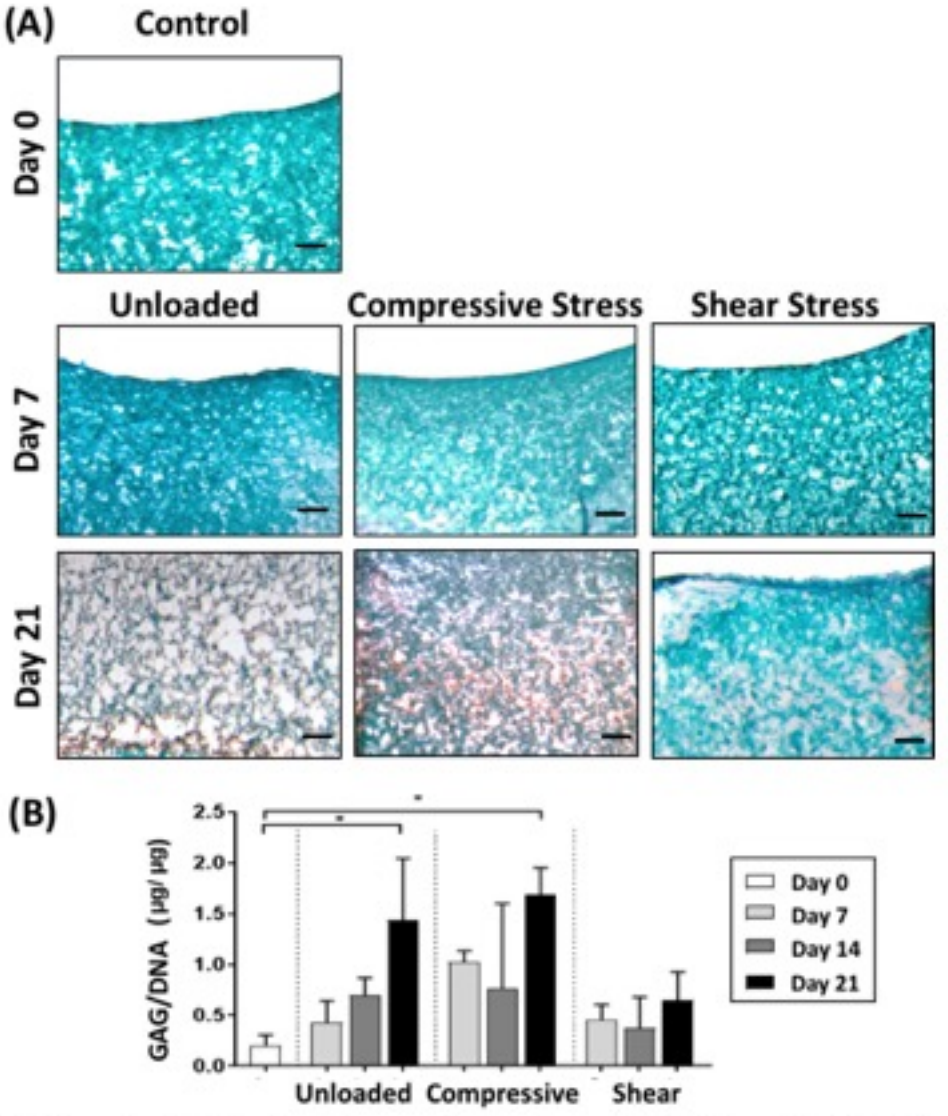
Results: Integration strengths between the outer chondral ring and central neotissue of the cartilage plug and fibrin + BM-MSC groups significantly increased upon exposure to compressive loading, compared to day 0 controls ($p= 0.007$). Compressive loading upregulated expression of chondrogenesis-associated genes (SOX9, collagen type II, and collagen type II:I, an indicator of more hyaline phenotype) in the neotissue of the fibrin + BM-MSC group, as compared to the unloaded group at day 21 (SOX9, $p =0.0032$; COL2A1, $p <0.0001$; COL2A1/COL1A1, $p = 0.0308$,). Fibrin + BM-MSC constructs exposed to shear loading expressed higher levels of chondrogenic genes as compared to the unloaded condition, but not as high as the compressive loading condition. Furthermore, catabolic markers (MMP3 and ADAMTS 5) were significantly upregulated by shear loading ($p = 0.0234$ and $p< 0.0001$, respectively) at day 21, as compared to day 0.

Conclusion: Dynamic compressive loading enhanced neotissue chondrogenesis and maturation in a simulated in vitro model of microfracture, with generation of more hyaline-like cartilage and improved integration with the surrounding tissue. Early weight-bearing after microfracture may be beneficial in promoting the formation of more hyaline-like cartilage repair tissue, whereas range of motion exercise by continuous passive motion without weight-bearing might not be as effective, or even negatively affect the formation of the repair tissue during post-surgery rehabilitation.

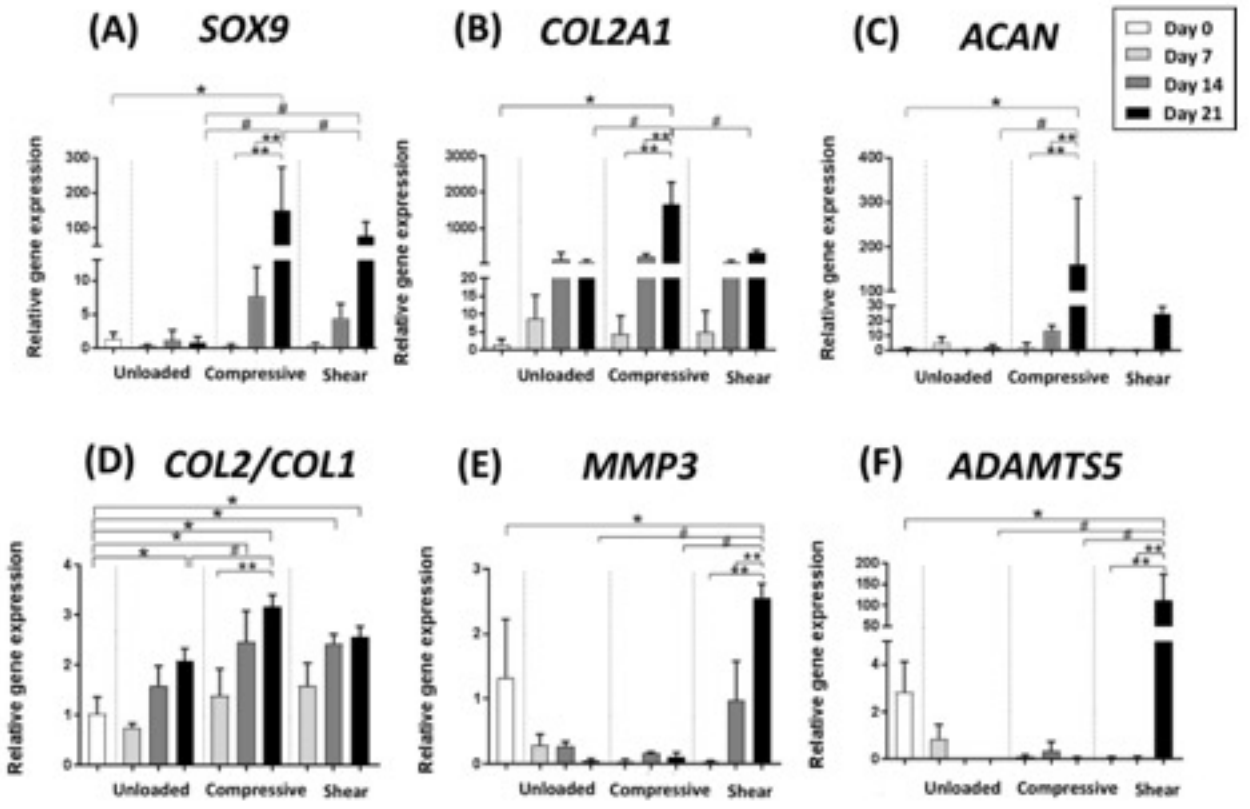

In vitro model of Microfracture
Mechanical loading
Analysis
Juvenile bovine knee
6mm
4mm
Hyaline cartilage plug
3mm
Local cartilage defect area
(1) Positive control
Cartilage Plug
(2) Negative control
Fibrin only
(3) Microfracture Mimic
Fibrin+BM-MSCs
(1) Unloaded
(2) Compressive stress (MATE) (Weight-bearing)
(3) Shear stress (RCCS) (ROM exercise)
✓ Pre-culture for 24 hour
✓ Chondrogenic medium with 10ng/ml TGF-β3
✓ Day 0
✓ Day 7
✓ Day 14
✓ Day 21
✓ Integration strength
✓ Material properties
✓ Biochemical analysis
✓ Gene expression
✓ Histology



Integration between outer cartilage ring and central insert. The integration strength between outer hyaline chondral ring and central insert of (A) cartilage plug, (B) fibrin only, and (C) fibrin + BM-MSCs, compared to day 0 (control), $p < 0.05$. Safranin O/Fast Green histological staining of the interface between outer hyaline chondral ring and central insert: (D) cartilage plug, (E) fibrin only, and (F) fibrin + BM-MSCs at day 14 in the compressive loading condition. Arrowheads indicate pericellular deposition of proteoglycan. Bar, 100 μm; n =12, combined from three independent trials.



Safranin-O/Fast Green staining of fibrin with BM-MSCs. (A) Day 0 controls and Days 7 and 21, by loading condition; Scale bar = 200µm. (B) Normalized glycosaminoglycan content (GAG/DNA) across time, by loading condition. * = significantly higher than day 0 controls ($p < 0.05$).



Gene expression of fibrin + BM-MSC group cultured under three mechanical loading conditions. Gene expression was analyzed by qRT-PCR for (A) *SOX9*, (B) *COL2A1*, (C) *ACAN*, (D) *COL2/COL1* (ratio of collagen type II to collagen type I), (E) *MMP3*, and (F) *ADAMTS5*. All expression levels are expressed relative to day 0 controls. *, comparison with day 0 controls ($p < 0.05$); **, comparison with other time points within the same activation group ($p < 0.05$); #, comparison between activation groups at the same time point ($p < 0.05$).

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