## Comparative Analysis of Shaver and Scalpel Mincing Techniques: Evaluating the Effect on Chondrocyte Viability in Bovine Articular Cartilage

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INTRODUCTION: Focal articular cartilage lesions in young patients can lead to early osteoarthritis. Current joint-preserving treatments include microfracture, osteochondral transplantation, and autologous chondrocyte implantation (ACI), with the latter being considered the gold standard for mid-sized defects but having limitations like cost and accessibility. Recently, the minced cartilage procedure has become popular, wherein cartilage is harvested, minced, and reimplanted in one operation, promising to address ACI's drawbacks. Despite the increasing use of shavers in mincing, there needs to be a consensus on their effect on chondrocyte viability. This study examines the impact of shaver mincing on chondrocyte viability and whether the shaver's size influences outcomes.

METHODS: A commercial shaver procured articular cartilage from bovines using a scalpel and three distinct shaver blades (2.5mm, 3.5mm, and 4.2mm). Cartilage sourced via the scalpel was further minced into fragments smaller than 1mm<sup>3</sup>. All samples were then cultivated in a culture medium for a week. Metabolic activity, RNA isolation, and the gene expression of anabolic (COL2A1 and ACAN) and catabolic markers (MMP1 and MMP13) were gauged on Day 1 and Day 7. Live/Dead staining was assessed through confocal microscopy with subsequent image analysis, and flow cytometry was employed to determine apoptosis of the chondrocytes in the minced cartilage. The statistical analysis was carried out using a two-way analysis of variance (ANOVA). Multiple comparisons were performed, followed by the Tukey test to correct multiple comparisons. Statistical significance was set at p < 0.05.

RESULTS SECTION: Shaver-minced cartilage displayed a statistically significant decline in metabolic activity on Day 1 and Day 7 compared to the scalpel-minced counterpart (p<0.001). Anabolic gene expression (COL2A1 and ACAN) was suppressed, while the expression of catabolic genes (MMP1 and MMP13) surged in the shaver-minced samples by Day 7. A rise in the MMP13/COL2A1 ratio was evident across all shaver samples. Confocal imaging revealed a distinct layer of deceased cells on the lesion surface in scalpel-minced samples, with living cells below. The percentage of viable cells showed a significant decrease in all shaver conditions compared to the minced scalpel group (p<0.05 to p<0.01). Flow cytometric data underscored the presence of more dead cells and fewer intact cells in the shaver-minced cartilage, in contrast to scalpel mincing.

DISCUSSION: Mincing cartilage with a shaver has been shown to negatively impact cell viability compared to using a scalpel. While studies by Redman et al. and Levinson et al. examined trauma types and mincing devices' impact on chondrocytes, the ideal cartilage fragment size for effective ECM production remains uncertain. Some evidence suggests that smaller fragments yield better outcomes. However, the effects of shaver-mincing, increasingly adopted in clinics, have yet to be extensively investigated in animal or clinical trials. The study's limitations include that it was conducted on bovine cartilage, which does not directly equate to human conditions, and was limited by its in vitro setup, lack of 3D cultures, and absence of mechanical loading. No significant differences were noted, with only four samples observed for short-term effects, but distinct trends were apparent. Mincing bovine articular cartilage with commercially available shavers compromises chondrocyte viability compared to traditional scalpel mincing. This insinuates that shaver-minced cartilage is better suited as a matrix than a cellular therapy. There is an immediate need for further standardized research, both experimental and clinical, to delve deeper into the impact of the shaver-based mincing procedure.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): The minced cartilage procedure has recently gained traction as a solution to the shortcomings of traditional methods, leading medical companies to invest in standardized tools like shavers and harvesting devices. Currently, no scientific consensus exists on the best mincing method, be it manual or with commercial devices.

## IMAGES AND TABLES:

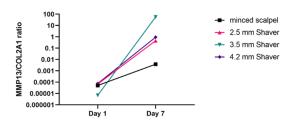


Image 1: MMP13 to COL2A1 ratio of the different conditions.

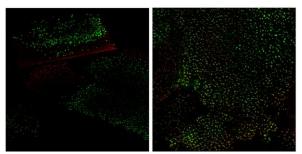


Image 2: Confocal microscope images on Day 7 from Scalpel-minced cartilage (left) and by using a 3.5 mm shaver (right).