EFFECTS OF RESECTION TECHNIQUE ON CELL VIABILITY, METABOLISM, AND TISSUE ARCHITECTURE OF NORMAL MENISCI

Nagel C, Jayabalan P, Kuroki K, Stoker A, Whitener D, and Cook JL
Comparative Orthopaedic Laboratory, University of Missouri, Columbia, MO
www.columc.missouri.edu

INTRODUCTION

Nearly one millions surgeries are performed every year in the US to remove damaged portions of the knee menisci to relieve associated mechanical symptoms and pain. Due to the direct relationship between the amount of meniscal tissue removed and the degree and progression of secondary osteoarthritis, preservation of as much functional meniscal tissue as possible is an important goal of surgery. Currently, mechanical instruments (e.g. basket forceps) or radiofrequency devices are most often used for arthroscopic partial meniscectomy. The Harmonic Scalpel™, is an ultrasound based heat generating tool commonly used in other surgical procedures. It could be used for partial meniscectomy since in theory it would allow for precise and efficient removal of tissue. Each device has potential advantages and disadvantages; however, they have not been comprehensively tested, or compared, with respect to effects on meniscal tissue viability, metabolism, and tissue architecture when used for partial meniscectomy.

Objectives

To determine the effects of each resection method on cell viability, metabolism, tissue composition and architecture of remaining *ex-vivo* meniscal tissue when performing a partial meniscectomy.

Hypothesis

The Harmonic Scalpel™ will allow for accurate and efficient performance of partial meniscectomy without significant differences in adverse effects on cell viability, metabolism, or tissue architecture of remaining meniscus compared to mechanical resection or radiofrequency.

MATERIALS & METHODS

Canine menisci (n=45) were randomly divided into 3 treatment groups:

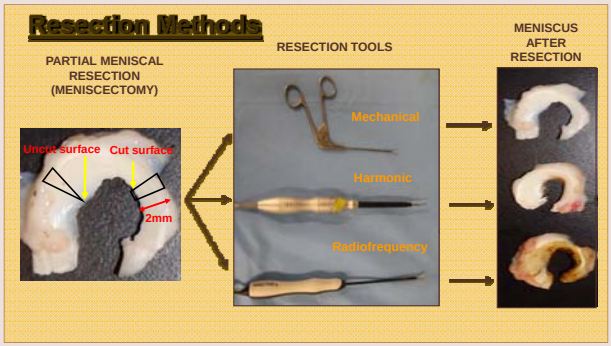
1. Harmonic Scalpel™ (HC)
2. Radiofrequency (RC)
3. Mechanical (MC)

Arthroscopic conditions were simulated by immersing the menisci in a bath of saline at room temperature with a constant fluid flow of 100mL/min. Post-resection the remaining meniscal tissue was cultured for 0, 3, or 15 days in DMEM+ITS media at 37 ºC with 5% CO_2 and 95% humidity. On the respective tissue harvest day, meniscal tissue was evaluated for tissue morphology histologically, for cell viability using a live/dead assay and glycosaminoglycan and collagen content using biochemical assays. Degree of cell viability in each explant was subjectively determined from the live/dead images using a stereomicroscope. Histological assessment was made by staining the tissue with haemotoxylin and eosin for tissue architecture and trichrome for the collagen matrix.

Culture media was collected on days 3, 6, 9, 12, and 15 and assayed for:

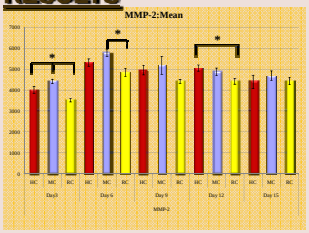
1. Total collagen content using an absorbance assay measuring hydroxyproline (HP).
2. Total sulfated glycosaminoglycan (GAG) content using the dimethylmethylene blue (DMMB) assay
3. Nitric Oxide (NO) production calculated from nitrite concentration using the Greiss assay
4. Prostaglandin E_2 (PGE_2) using an enzyme immunoassay
5. Matrix metalloproteinases (MMP-1, -2, -3, -9 and -13) using a multiplex Luminex assay

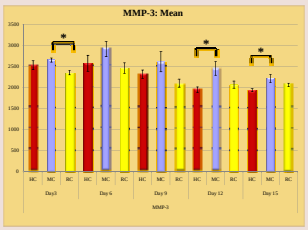
Data were analyzed for differences between45 groups with significance set at $p < 0.05$

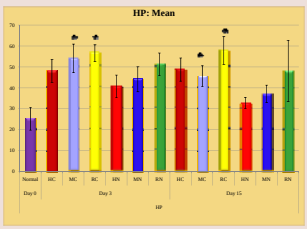


RESULTS

Biochemical Assessment

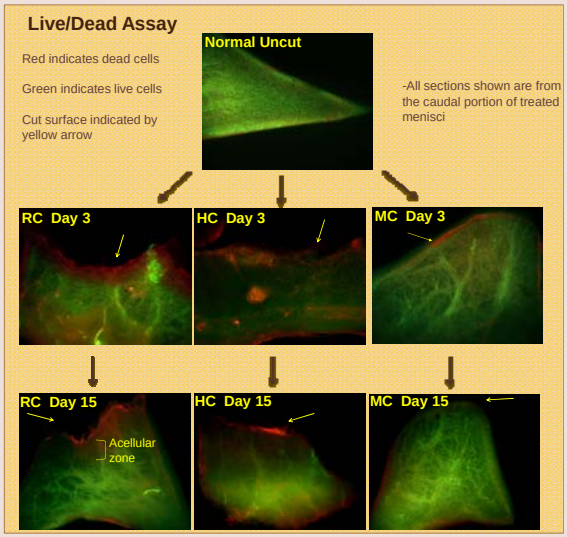




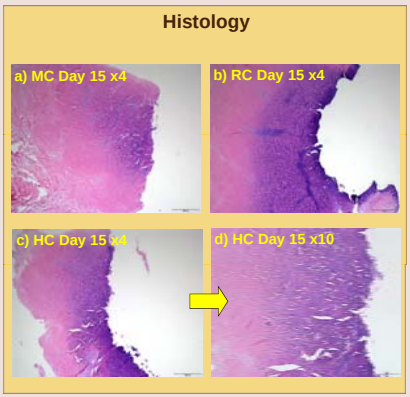


Biochemical Assessment: On days 3 and 6, the explant media of menisci cut by mechanical resection contained higher levels of MMP-2 than radiofrequency. Days 3 and 12 revealed higher levels of MMP-2 in the Harmonic Scalpel™ group than the radiofrequency group. MMP-3 levels were greater in media from mechanical resection than radiofrequency on day 3 and Harmonic Scalpel™ in days 12 and 15. The cut Harmonic tissue contained a greater amount of HP (collagen) content than the uncut Harmonic tissue. When compared to a normal uncut meniscus, on day 3 and 15 both radiofrequency and mechanical resection displayed a significant increase in HP.
*= $p < 0.05$; HC = harmonic scalpel cut; MC = mechanical cut; RC = radiofrequency cut

Live/Dead Assay



Histology



Histology: These histological sections are representative of the 3 different treatment groups on day 15. Subjective assessment was made of the degree of cellular and tissue damage and tissue architecture disruption. The increased amount of space between cells shown in both Harmonic Scalpel™ and radiofrequency suggests tissue damage (b&c). The larger number of pyknotic cells and empty lacunae suggest cell death in tissue samples following resection with Harmonic Scalpel™ (d) and radiofrequency instruments (data not shown).

DISCUSSION

These data indicate that mechanical resection has potential clinical advantages over the other two methods tested when comparing live/dead and histologic outcome measures. Harmonic Scalpel™ and Radiofrequency partial meniscectomies were associated with significantly lower cell viability and greater cell and tissue damage in the remaining meniscal tissue when compared to Mechanical resection at all time points. The most severe changes were consistently noticed in the Radiofrequency group. The adverse effects seen in Harmonic Scalpel™ and Radiofrequency groups are most likely due to heat produced by these instruments. This could have important clinical ramifications for our goal of preservation of functional meniscal tissue. Interestingly, mechanical partial meniscectomy was associated with significantly higher levels of MMP production early in culture. This is likely an initial remodeling response of the tissue to the direct trauma as it was seen to dissipate with time. The higher levels seen in this group compared to the other groups may be related to cell viability and/or type of insult. No significant differences were seen among groups for NO, GAG or PGE_2 production suggesting that these components of tissue metabolism were not differentially affected by treatments as assessed in this study. Collagen loss to the media was noted in all treatment groups and may be an important marker to investigate in future studies. Further investigation is needed to fully delineate the long term clinical effects of these methods for performing partial meniscectomy such that clear recommendations can be made regarding optimal treatment of meniscal pathology.

Conclusions

- **Mechanical resection had less severe effects on cell viability and tissue architecture of remaining meniscus compared to radiofrequency or Harmonic Scalpel™ techniques for partial meniscectomy, but was associated with higher levels of MMPs early in explant culture**
- **Harmonic Scalpel™ resection had potential advantages with respect to collagen turnover and MMP production**
- **Mechanical and Harmonic Scalpel™ techniques should be further assessed to determine optimal clinical techniques for partial meniscectomy**

Acknowledgements: Student support: Merck/Merial/CVM;
Research support: Pfizer & Ethicon