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ARTICULAR CARTILAGE AND SYNOVIAL TISSUE

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INTRODUCTION

Standard histoprocessing for hard tissue or sensitive soft tissues typically involves a lengthy manual process. Advances in microwave (MW) technology offer new opportunities to facilitate histoprocessing without compromising results.[1] In 1998, a new one-step dehydration/clearing agent called JFC solution (Milestone, Italy) was developed specifically for use in MW histoprocessing. JFC solution consists of absolute ethanol, and isopropyl alcohol with long-chain hydrocarbons and has transformed histoprocessing into a 2-step process: JFC and paraffin, and effectively reduced processing time to between 30 minutes and 3 hrs (depending on size and nature of tissue).

Here we compared the histological quality of rabbit femoral articular cartilage and patellar synovial tissue following histoprocessing using two different methods: a) routine manual and b) MW processing with JFC solution for dehydration and clearing in one step.

MATERIALS AND METHODS

Tissues: Retrieval and Fixation

Tissues retrieved from euthanized animals included:

- 8 samples of rabbit femoral articular cartilage
- 8 samples of the rabbit synovial tissue that was attached to the patella in an area between bone and tendon

All tissues fixed in 10% neutral buffered formalin, except for 2 samples of articular cartilage intended for immunohistochemistry which were fixed in 4% paraformaldehyde.

Prior to histoprocessing all samples of articular cartilage were decalcified in 0,5M HCL/0,1% glutaraldehyde

Tissue sample dimensions used for both manual and MW histoprocessing after trimming:

- 1.0 X 0.9 X 0.7 cm for articular cartilage
- 0.8 X 0.5 X 0.2cm for synovial tissue

Microwave (MW)

The MW used was an Advanced Multifunction Microwave Labstation (MicroMed T/T Mega, Milestone Inc,Italy).

Histology

Following histoprocessing, all paraffin-embedded samples were cut into 5 μm sections on a Leica RM2155 microtome (Nussloch,Germany).

Articular cartilage was stained using Safranin-O/Fast Green (0.1% Safranin-O, 0.01% Fast Green) for glycosaminoglycan (GAG) content. Immunostaining was completed on some samples for collagen Type II (mAb II-II6B3, DSHB), using primary antibody diluted 1:10 in 10% Goat serum/ PBS, and incubated for 1 hr at room temperature. An ABC-AP kit was used for visualization.

Synovial tissue sections were stained with Hematoxylin- Eosin.

Manual Histoprocessing Times

Articular Cartilage		*Synovial Tissue*	
Rinsing		Rinsing	
PBS:	45 minutes	PBS:	40 minutes
Dehydration:		Dehydration:	
70% Ethanol:	overnight	70% Ethanol:	30 minutes
95% Ethanol:	4 hrs	80% Ethanol:	30 minutes
100% Ethanol:	4.5 hrs	95% Ethanol:	60 minutes
Clearing:		100% Ethanol:	80 minutes
Cedar wood oil:	overnight	Clearing:	
Toluene:	20 minutes	Xylene:	60 minutes
Paraffin impregnation:	6 hrs at 62°C	Paraffin impregnation:	3 hrs at 62°C
TOTAL TIME:	**~48 hrs**	**TOTAL TIME:**	**~8 hrs**

MW Histoprocessing Times

Articular Cartilage		*Synovial Tissue*	
Rinsing		Rinsing	
PBS:	45 minutes	PBS:	45 minutes
Dehydration and clearing one-step:	2 hrs	Dehydration and clearing one-step:	2 hrs
Wax impregnation:	30 minutes	Wax impregnation:	30 minutes
TOTAL TIME:	**~3hrs, 15minutes**	**TOTAL TIME:**	**~3hrs, 15minutes**

Microwave Protocol

DEHYDRATION STEP				WAX IMPREGNATION STEP			
Step	Time	Power (W)	Temperature	Step	Time	Power (W)	Temperature
1	00:20:00	400	40°C	1	00:20:00	600	79°C
2	00:03:00	200	45°C	2	00:05:00	300	79°C
3	00:20:00	300	50°C	3	00:20:00	600	83°C
4	00:03:00	200	55°C	4	00:05:00	300	83°C
5	00:20:00	350	65°C	5	00:20:00	500	85°C
6	01:15:00	250	70°C	6	00:30:00	300	85°C

RESULTS

These evaluations demonstrated that MW histoprocessing of rabbit articular cartilage and synovial tissue using one step dehydration and clearance with JFC solution consistently gave high quality, reproducible and equivalent histological results when compared with routine manual histoprocessing.

MW-processed paraffin blocks of cartilage and synovial tissue samples were neither hard nor brittle and cut well. Some residual hydrocarbon chains from the JFC solution likely remains in the paraffin, making the blocks very easy to cut.

As demonstrated in the Figures 1, 2 and 3, there was no evidence of unacceptable shrinkage, alteration of cells or swelling of connective tissue fibers. Furthermore, MW histoprocessing permitted easy identification of cartilage layers, different cell types like osteocytes, osteoclasts, and characteristics associated with the growth plate. Immunostaining was not affected.

Manual *MW*

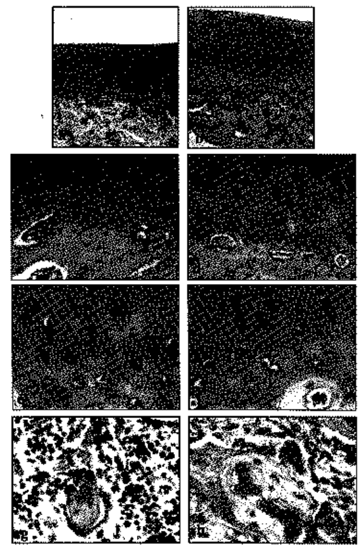

Figure 1: Histology of articular cartilage samples processed using either routine manual processing or MW processing and stained using the Safranin-O method. (a,b) 10X mag (c,d) subchondral area, 40 X mag (e,f) accumulation of GAG in vicinity of columnar chondrocytes, 40X mag (g, h) osteoclasts in region of growth plate, 60X mag.

Manual *MW*

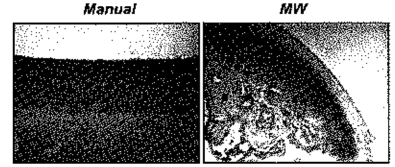

Figure 2: Immunostaining of collagen Type II in articular cartilage samples processed using either routine manual processing or MW processing and visualized using ABC-AP. 10X mag

Manual *MW*

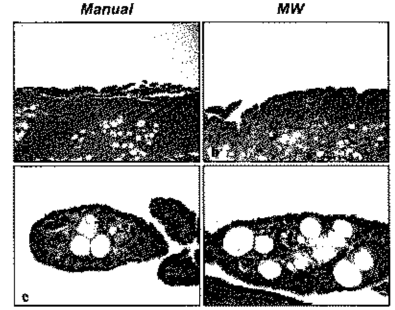

Figure 3: Histology of rabbit synovial tissue samples processed using either routine manual processing or MW processing and stained using the Hematoxylin-Eosin. (a,b) 10X mag (c,d) 40 X mag

CONCLUSIONS

In this study, it was demonstrated that MW histoprocessing:

-significantly reduced the time for histoprocessing of articular cartilage from 48 hrs (manual) to 3 hrs, and from 8 hrs to 3 hrs for synovial tissue

-gave high quality, reproducible and equivalent histological results in samples of rabbit articular cartilage and synovial tissue

-had no effect on the GAG distribution (histology) nor the identification of collagen Type II (immunohistochemistry) in rabbit articular cartilage

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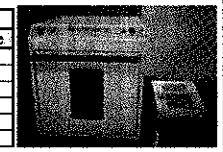

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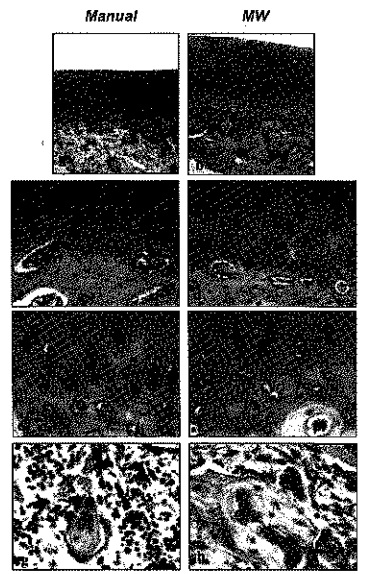

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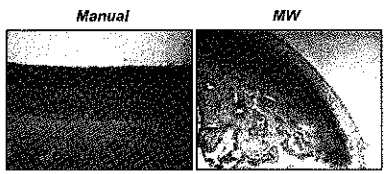

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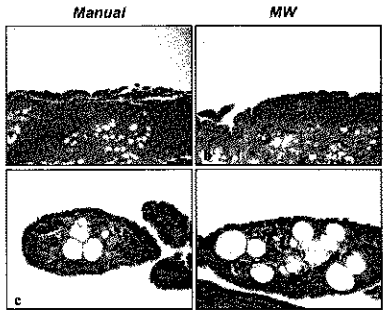

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REFERENCES

1 Boix J R, Layfield L J, Wallis D, Hardy DA. "A Comparison of routine and rapid microwave tissue processing in surgical pathology laboratory