HISTOLOGIC®
Technical Bulletin for Histotechnology

SAKURA

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Vol. XLV, No. 1 June 2012

A Decemented, Decalcified Paraffin Processing Option for Resurfaced Femoral Head Implant Specimens

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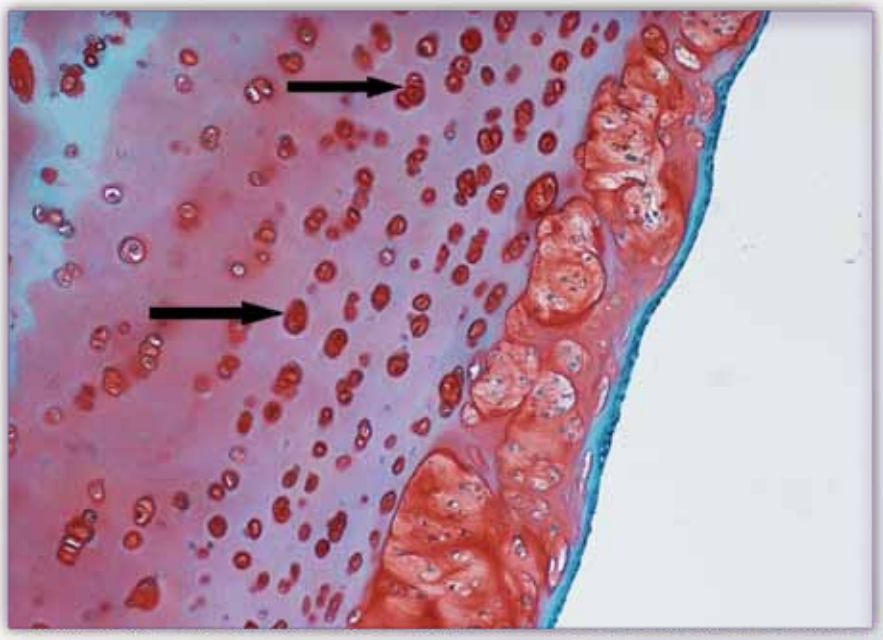
Abstract

The field of histotechnology in an orthopaedic research setting encompasses a wide variety of procedures and instrumentation. Crossover versatility of both personnel and equipment is key, not only to gathering information, but also for securing extramural funding. The study of bone or components of bone, which may be optimally served by undecalcified analysis in a methacrylate embedding medium, could possibly be adequately served by a decalcified paraffin workup that is almost always less expensive and can provide a platform to support a wider range of downstream analyses. A particular dilemma arises when the bone to be studied has been integrated with biomaterials or orthopaedic appliances. This report describes a method for preparing paraffin sections of decalcified bone following removal of the cement used to retain the prosthesis that allows for the investigation of cellular changes in metal cap resurfaced femoral heads.



Optimal safranin O-fast green (SOFG) staining of formalin-fixed, formic acid decalcified, and paraffin-processed articular cartilage in a pathologic femoral head. Crisp orange-red staining of the proteoglycan clearly illustrates the differentiating chondrocytes (arrows). 100X

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Fig. 1. "Sawbones" (Sawbones, Vashon, WA 98070) mock-up of a femoral head that has been debrided of necrotic articular tissue and coated with methyl methacrylate bone cement in preparation to receive the metallic cap as the new articular surface.

Fig. 2. Second stage of Fig. 1 where the metallic cap has been pressed into place atop the debrided femoral head. The flowable consistency of the cement will fill subcapital voids resulting from the debridement and the excess will ooze out and be removed prior to implantation.

Fig. 3. X-ray of representative resurfaced femoral head in situ.

Introduction

Metal cap resurfacing of the femoral head was originally developed as a treatment option for young, active orthopaedic patients who presented with delamination of the articular cartilage as a result of osteonecrosis. The affected or damaged tissue from the surface of the femoral head (ball) was removed and the patient's remaining underlying bone stock was shaped to accommodate a metal cap that interfaced with the patient's acetabulum (socket). Any potential voids in contact between the inner surface of the cap and the patient's bone were mitigated by the application of a methacrylate "bone cement," which acts both as a filler as well as an adhesive (Figs. 1, 2, 3). This procedure was developed as a preferred temporary treatment option for younger patients in advance of what most likely would result in eventual total joint replacement. It was felt that, while joint replacement technology and materials would continue to be improved, the temporary metal cap procedure would preserve joint mobility and allow for a relatively streamlined removal procedure with eventual total hip arthroplasty. Results for these original surgeries did not, however, live up to expectations and the original practices quickly fell out of favor.

Over the years, advances in component materials and metal-on-metal technology sparked a resurgence in metal cap resurfacing applications. The original designs have been revised so that a central post from the highest portion of the inner dome of the cap can be driven into the interior femoral neck to theoretically enhance stability and reduce the type of femoral neck fractures that plagued the original procedures.

At the Center for Orthopaedic Research facility at the University of Arkansas for Medical Sciences (UAMS), the availability of archived metal-capped femoral heads, some with the 1980s design and others with the newer central post design, raised questions regarding their value for microscopic study. Stogiannidis et al reported that such specimens, processed with the biomaterials remaining in situ using methyl methacrylate (MMA) resin embedment and prepared for microscopic evaluation via the cut-and-grind method, would yield the most information.[1] However, a lack of funds and necessary equipment for such a study in our lab made this approach prohibitive. As an alternative, we proposed a procedure for removing the metal and bone cement from the bone, decalcifying the remaining bone tissue, and then preparing routine paraffin-embedded sections to assess architectural and cellular integrity as well as suitability for additional studies, such as special stains and immunohistochemistry (IHC). The results, if favorable, might then be used to justify the more costly and extensive resin-based evaluation.

Our workup was based on work performed in the lab of Dr. Leon Sokoloff at the Department of Pathology, SUNY Stony Brook, in the early 1980s. At that time, serial 5-mm slabs of resected femoral heads were cut on a band saw for the study of arthritis and osteonecrosis. The bone slabs were decalcified slowly in a dilution of formic acid and then hand processed and cleared in methyl salicylate to facilitate paraffin infiltration. But this process was slow and labor intensive, which did not allow for a rapid pathologic diagnosis. Generally, during the slabbing or grossing of the specimen, we would identify "mirror-face" slabs containing similar significant pathology. One slab would go through rapid decalcification (using commercial hydrochloric acid decalcifying solutions) and then be conventionally processed with the clearing agent xylene in order to achieve an acceptable specimen suitable for rapid diagnosis. The mirror-face slab would go through full-thickness decalcification in 5% formic acid with methyl salicylate clearing, achieving a sample optimally suited for whatever histologic analyses might be needed. This strategy for sample preparation yielded specimens of value to both diagnostic and research laboratories.

Over the years, pathologists would sporadically receive bone and bone/soft tissue composite specimens containing bone cement dowels used for in situ bone fixation. In some cases the consistency and density of this cement would be so similar to bone that, during grossing and cutting on a conventional band saw, one might not be aware of its presence. Occasionally, these dowels would be situated within the region of pathologic interest. The optimal way to process this type of specimen, especially from the orthopaedic perspective, would be to embed it in a methacrylate medium without decalcification to allow for the assessment of cellular integrity and bone mineralization as well as visualization of the bone-to-cement dowel fixation interface.[2]

Since we were not equipped to perform this type of workup and the greatest benefit was in the analysis of the cellular detail, paraffin sections of decalcified samples were considered as an alternative. In cases where the cement dowel would interfere with post-decalcified paraffin sectioning, the cement would be dissolved in acetone prior to paraffin processing. Acetone immersion could be performed either prior to or after decalcification, and would also serve to remove lipids and water from the tissue.[3,4]

Materials and Methods

A femoral head was loaded into the appropriate clamp on the Exakt Macro Saw (Exakt Techologies, Oklahoma City, OK) (Fig. 4). Some specimens required 320 grit "scuffing" of the metal cap in order to facilitate an appropriately firm grasp of the ultrasmooth surface of the metal cap. The specimen was clamped so that a cutting would yield one sample at "midline minus 1 cm" and another at "midline plus 1 cm." In this way, the true midline of the specimen was well contacted by the jaws of the saw clamp for maximum stability. If necessary, a second "midline minus" sample could be cut leaving a central slab approximately 1 cm thick, which would be suitable for MMA preparations (Fig. 5).

Fig. 4. Metal resurfaced femoral head loaded onto Exakt Macro Saw. Note the scuffed cap surface to facilitate firm placement in the clamp and the offset alignment.

Fig. 5. Exakt Macro Saw grossed metal resurfaced femoral head revealing the underlying bone with cement mantle and central post as per recent design changes. A central core of brown vascularized tissue surrounded by pale yellow tissue with a sharp demarcation is noted. Microscopic evaluation of these sections (Fig. 6) revealed viable and nonviable tissue.

After sawing, the slab selected for decalcification was immersed in fresh formalin for up to 72 hours to ensure complete fixation of the newly exposed bone and tissue. The sample was briefly washed in tap water and then decemented via multiple changes of acetone until the last acetone change showed no visible signs of cement. The length of this procedure varied depending on the size of the sample, amount of cement in the sample, and composition of cement utilized. The length of time in acetone varied from 6 to 28 hours.

Following acetone treatment, the specimen was partially rehydrated through a series of graded ethanols to 70% and radiographed (if deemed appropriate) using an AXR Minishot 110 closed system cabinet X-ray machine (Associated X-Ray Corporation, East Haven, CT) with Kodak PPL film. The sample was then immersed in 5% formic acid with daily changes until the decalcifying solution was found to be clear of calcium using the 5% ammonium oxalate chemical test. When decalcification was completed, the sample was processed into paraffin as per our routine protocol. The embedded sample was then sectioned at 5 microns and stained with hematoxylin and eosin (H&E).[5]

Results

We knew from prior experience that samples stored for prolonged periods in formalin would still be suitable for evaluative staining.[6] However, it was unclear if prolonged immersion in acetone to remove bone cement would affect our results.

The samples utilized for this study had been stored in formalin for various periods of time (shortest duration was 30 days and the longest was 17+ years) and were selected based on areas of interest identified during gross examination (Figs. 6, 7, 8). The H&E stained slides were reviewed by a board-certified pathologist.

Sections from bones fixed in formalin for 30 days stained well with H&E and readily distinguished cell types with clear delineation of viable and necrotic bone and tissue. Bone marrow architecture was well preserved, with areas demonstrating intact trilineage hematopoiesis adjacent to necrotic tissue separated by a zone containing predominantly small lymphocytes (Figs. 9, 10).

H&E stained sections from samples stored in formalin since 1992 revealed focal amorphous material within a fibrotic matrix (Fig. 11). Importantly, bone marrow architecture was intact, with areas demonstrating unremarkable trilineage hematopoiesis similar to that seen in the 30-day fixed sample (Fig. 12). Interestingly, focal areas with numerous histiocytes admixed with fibrous bands were also identified (Fig. 13). A specimen stored in formalin since 1997 also showed unremarkable bone and marrow space adjacent to where the cement had been removed (Fig. 14). From these microscopic evaluations, we were confident that prolonged formalin fixation and extended time in acetone followed by formic acid decalcification did not adversely affect histologic evaluations of H&E stained sections. This result can be valuable to the orthopaedic researcher who must decide if additional study of archived samples will be fruitful.

Diminished staining intensity of safranin O-fast green (SOFG) in the articular cartilage was an unexpected consequence

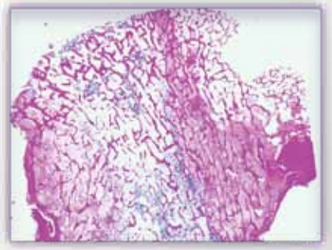

Fig. 6. H&E stained section from the decemented, decalcified, paraffin-processed block in Fig. 5. Note the irregularities about the original articular surface that was filled with bone cement before its removal with acetone. The peripheral portions show eosinophilic material in the intertrabecular spaces, which is relatively absent in the central regions. Specimen was retained in formalin for approximately 30 days prior to grossing. Scan of microscope slide, 600 dpi.

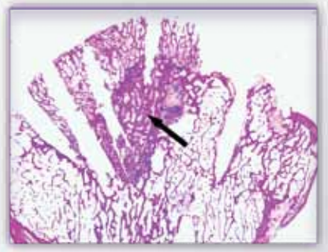

Fig. 7. H&E stained section from a decemented, decalcified, paraffin-processed block that had been stored in formalin since 1992. Note the numerous "tunnels" that were bored perpendicular to the articular surface in an effort to increase subcapital stability with bone cement. The preliminary region of interest is the noticeably more dense central region (arrow). Scan of microscope slide, 600 dpi.

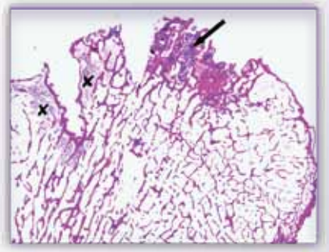

Fig. 8. H&E stained section from a decemented, decalcified, paraffin-processed block that had been stored in formalin since 1997. Primary regions of interest are the dense area on the upper right (arrow) and what grossly appeared to be regenerative tissue adjacent to the shaft-like void on the upper left (two stars). Scan of microscope slide, 600 dpi.

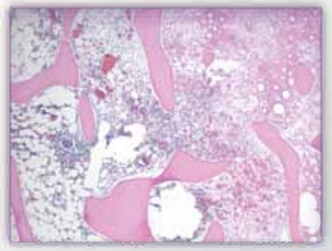

Fig. 9. H&E stained section of Fig. 6 showing intact trabecular bone and marrow space with a transitional zone between viable tissue with trilineage hematopoiesis and nonviable tissue. 40X

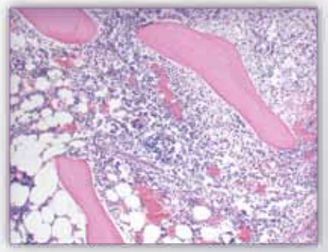

Fig. 10. Higher power view of Fig. 9 showing a transition zone of primarily lymphocytes. 100X

Fig. 11. H&E stained section of the dense area in Fig. 7 showing amorphous, acellular material located within a fibrotic matrix. 20X

of exposure of the bone samples to acetone. Safranin O stains proteoglycan present in proliferative and differentiating chondrocytes. These chondrocytes are found in growth plate cartilage and healing fracture sites as well as in articular cartilage. In orthopaedic research, this is a useful stain in osteoarthritis studies in the femoral head[7] as well as in the basic investigation of decalcified bone samples derived from fracture healing and osteogenesis models where the stain helps to differentiate between direct and endochondral bone formation in distraction osteogenesis (a limb-lengthening procedure).[8]

Based on published results, we fully expected to achieve acceptable SOFG staining of specimens stored in formalin for as long as 10 years.[6] However, when compared to staining in a non–acetone-treated specimen (Fig. 15), the SOFG staining of the cartilage in an acetone-treated sample (Fig. 16) was far from satisfactory or diagnostic.

Discussion

The purpose of this study was to identify a processing modality for metal-capped resurfaced femoral heads. We use the term "metal capped" because the preferred material for these resurfacing caps has varied between stainless steel, titanium, and cobalt chrome. Even bone cement has undergone an evolution in composition and consistency over time. Each of these metallic materials has its own properties for use and potential additional studies. Rigidity, retention surface resiliency, and potential for metallosis each play a part in material selection. Our purpose was not to evaluate these materials or their results but to develop the methodology to prepare these samples for microscopic study.

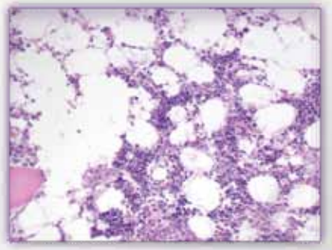

Fig. 12. H&E stained region of interest from a different specimen from 1992 showing intact marrow space with representative residual hematopoiesis. No amorphous material was noted in this specimen. 100X.

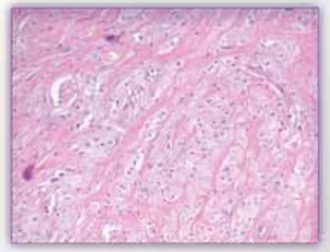

Fig. 13. Different H&E stained region of interest from the same 1992 specimen as in Fig. 12 showing a representative marrow space that focally contains numerous histiocytes admixed with fibrous bands. 100X

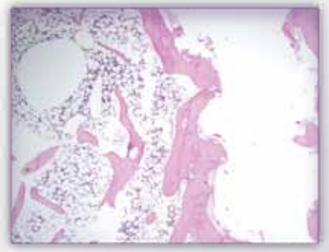

Fig. 14. H&E stained section from Fig. 8 reveals bony trabeculae and intact cellular marrow space adjacent to what was a shaft of bone cement. Possible "remodeling" on the inner aspect of the shaft could be inferred from the conformity of the thicker trabecular bone in response to where the cement had been forced during surgery. 20X

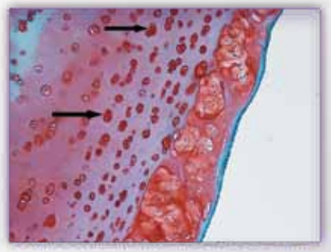

Fig. 15. Optimal SOFG staining of formalin-fixed, formic acid decalcified, and paraffin-processed articular cartilage in a pathologic femoral head. Crisp orange-red staining of the proteoglycan clearly illustrates the differentiating chondrocytes (arrows). 100X

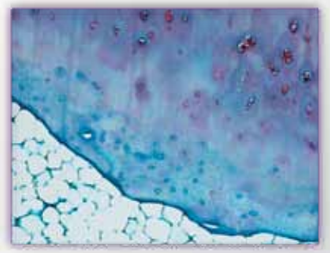

Fig. 16. The "best" field of what was deemed to be an inadequate SOFG stained section of articular cartilage in femoral head. This specimen was retained in formalin for approximately 60 days before being grossed, then immersed in acetone approximately 18 hours to remove methyl methacrylate bone cement prior to decalcification and paraffin processing. The acetone immersion appears to have leached out proteoglycans, thereby diminishing the intensity of the SOFG staining of the chondrocytes. 100X

Fig. 17. Decalcified slab from a resected hip acetabulum area. The central white zone is bone cement having been injected into the site to act as a stabilizer after previous local tumor resection. The cement cut well with a standard band saw at time of initial grossing and the cement was not discovered until this suspicious zone appeared after decalcification. Options are to manually remove the cement, jeopardizing interfaces, or to remove the cement with acetone immersion.

Histologic study of samples associated with biomaterials and/or metal prostheses is often out of reach for all but those select laboratories that have the specialized equipment needed to gross in these samples and prepare methyl methacrylate sections. The cost of this equipment is prohibitive unless there is a sufficient volume of this unique work to be cost effective. The cost of a full-blown Kulzer resin in situ metal, cut-and-grind histologic workup is significantly more expensive. Even a specialty orthopaedic research lab cannot afford to undertake this casually.

This study required the use of an expensive water-cooled, diamond-edged bladed band saw. Our particular unit is at the high end of precision. It may be possible for metal-capped femoral head samples to be "opened" or slabbed at 5 to 10 mm thickness with less precise models of water-cooled, diamond-edged units such as the Exakt Pathology Saw (Exakt Technologies, Oklahoma City, OK), which is nearly 50% of the cost of the Macro Saw we used.[9] The ability to utilize routine paraffin processing as an alternative to methyl methacrylate allows this type of specimen to be within reach of laboratories having less sophisticated equipment.

While the sample size of our study may be too small to draw sweeping conclusions, our results do illustrate that femoral head specimens stored for prolonged periods in formalin and/or treated with acetone for bone cement removal have preserved cytoarchitecture. These specimens may also be used to identify samples suitable for more elaborate evaluation of cement-to-bone and cement-to-metal interfaces as well as mineralization characteristics and microfracture occurrence using more expensive resin technologies.

While more sophisticated technologies are available for orthopaedic study, samples prepared in the manner described here will yield sufficient cellular detail to serve as a useful screening tool, reserving costly techniques for samples identified as most suitable for more detailed study. The described technique is also useful in working with bone specimens where cement may have been employed as either a "spacer" in tumor surgery or as an antibiotic delivery system in the treatment of acute orthopaedic infections (Fig. 17).

Acknowledgments

Figures 1, 2, and 3 first appeared in the following article and are being used with permission from the Arkansas Medical Society:

Nelson CL, Garrison RL, Walz BH, McLaren SG. Resurfacing of only the femoral head—treatment for young patients with osteonecrosis of the femoral head with collapse, delamination and significant head involvement. *J Ark Med Soc.* 2003;100(5):162-163.

Charles L. Stewart, UAMS Department of Orthopaedic Surgery (retired), performed the seminal work that laid the foundation for the development of resin embedment protocols at the Center for Orthopaedic Research.

The work of Dr. Carl Ilardi, SUNY Stony Brook Department of Pathology (retired), was important in the early management of bone cement-containing specimens and influenced the strategy used in our study .

The authors wish to thank William (Bill) R. Hogue, Center for Orthopaedic Research, for his photography and computer assistance.

Funding provided by the Carl L. Nelson Chair of Orthopaedic Creativity. Dr. Carl L. Nelson was an innovative surgeon, a respected researcher, an effective teacher, and a great colleague.

We appreciate the opportunity to share with you these unique specimens that prompted the ensuing thoughts and concepts.

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References

1. Stogiannidis I, Puolakka T, Pajamäki J, Moilanen T, Konttinin YT. Whole-mount specimens in the analysis of en bloc samples obtained from revisions of resurfacing hip implants: a report of 4 early failures. *Acta Orthop.* 2010;81(3):324-330.
2. Yaeger JA. Methacrylate embedding and sectioning of calcified bone. *Stain Technol.* 1958;33(5):229-239.
3. Jowsey J, Phil D, Kelly PJ, et al. Quantitative microradiographic studies of normal and osteoporotic bone. *J Bone Joint Surg Am.* 1965;47:785-806.
4. Huber M, Reinisch G, Zenz P, Zweymüller K, Lintner F. Postmortem study of femoral osteolysis associated with metal-on-metal articulation in total hip replacement: an analysis of nine cases. *J Bone Joint Surg Am.* 2010;92(8):1720-1731.
5. 37th Annual NSH Symposium/Convention Workshop 92. National Society for Histotechnology Web site. http://www.nsh.org/nshws92. Accessed May 11, 2012.
6. Skinner RA, Bellamy WT, Swain FL, Suva LJ. After 24 years in formalin, it *should* be fixed. *HistoLogic.* 2009;42(1):6-9.
7. Kalscheur VL. Bone and cartilage changes in osteoarthritis: a proteoglycan stain. *HistoLogic.* 2001;34(1):14-15.
8. Lumpkin CK Jr. Department of Pediatrics, UAMS. Unpublished data.
9. Durbin L, President, Exakt Technologies, Oklahoma City, OK. Personal communication.

The Effect of Temperature and Agitation on Adipose Tissue Fixation

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Abstract

Proper processing and fixation of fatty tissue continue to be a challenge in the histology laboratory. The ability to obtain optimal fixation varies depending on the thickness and fat content of the specimen, as well as the nature of fixative solution used. These are the main factors that may yield underprocessed tissue samples. The use of alcoholic formalin in preprocessing and processing specimens, often recommended as a solution to this problem, is not an option for some immunohistochemical (IHC) procedures, especially those typically used on breast samples. Although different ratios of alcohol/formalin solutions have been proposed, it is the most common practice to use 10% buffered formalin. It has been shown that temperature is a variable that affects tissue fixation. Usually, tissue fixation is carried out at room temperature, although it is expected that higher temperatures would increase the rate of fixative penetration; however, an increase in temperature might also increase the rate of autolysis and diffusion of cellular components.

The purpose of this study was to evaluate the effect that different temperatures and the use of agitation during the fixation process have on the histology of residual breast fat tissue samples. Several pieces of fatty tissue were taken from the same specimen and sectioned at 3 mm thickness, fixed for either 24 hours at room temperature or 29°C, with and without agitation with a stirring bar. These samples were then processed using the same fatty tissue settings (12 hour cycle with isopropyl alcohol, xylene, and paraffin) in the tissue processor. Hematoxylin and eosin (H&E) staining on 4 µm sections were reviewed by a pathologist. Although no major differences were observed, slightly better histology was noted in the samples fixed at room temperature with agitation compared to those fixed at 29°C. As a result, we concluded that the assistance of agitation alone improves the penetration process without the need to increase the temperature of the process, thus preserving cellular integrity.

Introduction

The process of fixation occurs by affecting the desired tissue so that it is resistant to further changes. There are several types of fixatives used in today's pathology laboratories, although the most popular broad-spectrum fixative is 10% neutral buffered formalin (NBF). Some of the most important features of a fixative are its ability to make tissue more receptive to dyes and stains, and also the ability to alter tissue properties in order to maintain the tissue's form throughout the steps of processing, which can be negatively affected in the final and subsequent steps of processing.

There are several factors that affect the process of fixation: time, size, temperature, and the ratio of tissue volume to fixative volume. In general, the size of the tissue and the volume ratio have fairly set pathological standards. The average size of tissue submitted for processing should be no thicker than 3 mm (about the width of a nickel), and the volume of fixative should be at least 15 to 20 times greater than that of the tissue volume; the use of an inadequate amount of fixative can yield poor staining results in the end. The most variable aspects of fixation are the time spent in the fixative and also the temperature at which the fixation is carried out. In general, an increase in both temperature and time increases the rate of fixation, but can also increase the rate of cellular breakdown. Knowing what tissue type is being fixed will determine the fixation time and fixative type; for example, breast tissues can only be in 10% NBF a minimum of 6 hours and a maximum of 48 hours; leaving them longer can cause false negative results in the detection of the HER-2/*neu* gene by fluorescence in situ hybridization (FISH). It has been noted that the addition of agitation or shaking of the tissue at the same time that fixation is taking place causes an increased rate of fixation, resulting in less time necessary in the fixative before processing.

The ideal situation is to find an optimal fixation time to yield the best outcome. The purpose of this study was to evaluate the effect of different temperatures and the use of agitation during the fixation process on the histology of residual breast fat tissue samples. Although no major differences were observed, slightly better histology was noted in the sections obtained from the samples fixed at room temperature with agitation in comparison with those at 29°C. In addition, the assistance of agitation alone was shown to improve the penetration process without the need to increase the temperature, thus preserving cellular integrity.

Materials and Methods

Residual breast fat tissue samples were collected from two reduction mammoplasty cases. The tissues were selected based on the content of fibrous and fat tissue. The tissue was trimmed to fit the suggested guidelines for optimal size to allow the best fixation (3 mm), and six cassettes per case were created. All the cassettes were placed in a basket submerged in 10% NBF for a period of 24 hours—two cassettes were placed at room temperature (RT, approximately 19°C), two other cassettes were placed at RT under continuous agitation using a magnetic stirring bar underneath, and the final two cassettes were placed in 10% NBF at 29°C, also with a stirring bar underneath to provide agitation. In a separate experiment, the same conditions were repeated with the addition of a shaker/rotator system while keeping the samples at RT. After the 24-hour time period, the cassettes were removed from their respective formalin tubs and placed on an overnight fatty tissue processing run on a closed system processor; the samples were processed for a total of 14 hours. After processing was completed, the tissues were embedded in paraffin, and sections were cut at 4 microns for

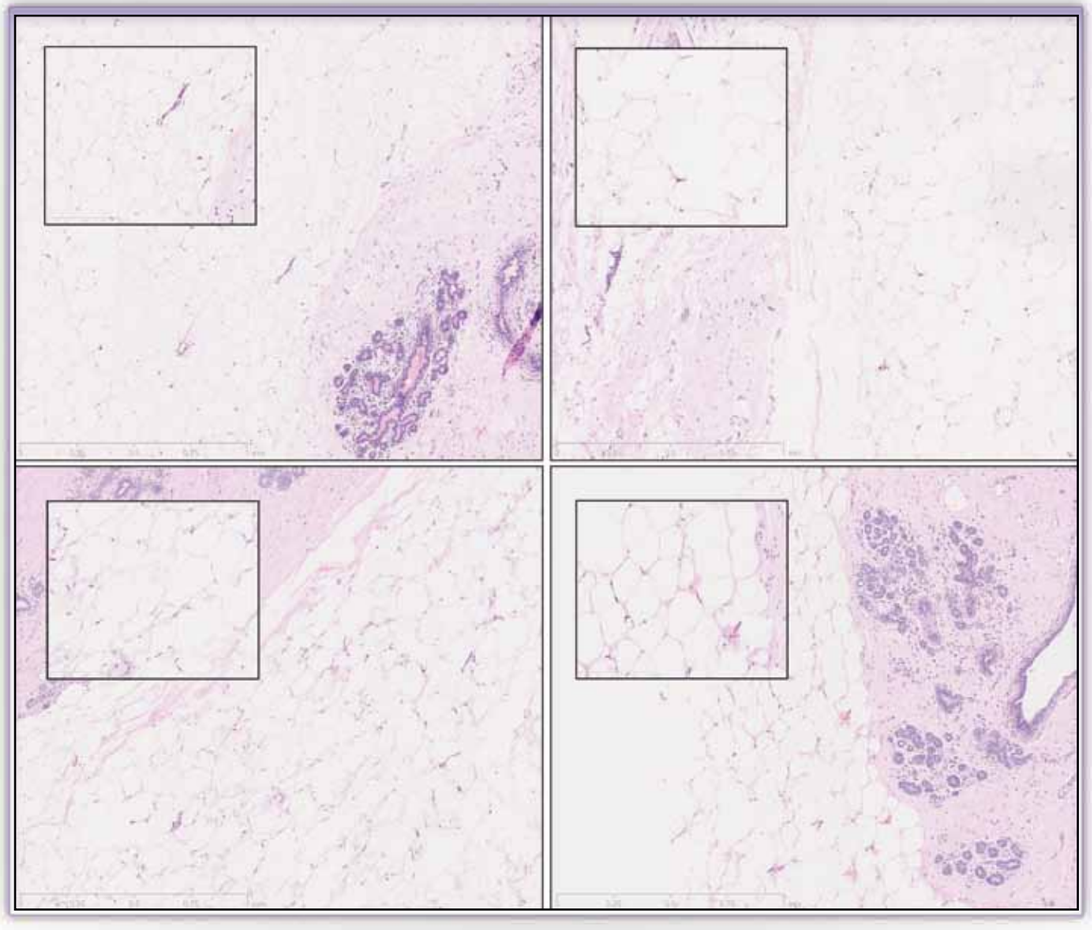

Fig. 1. Formalin-fixed breast tissue samples stained with H&E under the 4 conditions noted above. The focus of this comparison is the adipose tissue, which is best demonstrated in the inset under the conditions of room temperature + rotator. 40X (insets 100X)

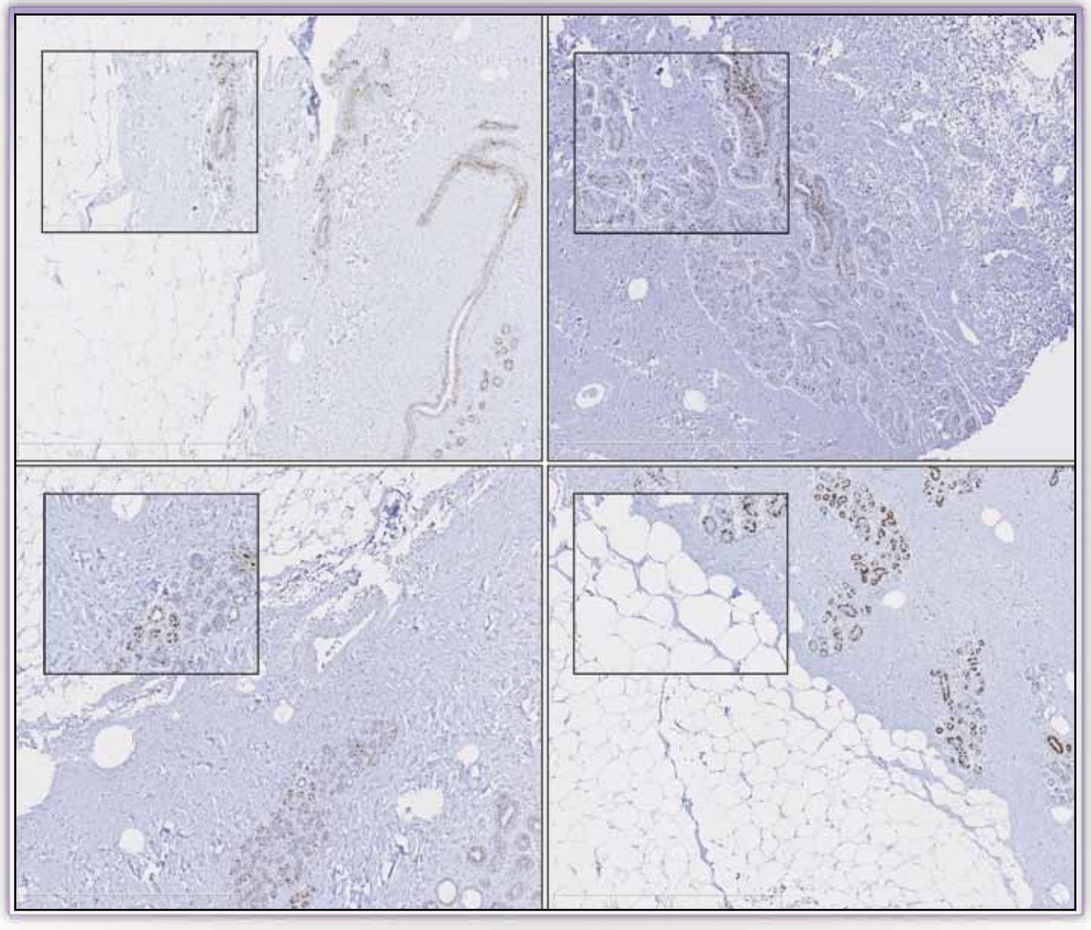

Fig. 2. Breast tissue samples stained by IHC for estrogen receptors under the 4 conditions noted above. The focus of this comparison is on the glandular tissue, which is where the estrogen receptors are found (visualized by the clusters of small dark dots). Room temperature + rotator demonstrated the optimal conditions for this comparison. IHC for progesterone yielded similar results (not shown here). 40X (insets 100X)

staining. Routine H&E staining was performed on each block, and further immunostaining for estrogen and progesterone receptors (ER/PR, clone 1D5 and PgR636, respectively) was later performed (Autostainer link 48, Dako, Carpinteria, CA).

Results

Fatty tissues, such as breast tissue, can be especially challenging to prepare for most laboratories as the high lipid content requires prolonged time in fixatives and processing solutions in order to achieve sections of adequate quality. Incomplete removal of lipid can prevent sufficient infiltration with paraffin and the resulting tissue blocks will section poorly. However, it has been our experience that fatty tissues that have been well fixed seem to process more thoroughly, yielding higher-quality sections.

In this study, we compared fixation of breast tissue with 10% NBF both at room temperature and elevated temperature (29°C) as well as with and without various forms of agitation (stirring fixative with a stir bar or placing specimens on a laboratory rotator).

It is generally accepted that fixation can be accomplished more quickly when carried out at elevated temperatures. As a result, we expected that tissues fixed at 29°C would receive more thorough penetration of formalin, which in turn was expected to yield the best results. In one study group, the heated fixative solution was also stirred with a stir bar to increase the fixative's exposure to the tissues.

It was no surprise that tissues fixed at room temperature without any form of agitation yielded the lowest quality slides. What was surprising, however, was that the tissues fixed at elevated temperature (29°C) did not yield the best outcome as we had expected. We discovered that tissues fixed at room temperature on a rotator achieved the best result (Figs. 1, 2). Tissues that were fixed in a stirred solution of formalin were superior in quality to slides fixed with no agitation at all, but tissues placed on the rotator during formalin fixation demonstrated the highest stain quality of all the conditions tested.

Conclusion

The aim of this study was to optimize the temperature and fixation time for large, fatty breast tissue samples. It was expected that the addition of heat and agitation would increase the rate of formalin fixation, ultimately improving the quality of the outcome of slides and stains. The experimental data show that the use of the rotator/shaker provides better fixation than agitation using magnetic stirring bars or than no agitation at all (Fig. 1).

Also, the data demonstrate that, even though fixing the tissue samples at 29°C did not improve the fixation process compared to room temperature fixation, the addition of heat did not affect the antigen expression, proven by the quality and crispness of the ER/PR stains by IHC (Fig. 2).

Although there were no differences in the histology of the sections, the blocks that were fixed with the use of the rotator/shaker were cut smoothly, and the ribbons that were retrieved contained fewer holes and did not erupt on the surface of the water flotation bath.

Overall, our results demonstrate that increasing the fixation temperature to 29°C does not improve the fixation process; however, the use of agitation with a rotator/shaker does dramatically improve the tissue sectioning process.

Bibliography

Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques.* 5th ed. New York, NY: Churchill-Livingstone; 2002:62-84.

Carson FL, Hladik C. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, IL: American Society for Clinical Pathology; 2009:2-12.

Chesnick IE, Mason JJ, O'Leary TJ, Fowler CB. Elevated pressure improves the rate of formalin penetration while preserving tissue morphology. *J Cancer.* 2010;1:178-183. http://www.jcancer.org/v01p0178.htm. Accessed February 16, 2011.

Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology.* 2nd ed. St. Louis, MO: CV Mosby Co; 1980:40-58.

Mounting of Paraffin Tissue Sections on Filter Paper for Storage, Mailing, and Microdissection

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Abstract

A method is described for mounting paraffin sections on filter paper instead of glass slides. The method has the advantages of being simple to perform, economical, unbreakable, requires less storage space, and the tissue sections can be mailed easily. Sections mounted in this manner can help in further processing of paraffin-embedded material for electron microscopy, molecular biology, and assembly of tissue microarrays.

Introduction

Histologic specimens have been used since the 18th century to help illustrate the structures and function of cells, tissues, and organs. The 20th century saw continued refinements in microscopy, laboratory technique, and instrumentation that have enhanced the information that can be obtained from microscopic preparations of tissues. Today, it is the norm to mount paraffin sections onto glass microscope slides.

Advances in molecular biology, biomarker validation studies, and disease diagnosis have generated a need for establishing a developed infrastructure for tissue collection, storage, and distribution for biomedical research and clinical practice. This has been especially valuable within a public healthcare service and in the pharmaceutical industry. Recovery of cells from sections by histological microdissection is being used more frequently for analysis by molecular biological methods or microchemical techniques. Archived paraffin sections are an important resource for DNA studies,[1] and can be processed for investigation by electron microscope.[2-6]

In this report, we describe a method for transferring and affixing a thin tissue section to filter paper instead of a glass slide. Use of a paper medium facilitates storage, mailing, and exchange of tissue specimens used in histological, histochemical, and histopathological laboratories, as well as receiving tissue specimens from biobanks and tissue banks for research purposes.

Materials and Methods

Tissues used for this study were chemically processed and embedded into paraffin blocks using conventional tissue processing methods.

Mounting Tissue Sections Onto Filter Paper Strips

Paper strips were prepared by cutting laboratory-grade filter paper (3" x 1" each) to mimic the size and shape of glass microscope slides. The strips were then labeled with the case number, type of organ, date, and other pertinent information using a pencil or waterproof marker.

Fig. 1. Mounting of paraffin section on filter paper; A) paper strip and glass slide before mounting; B) filter paper supported by glass slide after mounting.

Single sections of paraffin ribbons were sectioned at a suitable thickness (4-7 µ) with a rotary microtome. The sections were floated onto a warm water bath (45°C-50°C) to allow them to expand from the surface tension of the water. Ribbons were separated into individual sections using a fine needle and then transferred on to a clean glass slide covered with moistened, previously cut and labeled strips of filter paper (Fig. 1A). Avoid using albuminized slides—albumin, or egg white, is a sticky adhesive that will make removal of the paper strips very difficult after the slides have dried. The paper-covered slides (with sections of filter paper) were permitted to dry completely in an incubator (40°C-45°C) or slide dryer for about 1 hour. After drying was completed, the filter paper strips containing the paraffin sections were removed from the supporting glass slides (Fig. 1B) and stored either at room temperature or in a refrigerator (4°C), depending on intended use, separating each one from the other with a clean paper strip. For storage, empty glass slide boxes are suitable for sections mounted on filter paper.

Recovery of Paraffin Tissue Sections

Strips of filter paper carrying the stored paraffin sections were gently spread on the surface of a warm water bath (use plain water—avoid alcohol:water for spreading) at a temperature of 45°C-50°C. After a few minutes, the filter paper strips settled to the bottom of the water bath, leaving the thin paraffin sections floating on the surface (Fig. 2).

Each section was then transferred to a clean glass microscope slide, on which a little egg albumin or other adhesive had been smeared. The slide was passed under the surface of the water and brought up under the tissue section, lifting the glass slide with the tissue oriented where desired. The slide was placed vertically to allow water to drain from it, and then permitted to dry. Recovered thin tissue sections adhered firmly to the slides and were ready for further processing (eg, staining, application of immunohistochemical reactions, etc).

Separation of Areas of Interest (AOI) for Extraction of DNA or Investigation by Electron Microscopy

The filter paper storage technique is especially useful for collecting tissues for molecular studies or electron microscopy. The AOI was identified by matching a hematoxylin and eosin (H&E) stained section with the paraffin block. Material required for molecular biological studies (DNA extraction) and investigation by electron microscopy was usually cut at a thickness up to 20 microns. These sections were mounted onto filter paper strips as described above. Using a punch of suitable diameter (or a fine scalpel or razor blade), the sections were cut around the AOI and crosswise through their borders to the margins of each section (as shown in Fig. 3); it is acceptable to go deep in the filter paper thickness but do not separate the strip into pieces. After placing the filter paper onto the surface of a warm water bath, the AOI floated separately from the surrounding paraffin section. A glass slide, coverslip, or fine brush was used to transfer the AOI to an Eppendorf tube or glass vial for extraction of DNA, processing for electron microscopy, or assembly of tissue microarrays.

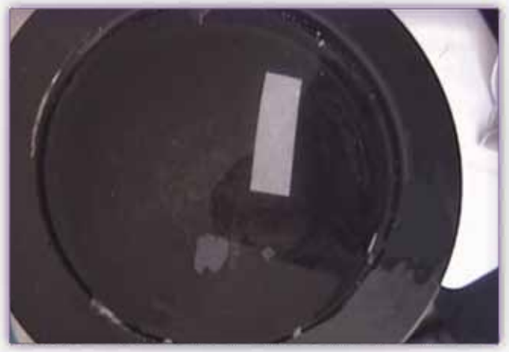

Fig. 2. Filter paper strip settles to the bottom of the water bath leaving the paraffin section floating on the surface.

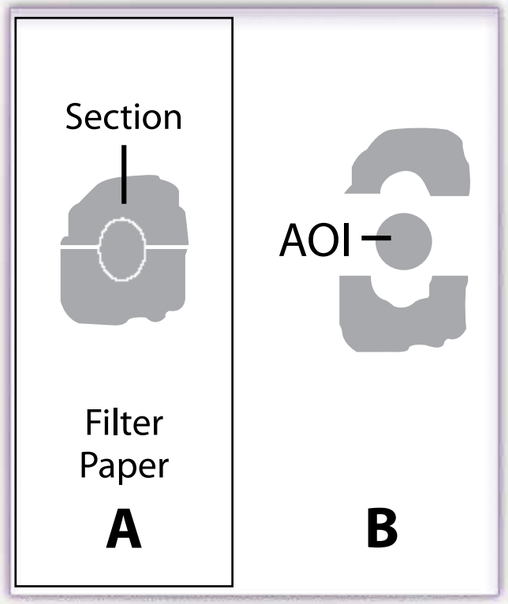

Fig. 3. Microdissection; A) before insertion in water bath; B) paraffin section separates from the paper and floats on the water surface.

Results and Discussion

Tissue sections mounted on filter paper separate easily from the filter paper when floated onto a warm water bath and can easily be mounted again on new glass slides for further preparation. Sections mounted on filter paper are cost-effective, and can easily be mailed as paper strips. They are not fragile compared to glass slides, and are good for 1 year or more without risk of mold growth if stored properly. The material occupies minimum storage space, is easily labeled, and can be conveniently archived.

In the field of histochemistry, from a practical point of view, one of the most difficult issues in the standardization of results is the availability of control material. Interlaboratory exchange of tissue sections is simple through regular mail, allowing any laboratory to achieve reliable results by mounting reference control material onto the slide containing the test specimen.

Mounting of tissue sections on paper is a good method for isolation (microdissection) and collection of areas of interest for molecular biology and pathology studies.

Many tissue banks (bio-banks) have been established worldwide.[7-12] These tissue banks usually deal with a large number of slides each year, which oftentimes creates problems with storage and archiving. For example, a tissue bank has been established in Austria (Genome Austria Tissue Bank, GATiB) that specifically collects diseased tissues and corresponding normal tissues from a nonselected Central European population of more than 700,000 patients. Tissue banks, moreover, provide quality-controlled tissue specimens and sections with standardized clinical annotation for cancer research.

Extraction of DNA from archival formalin-fixed, paraffin-embedded tissue was accomplished as early as 1985 using proteinase K and SDS as the major reagents.[13,14]

Tissue sections stored on filter paper can be utilized for electron microscopy studies. There are always cases where tissue retrieved from formalin-fixed, paraffin-embedded material for further studies of ultrastructure is necessary. Although structural preservation from formalin-fixed material is variable, Jing described a simple method of sample preparation for electron microscopy.[5] After paraffin-embedded tissues that were fresh and fixed on time were deparaffinized, the tissues were post fixed in 4% gluteraldehyde for 24 hours, 1% osmic acid (osmium tetroxide) for 1 to 2 hours, dehydrated with acetone, and embedded in Epon 812 (Shell Chemical Co., U.S.A.) before being cut into ultrathin sections. This method demonstrated better ultrastructure of paraffin-embedded tissues and satisfied the standards for observation with electron microscopy.

References

1. Shi SR, Cote RJ, Wu L, et al. DNA extraction from archival formalin-fixed, paraffin-embedded tissue sections based on the antigen retrieval principle: heating under the influence of pH. *J Histochem Cytochem.* 2002;50(8):1005-1011.
2. Gonzalez-Angulo A, Ruiz de Chavez I, Castaneda M. A reliable method for electron microscopic examination of specific areas from paraffin-embedded tissue mounted on glass slides. *Am J Clin Pathol.* 1978;70(4):697-699.
3. Klochkov ND. Electron microscopic study of archival material fixed in formalin [in Russian]. *Arkh Patol.* 1979;41(3):64-66.
4. Yang G, Tang X. A simple and rapid method of preparing cell or tissue embedded in situ for transmission electron microscopy [in Chinese]. *Hua Xi Yi Ke Da Xue Xue Bao.* 1997;28(2):229-231.
5. Jing J. Sample preparation for electron microscope with tissues fixed with formalin and embedded in paraffin [in Chinese]. *Hunan Yi Ke Da Xue Xue Bao.* 1999;24(6):581-582.
6. Ertault-Daneshpouy M, Deschaumes C, Leboeuf C, et al. Histochemical and immunohistochemical protocols for routine biopsies embedded in Lowicryl resin. *Biotech Histochem.* 2003;78(1):35-42.
7. Hewitt R, Hainaut P. Biobanking in a fast moving world: an international perspective. *J Natl Cancer Inst Monogr.* 2011;2011(42):50-51.
8. Murtagh MJ, Demir I, Harris JR, Burton PR. Realizing the promise of population biobanks: a new model for translation. *Hum Genet.* 2011;130(3):333-345.
9. Malin B, Loukides G, Benitez K, Clayton EW. Identifiability in biobanks: models, measures, and mitigation strategies. *Hum Genet.* 2011;130(3):383-392.
10. Kugler KG, Hackl WO, Mueller LAJ, Fiegl H, Graber A, Pfeiffer RM. The impact of sample storage time on estimates of association in biomarker discovery studies. *J Clin Bioinforma.* 2011;1:9.
11. Sterckx S, Van Assche K. The new Belgian law on biobanks: some comments from an ethical perspective. *Health Care Anal.* 2011;19(3):247-258.
12. Kiehntopf M, Krawczak M. Biobanking and international interoperability: samples. *Hum Genet.* 2011;130(3):369-376.
13. Goelz SE, Hamilton SR, Vogelstein B. Purification of DNA from formaldehyde-fixed and paraffin-embedded human tissue. *Biochem Biophys Res Commun.* 1985;130(1):118-126.
14. Dubeau L, Chandler LA, Gralow JR, Nichols PW, Jones PA. Southern blot analysis of DNA extracted from formalin-fixed pathology specimens. *Cancer Res.* 1986;46(6):2964-2969.

Modified Movat Stain Is Efficient and Cost-effective

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Introduction

Movat pentachrome stain is one of the most useful stains used in cardiovascular and pulmonary pathology to highlight the various tissue components and their pathologic alterations. This stain is technically difficult to perform, labor intensive, and, to a certain degree, histotechnician dependent. For several years, we have encountered various problems in sustaining the quality and accuracy of performing this stain. Histotechnologists assigned at the special stain area spend a considerable amount of time performing the stain; on occasion, it is necessary to redo the staining, which requires even more histotechnician time.

The aim of modifying our current laboratory Movat protocol was not only to reduce the number of stains that had to be repeated due to suboptimal collagen staining, but also to attain more distinct differentiation in various connective tissues and to develop a protocol that allows for reproducible staining results.

Materials and Methods

In modifying our Movat procedure, we first developed a "combo" control block composed of aorta, heart, and lung tissue (Fig. 1). Since one of the most crucial steps in the Movat procedure is the differentiation from Verhoeff hematoxylin, our combo control block gives us a good representation of all the different tissue components that should be demonstrated with this stain. This resulted in complete differentiation and easy penetration of the succeeding stains used in the procedure.

Next, we evaluated a more concentrated commercially prepared alcoholic saffron reagent (American MasterTech Scientific, Inc., Lodi, CA). This saffron stain solution is actually a component of their Russell-Movat pentachrome stain kit, but we used it with our own original reagents and stains. The different time parameters for the rest of the reagents and solutions were adjusted to obtain optimum differentiation. Specifically, we

Original Movat Staining Procedure: Approximately 80 minutes	Modified Movat Staining Procedure: Approximately 45 minutes
• Deparaffinize and hydrate slides to water (approximately 15 min for all steps)	• Deparaffinize and hydrate slides to water (approximately 15 min for all steps)
• Rinse in distilled or deionized water for 30 sec	• Rinse in distilled or deionized water for 30 sec
• Stain in alcian blue (microwave slides for **30 sec** in 250 mL solution)	• Stain in alcian blue (microwave slides for **20 sec** in 250 mL solution)
• Wash in running tap water for 5 min	• Wash in running tap water for 5 min
• Stain in Verhoeff hematoxylin for **12 min** (solution good for 1 week)	• Stain in Verhoeff hematoxylin for **8 min** (solution good for 1 week)
• Rinse well in running distilled water until water turns clear	• Rinse well in running distilled water until water turns clear
• Differentiate with 2% aqueous ferric chloride (rinse in distilled water and **check microscopically for black staining of elastin fibers**)	• Differentiate with 2% aqueous ferric chloride (rinse in distilled water and **check microscopically for the elastin fibers to contrast sharply with the background**)
• Place slide in 5% aqueous sodium thiosulfate for 1 min	• Place slide in 5% aqueous sodium thiosulfate for 1 min
• Wash in running tap water for **5 min** and rinse in distilled water	• Wash in running tap water for **1 min** and rinse in distilled water
• Stain in crocein scarlet-acid fuchsin for 5 min	• Stain in crocein scarlet-acid fuchsin for 5 min
• Rinse in distilled water	• Rinse in **3 changes of distilled water**
• Rinse in 0.5% acetic acid	• **Place slide in 0.5% acetic acid for 30 sec**
• Place slide in 5% phosphotungstic acid for **8 min**	• Place slide in 5% phosphotungstic acid for **5 min**
• Rinse in 0.5% acetic acid	• **Place slide in 0.5% acetic acid for 30 sec**
• Place slide in absolute ethyl alcohol for **1 min**	• Place slide in absolute ethyl alcohol for **30 sec**
• Stain slide in alcoholic saffron for **15 min**	• Stain slide in alcoholic saffron for **4 min** (stir before use)
• Dehydrate in 3 changes of absolute ethyl alcohol	• Place slide in absolute ethyl alcohol for **30 sec** • Dehydrate in 3 changes of absolute ethyl alcohol
• Clear in xylene and mount	• Clear in xylene and mount

Reagents

Alcian Blue – pH 2.5 (use commercial stain)

Verhoeff Hematoxylin Solution

5% hematoxylin in absolute alcohol (see prep below)	160 mL
10% aqueous ferric chloride (use commercial solution)	80 mL
Iodine solution (see prep below)	80 mL

Iodine Solution

Iodine	16 g
Potassium iodide	32 g
Distilled water	800 mL

Dissolve potassium iodide in a small amount of water. Add iodine and dissolve. Add remaining volume of water.

5% Hematoxylin

Hematoxylin	40 g
Absolute ethyl alcohol (flammable)	800 mL

10% Ferric Chloride (use commercial solution)

Alcoholic Saffron (use commercial solution)

0.5% Acetic Acid (add acid to water)

5% Sodium Thiosulfate (use commercial solution)

5% Phosphotungstic Acid (use commercial solution)

2% Ferric Chloride (prepare from 10% ferric chloride solution)

Crocein Scarlet-Acid Fuchsin

Stock Solution A

Crocein scarlet	0.5 g
Distilled water	497.5 mL
Acetic acid	2.5 mL

Stock Solution B

Acid fuchsin	0.2 g
Distilled water	199 mL
Acetic acid	1 mL

Working Solution (1:4 concentration)

Stock Solution A	200 mL
Stock Solution B	50 mL

Fig. 1. Combination control block composed of aorta, heart, and lung tissue.

shortened the staining in Verhoeff solution as well as in crocein scarlet and saffron. These alterations in time were recorded and developed into the modified protocol. An important step to emphasize is the washing after each differentiation of stains. We observed that incomplete washing off or failure to remove excess reagents or stain with running water and then deionized water before moving forward will inhibit the subsequent staining steps.

Results

From deparaffinization to the finished slide, the addition of new saffron shortened the staining method from 80 minutes with the old procedure to 45 minutes with our modified procedure. This new procedure was easily performed by all histotechnicians rotating in the special stains area. This is not only easier to perform but also gives us more precise and accurate staining results (Figs. 2, 3). Since the procedure is easier and quicker to do, it also lessens the billable technical time component in doing the staining procedure, which in turn lowers our stain cost. More histotechnician time was saved and is now spent in busier areas, such as embedding and microtomy, which increases laboratory productivity.

One of the most significant outcomes in implementing the modified protocol was a decrease of at least 75% in the need for repeat staining (Fig. 4). It also demonstrated better differentiation of various tissues needed for diagnostic purposes with improved satisfaction of the pathologists.

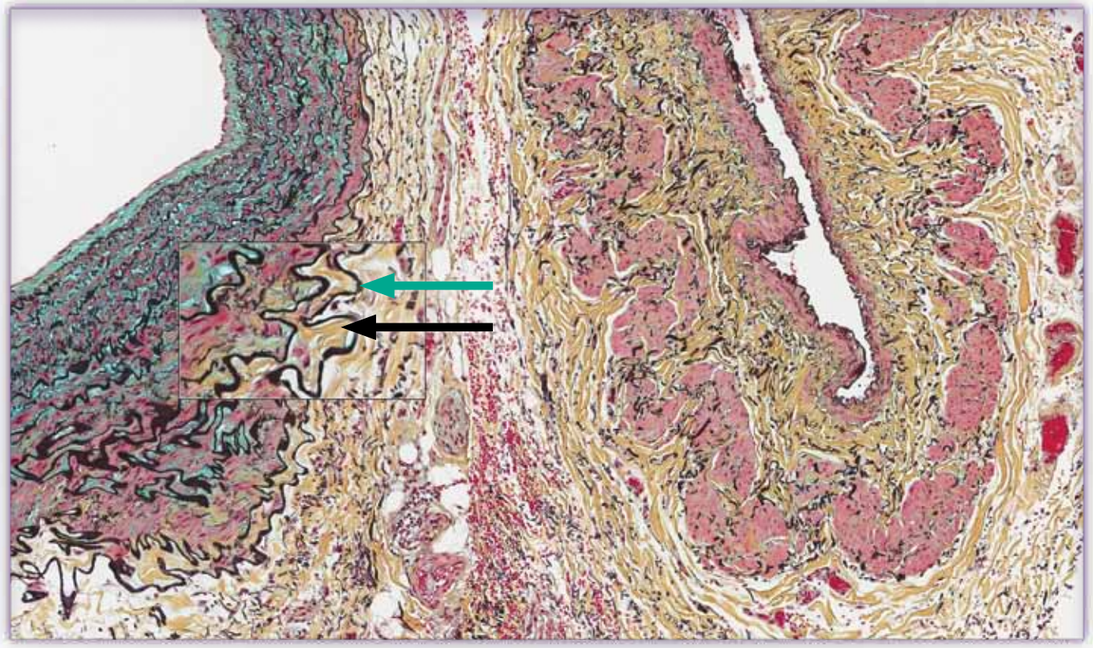

Fig. 2. Lung tissue stained using the modified Movat procedure. Note the well-defined structures including elastin (black structures denoted by green arrow) and collagen (yellow-orange structures denoted by black arrow). 100X (inset 200X)

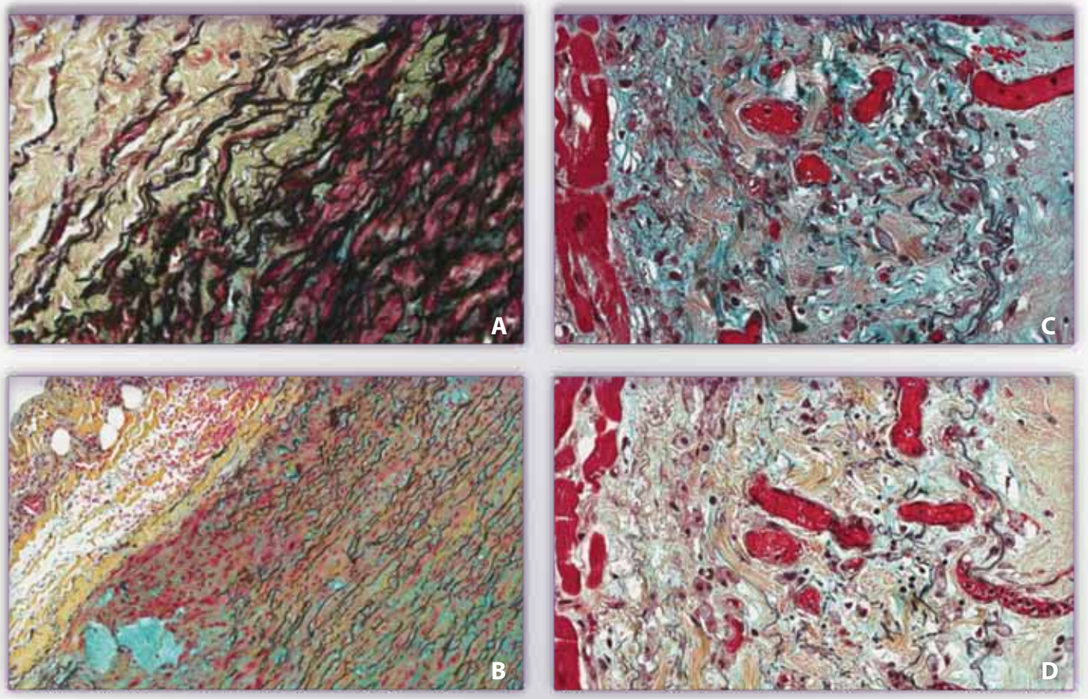

Fig. 3. Slide comparison using the original Movat staining procedure (A, C) and the modified Movat staining procedure (B, D); A) aorta, 200X; B) aorta, 100X; C) heart, 100X; D) heart, 100X. The modified procedure yielded more consistent results with better differentiation of tissue structures.

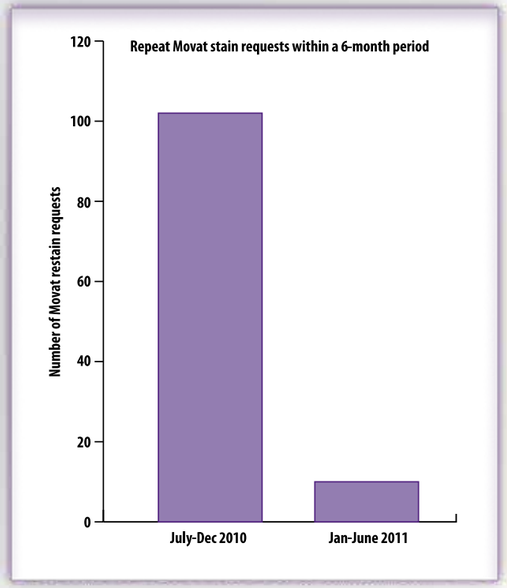

Fig. 4. Implementation of the modified protocol decreased the number of repeat staining from 3% (110 out of 3538 stains) to 0.5% (19 out of 3615 stains) over a 6-month period. In addition, there is significant improvement in satisfaction of pathologists reviewing the stains.

Conclusion

This modified Movat staining procedure with the use of a more concentrated saffron reagent is time saving because of the shortened procedure time. It is highly reproducible and decreased the number of cases that required repeat staining.

Bibliography

Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques.* 6th ed. New York, NY: Churchill-Livingstone; 2008:157-158.

Mikel UV. *AFIP Advanced Laboratory Methods in Histology and Pathology.* Washington, DC: Armed Forces Institute of Pathology, American Registry of Pathology; 1994:127-129.

Russell HK Jr. A modification of Movat's pentachrome stain. *Arch Pathol.* 1972;94(2):187-191.

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Title: **Process Improvement in the Histology Laboratory: LEAN**
Speaker: William DeSalvo, BS, HTL(ASCP)
Laboratory Sciences of Arizona
Tempe, AZ

27 NSH Teleconference 1:00 pm Eastern Time
Title: **Controlling Your IHC Stains**
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McKinney, TX and
Kathleen A. Dwyer, HT(ASCP)
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20 University of Texas Health Sciences Ctr/San Antonio
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Title: **Why Do the H&Es Look Different Today?**
Speaker: Ada Feldman, MS, HTL(ASCP)
Anatech Ltd
Battle Creek, MI

25 NSH Teleconference 1:00 pm Eastern Time
Title: **Sentinel Lymph Nodes: A Look at Their Significance From the Histotech's Perspective**
Speaker: Heather Renko-Montes, BS, HT(ASCP)QIHC
Leica Microsystems
Bannockburn, IL
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AUGUST

17 University of Texas Health Sciences Ctr/San Antonio
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Efficiency in Anatomic Pathology**
Speaker: William DeSalvo, BS, HTL(ASCP)
Laboratory Sciences of Arizona
Tempe, AZ

22 NSH Teleconference 1:00 pm Eastern Time
Title: **Hirschsprung's Disease: Histologic Techniques for Diagnosis**
Speaker: Sarah Bajer, HTL(ASCP)
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

SEPTEMBER

19 NSH Teleconference 1:00 pm Eastern Time
Title: **Assessing Competency in the Histology Laboratory**
Speaker: Joelle Weaver, MAOM, HTL(ASCP)
Vanderbilt Medical Center
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Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

21 University of Texas Health Sciences Ctr/San Antonio
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **The Role of the Pathologist in the Management of Breast Cancer**
Speaker: Prashant A. Jani, MD, FCAP, FRCPC
Thunder Bay Regional Health Sciences Centre
Thunder Bay, Ontario, Canada

28-Oct 3 National Society for Histotechnology Symposium/Convention
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Vancouver, British Columbia, Canada
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OCTOBER

19 University of Texas Health Sciences Ctr/San Antonio
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Title: **Applications of Molecular Pathology in Colon and Lung Cancer**
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Department of Medical Laboratory Sciences
Tarleton State University
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24 NSH Teleconference 1:00 pm Eastern Time
Title: **Emergency Preparedness: Would Your Histology Lab Survive a Disaster?**
Speaker: Marcia Fisher, MEd, HT(ASCP)
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NOVEMBER

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Emory University Hospital
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28 NSH Teleconference 1:00 pm Eastern Time
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DECEMBER

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