Use of softening agents to improve the production of formalin-fixed, paraffin-embedded sections of nail tissue: an assessment

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ABSTRACT

The use of tissue softeners to enhance the quality of tissue sections of heavily keratotic tissue is not widely published. There are very few indicators in the scientific literature that attempt to compare and contrast the benefits and disadvantages of such techniques, as most are passed down through word of mouth rather than through published data. This study attempts to present a preliminary evaluation of several methods employing tissue softeners to facilitate the preparation of reproducible, good-quality formalin-fixed, paraffin-embedded sections of nail tissue. A standard 10-minute surface application of each softener is employed for all paraffin-embedded tissue in order to ensure consistency. The results show that the use of Veet (hair remover), Fairy Liquid or fabric conditioner provides the most beneficial results. Thus, widely available products can be used in preference to specific commercially produced reagents that have no clear benefits and can cost considerably more to purchase. This study will form the basis of a more in-depth evaluation of the most beneficial softeners, in an attempt to determine optimal parameters for their use in routine histopathology laboratories.

KEY WORDS: Baker's fluid.
Detergents.
Fabric softener.
Molliflex.
Potassium hydroxide.

Introduction

The evaluation of reagents to enhance the softening of heavily keratotic tissues is not widely published. An understanding of which compound constituents enhance this process is generally poorly understood. A search through the literature reveals scant reference to beneficial reagents that can be used for this purpose. Very few indicate any comparative appraisal of different techniques in an attempt to standardise the most appropriate choice of keratin softener in most circumstances.

The use of fabric conditioners[1,2] is cited. Turner's paper highlights the use of fabric conditioner in the reconstitution of mummified tissue prior to paraffin-wax sectioning, the rationale being one of rehydration of the mummified remains. Cutuly and Cutuly,[3] in 1934, and later Tahmisian and Slifer,[4] in 1942, demonstrated that paraffin wax is penetrated by aqueous solutions and that storing blocks in water obviates brittleness.

Baker,[5] in 1941, proposed the use of a mixture of nine volumes of 60% ethanol and one volume of glycerol in preference to water alone. However, Pearlman and Cole,[6] in 1950, made the observation that water alone only resulted in superficial softening, and recommended the use of soaking in wetting agents, presumably to ensure adequate penetration of the keratinised tissue.

However, a plethora of other reagents based on widely available and inexpensive products have achieved some popularity more recently (e.g., Fairy Liquid or hair removal creams such as Veet). How such substances achieve the desired effect of softening keratin is not understood because not all the constituent components of such substances are widely known.

Other substances such as phenol-based softeners,[7,8] used in studies involving the sectioning of grasshopper eggs, have proved popular, but they have significant health and safety drawbacks and do not represent a realistic option for broad use. Potassium hydroxide is widely advocated in mycology investigations for softening keratin in order to enable the production of a monolayer of keratinocytes for light microscopy evaluation.[9] However, in the primary author's opinion, this may represent an excessive form of pretreatment for histologically prepared tissue and may result in adverse tissue disaggregation and loss of morphological detail.

The aim of the present study is to evaluate a series of keratin softening reagents on human nail tissue in a standardised trial in order to provide an indication of which reagents provide the best option for widespread use. It will also form the basis of a more extensive appraisal of the softeners that demonstrate beneficial effects.

Materials and methods

Nail clippings were taken from the authors and fixed in 10% neutral buffered formalin for 12 hours. It was then processed on a 17-hour processing schedule (Leica TP10/50 enclosed tissue processing machine).

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The softeners assessed were Baker's fluid, Fairy Liquid (Procter and Gamble), Molliflex (VWR), Easy fabric conditioner (Powder and Liquid Products), distilled water, 30% (w/v) potassium hydroxide and Veet hair removal cream (Reckitt Benckiser). All reagents used were applied for just 10 minutes each following paraffin wax embedding, in order to ensure consistency. The same paraffin blocks were used to appraise all the softeners, and the surfaces were washing in water and wiped before application of each softener. In addition, each block was trimmed between each application of softening agent to ensure that no cumulative benefit was achieved.

Paraffin blocks were placed on a cold plate prior to sectioning and also following softening with the above reagents. All sections were cut on a Leica rotary microtome with the section thickness set at 4 µm. All sections were mounted on Superfrost Plus charged slides (VWR) and dried on a hot plate. All sections were stained with haematoxylin and eosin (H&E) using the department's standard staining protocol on a Leica Autostainer XL staining machine.

Section quality was determined microscopically for each reagent used by two of the authors (GO and JT). Technical notes were prepared for each assessment to determine whether or not difficulties were experienced during microtomy (e.g., juddering or shattering of tissue material) (Table 1).

Results

Results showed that Easy fabric conditioner, Fairy Liquid and Veet proved to be the most effective keratin softeners (Figs. 1, 2 and 3). Observations from the sectioning of paraffin blocks indicated that the less-viscous softeners penetrated the tissue blocks more effectively and therefore enabled the production of several serial sections before juddering or shattering of the tissue became apparent.

Discussion

This study demonstrates that a host of tissue softeners have benefit in the sectioning of keratotic nail tissue. Furthermore, it has been possible to demonstrate, through light microscope examination and observations of microtomy, that Easy fabric softener and Veet hair removal cream are the two most effective softeners, closely followed by Fairy Liquid.

All other reagents used did not produce good section quality or prevent juddering and/or shattering of nail tissue during microtomy. These softeners seemed only able to penetrate the paraffin block sufficiently to enable just one or two sections to be cut before juddering and shattering returned (e.g., distilled water, Molliflex and Baker's fluid).. This indicates that the fabric softener and Veet were able to penetrate the tissue blocks more effectively and enable more consistent tissue sectioning.

Potassium hydroxide (30% [w/v]) did soften the nail tissue but appeared excessive in some cases, as the resulting disaggregation of nail tissue implied that its use may be harder to standardise for histological tissue preparations.

The present study was standardised throughout by the

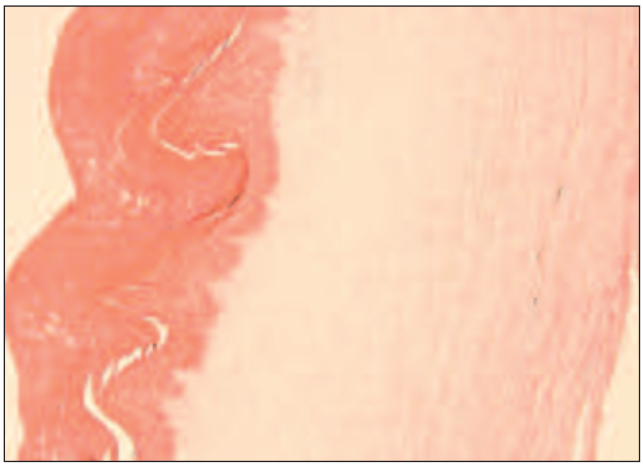

Fig. 1. Neutral buffered formalin-fixed and paraffin-embedded section of toe nail tissue showing uniform, undisrupted preservation of the keratin and nail bed, following the application of Easy fabric conditioner. Note there is no evidence of shattering or knife mark scores (original magnification x100).
See the images in colour at www.bjbs-online.org

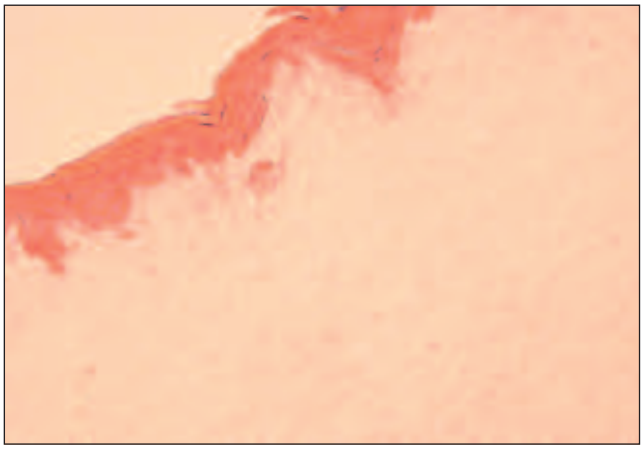

Fig. 2. Neutral buffered formalin-fixed and paraffin-embedded section of toe nail tissue showing uniform, undisrupted preservation of the keratin and nail bed, following the application of Veet (original magnification x100).

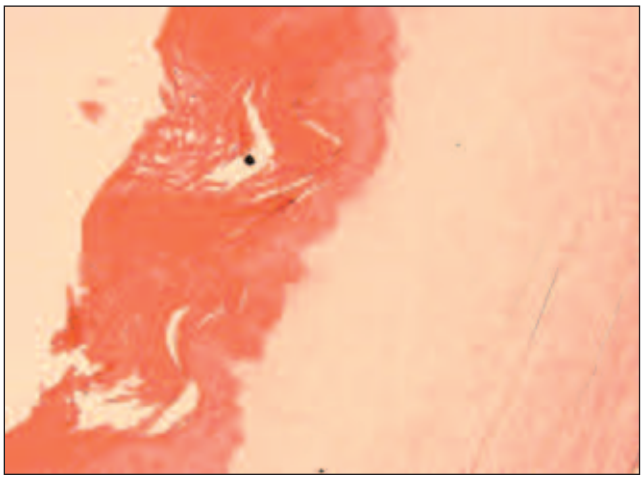

Fig. 3. Neutral buffered formalin-fixed and paraffin-embedded section of toe nail tissue showing uniform, undisrupted preservation of the keratin and nail bed, following the application of Fairy Liquid (original magnification x100).

Table 1. Technical notes.

	Distilled H_2O	30% (w/v) KOH	Easy fabric conditioner	Molliflex	Fairy Liquid	Baker's fluid	Veet hair removal cream
Section quality	Adequate	Adequate	Good	Generally poor	Good	Adequate	Good
Technical notes	First few sections reasonable then juddering experienced	First few sections reasonable then juddering experienced	No juddering. Sections comparatively easy too cut	Juddering	First few sections reasonable then juddering experienced	Juddering	Slight juddering following first few sections

fact that all reagents used to soften nail tissue were used as paraffin block surface softeners for exactly 10 minutes each. The same blocks were used for all the softeners, thereby ensuring consistency of observation with all the test reagents.

Easy fabric softener, Veet and Fairy Liquid are all readily available and inexpensive consumable products. They have the added advantage that their use poses very few health and safety issues. This is not the case with some phenol-based softeners.

One consistent problem encountered during this study was that of ensuring that the keratotic tissue remained firmly attached to the Superfrost Plus charged slides. This was encountered with all the reagents used in the study. The nature of the nail tissue used meant that it was almost entirely keratin, with very little cellular material present. This will form the basis of a further assessment designed to improve keratin attachment to the slides.

In summary, this study highlights the use of inexpensive tissue softeners as an aid to sectioning nail tissue. The study was controlled in order to determine which softener was most effective in dealing with nail tissue. It is clear from these preliminary studies that a more detailed appraisal is needed to optimise the application of these agents. This will include consideration of pre-processing softener application as opposed to post-processing application (or both), incubation time, variation in temperature, and consideration of the use of additional reagents to enhance the penetration of some of the softeners. □

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