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Trial of Ultraviolet Clear PermaSlip Mounting Medium and Ultraviolet Light as New Clearing Agent in Mohs Micrographic Surgery Tissue Processing

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Abstract

Xylene and xylene substitutes are commonly used as clearing agents when processing tissue samples during Mohs micrographic surgery (MMS). However, there are several concerns with these solvents. Xylene is expensive, malodorous, and can be toxic depending on exposure levels. While xylene substitutes are less toxic, some of these are more expensive than xylene itself or do not biodegrade easily and require specific waste disposal. Here, we discuss an alternative using Ultraviolet (UV) Clear PermaSlip Mounting Medium and a UV light. This technique is advantageous in that it is non-toxic, has no odor, and does not have the significant costs of purchase and disposal that xylene and xylene substitute typically have. However, we found this technique can add up to 2 minutes to the process, and more importantly, may reduce the quality of the slide. Upon slide review with this method, we found more intracellular lacunae artifact with keratinocytes in the epidermis compared to the same tissue on consecutive cuts where traditional xylene substitute was used. Future studies could look at ways to minimize lacunae with this method. Ultimately, we must weigh the advantages and disadvantages of using a UV mounting medium and UV light and utilize our different options to provide the best quality patient care

Abbreviations

MMS: Mohs Micrographic Surgery; UV: Ultraviolet

Introduction

MMS is a specialized form of skin cancer surgery resulting in a nearly 100% cure rate while minimizing lost tissue and is particularly useful in cosmetically sensitive areas [1]. The surgeon first removes a thin layer of skin from the skin cancer. The tissue is then frozen, cut into thin slices, stained, and evaluated under a microscope for circumferential and deep tissue margin clearance of the skin cancer. If the margins are not clear, the process is repeated until they are clear [2]. During this tissue processing, speed and efficiency are pertinent in providing quality care. The histologicfrozen-tissue stains commonly used are generally based on preference and training, but three commonly used stains are toluidine blue, thionine, and hematoxylin &eosin [3]. Today, many Mohs clinics use automated slide stainers. Afterwards, a clearing agent is used to make biological tissues transparent while preserving tissue structure, allowing for visualization of the tissue under a microscope [4].

Currently, there are three main categories of tissue clearing techniques: solvents, hyperhydration, and hydrogel embedding techniques [4]. However, hyperhydration and hydrogel embedding technique are very time consuming and can take days. One such solvent used as a clearing agent is xylene. Xylene is an aromatic hydrocarbon commonly used during tissue processing in MMS. Advantages of xylene usage include that it is biodegradable, noncorrosive, nonflammable, soluble in alcohol and mounting media, reasonably fast drying, and doesn't leave a residue. However, xylene is also expensive, malodorous, and can be toxic depending on exposure levels. Some literature even recommends the use of a fume hood or some local exhaust ventilation in addition to personal protective equipment to reduce toxicity [5] .MMS labs now commonly use solvents such as Naphthenic solvent and d-limonenes chemicals that can be used a xylene substitute, but some of these are more expensive than xylene itself or do not biodegrade easily and require specific waste disposal.

Materials and Methods

As a result, in our clinic, we trialed the use of UV Clear PermaSlip Mounting Medium and a UV light as a new clearing agent (Figure 1). Using the Leica ST4020 linear slide stainer, we utilized a combination of alcohol, H_2O, Gill's Hematoxylin I, Gill's Hematoxylin II, Toluidine blue, and Eosin Y during the staining process (Figure 2). In order to use this mounting medium, the slide must first dry after going through the staining process. Then a small amount of the mounting medium is applied to the slide and cover slipped. Finally, the slide must sit under a UV light for approximately 30 seconds. This whole process can take up to 2 minutes.

Results

We found that this technique is advantageous in that it is non-toxic, has no odor, and does not have the significant costs of purchase

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Figure 1: UV Clear PermaSlip Mounting Medium and a UV light trialed as a new clearing agent in our clinic.

Figure 2: Leica ST4020 linear slide stainer and stains used in our clinic.

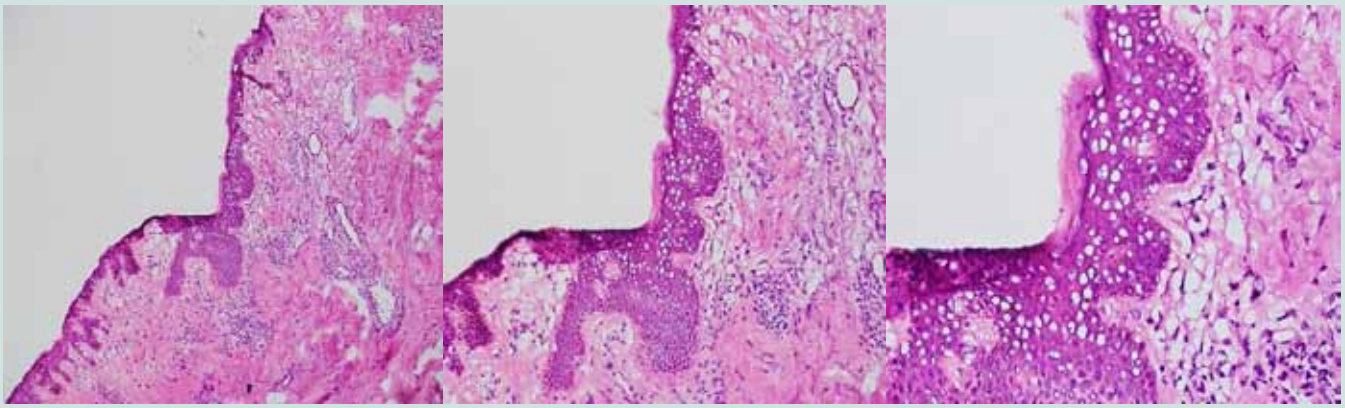

Figure 3: Tissue sample using UV mouting medium and UV light under the miscroscope at (from left to right) 10X, 20X, and 40X.

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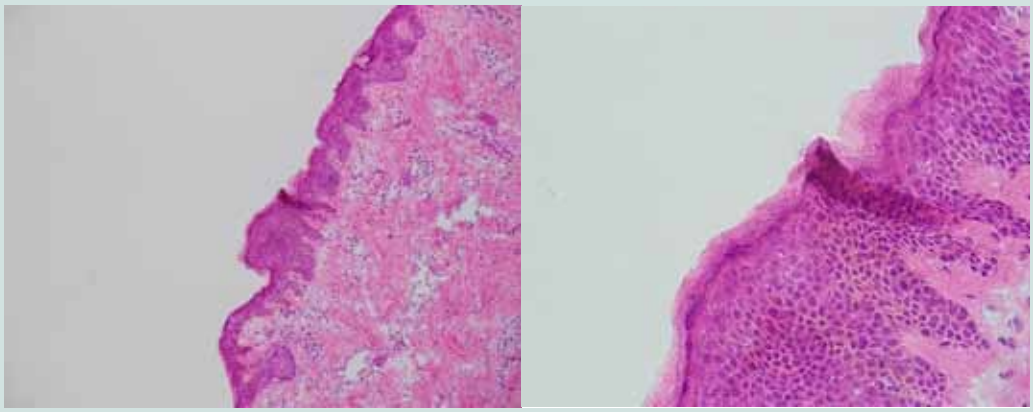

Figure 4: Same tissue sample using xylene substitiute under the miscroscope at 10X (left) and 40X (right).

and disposal that xylene and xylene substitute typically have. However, we found this technique can add up to 2 minutes to the process, and more importantly, may reduce the quality of the slide. Upon slide review with this method, we found more intracellular lacunae artifact with keratinocytes in the epidermis (Figure 3) compared to the same tissue on consecutive cuts where traditional xylene substitute was used (Figure 4). These intracellular lacunae are likely the result of focal evaporation and are important as melanocytic lesions, some inflammatory conditions, and extramammary Paget's disease may be confused with this artifact.

We present another viable clearing method that can be utilized in MMS tissue processing. It is important to weigh the advantages and disadvantages of using a UV mounting medium and UV light in comparison to xylene or xylene substitutes. For example, in a clinic trying to cut the use of xylene substitutes or cost, UV mounting medium in conjunction with a UV light may be beneficial. Future studies could look at ways to minimize lacunaewith this method. Drying the slides for a longer duration, using a different drying method, and/or changing the amount of time spent under UV light may reduce this artifact. Ultimately, we must utilize our different options toprovide the best quality patient care.

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