The Use of Antimicrobial Solutions for the Preservation of Toolmarks on Bone

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ABSTRACT

Body dismemberment by the use of sharp-edged instruments, hacking implements, and saws often leave toolmarks on bone. Long-term dry storage of bone results in shrinkage that can have an adverse effect on the toolmark striations. To prevent shrinkage, fourteen antimicrobial solutions were tested as preservatives on the bone and toolmark striations. Seven preservative solutions were identified as possible alternatives to dry storage. They include: Buffered 10% formalin, fixed in formalin then transferred to ethyl alcohol, 70% Isopropyl alcohol, 93% Ethyl alcohol, 5% Iodine Solution, 10% Iodine Solution and 6% Sodium Hypochlorite.

Introduction

The purpose of this study was to identify an effective antimicrobial solution for the preservation of toolmarks on bone. Various sharp edged instruments, hacking implements and saws can leave toolmarks on bone in dismemberment cases. Long-term dry storage of bones is prone to result in shrinkage that may distort microscopic toolmarks. One study suggests that the vertical and horizontal diameters of dried femoral bone lose about 5-7% in size [1]. Therefore, the use of a preservative could prevent bone shrinkage and the degradation of toolmarks. The preservative had to have characteristics that would inhibit the growth of bacteria, fungi, and molds which could also damage or destroy toolmarks by interacting with the bone.

Forensic pathologists routinely use buffered 10% formalin to preserve tissue collected at autopsy. This solution is effective as a preservative; however, the Occupational Safety and Health Administration (OSHA) classified formalin as a hazardous agent with carcinogenic properties [2, 3, 4]. Consequently, some selected preservative solutions were tested to determine their suitability for preserving toolmarks on bone.

In some cases, toolmark striations on bone from single edged cutting implements can be matched to a specific cutting implement. Even though toolmark striations on bone from saws only provide class characteristics, they are useful in identifying certain types of saws or saw blades [5, 6, 7]. Identifying or eliminating possible types of saws in a dismemberment cases can aid the investigation. From 1978 to 2008 a review of twenty-five dismemberment cases revealed a variety of edged weapons and saws. The types of saws identified were: handsaw, chainsaw, hacksaw, bow saw, band saw, circular saw, jigsaw, electric table saw, and one unknown type of power saw.

Consequently, all types of toolmarks on bone should be preserved for future examination even after photomicrographs and Mikrosil® casts are made of the toolmarks.

Material and Methods

Fourteen recently butchered samples of porcine rib bones were obtained and used as a human simulant in the study. A hack saw with a 25.4 cm (10.00 in) length blade containing 18 teeth per inch (tpi) was used to cut the bone into ~ 1.27 cm (0.50 in) sections leaving toolmarks on the sawed end of the cortical bone **(Figure 1)**. Similar pressure was exerted on the saw and reciprocating speed to prepare each of the bones samples. All samples were examined at about 20X using a Dino-Lite digital microscope. Digital photomicrographs were made for each of the bone samples prior to storage and after 6 months of

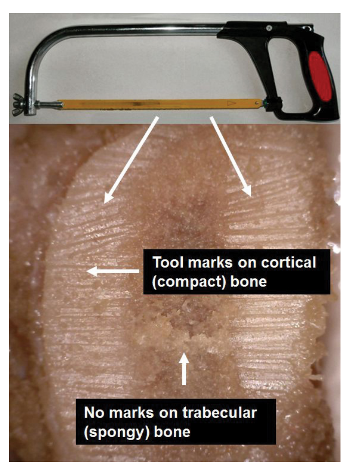

Figure 1: Toolmarks in sawed end of bone

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storage in the preservative solutions **(Figure 2)**. A six-month period was selected to determine if the preservatives had any adverse effects on the toolmark striations or bone.

Fourteen solutions were tested as possible preservatives. One bone section was stored in buffered 10% formalin for the six month period while another section was stored in the buffered 10% formalin for 12 days and then transferred to ethyl alcohol. The other bone sections were stored individually in the following solutions: 70% isopropyl alcohol, 93% ethyl alcohol, 5% acetic acid, 5% iodine solution, 10% iodine solution, 6% sodium hypochlorite, 10% sodium chloride with iodide, 10% sodium chloride with no iodide, 20% sodium chloride with iodide, 20% sodium chloride with no iodide, 26.4% sodium chloride with iodide and 26.4% sodium chloride with no iodide. The samples were placed in individual glass containers with approximately 100 ml of preservative solution and stored at 70° F. (21 C) for the six months **(Figures 3 through 30)**.

Results and Discussion

Hack saw blades are commonly available in 18, 24 and 32 tpi. A blade with 18 tpi was used in the experiment because of the three blades it would typically produce thicker striations than 24 and 32 tpi. The buffered 10% solution was tested because pathologists routinely use it as a preservative. If investigators request bone sections with toolmarks from pathologists, the samples will likely be preserved in formalin unless another preservative is requested.

The disadvantage of using this solution is its hazardous properties. To control exposure to this agent, examiners must wear protective gloves, face shield, mask and examine the bone sample under a vent hood. Even with these precautions, some safety officials suggest that, if the odor of formalin is detected, the exposure is too great [8]. If formalin is used, an alternative to long-term storage in buffered 10% formalin would be to fix the sample for 12 days in formalin then transfer it to ethyl alcohol. There would be trace quantities of formalin in the ethyl alcohol; however, the concentration of formalin would be significantly reduced.

Isopropyl and ethyl alcohol solutions have been used to control microorganism in various applications [9]. Isopropyl alcohol is commonly available and some forensic entomologist use isopropyl alcohol to preserve arthropods when ethyl alcohol is not available [10, 11]. There were no microorganisms such as bacteria, fungi or mold present in either solution after 6 months. Either of the alcohols could be used as a preservative and for long-term storage of bone with toolmarks.

Figure 2: Stored bone sections

A 5% solution of acetic acid also controls the growth of microorganisms and is commonly available [12, 13]. There were no microorganisms in the acetic acid solution; however, crystals formed on the bone, thereby making it unsuitable for long-term storage **(Figure 12).**

Both 5% and 10% iodine solutions were prepared from 10% Povidone. Iodine has been recognized as an effective broad-spectrum bactericide. There were no microorganisms present in the iodine solutions [13]. One benefit for using an iodine solution as a preservative was it stained the bone a light orange color (**Figures 14 and 16)**. Visualization of the toolmark striations was enhanced by the staining.

Sodium Hypochlorite is typically used as a disinfectant and is effective agent for killing microorganisms. A 6% solution of Sodium Hypochlorite was tested and determined to be an effective preservative.

The last six preservatives tested were saline solutions. Brine solutions were used by 19th century medical schools as a preservative for cadavers [14]. This type of solution would be the most eco-friendly and economical, compared to the other preservatives tested. Three different concentrations of sodium chloride were tested with and without iodide. There was no observable difference between the sodium chloride with and without iodide. All of the sodium chloride solutions contained fungi after 21 days. The fungi were identified as Cladosporium based on DNA sequencing of the gene. Cladosporium is a genus of fungi that includes some of the most common indoor and outdoor molds **(Figure 31)**. The species produce olive-green to brown or black colonies **(Figure 32).** The spores are wind-dispersed and they are often extremely abundant outdoors. They may also occur indoors and grow on moist surfaces. There are over 30 species of Cladosporium found on living and dead plant material. They were probably introduced into the preservation solutions as an airborne contaminant. The sodium chloride solutions are not recommended as a preservative for toolmarks on bone because sodium chloride solutions permit fungal growth. Even with no visual degradation to the bone or toolmark striations in 6 months, fungi are parasitic and will eventually digest the host tissue using extracellular activity and absorb the nutrients through the cell wall of the fungi.

Conclusion

One benefit of using a preservative is the elimination of bone shrinkage. Of the preservative solutions tested, the most hazardous contain formalin or traces of formalin. When using these solutions, toolmark examiners should use appropriate safety measures. Five of the solutions tested are less hazardous than formalin and could be considered as an alternative preservative for storing toolmarks on bone. They include the 70% isopropyl alcohol, 93% ethyl alcohol, a 5% or 10% iodine solution or the 6% sodium hypochlorite solution. Crystal formation on the bone prevents the acetic acid from being an effective preservative. Finally, the growth of fungi precludes use of any sodium chloride solution as a preservative for long-term storage **(Table 1)**.

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Solutions	Remarks	Long-Term Storage
Buffered 10% Formalin	Carcinogenic	R
Fixed in Formalin / Ethyl alcohol		R
70% Isopropyl		R
93% Ethyl alcohol		R
5% Acidic acid	Crystal growth	NR
5% Iodine Solution	Stained section	R
10% Iodine Solution	Stained section	R

Solutions	Remarks	Long-Term Storage
6% Sodium Hypochlorite		R
10% NaCl with iodide	Fungi growth	NR
10% NaCl no iodide	Fungi growth	NR
20% NaCl with iodide	Fungi growth	NR
20% NaCl, no iodide	Fungi growth	NR
26.4% NaCl w/ iodide	Fungi growth	NR
26.4% NaCl, no iodide	Fungi growth	NR

Table 1: Antimicrobial solutions tested (R = Recommended and NR = Not Recommended)

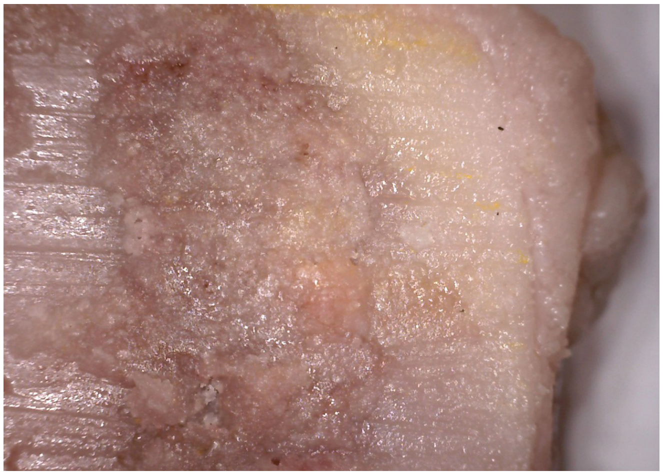

Figure 3: Sample before treatment in buffered 10% formalin

Figure 4: Sample after 6 months in buffered 10% formalin

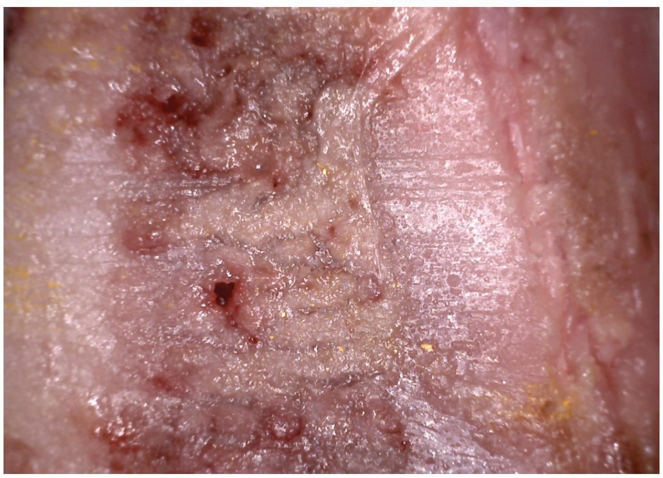

Figure 5: Sample before treatment in buffered 10% formalin and 93% ethyl alcohol

Figure 6: Sample after 6 months in 93% ethyl alcohol (fixed in buffered 10% formalin for 12 days)

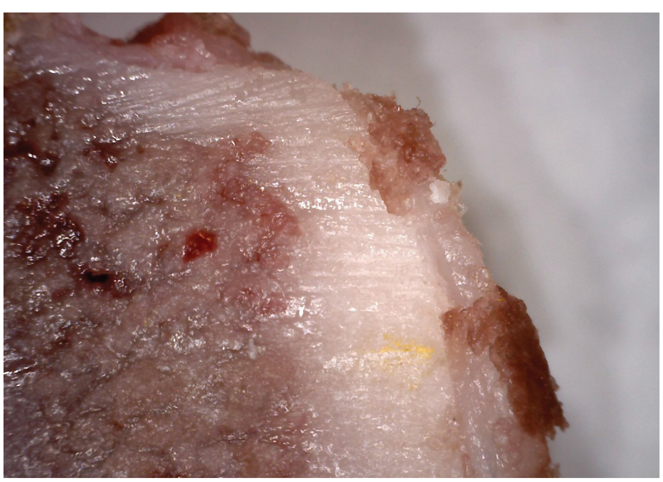

Figure 7: Sample before treatment in 70% isopropyl alcohol

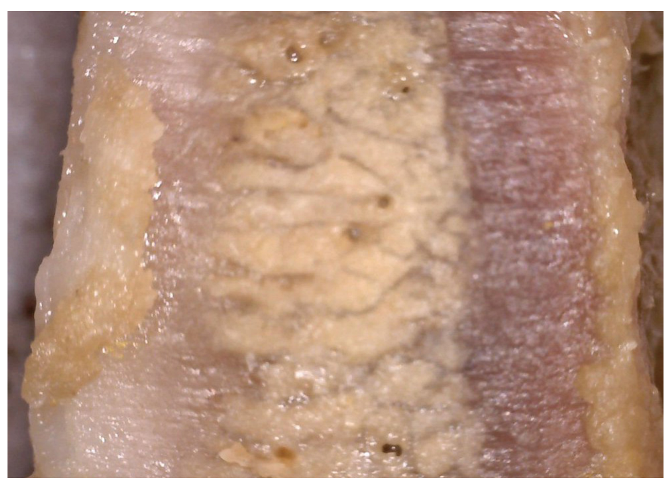

Figure 8: Sample after 6 months in 70% isopropyl alcohol

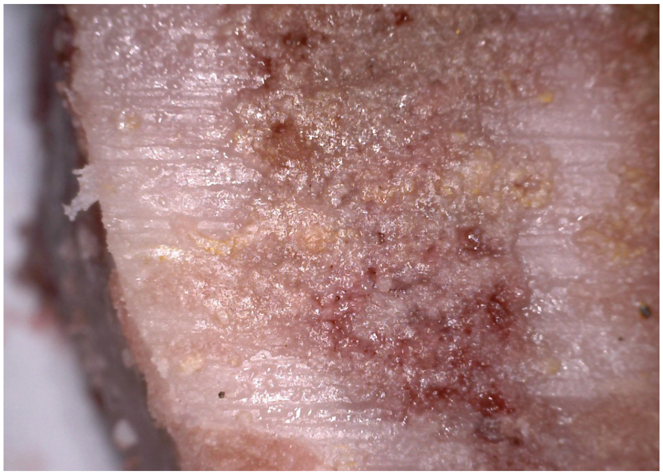

Figure 9: Sample before treatment in 93% ethyl alcohol

Figure 10: Sample after 6 months in 93% ethyl alcohol

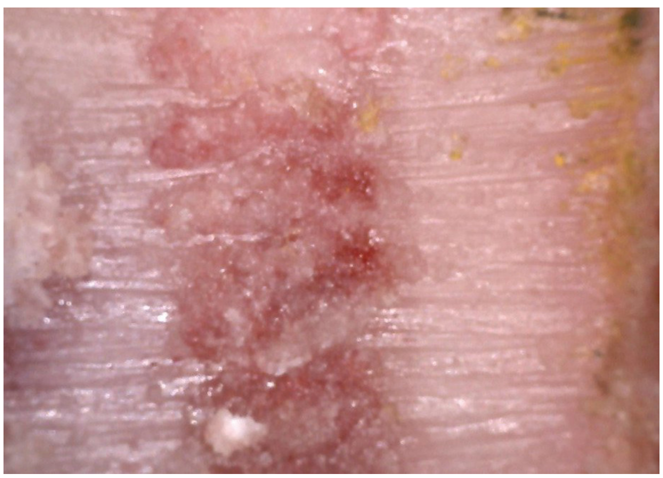

Figure 11: Sample before treatment in 5% acetic acid

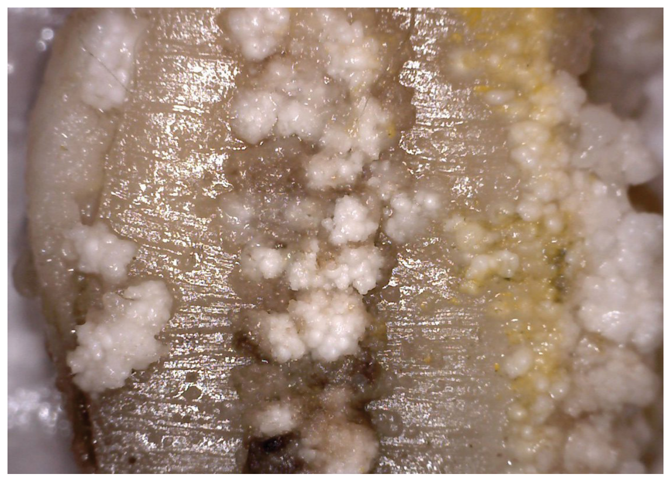

Figure 12: Sample after 6 months in 5% acetic acid

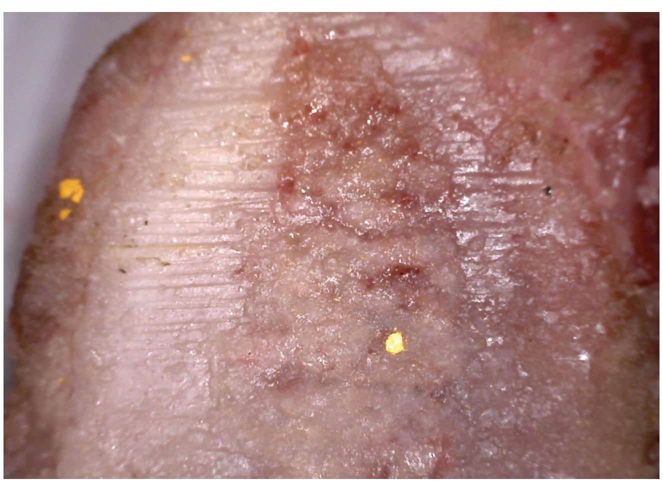

Figure 13: Sample before treatment in 5% iodine solution

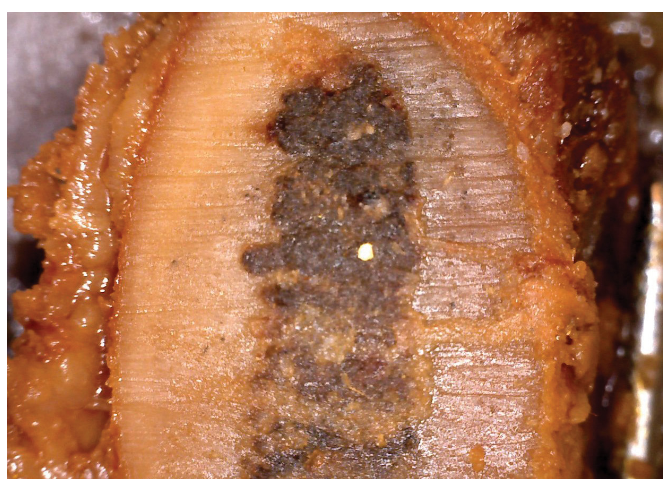

Figure 14: Sample after 6 months in 5% iodine solution

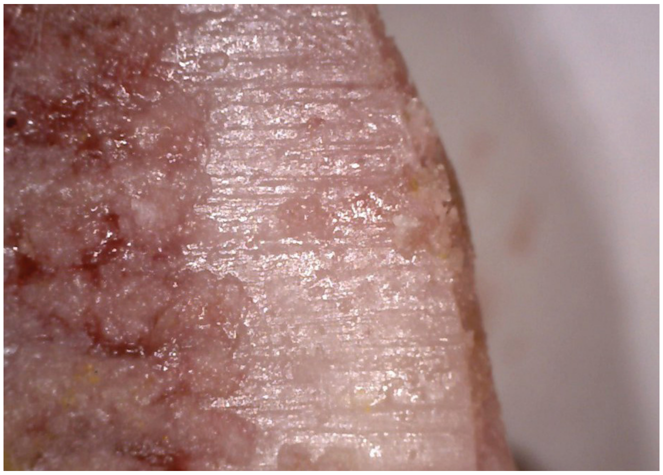

Figure 15: Sample before treatment in 10% iodine solution

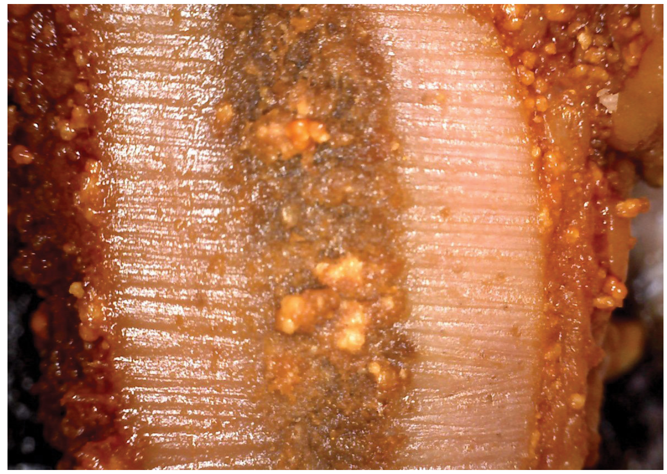

Figure 16: Sample after 6 months in 10% iodine solution

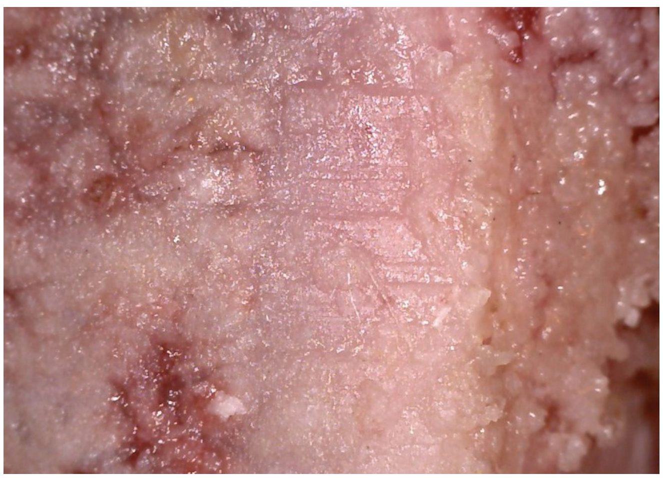

Figure 17: Sample before treatment in 6% sodium hypochlorite

Figure 18: Sample after 6 months in 6% sodium hypochlorite

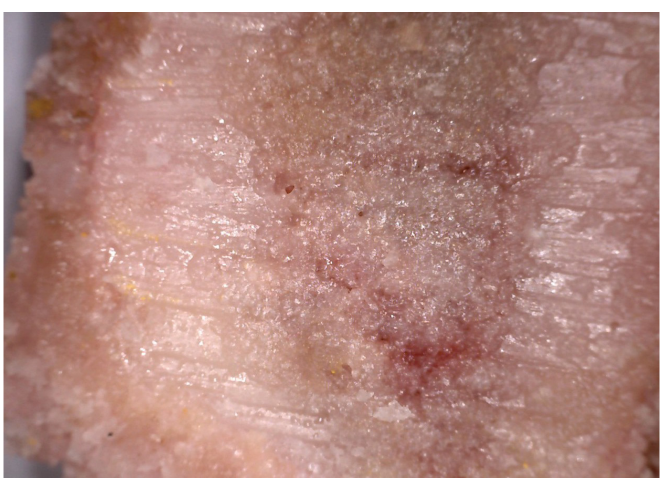

Figure 19: Sample before treatment in 10% NaCl with iodide

Figure 20: Sample after 6 months in 10% NaCl with iodide

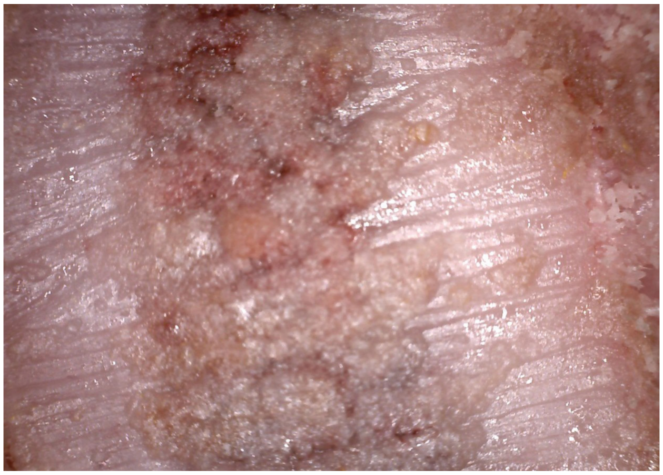

Figure 21: Sample before treatment in 10% NaCl, no iodide

Figure 22: Sample after 6 months in 10% NaCl, no iodide

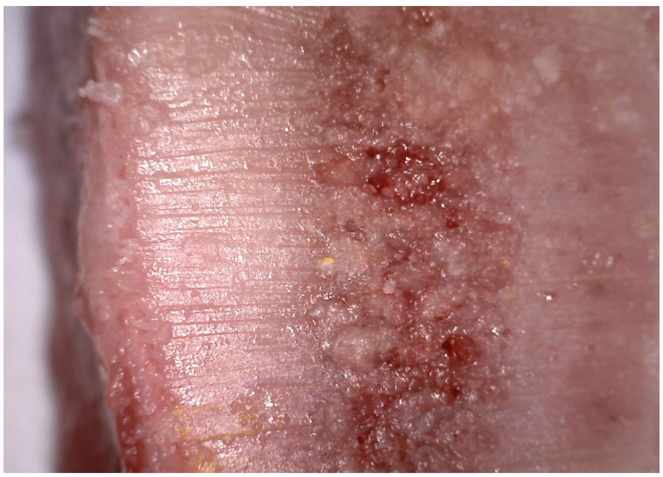

Figure 23: Sample before treatment in 20% NaCl with iodide

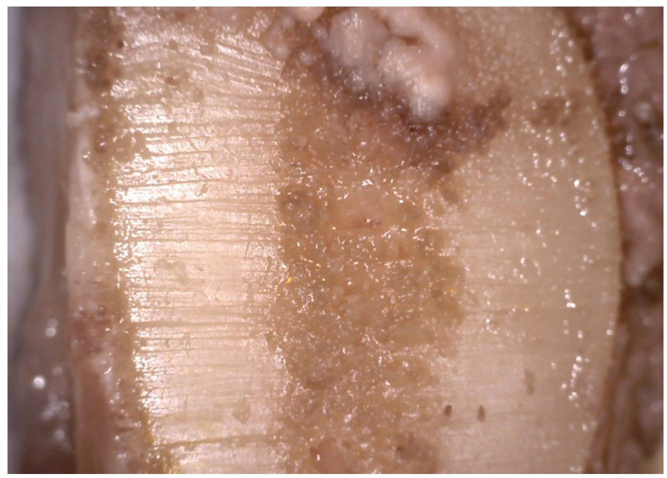

Figure 24: Sample after 6 months in 20% NaCl with iodide

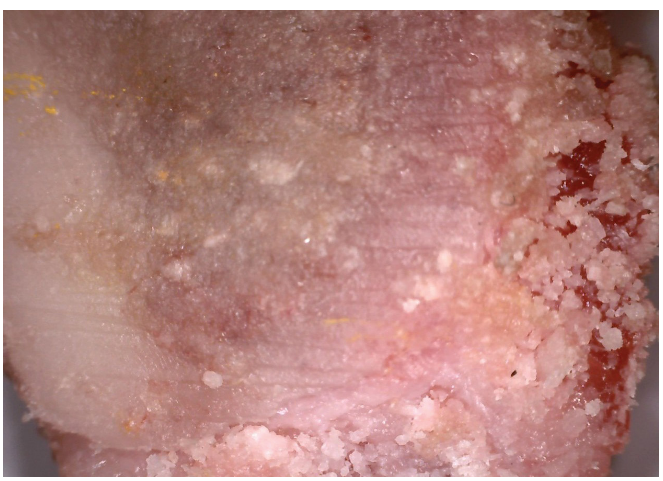

Figure 25: Sample before treatment in 20% NaCl, no iodide

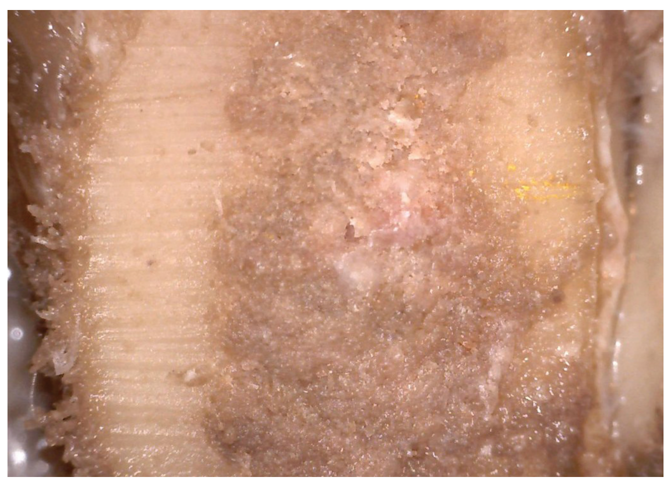

Figure 26: Sample after 6 months in 20% NaCl, no iodide

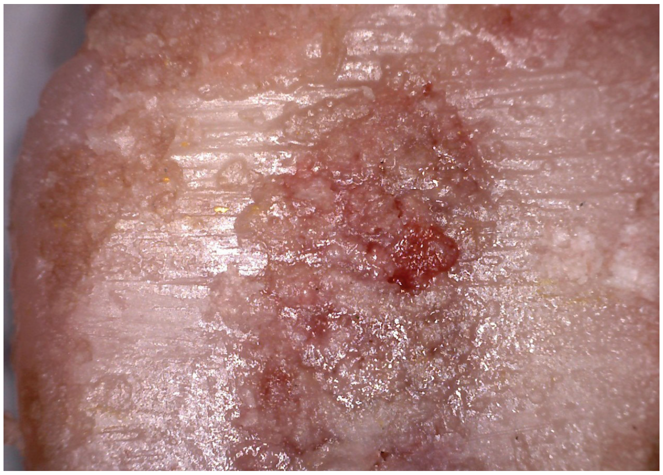

Figure 27: Sample before treatment in 26.4% NaCl with iodide

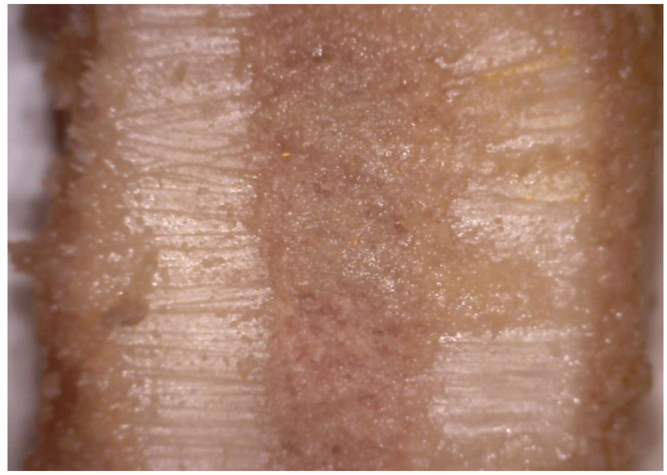

Figure 28: Sample after 6 months in 26.4% NaCl with iodide

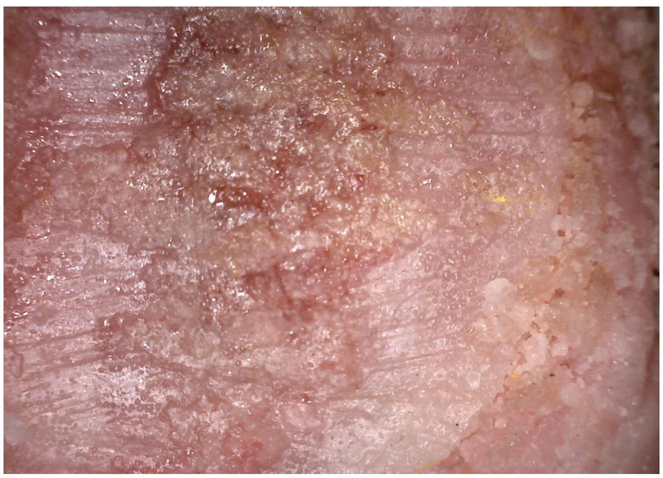

Figure 29: Sample before treatment in 26.4% NaCl no iodide

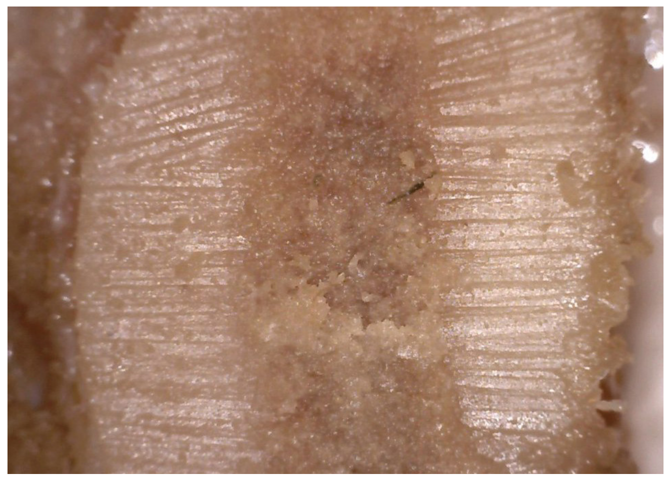

Figure 30: Sample after 6 months in 26.4% NaCl no iodide

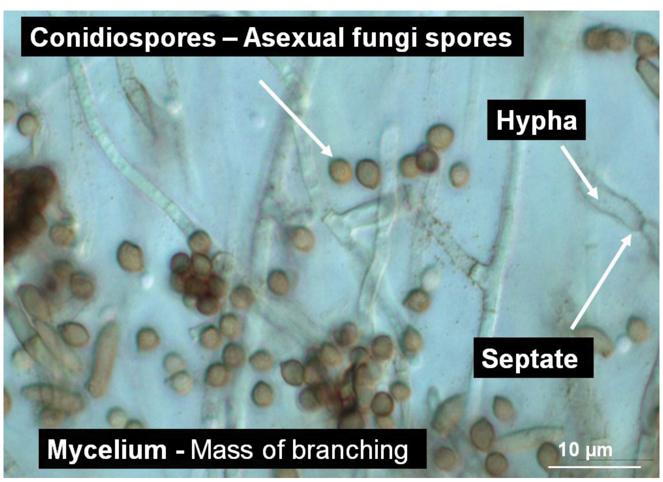

Figure 31: Microscopic morphology of Cladosporium

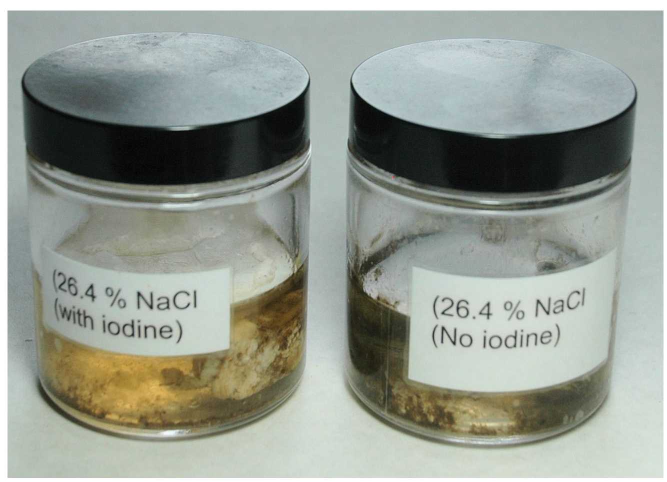

Figure 32: Olive-green to brown or black colonies of Cladosporium