Prosthetic Sanitizer and Deodorizer

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Sterilization Test

I. INTRODUCTION

The testing procedure described in the following report attempts to answer the question of the sterilizing benefits of ultraviolet radiation versus the conventional methods of mild soap, water, and alcohol in prosthetic liner cleansing. A common bacterium found on human skin that causes infection is Staphylococcus epidermidis, abbreviated S. epidermidis (Wikipedia). Therefore, we chose to use S. epidermidis in our microbial content test. The use of ultraviolet light as a sterilizer comes from its innate ability to disrupt the DNA of bacteria, effectively rendering the bacteria harmless (or killing it). Specifically, the ultraviolet radiation disrupts the thymine nucleotide in the DNA (Campbell 330). By comparing the sterilizing effects of ultraviolet radiation with those of the two common methods currently in practice – soap/water and alcohol – we hope to achieve results that will call for further development in the field of ultraviolet sterilization.

II. PROCEDURE

The materials required for this experiment were: 10 nutrient agar plates, an inoculating loop, a Bunsen burner, distilled water, a vial of living S. epidermidis, 9 2 X 2 inch squares of prosthetic liner, an incubator, a refrigerator, and 9 empty Petri dishes. Once all of the materials are secured, the first step is to culture as much of the S. epidermidis onto one of the nutrient agar plates as possible. This is done by dipping the inoculating loop into the vial of S. epidermidis and streaking the bacterium onto the agar. Prior to inoculation, the loop must be sterilized in the flame of the Bunsen burner. It should be sterilized again following the inoculation. The purpose of this step is to get a large living colony of bacteria to use as a "supply" in the following steps. The agar and the bacteria must be thawed to room temperature for this step if they were refrigerated prior to use. After streaking, the agar plate is put into the incubator at 37˚ Celsius.

After 120 hours we removed the agar plate and distributed the bacteria, along with some of the agar, onto the nine squares of liner with the inoculating loop, which one again was sterilized. Some agar came with the bacteria so that the S. epidermidis would have some "bacteria food" while living on the liners. Each infected liner was now placed in its own empty Petri dish and then returned to the incubator at 37˚ Celsius. This was to simulate actual living conditions on the prosthetic liner.

Next, after 216 more hours, the liners were removed from the incubator and sterilized. Two of the liners did not get sterilized, to serve as controls. These liners were simply gently rinsed with distilled water into their own thawed agar plate. The agar plates were then put in the incubator again at 37˚ Celsius. The other seven liners were sterilized seven different ways: with mild soap and water, alcohol, a combination of soap and alcohol, a minute of 9-Watt ultraviolet radiation (one with and one without titanium dioxide), and a minute of 5-Watt ultraviolet radiation (one with and one without titanium dioxide. After each sterilization method, each liner was gently rinsed with distilled water into separate thawed agar plates. The plates were numbered, in no particular order, with reference to the sterilization technique used. A key is shown at right. These plates were then put into the incubator at 37˚ Celsius with the controls.

Number	Method
C1	Control 1
2	Soap/Water
3	Alcohol
4	Soap/Alcohol
5	5-Watt UV
6	9-Watt UV
7	9-Watt UV/TiO_2
8	5-Watt UV/TiO_2
C9	Control 9

Fig 1. Numbering system for samples

Finally, after 96 hours, the agar plates were removed from the incubator and observed with the naked eye and microscope to observe bacterial growth. The resulting growths from each of the nine liners were compared with one another to determine sterilizing ability based on ranking. Theoretically, the more growth found over the 96 hours since sterilization, the less effective the sterilization. Above all, the controls would be expected to have much more growth than any of the other plates.

III. RESULTS AND DISCUSSION

After the final growth period following liner sterilization, the nine agar dishes were observed for bacterial colonies. This was done with regard to the area, individual size, and density of the colonies. Preference was given to density as the tell-tale factor in bacterial growth. The resulting growths are shown in *Appendix A*; however it will suffice to relay the rankings here. Although it was difficult to discern an *exact* ranking system based on visually analyzing the growths, we were able to gather some substantial data. First, to insure an unbiased test, we made sure to not know which samples corresponded to which sterilization methods. In effect, the order we agreed upon was, from worst to best sterilization: C9 → C1 → 4 → 7 → 5 → 2 → 3 → 8. Translated, this corresponds to: control 9 → control 1 → mild soap and alcohol mix → 9-Watt UV with TiO_2 → 5-Watt UV → mild soap and water → alcohol → 5-Watt with TiO_2.

An important observation to make is that sample 6, the 9-Watt ultraviolet radiation without TiO_2 sterilization technique, is missing from the rankings. This is because the growth that appeared on the agar plate of sample 6 was very different from the growths of the other liners. A picture of the resulting sample 6 growth is shown in *Appendix B*. Specifically, it was unclear whether sample 6 should be ranked 2nd behind sample 8 or 5th behind sample 5, or anywhere between for that matter. The reasoning behind this inconsistency came as a result of the unusually large colonies present on sample 6, while there being relatively small area and low-density colonies all the same. These qualities were in sharp contrast to the other eight samples, in which the three qualities (area, density, and size) used to determine quality of sterilization were much more consistent with each other. Since the results were unclear with respect to the other samples, sample 6 was left out of the rankings. However, it should suffice to say that it was clearly better than either control, as well as the soap and alcohol combination. This strongly suggests that the 9-Watt ultraviolet radiation had some sterilizing effect.

The rankings provided were in hopes of developing some relationship between ultraviolet light and the current methods, although clearly this was not presented. However, the differences between growth colonies were so subtle, as can be seen in *Appendix A*, that it is more apt to conclude that ultraviolet radiation works just as well as the current methods in disinfecting the liners, based on the results. Although further, larger-scale testing at the microbiological level is necessary to determine more definite conclusions, it is sufficient to note that all sterilization methods (perhaps with the exception of the soap and alcohol combination) were effective in killing the Staphylococcus epidermidis bacterium. However, it is also interesting that none of the methods were able to completely eradicate the strains.

The effects of titanium dioxide, however, are not as clear. Although the clear-cut best sterilizer was the 5-Watt ultraviolet bulb with titanium dioxide, the 9-Watt bulb with titanium dioxide was at the other end of the spectrum as the second-worst sterilizer. This inconsistent and unexpected result inhibits any conclusions surrounding titanium dioxide. However, it is adequate to postulate that the effects of titanium dioxide offer little added benefit to the natural sterilizing

effects of ultraviolet radiation, if any. At the same time, it is impossible to hypothesize any differences in sterilization based on ultraviolet wattage. This is because, sample 6 withholding, the sterilizing effect of the ultraviolet bulbs appeared better for the 5-Watt bulbs than the 9-Watt bulbs, which is the opposite of what one would expect, granted the difference is for the most part minimal.

IV. CONCLUSION

The primary conclusion to be drawn from the Sterilization Test is that ultraviolet radiation is about as effective in disinfecting silicone prosthetic liners as the current methods in practice. This conclusion can be drawn generally based on observing all of the bacterial growths of the sterilized liners versus the controls, where there is a clear difference in bacterial accumulation. Unfortunately, we can only speculate on the effects of ultraviolet coupling with titanium dioxide and wattage. A reasonable assumption is that titanium dioxide provides little, if any, added sterilizing effects to the ultraviolet radiation based on the data collected. Wattage effect is even more obscure, and further testing is definitely needed before making any postulations.

Due to budget constraints, we were only able to use nine agar dishes, and subsequently nine liners. With only nine samples, and only the control as a duplicate, there is a high probability of error. In order to obtain more reliable results, this experiment should be repeated with many more samples. The test should repeat the seven sterilizing methods at least ten times and compare results, along with the implication of more wattages and exposure times. Consistency is an issue when seven samples, independent of one another, are used since there is no guaranteed way to insure that all liners are exposed to equal amounts of bacteria. For this reason, some sterilizing methods may have been at an unfair disadvantage in trying to disinfect larger colonies of bacteria. Another potential source of error could have occurred between the independent sterilizations and the rinsing into the agar gels. Perhaps, if there was an insufficient time elapse between the two occurrences, the bacteria would not have completely died off before escaping the hazardous conditions and entering the nutrient agar. This could explain the fact that none of the methods were able to completely kill all colonies of the S. epidermidis.

V. REFERENCES

1. Campbell, Neil A., and Jane B. Reece. "Mutagens." Biology. Ed. Beth Wilbur, et al. 7th ed. San Francisco: Pearson - Benjamin Cummings, 2005. 329-30.

2. "Staphylococcus epidermidis." *Wikipedia, The Free Encyclopedia*. 28 Nov 2007, 11:22 UTC. Wikimedia Foundation, Inc. 3 Dec 2007 <http://en.wikipedia.org/w/index.php?title=Staphylococcus_epidermidis&oldid=174347018>.

VI. APPENDICES

APPENDIX A

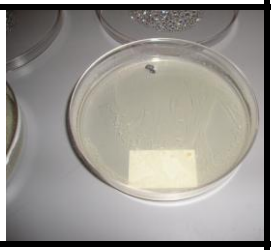
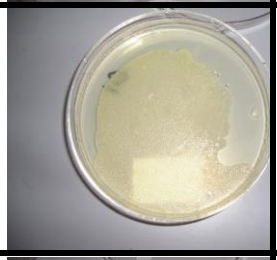
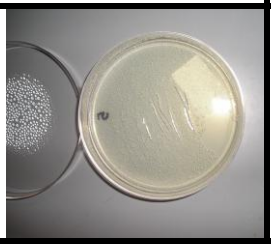
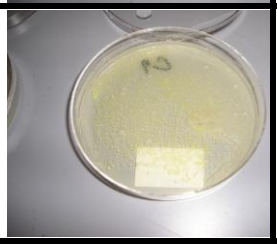
Ranking	Image	Sample #	Method		Ranking	Image	Sample #	Method
1		8	5-Watt UV with TiO_2		5		7	9-Watt UV with TiO_2
2		3	Alcohol		6		4	Mild Soap and Alcohol
3		2	Mild Soap and Water		7		C1	Control 1
4		5	5-Watt UV		8		C9	Control 9

Fig 2. Table of ranked sterilization methods based on visual S. epi growth.

APPENDIX B

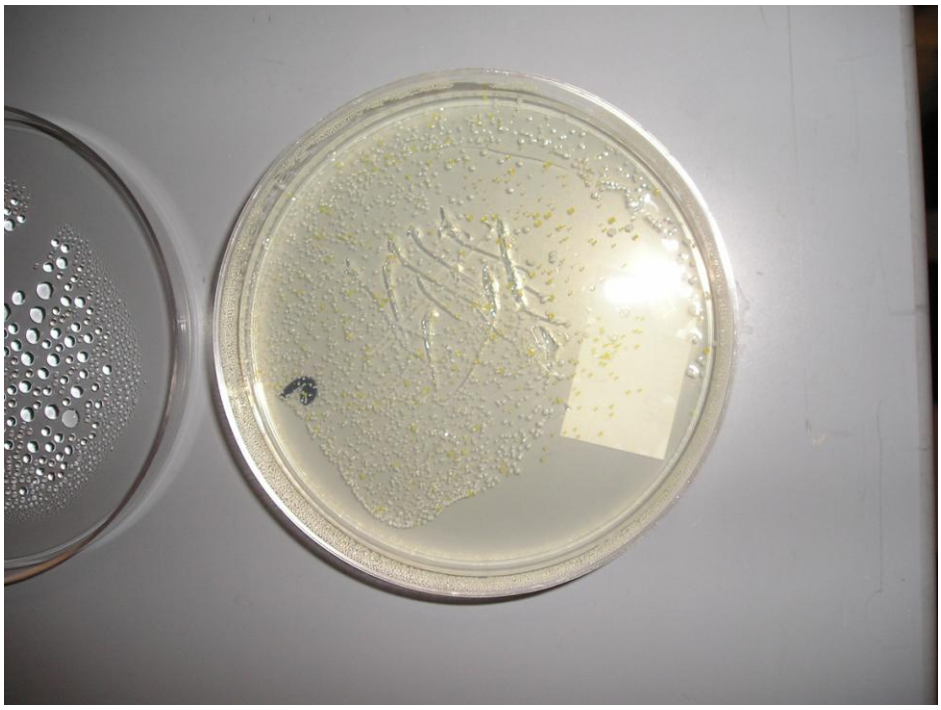


Fig 3. Visual growth after 9-Watt ultraviolet radiation (without TiO_2)