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Study Type- Original Research

COMPARATIVE EVALUATION OF INHERENT ANTIMICROBIAL PROPERTIES AND BACTERIAL SURFACE ADHERENCE BETWEEN COPPER (Cu) AND STAINLESS STEEL (SS) SUCTION TUBE

Running title – Anti microbial properties of copper vs stainless steel suction tube.

Abstract

Aim of the present study is comparative evaluation of the inherent antimicrobial properties of the copper and stainless steel suction tube also evaluate bacterial adherence and reduction with time inside suction the suction tube. Present study study was performed in an in vitro study setting in saveetha dental college and hospital. To compare the inherent antimicrobial property of both the metals both suction tubes were cut into equal lengths of 2.3 inches and dipped in a sterile container which had bacterial suspension of *Streptococcus Mutans* for 5 minutes. Each group included three samples of copper and stainless steel suction tubes. Control group was taken out at 0 mins post initial dipping period de-coated in a sterile container and immediately cultured on Brain heart infusion agar, followed by group 1 at 15 minutes group 2 at 60 minutes and group 3 at 180 minutes. Bacterial colonies were counted after overnight incubation. Copper suction tubes demonstrated less initial adhesion of bacterial colonies than that of stainless steel suction tubes. Percentage reduction in bacterial colonies was observed more in stainless steel suction tubes with respect to time. Spite of high initial adhesion stainless steel suction tube demonstrated higher degree of percentage reduction in bacterial colonies with time hence proven better for antimicrobial properties than copper suction tube. (Re-write, not clear)

Key words: antimicrobial property, cross infection, copper, contact killing, stainless steel, suction tube, *S. Mutans*

1.Introduction :

When certain bacteria come in contact with the uncoated copper surface it results in formation of intracellular oxidative stress in the bacterial cell wall due to release of ions from the copper surface which results in bacterial cell lysis ^{1,2}. This phenomenon was well known since ancient times but, recently it has gotten renewed attention from the researchers. The term ‘contact killing’ was coined for the above phenomenon. United States Environmental Protection Agency (US EPA) recognized copper as the first antimicrobial metal in the year of 2008³. One of the most important advantages of copper as an antimicrobial agent is its low levels of resistance in the microorganisms³.

Abbreviation name HAI stands for healthcare associated infection or iatrogenic infections are one of the most important causes of increased hospitalization and complications post procedures⁴⁻⁶. Factors playing a role in the spread of these infections include patient condition, hospital or dental clinic environment and mainly contaminated surfaces and instruments pre procedure and even during the procedure due to improper sterilization protocol ^{2,7-11}. Therefore meticulous sterilization protocol and surface disinfection is of utmost importance to avoid iatrogenic propagation of infection and postoperative complications¹². In recent years coating the surfaces with solid copper or mixing copper alloys has been practiced in hospitals to reduce the number of HIA's. While some studies show promising results or reduction in infections others find the use of copper to be non significant. As most of the studies have highly variable study design and setting it is hard to compare different studies although use of copper in medical devices and workplaces shows a promising future ^{3,13-15}.

Removal of the body fluids and **irrigates** is of high importance in modern dental as well as medical practice. Potent evacuation of the fluids aids in improving the quality of the treatment also in comfort of the operator as well as the patient. The suction unit becomes contaminated by residual microbes and is considered the most common cause of the HAI's.

Suction in the dental chair works on the vacuum⁴⁻⁶, This unit is repeatedly used only with a change of the suction tip for consecutive patients. Such practice although not considered safe and risk free by the researchers **and** is yet followed all over the world ^{3,13-15}. Disposable plastic suction tubes are available in pre sterilized packs but to reduce the risk during the major surgical procedures **(autoclaved) metal suction tubes are preferred over the plastic suction tubes³.(not clear)**

Chances of cross contamination although low still hold a threat for the major surgical procedures and can cause postoperative complications³. First case of cross contamination due to back flow of fluids from the suction tube was recorded in the year of 1993 and a recent one in the year of 2006¹⁶.

Hence in this study we decided to compare suction tubes made with solid copper against the conventional stainless steel suction tube to evaluate whether the intrinsic antimicrobial property of the copper has any significance in the reduction in the risk of the procedures ¹⁷⁻¹⁹.

Previously we have worked on plenty of topics in periodontology ²⁰⁻³²(is the works in Refe.20-32 belongs to you? when not the senescens we have worked should be deleted). Now we are planning to do a comparative evaluation of intrinsic antibacterial activity of copper suction tube vs stainless steel suction tube against the *S.mutans* strains.

2. Material And Methods:

Present study is an in vitro study carried out in saveetha dental college and hospital chennai. *Streptococcus Mutans* (figure 3) was selected to evaluate the antimicrobial properties of both the metal suction tubes (figure 1). Study was carried out under expert supervision of a guide in the microbiology department of Saveetha dental college and hospital. Study was done after acquiring all necessary clearances from the college review board and research department. Both copper and stainless steel suction tubes were sectioned into 2.3 inches pieces so that they fit in the selected sterile airtight containers. The tubes were autoclaved separately and divided into 4 groups (control group 0(0 mins) , group 1(15mins), group 2(60 mins) , group 3(180 mins) according to the time intervals decided previously each group included 3 tubes of copper and stainless steel. Bacterial suspension of *S. mutans* was made in a sterile airtight container and all the tubes were completely immersed in the suspension for 5 mins and then were segregated into appropriate groups according to time interval in separate containers. Control group tubes were immediately de-coated in separate eppendorf tubes and 0.2ml lawned using the zig-zag strokes (figure 4) on brain heart infusion agar culture media. Similar protocol was followed for the remaining groups. Group 1 group 2 and group 3 were de-coated and cultured on the same media (separate petri plates) and incubated overnight in an automated incubator. Bacterial colonies were counted using Scan 300 - Colony counter for each group post 24 hours of incubation by the primary examiner and recounted by a third person who was not part of the study and average was taken to calculate the results to minimize the assessment bias.

3. Results And Discussion :

Result table :

Metal /Time	Control	!!!!	Group 1 (15 mins.)	!!!!	Group 2 (60 mins.)	!!!!	Group 3 (180 mins.)	Mean
Copper	730	738	140	120	40	32	17	17
	772		112		22		13	
	700		128		34		22	

Stainless steel	1000	1189	127	137	37	47	10	18
	1378		147		57		18	
	1189		137		47		28	

!!!= mins=minutes

Table 1: Showing (delete). The number of *S.Mutans* colonies at different time intervals in copper and stainless steel suction tubes. (Table title should be in the top of the table)

	Percentage of remaining colonies after each time interval (delete)		
Metal/Time	From control to group 1	From group 1 to group 2	From group 2 to group 3
Copper suction tube	16.26%	4.33%	2.30%
Stainless steel suction tube	11.52%	3.98%	1.51%

Table 2: Showing (delete) The percentage of the remaining number of bacterial colonies with respect to time seen in each time interval in copper and stainless steel suction tubes. (Table title should be in the top of the table)

	Percentage of reduction in the total number of bacterial colonies after each time interval (delete)		
Metal/Time	From control to group 1 (15 mins)	From group 1 to group 2 (60 mins)	From group 2 to group 3 (180 mins)

Copper suction tube	83.74%	95.67%	97.70%
Stainless steel suction tube	88.48%	96.02%	98.49%

Table 3: **Showing(delete)** The percentage reduction in the number of bacterial colonies (from bacterial colony count at 0 mins.) with respect to time seen in each time interval in copper and stainless steel suction tubes. **.(Table title should be in the top of the table)**



Figure 1 : Stainless steel and copper suction tubes in separate autoclaved pouches **ready to be immersed in the bacterial suspension.**



Figure 2: Sterile airtight holding containers with labels according to the groups.



Figure 3: *Streptococcus Mutans* strain used for the study.

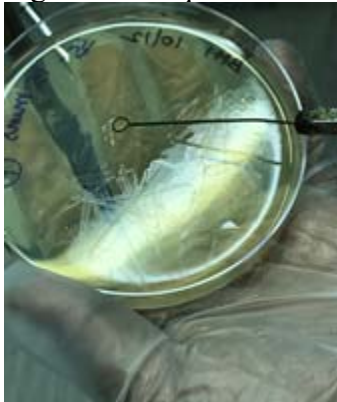


Figure 4: ~~showing(delete).~~ The process of lawn culturing on the brain heart infusion agar plate.

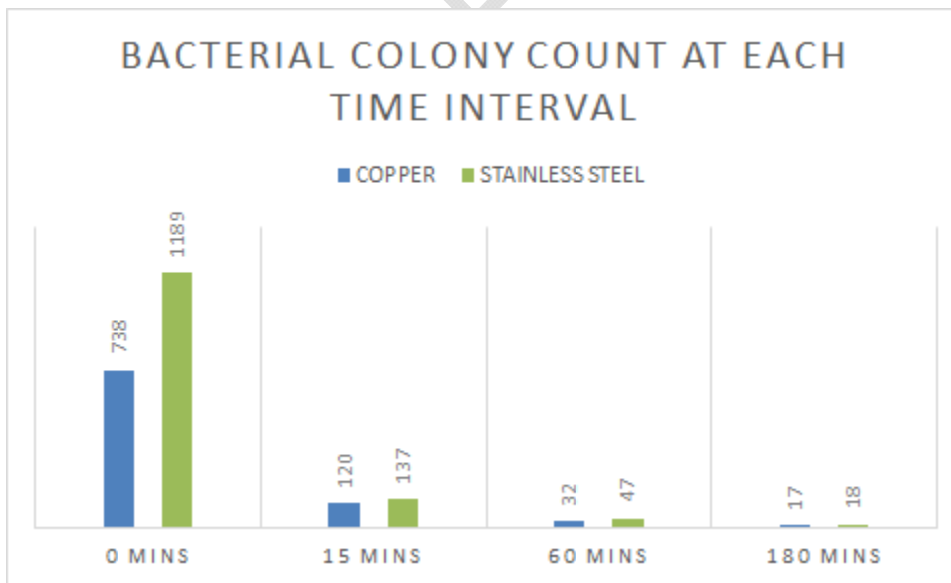


Figure 5: Bar diagram showing number of bacterial colonies at each time interval. Blue colour bar represents values of copper group and green colour bar represents the values of stainless steel group. Where x axis represents the time intervals and colour bars represent the two different

material groups included in the study.

As we can see (delete) At 0 mins the number of colonies in the copper group are significantly less than that of the SS group which implies less initial adhesion of bacteria to Cu suction tubes than that of copper. (738 in Cu and 1189 in SS respectively.) We can also see that the percentage reduction in the SS group at each time interval is higher than that of the Cu group. T 180 mins both the groups show 17 and 18 number of colonies in Cu and SS groups respectively.

As seen in the table 1 (figure 5) above initial bacterial adhesion on the stainless steel suction tube was significantly higher than that of the copper suction tubes at 0 minutes (control group) which was 738 colonies for copper suction tube and 1189 colonies for stainless steel suction tubes. Group 1 which was cultured at 15 mins showed 52 and 137 colonies in copper and stainless steel suction tubes. Group 2 which was cultured at 60 mins showed 21 and 47 colonies in copper and stainless steel suction tubes followed by group 3 which was cultured at 180 mins and showed 11 and 18 colonies in copper and stainless steel suction tubes respectively. It was observed We can clearly see that the initial adhesion on stainless steel tubes was significantly higher but the percentage drop between the time interval in the number of colonies was much higher than that of the copper suction tubes as well this implies that stainless steel suction tube showed better antimicrobial effect on *S.Mutans* in the present study than that of the copper suction tube. (delete)

In the present study we observed that the initial adhesion on stainless steel tubes was significantly higher and the time interval in the number of colonies was much higher than that of the copper suction tubes as well this implies that stainless steel suction tube showed better antimicrobial effect on *S.Mutans*.

It can be concluded from the results in table 2 that the drop in the number of colonies in case of stainless steel suction tubes was higher than that of the copper suction tubes with respect to time; with maximum of 88.48% drop in the at 0 min to 15 mins interval in stainless steel suction tube which was 83.74% for copper suction tubes respectively for the same interval.

It can be concluded from the results in table 2 that the drop in the number of colonies in case of stainless steel suction tubes was higher than that of the copper suction tubes at 0 min to 15 mins interval which was 88.48% drop in stainless steel suction and 83.74% for copper suction tubes respectively.

Suction lines in the sterile dental clinical areas should be cleaned every day and flushed using antiseptic solution after every patient even after flushing the dental suction unit meticulously after each patient with non foaming antiseptic solution there is evidence that shows some amount of residual bioburden still remains within the suction unit. Changing the suction tip for every patient is mandatory¹². To further minimize the contamination due to the dental suction unit and the backflow, newer material with intrinsic antimicrobial properties should be promoted such as copper. A study shows that type of suction used is equally important that high vacuum suction shows less number of residual bioburden and less number of viable bacterial mass even after regular flushing with the antiseptic solution^{16,17}. (Re-write)

It has been proven that frequently touched surfaces in the dental clinics and hospitals act as reservoirs of the residual microbial flora and are the main causes of the HAI's and postoperative complications ^{2,33-35}. Multiple studies have been done in the medical field which shows a great level of evidence that the coated surfaces with metals containing intrinsic antimicrobial activity result in reduced microbial burden in the hospitals and in turn reduce the chances of postoperative as well as intra operative complications ^{2,36-39}. Covering the bed handles, bedside table surfaces, door handles with copper has helped to significantly to reduce the bacterial load⁴⁰. Very few studies have been carried out in dental practice to incorporate such metals used for instruments used on a daily basis to reduce the bacterial load in the environment, chances of cross contamination during procedures and post procedures. Contact killing property of copper metal was discovered in the early year of 2008 although it has been used widely since ancient times for its other beneficial mineral contents. Prior to discovery of antibiotic agents in 1930's copper iodine and other substances with antimicrobial properties were the epicenter of the research ^{3,13-15}. Post discovery of antibiotics research about such elements was hampered but evidence of multi drug resistant bacteria's in 1980-1990 forced the research community to drive the research back to the naturally available antimicrobial elements such as copper which showed low levels of bacterial resistance³⁶.

Some of the authors found highly significant results for the intrinsic antimicrobial properties and its merits in the medical field whereas some did not^{2,7-11}. Due to variability in the study designs and lack of research in the dental field regarding use of copper incorporated materials it is difficult to formulate any hypotheses which have been proven. More research in the field is necessary to find better ways of minimizing the chances of cross contamination and postoperative complications due to iatrogenic infections ^{3,13-15}. Multiple factors play a role in the outcome of any study such as surface texture of the internal surface of the suction tube, operator bias ect. which can hamper the results.

Most of the regular dental procedures are finished within 40-60 minutes hence reducing the bacterial load in the suction tube within this period is of high importance to avoid infection and cross contamination ^{3,13-15}. As we can see in the results of this study (table 1) copper suction tube shows fairly less number of initial bacterial adhesion, we can imply that copper suction tubes can be used for short procedures. Further research using different strains of oral pathogenic bacteria has to be done to prove copper suction tubes as a better substitute for stainless steel as well as plastic suction tubes ^{2,7-11}. It will also aid in reducing the amount of dental non-degradable waste of plastic suction tubes created.

4. Conclusion :

In Spite of lesser initial adhesion on the copper surface at 0 minutes which was 738 colonies and that for SS group was 1189 colonies the percentage reduction seen on the surface of stainless steel suction tubes was higher than that of the copper suction tubes at each time interval as seen in the results (figure 5). Further research using different strains of oral pathogenic bacteria has to be done to prove copper suction tubes as a better substitute for stainless steel as well as plastic suction tubes.

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