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Anti-bacterial efficacy of the elements of amalgam restoration- An *in vitro* study

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Abstract

The interaction between bacteria and solid surfaces often results in the attachment and subsequent proliferation of the microorganisms, culminating in a well-defined biofilm. Restorative materials with long lasting antibacterial surface properties may reduce the biofilm and thus disease recurrence. Dental amalgams are widely used as filling materials in restorative dentistry. This study was done to evaluate and compare the *in vitro* antibacterial activity of different elements in an amalgam restoration against *S. mutans* which is an important cariogenic bacteria present in dental plaque. Each test materials were placed in an eppendorf tube and the materials were placed until the standardized marking. Direct contact test was done to evaluate the bacterial colonies. After incubation, the bacterial colonies were counted from the plates using digital colony counter and the CFU/ml was calculated based on the dilutions performed. The results concluded that silver has a superior antibacterial activity compared to other metals.

Keywords: Amalgam, copper, silver, tin, mercury, zinc, streptococcus mutans, direct contact test

1. Introduction

The development of secondary caries, pulpal inflammation and gingivitis resulting from bacterial invasion under and/or around dental restorations is a major clinical problem in operative dentistry [1]. Dental plaque deposited on and around fillings is the source of the recurrence of human dental decay. The existence of a microspace (at the interface between the restorative material and the cavity wall) along which micro-organisms can penetrate promotes this recurrence of caries [2].

Acidogenic bacteria play the main role in the development of dental caries. Streptococcus mutans is the single most important organism in the initiation of dental caries. *Streptococcus mutans* (*S. mutans*) is the bacteria which is involved in the transition from nonpathogenic to cariogenic biofilms, although many other microorganisms also take a role in the pathogenesis of the dental caries [3]. Therefore, antibacterial agent which is effective against *S. mutans* might be considered as an anticariogenic agent

The interaction between bacteria and solid surfaces often results in the attachment and subsequent proliferation of the microorganisms, culminating in a well-defined biofilm [4].

Restorative materials with long lasting antibacterial surface properties may reduce the biofilm and thus disease recurrence. Due to this potentiality for leakage, any restorative/ base/liner material used in the mouth should have anti-bacterial properties in defense against bacterial migration [5].

Dental amalgams are widely used as filling materials in restorative dentistry. In recent years, an increasing number of new amalgams, with different copper content, have become commercially available. These variations probably arise from differing bacterial sensitivity to the materials, although the precise source of the antibacterial effect is difficult to ascertain because of the complexity of amalgam structure and composition [6].

So the purpose of this study was to evaluate and compare the *in vitro* antibacterial activity of different elements in an amalgam restoration against *S. mutans* which is an important cariogenic bacteria present in dental plaque.

2. Methodology

Streptococcus mutans was isolated from human saliva from a patient with carious lesion and were used in this study. The sample was inoculated in Mitis Salivarius Bacitracin agar (MSB Agar - HiMedia, Mumbai) and incubated at 37 °C in Candle Extinction Jar for 24 hours. Colonies resembling Streptococcus Mutans were identified by biochemical tests. The identified Streptococcus mutans bacteria strains were cultured aerobically overnight at 37 °C in 5 ml of brain–heart infusion broth (BHI) (Hi Media, Mumbai). Streptococcus mutans culture was adjusted to 0.5 McFarland standard (bacterial count 1.5×10^8) from overnight growth of S. mutans on brain–heart infusion broth (Himedia, Mumbai). Five test materials were used in the study namely metallic powders of zinc, copper, tin, silver and mercury (Industrial metal powders- IML, pune) including a positive and a negative control.

Each test materials were placed in an eppendorf tube and the materials were placed until the standardized marking.

- Group 1-Copper
- Group 2-Zinc
- Group 3-Silver
- Group 4-Tin

- Group 5-Mercury
- Group 6-Positive control (CHX)
- Group 7-Negative control (Saline)

Sterile eppendorf’s tube (Hi Media, Mumbai) were obtained and a 3mm marking from the base was done on it and all the sterile test materials were placed until the marking. 50µl of adjusted culture was added over test materials placed in the eppendorf’s tube and they were incubated at 37 °C in moist chamber. Each day 100µl of sterile BHI broth added to each eppendorf’s tube and mixed and 10µl transferred to 990µl of BHI broth and vortexed. 100µl of the dilution is transferred to BHI agar and spread using sterile L spreader. The incubation of all the plates was carried out in Candle Extinction jar for 24 h at 37 °C. 240µl of culture is discarded from the eppendorf ’s tube containing the test materials and 250µl of fresh BHI broth was added and incubated at 37 °C in Candle extinction jar. After incubation, the bacterial colonies were counted from the plates using digital colony counter and the CFU/ml was calculated based on the dilutions performed. Statistical analysis was done using Chi-Square test /Fisher Exact test.

3. Results

3.1 Study design: Comparative study

Table 1: Various groups of test materials

Various groups of test materials	Number of colonies after 24 hrs (n=92)	Number of colonies after 7days (n=90)	P value
Group 1-Copper	3(3.3%)	2(2.2%)	1.000
Group 2-Zinc	12(13%)	12(13.3%)	0.954
Group 3-Silver	8(8.7%)	8(8.9%)	0.963
Group 4-Tin	31(33.7%)	31(34.4%)	0.915
Group 5-Mercury	3(3.3%)	2(2.2%)	1.000
Group 6-Positive control	0(0%)	0(0%)	1.000
Group 7-Negative control	35(38%)	35(38.9%)	0.907

Chi-Square test Fisher Exact test

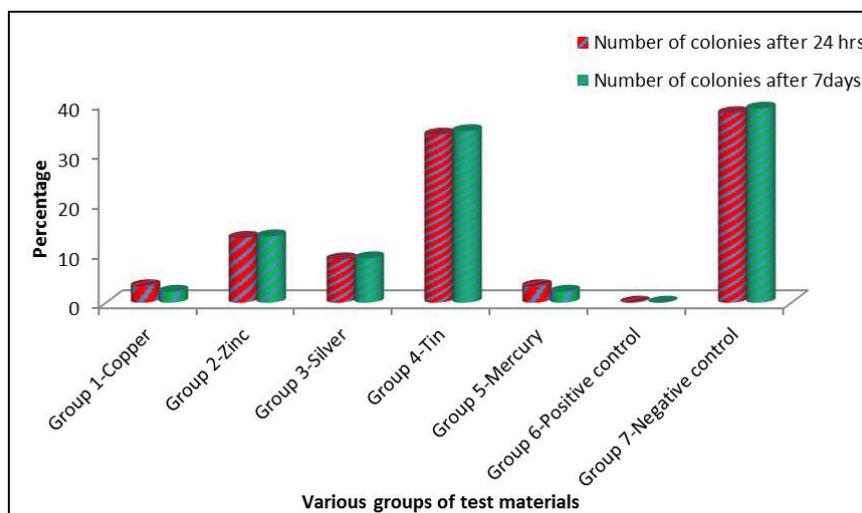


Fig 1

4. Discussion

The present study was conducted to evaluate the antimicrobial effectiveness of various elements potentially released from amalgam.

The most frequently employed methods for testing the antimicrobial effect of dental materials are those based on direct contact test (DCT) [7]. The direct contact test is a relatively new method that provides the information on the bacterial viability and growth rate and quantitatively measures

the effect of direct and close contact between the microorganisms and the tested materials, regardless of the solubility and diffusibility of their components. Metallic silver is element commonly used in dentistry. Silver has a superior antibacterial activity compared to other metals; it has a strong cytotoxic effect on a broad range of microorganisms in both metallic and ionic forms. This antibacterial activity has a large number of medical and hygienic applications [8, 9, 10]. The lack of antibacterial activity of pure silver and tin,

together with the absence of bacterial inhibition by the gamma phase indicate that these metals contribute minimally, if at all, towards the antibacterial properties of amalgams. According to Kaga *et al.* tin and silver did not contribute to the cytotoxicity of amalgams. Further, it was suggested that elements such as tin could affect the dissolution behaviour of other elements released from amalgams by diffusing to the surface which then becomes coated with a tin oxide surface film [11, 12, 13, 14].

Table 1 suggests that the order of antimicrobial potential of elements in amalgams would be Hg, Cu, Zn. However, based upon these results, it is believed that mercury rather than copper is a major contributor to the antimicrobial activity of dental amalgams but this activity may probably depend on the release of these components from the amalgam and the presence of these components at the surface of the sample.

The anti bacterial property of copper amalgam was substantially greater than that of the other materials. This strong antibacterial effect confirms the observations of some previous studies. Recent *in vitro* studies suggest that mercury could inhibit bacterial protein and carbohydrate metabolism in a biofilm forming on the surface of an amalgam [15]. There is considerable evidence that none of the materials currently used in dental practice, with the possible exception of the glass ionomer cements, adhere to the cavity walls. As a consequence, a microspace quickly forms at the interface of the material and the cavity wall after insertion of the material, within which bacteria may become established [16].

Copper ions have been reported to have an antibacterial effect both *in vitro* and *in vivo*. Copper reduces the number of bacteria on tooth surfaces [17, 18]. The suggested mode of the action of copper is the limitation of bacterial growth and the inhibition of glycolysis, leading to a decrease in acid production [19, 20].

The study of on the bacteriostatic properties of pure metals demonstrated that Ag and Sn did not inhibit the growth of *Streptococcus mutans* whereas Cu displayed inhibitory effects. The lack of antibacterial activity of pure silver and tin, together with the absence of bacterial inhibition by the gamma phase indicate that these metals contribute minimally, if at all, towards the antibacterial properties of amalgams. In agreement with the results of Bundy *et al.*, we found that of all the pure metals tested, mercury and copper had the two best inhibitory activities over the 24 h experimental period. For both the pure metals and aqueous solutions mercury had greater antibacterial properties than copper [11]. It was shown that zinc ions inhibit glucose metabolism by *S. mutans* [21]. Recent *in vitro* studies suggest that mercury could inhibit bacterial protein and carbohydrate metabolism in a biofilm forming on the surface of an amalgam [22]. From a clinical point of view, although amalgams with antibacterial properties are desirable in order to circumvent the risk of secondary caries, pulpal inflammation and gingivitis, the possible deleterious effects of such materials towards host cells and tissues must be thoroughly investigated and assessed.

5. Conclusion

Based upon these results, it is believed that mercury and copper might be major contributors to the antimicrobial activity of dental amalgams but this activity may probably depend on the release of these components from the amalgam and the presence of these components at the surface of the sample.

6. References

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