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Effect of Selected Metal Salts on the Microscopical Feature of Initial Carious - Like Lesion of Permanent Teeth (*AN IN VITRO STUDY*)

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Abstract

This study was designed to assess the microscopical changes of the enamel surface before and following the treatment with different concentration of metal salts, involving zinc, strontium, magnesium, in addition to stannous fluoride and de-ionized water as the control.

Microscopic examination was conducted on teeth samples before and following pH-cycling procedure and following the treatment with the selected metal salt under polarized microscope (100X).

The best obvious remineralization was found following treatment with stannous fluoride, followed by zinc chloride. Samples treated with strontium chloride showed rather poorly mineralized surfaces. No signs of mineralization were observed for samples treated with magnesium chloride.

Although stannous fluoride was the best in the remineralization of initial caries, results for other metals seem to be promising if used for prevention of dental caries.

Introduction

Teeth play an important role in determining the resistance or susceptibility to caries process [(1)]. Mineralization of teeth is determined by major inorganic elements as calcium, phosphorous in addition to other elements that occur in traces within tooth structure [(1, 2, 3)]. Fluoride now a day is widely used either systemically or topically to increase resistance of teeth surface, it has been well documented as a major contributing factor in the decline of the incidence and severity of dental caries in many of countries [(2, 4)]. So far fluoride has been considered to play an almost unique role in the prevention of dental caries; however there is no reason to dismiss the role of other element as being potential cariostatic agents. Different concentrations of different metal ions as zinc and copper have been tested for better effect on remineralization *in vitro* [(5,6)]. A controversy is present in the literature concerning their effectiveness in prevention of dental caries when used topically at effective concentrations [(7-9)].

In order to increase the knowledge in regards to the use of metal ions in the prevention of dental caries and remineralization of initial caries, this laboratory study was conducted, to test

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the effect of different concentrations of zinc, strontium, magnesium, chloride on remineralization of initial carious lesion.

Materials and Methods

aTeeth selected for this experiment were first permanent premolars extracted from 12-14 year old patients referred from orthodontics in the College of Dentistry/ University of Baghdad. Buccal and lingual surfaces of each tooth were coated with an acid resistant nail varnish leaving a circular window six mm in diameter. The position of each window on tooth surface was standardized using orthodontic ruler, an imaginary line was drown from the tip of the buccal cusps to the cervical line and another one between the most prominent curvature of mesial and distal surfaces. Therefore the middle area of each surface was identified.

Caries-Like Lesion Induction in Enamel Specimens:

The pH-cycling procedure was followed for the induction of caries lesion on enamel surface [10]. The preparation of de mineralized and re mineralized solution was conducted by mixing the following and adjusting the pH:-

De mineralizing solution:

This solution consisted of 0.075 M/L acetic acid, 1.0 M/L calcium chloride, and 2.0 mM/L phosphate chloride. The pH was adjusted to 4.3 at 37^oC,

Re mineralizing solution:

This solution consisted of I50 m M/L potassium chloride, 0.9 m M/L potassium phosphate and 1.5 m M/L calcium nitrate. The pH was adjusted to 7 at 37^oC.

The Cycling Steps:

Each tooth was immersed in 20 ml of the demineralization solution and kept for 6 hours at 37°C in the incubator. Teeth were then with drawn and rinsed with running de-ionized water for one minute. There after each tooth was immersed in a re mineralizing solution for 17 hours and kept in an incubator at 37°C. This procedure was repeated daily for a period of ten days.

Each enamel surface was examined microscopically using polarized light microscope for the detection of any microscopic changes of the outer enamel surface.

Metal salt preparation:

Zinc ion (Zn^{2+}): 5.5 mmol/L by dissolving 0.7423 gm, $ZnCl_2$ 7.5 mmol/L by dissolving 1.0125 gm, $ZnCl_2$, 9.5 mmol/L by dissolving 1.2825 gm $ZnCl_2$.

Strontium ion (Sr^{2+}): 50 mmol/L by dissolving 13.25 gm, $SrCl_2.6H_2O$, 100 mmol/L by dissolving 26.50 gm, $SrCl_2.6H_2O$,. 150 mmol/L by dissolving 39.75 gm $SrCl_2.6H_2O$.

Magnesium ions (Mg^{2+}): 50 mmol/L by dissolving 4.70 gm, $MgCl_2$ 100 mmol/L by dissolving 9.40 gm, $MgCl_2$ 150 mmol/L by dissolving 14.10 gm $MgCl_2$.

Stannous Fluoride (SnF_2): 8gm of SnF_2 in 100 ml de-ionized water, 10 gm of SnF_2 in 100 ml de-ionized water.

Enamel ground sections were prepared following the procedure described by Guay-Fen et al [11]. Cutting and preparation of these samples were carried in the Department of Geology in Baghdad University. Each enamel section was fixed on the middle of glass slide using Canada balsam dye, and enamel slab examined under polarized microscope (100 X). Microscopic examination involves sound enamel surfaces, following demineralization by acid and after application of each selected concentration of metal salts.

Results

Figure (1) demonstrates the microscopic features of a sound enamel surface under polarized microscope (100X). A highly mineralized enamel surface is shown in the picture with a clear incremental line.

The ground section of the enamel surface following pH- cycling technique is shown in Figure (2). This section indicates a high loss of minerals in the outer most layers of the enamel surface. The inner layers indicate a less hypo mineralized area, which is surrounded by a well mineralized enamel layer.

Microscopic features of enamel ground sections following the treatment with different metal salt are shown in the Figures (3, 4, 5, and 6). A various degree of remineralization was observed for different metal salts. A remineralization of the outer enamel surface is shown following the treatment with both concentration of stannous fluoride as no area of demineralization were seen for the whole section of the enamel. Enamel sample with the zinc chloride also appeared with remineralization but relatively less than samples treated with stannous fluoride as a relatively thin and poorly mineralization of enamel was detected.

For enamel samples treated with strontium chloride the microscopic examination revealed a remineralization in the inner layers of enamel with still a hypo mineralized outer enamel layer. In regards to samples treated with magnesium chloride no remineralization layers were detected at all and still layers of demineralization were present in the inner and outer enamel surface.

Discussion

The primary prevention of dental caries involves the increase in the resistance of the outer enamel surface to acid dissolution and enhancement of remineralization [(1)]. Fluoride has been widely used since the thirties of last century for the prevention of dental caries [(3, 10,11)]. However the effect of other elements in relation to dental caries is not well substantiated. In the present study certain metal ions were chosen in order to study their affect on remineralization of the initial carious lesion. The selections of zinc and strontium chloride were based on the knowledge regarding their inverse relation to dental caries. Observational studies showed that there is an inverse co-efficient correlation between the level of zinc and strontium ions in teeth and/or saliva and severity of dental caries [(12-14)]. Experimental studies are also present regarding the cariostatic effect of zinc ion when used alone or in combination with others on the increase resistance of outer enamel surface to carious lesion [(7, 16)]. Magnesium on the other hand was reported to have a direct relation with dental caries. Observational studies showed a positive/or no correlation between magnesium level in teeth and dental caries [(14 – 16)]. It is well documented from the experimental study that stannous fluoride is effective in the remineralization of initial carious lesion and increase the resistance against caries attack [(17)]. For this reason stannous fluoride was chosen in the present experiment to be as a control positive while de-ionized water was applied here as a control negative

The PH-cycling method was successfully applied in the present experiment. Under polarized light microscope the ground section of enamel surface indicated a high loss of minerals. The hypo mineralization was found to decrease from the outer to

inner layers of enamel. However a clear demarcation between different zones of the carious lesion was not obvious. For natural carious lesion four zones are present starting from the translucent zone in the inner layer of enamel, dark zone, body lesion, and intact layer. Initiation of carious lesion in the present study was conducted in ten days [(10)]. Increasing the time of inserting media to weeks or months may allow for more obvious demarcation between zones of carious lesion in similarity to natural dental caries process.

In the present study the microscopic changes of enamel surface following application of metal ions were examined. A various degree of remineralization was observed for different metal salts. A higher remineralization was observed for stannous fluoride followed by zinc chloride while samples treated by strontium chloride showed a relatively a thin and poorly mineralized enamel layers. Enamel layers treated with magnesium chloride remained hypo mineralized confirming the cariogenic effect of this element.

The cariostatic potential of zinc chloride and strontium ions chloride need to be confirmed by further studies, (experimental as well as animal studies on an artificial as well as natural carious lesion) before considering them really as anti cariogenic element and before giving any recommendation for them to be used topically in the dental clinical practice.

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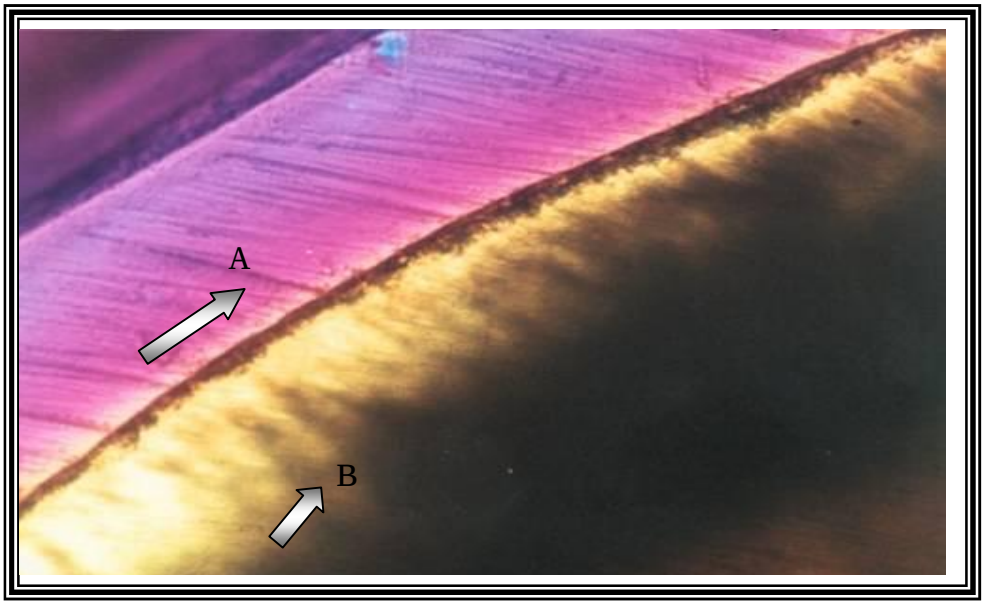
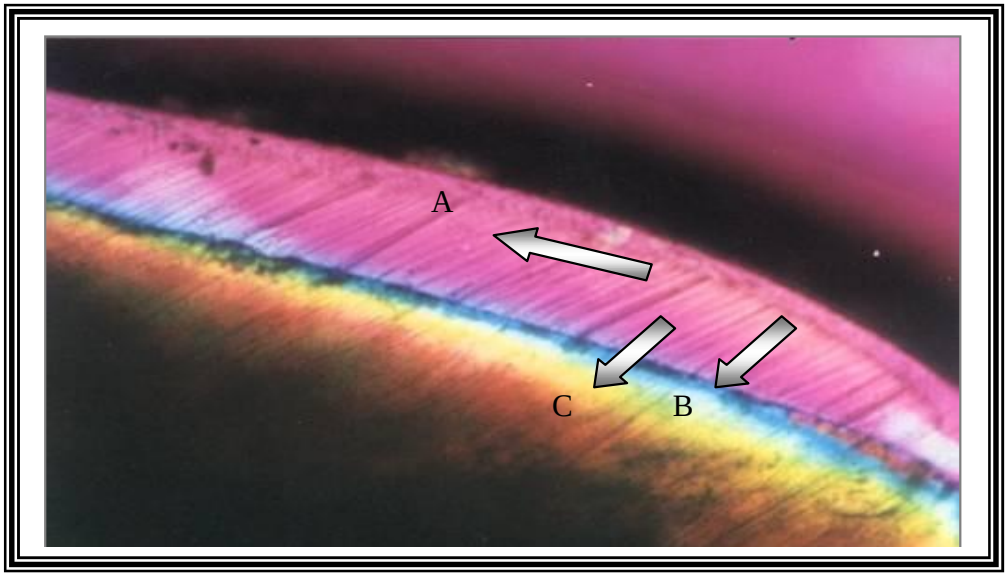


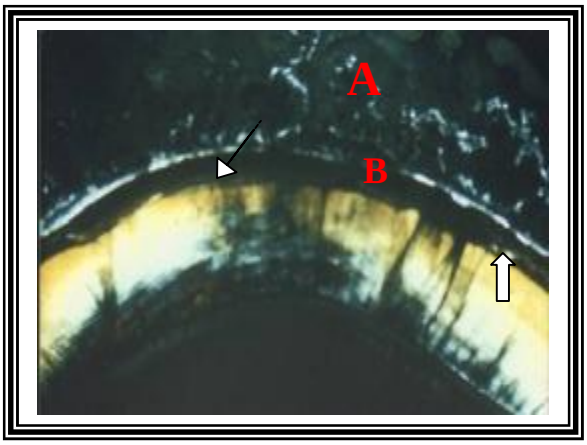
A. Canada Balsam
B. Sound Enamel Surface

Figure (1) Normal Sound Enamel Surface (100X)



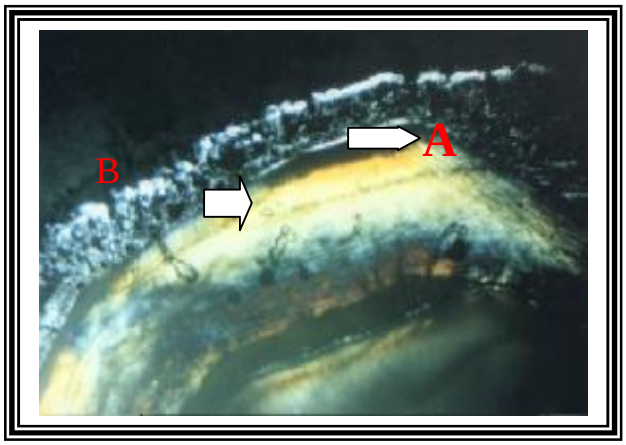
A. Canada Balsam
B. Deminerlized Enamel Surface
C. Sound Enamel Surface

Figure (2) De mineralized Enamel Surface (100X)



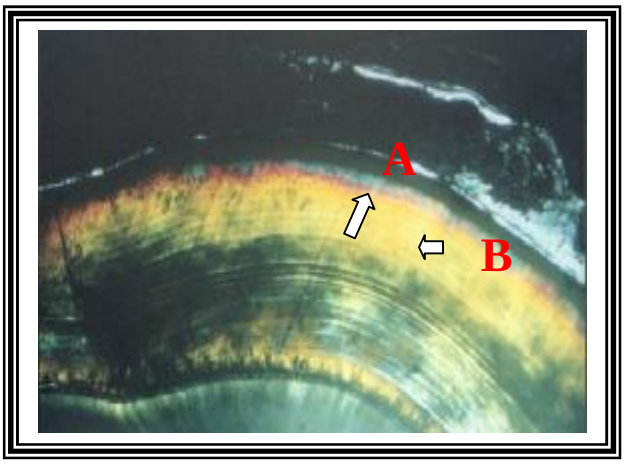
A. Demineralized enamel
B. Sound enamel

Figure (3)
Remineralized Enamel Surface after Treatment with SnF_2 . (100X)



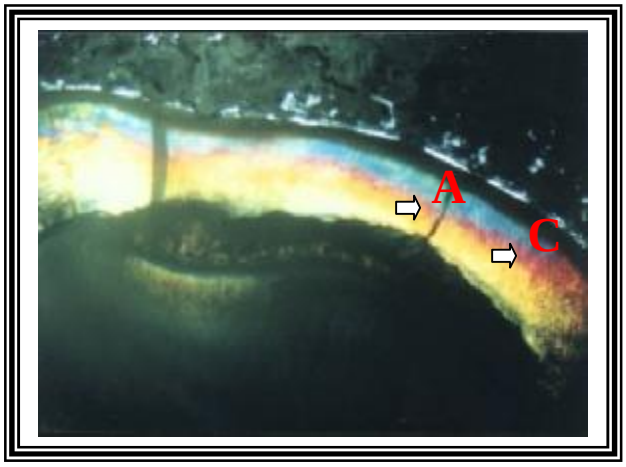
A.Sound enamel
B.Demineralizedenameln

Figure (4)
Remineralized E.namel Surface after Treatment with $ZnCl_2$. (100X)



A.Demineralized enamel
B.Sound enamel

Figure (5)
Enamel Surface after Treatment with $SrCl_2$. (100X)



A.Dmineralized enamel
C.Canada belsam

Figure (6)
Enamel Surface after Treatment with $MglCl_2$ (100X)