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ANNEALING EFFECT OF IRRADIATED TOOTH USING SURFACE MICRO HARDNESS TESTIN1G- AN INVITRO STUDY.

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ABSTRACT

Background: Dental complications is the common problem faced all over the world. Subjects with poor oral hygiene and patients undergoing radiotherapy for oral cancers are developing signs of decalcification of enamel, lesions and Caries. Hence a well-designed treatment is essential to protect the teeth among the patient population. Objective: To determine the annealing effect of radiated tooth by testing maximum micro hardness of the teeth before and after 30 days of radiation. Methodology: In our study, we collected 30 intact caries free human molar teeth and were sectioned mesio-distally. The sectioned teeth were divided into 3 groups as control, ACP CCP Coated teeth and ACP CCPF coated teeth. All the samples were maintained in artificial saliva before and after radiation to sustain oral environment. Samples were subjected to Radiation dosage of 70Gy and the microhardness profile was assessed before, after and after 30 days of radiation treatment. The results obtained were statistically analysed by performing bonferroni adjustment for pairwise comparisons and one way ANOVA for multiple comparison test at $P<0.05$ level of significance. Results: The microhardness test values defined that radiation had induced demineralisation which ensued the decrease in overall enamel hardness. It was also observed that teeth had acquired remineralising ability when tested for hardness after 30 days incubation period supplemented with artificial saliva from the day of radiation treatment.

Likewise, the teeth coated with ACP CCPF had the best outcome in comparison to the other groups and the results obtained were statistically significant. Conclusion: Teeth samples subjected to radiation undergo annealing (remineralisation) when maintained under oral hygienic environment over a period of time. Topical applications of fluoride has shown better remineralization efficacy.

KEYWORDS: Annealing effect, Hardness test, Remineralisation, Radiation therapy.

INTRODUCTION

One of the most common oral issues all over the world isDental caries.[1] Decalcification of the Enamel or the development of white spot lesions is the initial signs of caries, observable as chalky white patches on the surface of the tooth. These symptoms are mainly seen among subjects with poor oral health and hygiene as well with patients undergoing radiation therapy for head and neck cancers.

A well designed treatment is necessary to safeguard the tooth enamel surface before and after radiation, only then the caries on the enamel surface can be arrested by re-hardening it and ultimately converting them to the normal healthy condition. Only process that will help recover from the effects is self-remineralisation of enamel. At normal physiological conditions, the oral cavity components such as saliva and biofilm have calcium (Ca) and phosphate (Pi) in elevated concentrations in reference to the mineral elements of enamel. Hence these trace elements get deposited continuously on the enamel surface or redeposited in certain spots on enamel where they were dissolved. This is commenced as a natural defence mechanism induced by saliva which helps in maintaining the structure of enamel.[2] Therefore, remineralization can be the best process where enamel undergoes redeposition of the components that were disintegrated and this can be defined as enamel repair or rehardening.

Other such processes involve the usage of remineralizing agents that allows mineral diffusion into damaged or affected tooth structure. The treatment usually consists of remineralising agents containing fluoride or similar prophylactic agents.[3,4] In recent days, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) products are in use for the prevention of caries and also to induce remineralization among tooth tissues.[5,6] There is literature that proclaims incorporation of fluoride into the CPP-ACP compound (CPP-ACPF) has shown better remineralization properties in comparison to the former alone.[7,9] Mainly the studies using these agents has shown effectiveness on remineralization of white spot lesions, but very limited studies have been performed to analyse its anti caries benefits against the radiation treatment. The studies conducted by researchers have shown that CPP-ACP was successful in decreasing demineralization and thus increasing remineralization on enamel that was least sensitive to future degradation [10-14]. CCP ACP is known to be highly soluble and it is capable of rapid hydrolyzation to form apatite crystal molecules in the oral environment. Hence it is termed as an important product for remineralization treatment. [15] However, some other studies have reported that these products do not have supportive results without good oral health.

In several microhardness studies on enamel, it has been reported that the hardness rate depends on the degree of mineralization.[9,12] Even minute changes in the enamel surface during de/ re- mineralization and storage are considered very important for the analysis of micromechanical properties, which are precisely determined by nano indentations. As these nano indentations can examine a surface layer as thin as 1µm with a load as low as 10nN, it is considered as a standard technique for the determination of micro-mechanical properties of biological hard tissues.

Hence, an in vitro study was conducted to determine the annealing effect of radiated tooth by testing maximum micro hardness of the teeth before and after 30 days of radiation. The additive effect of remineralising agents such as CPP-ACP and CCP- ACPF maintained in artificial saliva were tested for enhancing the annealing process of the tooth before, after and 30 days after radiation.

MATERIALS AND METHODS

Tooth selection and sample preparation

In this In vitro study, 30intact caries-free, freshly extracted human molars were collected from AB Shetty Memorial Institute of Dental sciences, washed and stored as per OSHA regulations. The teeth were sectioned mesio-distally using carbondom disc dividing into 2 halves. They were cleaned to remove all the particulate debris, washed thoroughly with saline and double distilled water. For the invitro experiment, the teeth were divided randomly into 3 groups of 20 each.

Group 1: control group (C group), the teeth were introduced to artificial saliva maintained at room temperature

Group 2: CCP- ACP (GC Tooth Mousse, GC Corporation) coated teeth.

Group 3: CCP- ACPF (GC Tooth Mousse Plus, GC Corporation) coated teeth.

For the groups 2 and 3 teeth were dried and the exposed enamel is covered with proportionate amount of remineralising agent dried and maintained in artificial saliva at room temperature

Electron Beam Irradiation

All the 3 groups were maintained in artificial saliva at room temperature for 24 hours until radiation. The samples were then subjected to a cumulative radiation dosage of 70 Gy in Microtron Center (Mangalore University). The operation was performed by clamping the sample to a radiation board in 2 ml eppendorf tubes at a distance of 30 cm from the beam exit point.

Microhardness profile

After the radiation treatment, the teeth samples were thoroughly rinsed with normal saline and distilled water. Each specimen was fixed to the wooden blocks so that the cut surface was exposed. Microhardness profile of each sample were assessed using a microhardness tester (MATSUZAWA Co., Ltd. Model - MMT X7, Japan) with a Vickers diamond indenter under a 100g load for 10 sec. The indentations were made at the outer enamel surface. Three rows of indentations were made and then the mean of diagonal length of the 3 indentations at each depth was calculated for each sample.

Then, Vickers hardness (KHN) values were used for further statistical analysis.

Statistical analysis

We used analysis of variance (ANOVA) to determine whether there were any significant differences in the mean value between groups. A post-hoc Tukey comparison tests was performed to identify the statistically significant differences ($P < 0.05$) between the groups. The statistical analysis was performed by using SPSS software (version 16).

RESULTS

In this invitro study, the mean microhardness of the enamel as evaluated by Vickers microhardness tester showed variable results before (200 VHN) and after radiation (148VHN) denoting the occurrence of deminerilisation process. When examined the annealing effect of the demineralised teeth treated with ACP CCP, ACP CCPF and artificial saliva supplements, the mean hardness value of the enamel tend to increase to 162 VHN after

30 days [Table 1]. Thus the results obtained indicate that remineralisation process has been activated and these prophylactic agents play an important role in restoring teeth to healthy condition.

Table I: Distribution of Mean microhardness among the teeth sample.

Micro Hardness (Irrespective of groups)	Before radiation	After Radiation	30 days after Radiation
Mean	200.1667	148.1067	162.3100
Std. Deviation	7.57529	2.13750	6.67519

Further Repeated Measures of ANNOVA was used to compare the difference in micro hardness of between and within groups, (i.e Before, After and 30 days after radiation). The within group P value was 0.002 (Greenhouse-Geisser) and hence there was difference in mean microhardness within the groups. Bonferroni adjustment was used for pairwise comparison (table II).

Table II: Results of Pairwise Comparisons (irrespective of the Groups)

Pair		Mean Difference (Pairs)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
BR	AR	52.060*	1.408	<0.001	48.467	55.653
	Month AR	37.857*	1.493	<0.001	34.046	41.667
AR	MonthAR	14.203*	.613	<0.001	12.640	15.767

*. The mean difference is significant at the 0.05 level.

The p values for pairwise comparison are <0.05 (Table II), hence there is a difference in mean microhardness before and after radiation (52.06), Before and after 30 days of radiation (37.857) and after radiation and a month of radiation (14.203) at 5% level of significance.

The statistical analysis performed further was solely to determine differences in mean microhardness among control, ACP CCP and ACP CCPF treated groups. The values obtained clearly state that all the groups have differences in hardness before, after and 30 days after radiation. Fig.1 shows the graphical representation of the groups showing difference in hardness at 3 different time periods. Multiple comparison test was performed to find difference within groups (Table III).

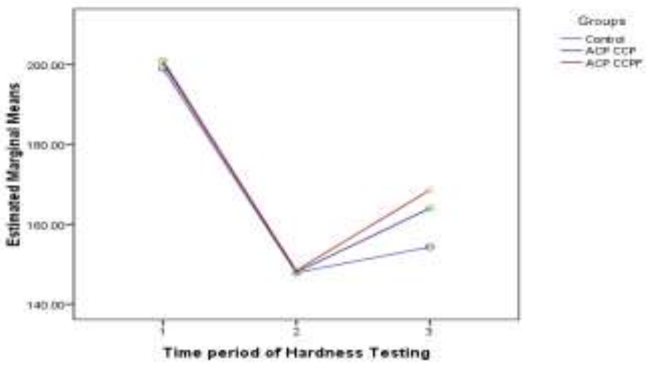

Fig 1: Mean hardness values of 3 groups (control, ACP CCP and ACP CCPF) at different intervals (Before, After and 30 days after radiation)

Table III: Results of Multiple comparison between groups.

Vickers Micro Hardness Test		Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	P value
					Lower Bound	Upper Bound		
Before radiation	Control	199.10	7.15	2.26	193.98	204.22	0.155	0.857
	ACP CCP	201.00	8.76	2.77	194.73	207.27		
	ACP CCPF	200.40	7.39	2.34	195.11	205.69		
After Radiation	Control	147.90	2.07	0.66	146.41	149.39	0.157	0.856
	ACP CCP	148.00	2.25	0.71	146.38	149.62		
	ACP CCPF	148.42	2.26	0.72	146.80	150.04		
30 days after Radiation	Control	154.32	3.28	1.04	151.97	156.67	62.293	<0.001
	ACP CCP	164.02	3.36	1.06	161.61	166.43		
	ACP CCPF	168.59	1.87	0.59	167.25	169.93		

Time period
1. Before radiation
2. After Radiation
3. 30 days after radiation

From the Table III, it is clear that the p values before radiation (0.857) and after radiation (0.856) among the groups is > 0.05. Hence there is no difference in the mean hardness among the groups before and after radiation.

The p value of the groups after 30 days of radiation was less than 0.001 and hence there was a difference among the micro hardness between the groups. Thus, explaining that remineralisation process has occurred at different rate at each group. ACP CCPF with VHN value of 168.59 has shown the maximum increase in mean microhardness in comparison to other groups. For more intricate analysis, Tukey's test was conducted for multiple comparison values among the group's after30 days of radiation (Table IV)

Table IV: Multiple Comparison by using Tukey's Test (30 days after Radiation)

	Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
30 days after Radiation	Control	ACP CCP	9.70000*	1.30572	.000	-12.9374	-6.4626
		ACP CCPF	14.27000*	1.30572	.000	-17.5074	-11.0326
	ACP CCP F	ACP CCP	4.57000*	1.30572	.005	1.3326	7.8074

*. The mean difference is significant at the 0.05 level.

Tukey's test connote that there is acceptable differences in mean micro hardness values among control and ACP CCP, ACP CCP and ACP CCPF and ACP CCPF and control at the level of 5% significance (p values < 0.05).

DISCUSSION

Tooth is composed of 3 calcified and a pulp tissue such as Enamel, Dentine, Cementum and Pulp respectively. Enamel is the hardest tissues and forms the outer protective covering of the anatomical crown of the tooth with a variable thickness of 1.0–2.5 mm.[16] The enamel is comprised of 96% inorganic matter (hydroxyapatite), 3% water and less than 1% of organic matter (protein matrix).[17] All the clinical investigations done on the enamel has said that once the enamel is formed and calcified, properties can be altered only by external chemical and physical action of its environment.[18] Most commonly they can undergo 3 possible reactions at aid of radiation, i.e polymerisation, breakage of hydroxyapatite crystals and no changes leading to damage.[19]

In this study, we examined the annealing effect of enamel of radiated teeth and additive effect of remineralizing agents for its capability of preventing enamel demineralization by performing Vickers microhardness testing. Annealing is a process where atoms undergo rearrangement to form stable molecules. In our previous studies we have clearly distinguished that radiation had deleterious effect on the enamel losing its integrity.[20] Hence self-annealing with supplementation of artificial saliva will be of clinical concern to motivate the future remedies. There are various methods to quantify and equate the mineralization process, which include microscopic examination, visualisation, microradiographs and microhardness testing. But in our study we opted for microhardness testing since enamel hardness is thought to vary proportionally by its mineral content.[21,22] Several preceding experiments have proclaimed that hardness testing is a reliable, easy, quantitative and a consistent technique [23]. Hence, in this study, Vickers micro indentation was used as a quantitative technique to ascertain the amount of enamel demineralization. The indentations were marked at a distance of 20 µm toward DEJ[24-26] and between the indentations to avoid possible inflictions of indentations. The averages of three indentations on each tooth at different depths on enamel were calculated to determine the overall Microhardness.

In recent days, it is likely common that cancer patient is getting subjected to radiotherapy. Though the treatment is known to reduce the progression of cancer cells, it can also have side effects on healthy tissues. One such effect is on teeth usually seen among Head and Neck cancer patients. The radiation energy interacts with biological materials of dental tissues which can result in the dissolution of the components. The above reaction is due to the thermal effect where the molecules of the tissue absorbs radiation energy and converts into heat, thereby causing microstructural and chemical changes within them. These changes are due to the elevated acid resistance and reduced solubility of enamel. The changes in dental tissues have been well explained by laser irradiation experiments [27-28]. On the basis of these studies we investigated the effects of Electron Beam Radiation on tooth enamel. The results attained in the study clearly signifies that the tissue have undergone changes which has resulted in decreased hardness after radiation dosage of 70 Gy. Thus the observed changes are attributed to the minified solubilisation of the enamel components leading to demineralisation of the tissues.[29] As well, the annealing of the tissue must have occurred within enamel which showed increase in Microhardness value after a period of 30 days when teeth were maintained in artificial saliva. A previous study on Electron Spin Resonance (ESR) Spectrometry has shown that the enamel undergoes thermal annealing at various temperatures.[18] but there are no studies yet on self-annealing property of enamel. Hence we can say that teeth can undergo remineralisation only when maintained under good oral hygienic condition.

Further we checked for additive action of prophylactic agents to render remineralisation of the tissues. The products (CPP-ACP and ACP CCPF) used in the study are commercially available and are known to prevent early demineralisation of the enamel.[30] It also has the ability to increase the buffering activity in saliva thus enabling remineralisation. The mechanism assisting the above process is stabilization of calcium phosphate molecules in the solution by increasing the binding affinity of ACP molecules to phosphoserine forming clusters of CPP – ACP.[8] Further combination of ACP CCP with fluoride (ACP CCPF), has shown higher potential for remineralisation than compared to ACP CCP alone.[31] Hence these products were selected for our study. Throughout the study, the samples were stored in artificial saliva before and after analysis to restore the oral environment and to check for remineralizing capability.

The study exclaims that the teeth have shown annealing effect at the interval of 30 days after radiation. This is an evidence that remineralisation has been activated in artificial oral environment. In comparison to control group and ACP CCP groups, the tooth treated with ACP CCPF has shown better remineralisation ability. This ACP CCPF is known to have very less amount of fluoride (0.2%), which enables free activities of calcium phosphate and fluoride ion in the solution, thus charging a supersaturated state of these ions and suppressing demineralization.[30] Many researchers have concluded that the Acidulated Phosphate Fluoride (APF)elevates the fluoride content [26], ACP CCPF is known prevent enamel caries [33], Sugar free chewing gums composed of CPP-ACP has shown higher remineralisation [10], and continuous use of APF is the only solution to increase the microhardness of the enamel surface.[32] In converse some studies suggest that these paste are ineffective in producing remineralisation.[22,23] However, maintenance of good oral hygiene plays an important role for beneficial output.

CONCLUSION

The results of the present study reciprocates that ACP CCPF showed better annealing effect in comparison to ACP CCP alone and Control samples maintained in artificial saliva. Hence we can conclude that the teeth subjected to radiation undergo annealing (remineralisation) when maintained under oral hygienic environment over a period of time. Topical applications of fluoride has shown better remineralisation efficacy.

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