ORIGINAL ARTICLE

AGE-RELATED CHANGES IN BLEACHED ENAMEL THROUGH SCANNING ELECTRON MICROSCOPY AND ATOMIC FORCE MICROSCOPY

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Objective:-To analyse the effect of bleaching on enamel morphology and roughness in different age groups. **Methods**: A-In-vitro experimental study was conducted in Pakistan Institute of Engineering and Applied Sciences (PIEAS) from 1st October to 31st December 2018. 20 recently extracted upper central incisors of four different age groups,21-30 years (n=5), 31–40 years (n=5), 41–50 years (n=5) and 51–60 years (n=5) werewere taken and divided into two parts where one part of 5mm × 5mm × 2.5 mm of enamel specimen served as a control group and the other counterpart as an experimental group. The control group was observed under AFM (Atomic Force Microscope) and then SEM (Scanning Electron Microscope). Enamel specimensof experimental groups were bleached with "Everbrite", in-officetooth whitening system and were again studiedunder the AFM (Atomic Force Microscope) and SEM (Scanning Electron Microscope). **Results:** There were significantly severe surface alterations and roughness observed in the young age group (21–30 years), moderate surface alterations and roughness found in the middle age group (31–40 years),whereas comparatively fewer surface alterations and unevenness was observed in the older age group, (41–50 years) and more elderly age group, (51–60 years) after the bleaching procedure. **Conclusions**: The detrimental effects of hydrogen peroxide were significantly evident in teeth of all the age groups after the bleaching procedure as compared tothose belonging to the controlage groups under the AFM and SEM analysis.

Keywords: Extracted Teeth, Bleaching, SEM, AFM, Age

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INTRODUCTION

Cosmetic dentistry is an essential part of today's therapeutic dental practice. Bleaching of vital teeth has obtained a specific benefitand teeth were also bleached as early as 1868, using oxalic acid and later with hydrogen peroxide.[1]

One of the most common products for bleaching procedures is those based on hydrogen peroxide at 35 or 38%, which is applied in dental offices under the supervision of dental professionals.[2] Hydrogen peroxide is the primary active ingredient of the bleaching gels. The breakdown of hydrogen peroxide results in theformation of oxygen and per-hydroxyl free radicals which oxidise the stained macromolecules and break them down into smaller lighter coloured fragments.[3] Then, the particles disperse across the tooth surface resulting in the bleaching effect.[4] Although, bleaching is a complex process and the mean reaction is oxidation.

Bleaching agents have generated microstructural changes on the bleached enamel surface.[5] Studies demonstrated demineralisation, degradation, and variations on surface micro-hardness and roughness of sound enamel surface.[6] Hardness is often described as closely spaced irregularities or with terms such as 'uneven', 'random', 'course in texture', 'broken by prominences', and other similar ones. The significance of surface roughness depends on the scale of measurement.[7]

Age-related changes occur in teeth between approximately ten weeks in utero to old age.[8] Lacassagne in 1889 was the first to characterise changes in fully formed teeth with ageing; however, the first scientific assessment was provided by Gustafson. He portrayed tooth ageing based on a scale of the severity of attrition, gingival recession, transparency of root, root resorption, apposition of secondary cementum at the root apex, and increasing secondary dentinal thickness.[9]

Researchers believe tooth bleaching has shown to increase the enamel surface roughness during or after treatment, which affects an increase in susceptibility to bacterial adhesion and subsequent carious lesion with following staining of the damaged enamel surfaceand comprising its surface morphology, which is evaluated through scanning electron microscope.[10,11] Additionally, since some authors, through scanning electron microscopy evaluation, have demonstrated demineralisation, surface defects and degradation of sound enamel as a consequence of bleaching, the effects of bleaching on teeth with variations in age warrants investigation.[8]

The current studies suggest that there is a need to study the relevance of the effect of bleaching with the ageing of patients. Therefore, the research will aim to assess the age-related changes on surface roughness and morphology of enamel as a consequence of bleaching

with in-office bleaching system containing 36% hydrogen peroxide.

MATERIAL AND METHODS

It is an in vitro experimental study which was undertaken at Pakistan Institute of Engineering and Applied Sciences (PIEAS) from 1st October to 31st December 2018. The sample consisted of 20 extracted central incisors about different age groups, 21–30 years (n=5), 31–40 years (n=5), 41–50 years (n=5) and 51–60 years (n=5) acquired by quota sampling. The research protocol was submitted for prior approval to the Board of Advanced and Secondary Education Research (BASR) and the ethics committee of Riphah International University.

Teeth included in this study were devoid of cracks and carious lesions. Moreover, no pre treatments were performed on these teeth with any chemical agents such as hydrogen peroxide. The labial surfaces of the central incisors used in this study were intact and free from any defects. Teeth with any defects such as cracks, caries, restorations and fractures were excluded from this study.

They were immediately stored after the extraction in thymol solution (buffered 0.1%, *p*H 7.00) for at least one week. Hard and soft deposits were removed with ultrasonic scaler (woodpecker) and teeth were finally polished.

Only crowns of teeth were required so the transversal sections of teeth were made at cemento-enamel junctions thusdividing the root and coronal portions with the help of low-speed digital cutting saw (MTI Corp, USA). Each crown was then longitudinally cut to get two parts of 5 mm×5 mm×2.5 mm enamel specimens of four different age groups, where one part of 5 mm×5 mm×2.5 mm enamel specimen was used as a control group and the other part of the same dimension as an experimental group.

The enamel specimens were embedded in polystyrene (Allied High Tech product inc, USA) by using 2.0 Cm diameter PVC moulds and thus leaving the external enamel surfaces uncovered by the resin. After 24 hours the specimens were removedfrom PVC moulds and then polished with silicon carbide papers 1200, 2400,4000 grit (English Abrasives Limited, London N17 0 x A England) in rotary polishing machine (Panasonic, Japan) and finally with 1 µm, 0.3 µm and 0.05 µm alumina polishing paste (Allied high tech product Inc, USA) to get a highly polished and smooth surface in rotary polishing machine (JP Studies Constructions; 27 rue Klock, 92 lucky 737.07.38).

Enamel specimens were examined under the stereomicroscope (Motic DMW–143-FBGC, Hongkong) at 20× magnification for the imperfections to be excluded. First, the sampleswere analysed using the AFM (Quesant Universal SPM, AmbiosTechnology, USA) for assessing the initial roughness of all the control group enamel specimens of different age groups.The enamel specimens were then sputter coated in a sputter coater (Imtech, ks550x) andthen photomicrographswere taken in SEM (Nova Nanosem 430; FEI company, 4022 26149391-Column flagAaron prep) at magnifications of 1,000 and 5,000. AFM is used to scan the surface morphology specially roughness of the surface in the micron scale and produces the 3D graphs of the surface. SEM is used for surface imaging and compositional analysis and can study the surface properties to the nanoscale.

Then the enamelspecimens were bleached with the Everbrite whitening system (1866 E San Jose CA 91748USA) and then again imaged through AFM (Quesant Universal SPM, AmbiosTechnology,USA) for the final analysisof roughness of all the experimental group enamel specimens ofdifferent age groups. The samples were again sputter coated in a sputter coater (Imtech, ks550x)and observed in SEM (Nova Nanosem 430; FEI company, 4022 261 49391-S column flag Aaron prep) for assessing the final surface morphology.

Classification of enamel changes[12]

No alteration	No alteration on enamel surface & no loss at enamel prisms.
A mild or slight alteration	A slight alteration in surface roughness and irregular patterns of conditioning and no loss of enamel prisms.
Moderate alteration	Distinct etching patterns were observed with loss of superficial structure & enamel prisms.
Severe surface alteration	Loss of superficial structure, deep irregularities with loss of prisms.

RESULTS

Enamel surface morphology and texture changes in different age groups ranging from 20–60 years were observed by scanning electron microscope are given in (Figure-1).Normal surface morphology is seen in all the control groups of different ages ranging from 20–60 years (Figure-3). After bleaching with 36% hydrogen peroxide, in-office whitening system increased porosity, loss of superficial enamel structure and erosionwas seen in young age, ranging from 21–30 years (Figure-4a).

Moderate irregularities in the form of distinct type I and II etching patterns were observed in middle age,a group ranging from 31–40 years (Figure-4b). Slight alterations exhibiting mild interprismatic dissolution on the surface were witnessed in the older generation,a group ranging 41–50 years and more oldage, ranging 51–60 years (Figure-4c,d).

The values of surface roughness for all the age groups were taken from AFM. Data were analyzed by performing the Paired *t*-test. The mean surface roughness values (Ra um) and standard deviation for all the four control groups and experimental groups ranging

from 21–30 years, 31–40 years, 41–50 years and 51–60 years were displayed in Table-1.

Significant changes were found in mean surface roughness which was increased in all the control groups with ageing. In healthy enamel, these changes were more prominent in old age group ranging from 51–60 years as compared to the young age group ranging from 21–30 years.

Mean surface roughness was also significantly increased in all the experimental groups after bleaching. In bleached enamel, these changes weremore profound in young age group ranging from 21–30 years while less in old age group ranging from 51–60 years with the mean difference 101.71±69.94 which was statistically significant (p<0.05).

Table-1: Mean changes in surface roughness levels for all age groups before and after bleaching (n=20)

Age Group	N	Mean Before	Mean After	Mean Difference	SD	SEM	*t*	*p*
21-30 Years	5	64.51	259.00	-194.49	0.10407	0.04654	-4179.04	0.000
31-40 Years	5	78.25	214.60	-136.35	0.11180	0.05000	-2727.00	0.000
41-50 Years	5	100.40	156.60	-56.20	0.44721	0.20000	-281.00	0.000
51-60 Years	5	114.70	134.50	-19.80	0.14755	0.06598	-300.040	0.000
Overall Test	20	89.46	191.17	-101.71	69.94	15.64	-6.50	0.000

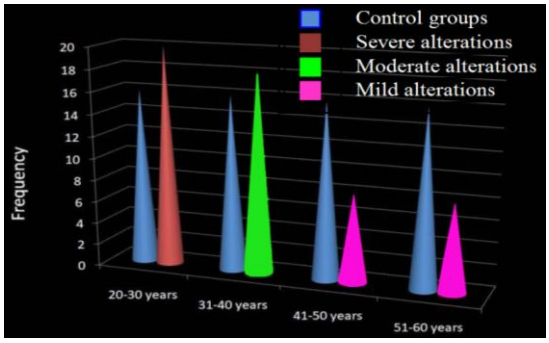


Figure-1: Surface morphology observed by scanning electron microscopy in all the age groups before and after bleaching.

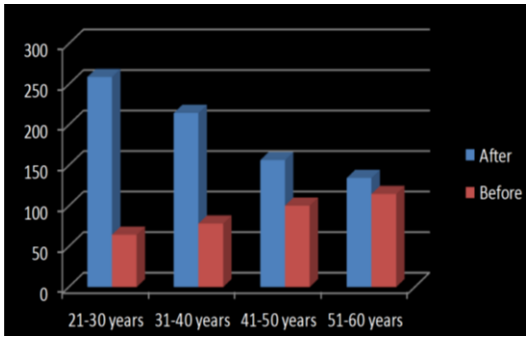
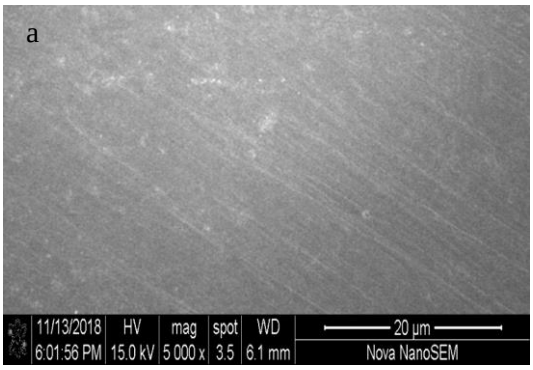
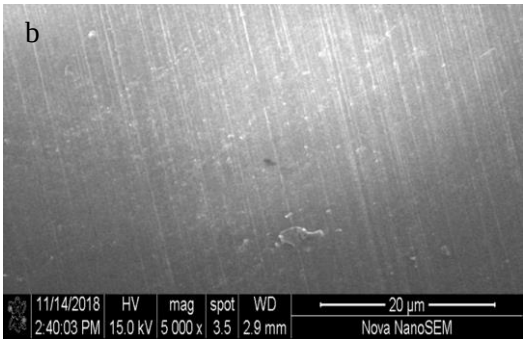
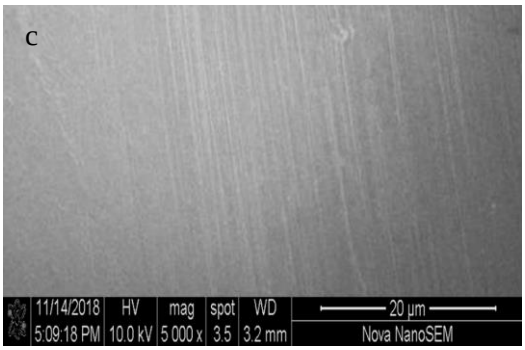


Figure-2: Mean surface roughness changes in all the age groups before and after bleaching.







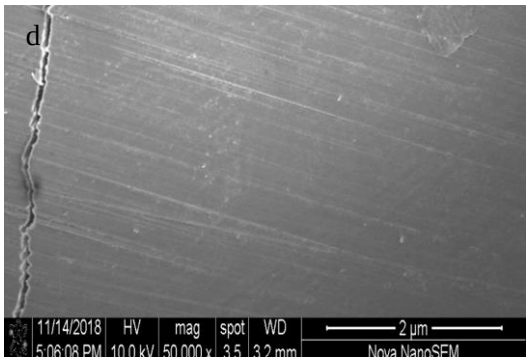


Figure-3: Enamel specimens of the control group (without bleaching) depicting different ages at 5000 mg. 21–30 years (a), 31–40 years (b), 41–50 years (c) and 51–60 years (d).

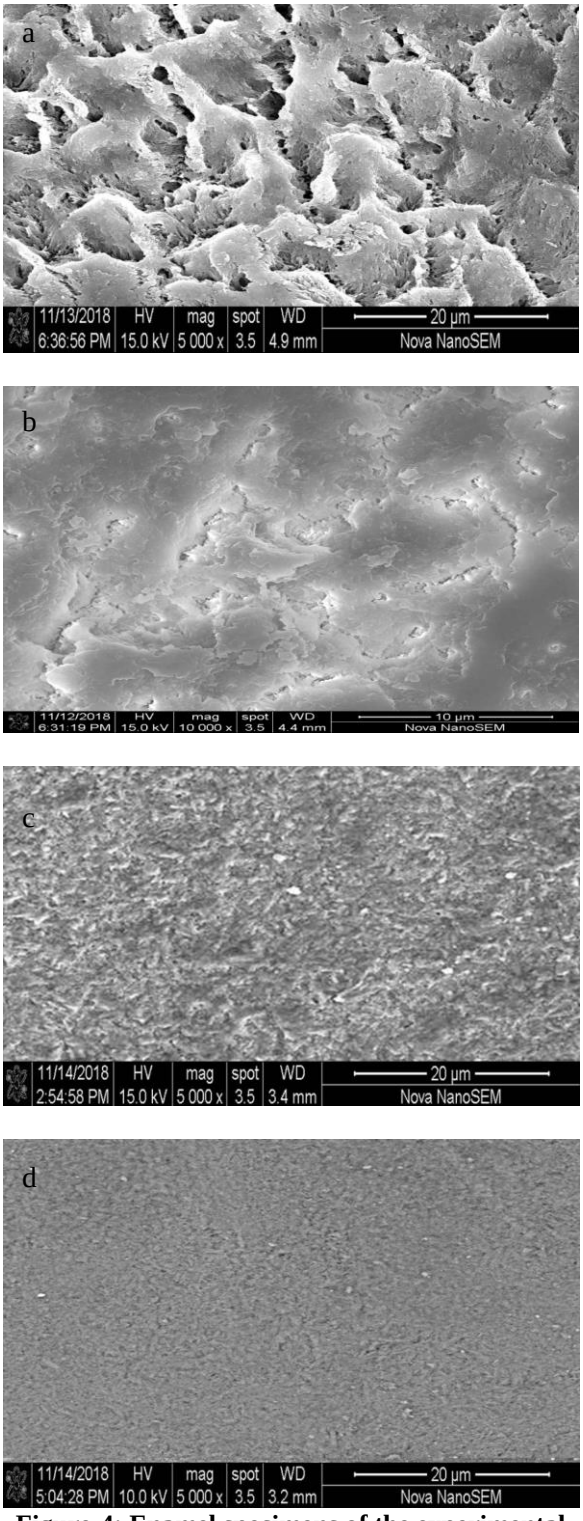


Figure-4: Enamel specimens of the experimental group (after bleaching) depicting different ages at 5000 mg. 21–30 years are showing sever changes (a), 31–40 years showing moderate changes (b), 41–50 years (c) and 51–60 years (d) and showing mild changes in surface morphology.

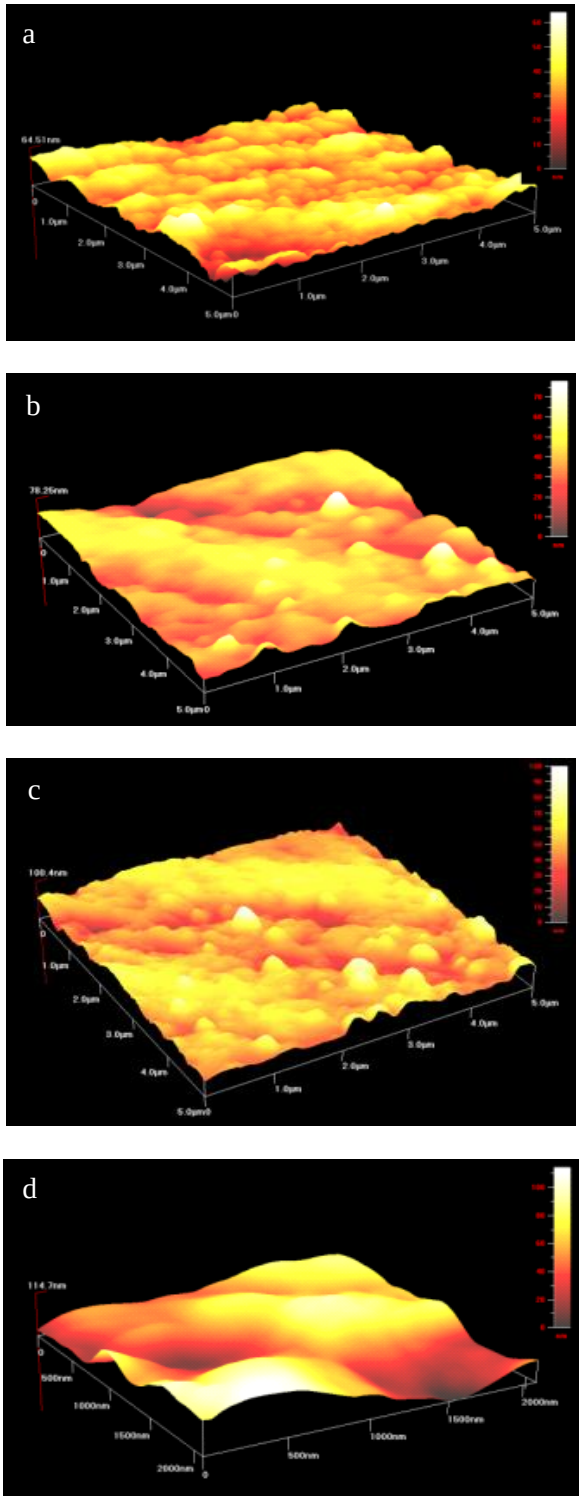


Figure-5: Enamel specimens of the control group (before bleaching) depicting different ages at 5000 mg. 21–30 years (a), 31–40 years (b), 41–50 years (c) and 51-60 years (d) showing a systematic increase in surface roughness with ageing.

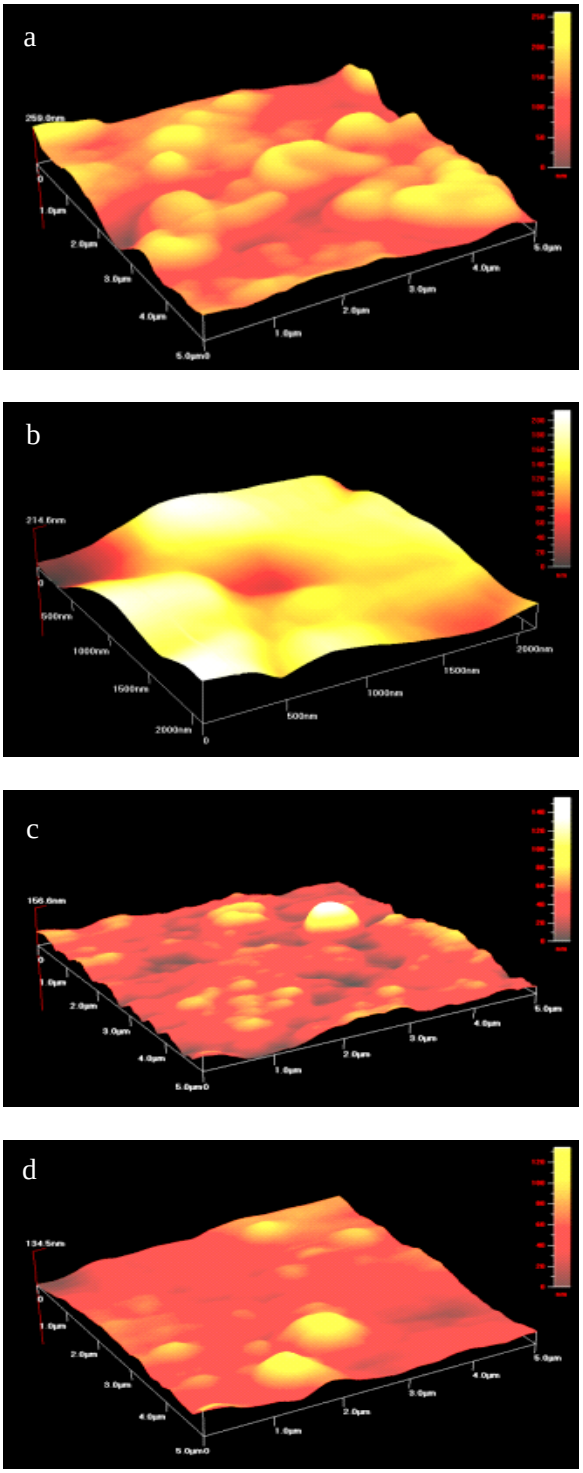


Figure-6: Enamel specimens of the experimental group (after bleaching) depicting different ages at 5000 mg. 21–30 years areshowing a severe increase in roughness (a), 31–40 years showing a moderate increase in roughness (b), 41–50 years (c) and 51–60 years (d) displaying a mild increase in surface roughness.

DISCUSSION

A wide range of results has been reported using Scanning Electron Microscopy which ranged from no changes in the surface morphology, mild surface pitting at specific areas and some enamel porosity to significant surface alterations relying on the study design, type of concentration of peroxide compound, phand duration of exposure.[13]

The scoring for external enamel morphology examined is given by O= enamel with smooth surface morphology, 1= enamel with slight irregularities, 2= enamel with moderate irregularities, 3 = enamel with accentuated irregularities.[14]

In the present study, severe changes in surface topography with increased porosity and loss of superficial structure were seen in young age group ranging from 21–30 years. Same differences were also observed by Shannon and colleagues and Zalkindand his colleagues after using 30% hydrogen peroxide.[15–17.] Moderate irregularities in the form of distinct type I and II etching patterns were observed in middle age group ranging from 31–40 years.[13,15,18]

Mild alterations with slight interprismatic dissolution on enamel surface were observed in old age group ranging from 41–50 years and more elderly age group ranging from 51–60 years.[13] Same changes in the enamel morphology were also reported by Yeh *et al*, in 2005.[19] Spalding and other colleagues demonstrated that alterations in tooth surface from the normal variations of enamel morphology might be higher than those alterations ascribed to the effect of peroxides on teeth.[20] However as age increases the process of erosion, attrition and abrasion cause the loss of enamel surface.[20]

Enamel structure has tunnels on its surface which are in the form of cracks, and these tunnels allow the easier penetration of the organic substances into the enamel. With the increase in the age, these enamel tunnels bend and decrease in number thus reducing the penetrating capacity of naturalmaterials into the enamel.[20] In the present study, the mean surface roughnessincreased by 89.46±69.94 as the age increased from young age group ranging between 21–30 years to old age group ranging between 51–60 years.

Previous studies showed that hydrogen peroxide used in 35% and 36% concentration increased the surface roughness.[21–23] In this study mean surface roughness increase from young age group ranging between 21–30 years to the old age group ranging between 51–60 years after bleaching was 191.17 ±69.94.[23]

The surface roughness was increased more in young age group ranging from 21–30 years as compared to old age group ranging from 51–60 years the mean difference in surface roughness before and after bleaching in this study was -101.71±69.94 which was

statistically significant because of *p*-value = 0.000 which is < 0.05.

Bleaching is an erosive process that affects the surface morphology largechromophore molecules are responsible for the enamel and dentine discolouration. The decomposition of hydrogen peroxide results in oxygen and per-hydroxyl free radicals that oxidise the staining molecules, breaking them down into smaller uncoloured fragments which are then converted into carbon dioxide and water.[24] The permeability of dental structures and the low molecular weight of bleaching agents provide free access of hydrogen peroxide through the enamel & dentin organic matrix.[25]

CONCLUSION

This study concluded that young age group, 21–30 years showed severe alterations and surface roughness due to increased permeability and diffusion of the bleaching agent. The middle age groups,31–40 years showed moderate changes and surface roughness whereas old age group, 41–50 years and more elderly age group, 51–60 years showed mild changes and surface roughness due to decreased permeability and diffusion of the bleaching agent.

CONFLICT OF INTEREST

There is no conflict of interest regarding this study

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