

# IN-VIVO ANALYSIS OF OXIDATIVE STRESS IN AESTHETIC COATED ORTHODONTIC ARCH-WIRES & BRACKETS

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## ABSTRACT

**Objectives:** To assess *in vivo* the oxidative damage that aesthetic coated arch-wires causes by measuring 8OHdG (8-hydroxydeoxyguanosine) as an oxidative marker to detect the level of oxidative stress generation.

**Materials and Methods:** About 80 patients of ages 15-30 years enrolled in the study having fixed orthodontic treatment were divided into control (patients with NiTi wire) and experimental groups including patients with wires coated in PTFE, epoxy resin, and rhodium. Saliva samples were collected with an unstimulated saliva collection method at two intervals. The first sample was collected before the bonding process and insertion of the arch-wires and the second sample was collected four weeks after the insertion of arch-wires. The samples were kept in storage at -80°C, and the amounts of 8-OHdG were measured by ELISA analysis.

**Results:** The salivary 8-OHdG level in all groups was measured at day 0 and the end of week 4. At day 0, it was 1.60±0.43 ng/ml in the NiTi group, 1.60±0.52 ng/ml in the epoxy resin group, 1.70±0.12 ng/ml in the rhodium group and 1.63±0.40 ng/ml in PTFE group. After 4 weeks, it was 3.68±0.47 ng/ml in the NiTi group, 3.50±0.77 ng/ml in the epoxy resin group, 3.06±0.70 ng/ml in the rhodium group and 2.75±0.72 ng/ml in PTFE group,  $p = 0.812$ ) This demonstrated that while there was no noteworthy variation in the groups' mean 8-OHdG levels at day 0, there was at week 4. Post hoc Tukey test revealed that PTFE-coated arch-wires and Rhodium group had significantly lower 8-OHdG levels compared to NiTi and Epoxy resin-coated arch-wire. Salivary 8-OHdG levels increased significantly after 4 weeks of arch-wire use in all groups.

**Conclusion:** This study suggests that PTFE-coated arch-wires are the most biocompatible among the coated arch-wires studied.

**Key words:** Ceramic brackets, esthetic, mechanical phenomena, orthodontic arch-wires

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## INTRODUCTION

"Aesthetics" is defined as "sensitive to, appreciative of, or enthusiastic about the beautiful." It recognizes how much an individual's own articulation, perception, and awareness impact their perceptions of beauty, and how society and one's own self-image further shape these perceptions. Advancements in orthodontic technology and biomechanics have

led adults to be aware of the need of orthodontic treatment and maintenance of good oral hygiene. However, these adult orthodontic patients have increased their cosmetic treatment expectations during and after treatment. Esthetic conscious patients often hesitate and refuse their orthodontic treatment because of the visibility of their braces for personal, professional and social reasons. The aesthetic arsenal includes ceramic brackets, lingual braces, clear invisible aligners (Invisalign), and coated arch-wires<sup>1</sup>.

Aesthetic coated arch-wires are a category of orthodontic wires that have been modified to improve their visual appeal. However, there are also worries about the cosmetic breaking off and greater frictional resistance. One type of decorative dental arch-wires is the metallic coated arch-wire, which is made of nickel-titanium or stainless-steel wires coated with rhodium, epoxy resin, polytetrafluoroethylene (PTFE), and parylene-polymer to give an enamel color.

To enhance aesthetic appeal, coating durability, and biomechanical reliability, manufacturers utilize a variety of coating materials, coating thicknesses, and treatment procedures<sup>2</sup>. Orthodontic wire coatings have the potential to change the external characteristics of the wires, which impacts properties like thickness, corrosion resistance, microbial adhesion, biomechanical and frictional properties, and exterior roughness<sup>3</sup>. Compared to their metallic equivalents, research shows that coated arch-wires generate less loading and unloading force, both when new and when retrieved<sup>4</sup>. Studies conducted by scientist recorded same findings and reported poor color constancy<sup>4-6</sup>. Scientist also recorded 25% coating loss after 33 days of use In-Vivo<sup>7-9</sup>.

Many factors within the mouth, like as saliva production, eating habits, ambient temperature, pH levels, chewing pressures, and the strength of orthodontic appliances, might affect orthodontic arch-wires with cosmetic coatings. The aforementioned conditions have the potential to affect the coated arch-wires structural integrity and longevity. They may also cause the surface coating to deteriorate and expose the underlying metal wire to corrosion. As a result of the metallic wire underneath corroding on the surface, different metal ions including nickel, chromium, cobalt, iron, and manganese are released. These metals can cause major health issues

such mucosal erythema, allergic contact dermatitis, stomatitis, periodontitis, and gingivitis in certain people who are sensitive to them or allergic to them<sup>10</sup>.

One of the biggest challenges facing orthodontic therapy is oxidative stress, which is brought on by an overabundance of reactive oxygen species (ROS) and the body's incapacity to neutralize their deleterious effects. ROS pose a threat to essential biological components such as proteins, lipids, cell membranes, and DNA. They may also cause apoptosis or cellular malfunction. Examples of ROS include hydrogen peroxide, superoxide, and hydroxyl radicals. Moreover, the mechanical strains brought on by orthodontic procedures may provoke inflammation and tissue damage, which will increase the level of oxidative stress in the tissues around them. A slower rate of tooth adjustment results from the disruption of vital cellular processes required for bone remodeling and tooth movement by this oxidative environment. Furthermore, increased pain and suffering before and after surgery may be brought on by oxidative stress, which raises the production of cytokines that worsen tissue damage and promote inflammation<sup>11</sup>.

Several researchers have evaluated oxidative stress using different markers including Nitric Oxide (NO) and Malondialdehyde (MDA)<sup>12,13</sup>. Thiobarbituric acid reactive substance (TBARS) 8-Hydroxydeoxyguanosine (8-OHdG) as a key marker<sup>14</sup>. This marker is important for evaluating how orthodontic therapy affects cellular health since it is linked to oxidative stress-induced DNA damage. It is produced by the attack of the hydroxyl radical (HO) on the C8 site of guanine and results in the transverse mutation of guanine to thymine. According to a study, the effects of self-ligating and traditional brackets on the levels of 8-OHdG in L929 murine fibroblast cells were evaluated in an in vitro investigation. According to the study, ceramic brackets had the least impact on 8-OHdG generation, whereas complete metal and polyurethane brackets caused the most amount<sup>15</sup>.

During orthodontic treatment, it's critical to develop plans and put particular preventive measures in place in light of the oxidative stress. In order to help the orthodontist choose the optimal arch-wire and enhance the standard of care, we aimed to assess in-vivo the levels of oxidative stressors and the biocompatibility of several cosmetic coated arch-wires

in this clinical experiment.

## MATERIALS AND METHODS

This In Vivo-Study was conducted after taking institutional ethics approval, at Orthodontic department of Akhtar Saeed Medical and Dental College, Lahore over One year and three months. The sample size, 20 in each group, was calculated using health studies version 2.0.21 WHO, by the formula keeping the power of the study equal to 90% and the level of significance equal to 5%, assuming anticipated mean of OHdG levels in NiTi Group  $2.39 \pm 0.83$  and mean of OHdG levels in Rh coated Group  $1.79 \pm 0.08$ . (11)

The sampling technique was non-probability, purposive sampling technique. The inclusion criteria was: patients who gave voluntary consent to participate in the study, patients seeking fixed orthodontic treatment, either gender, aged 15-30 years, patients with good periodontal status and oral hygiene, plaque index (PI) score of 0 or 1, gingival index (GI) score of 0 or 1, bleeding on probing score of 0. The exclusion criteria was patients suffering from any systemic disease, patients suffering from periodontal disease, patients working in any setting that exposes them to occupational hazards of chemical and biological nature and patients unwilling to participate in the study.

Eighty patients who met the inclusion criteria and agreed to take part in the research were selected for fixed orthodontic treatment. The patient and his or her legal guardian provided written informed consent (if required). They were divided randomly into four groups using the sealed envelope method. The groups were: Group 1 (Niti wire, n=20), Group 2 (Epoxy coated wire, n=20), Group 3 (Rhodium Coated wire, n=20), and Group 4 (PTFE Coated wire, n=20).

Each pair of arch-wire was enclosed in a white opaque envelope with a unique code. This was performed by a researcher. The patients chose only one of the sealed envelopes which contained coated arch-wires as shown in figure 2.

The following demographic information was collected: name, age, gender, and occupation. Their orthodontist gave them written and spoken information, giving them time to consider it and ask questions before they decided to take part in the study.

Three coated (PTFE, Epoxy, and Rhodium) and one bare NiTi wire were used for this experiment. The four selected arch-wire systems were: Uncoated

Archwire: Superelastic and uncoated is 3M Unitek 'Nitinol Superelastic Arch-wire'. It served as a cable for control. Coated Arch-wires: First-generation Rhodium Aesthetic and BioActive Arch-wires: this arch-wire has a 0.014-inch NiTi core that is plated in rhodium. (In Aspin, Illinois, the United States, GC Orthodontics America Inc.). FLI® Wire Nickel Titanium Preformed Natural Arch Aesthetic Coated Arch-wires - this arch-wire is PTFE coated with a core of 0.014" NiTi (Rocky Mountain Orthodontics Inc, Denver, Colorado, USA). G4™M Tooth-Colored Nickel Titanium Arch-wires - this arch-wire has an epoxy resin coating with a core of 0.014" NiTi (G&H Orthodontics Franklin, Indiana, USA).

Biocomma Saliva Collectors from Biocomma Limited Shenzhen, China, were used. It consists of a collection-funnel, collection-tube and saliva-preservation solution. Human 8-Hydroxy-deoxyguanosine (8-OHdG) ELISA Kit was used by Sunlong Biotech Co.LTD, Zhejiang, China.

An MBT bracket and a straight wire appliance were used to bond the teeth of each patient. The bracket that was utilized has slot dimensions of 0.022" × 0.028". To prime the teeth, 3M Unite Transbond self-etching primer was applied. Every tooth surface received three to five seconds of primer rubbing. After that, non-compressed air was used to gently whisk it away from the gingiva. After applying composite coating to each bracket, they were all positioned on the face axis of the patient's clinical crown of teeth and exposed to light for a duration of 20 seconds. DB Orthodontic elastomeric modules were utilized to precisely position the archwires.

All participating patients received comprehensive instructions on dental hygiene that they were required to follow for the length of the trial. Every patient had a clinical checkup. Before the trial began and the archwire was placed, as well as four weeks afterward, each patient's Silness Loe Plaque-Index (PI), Silness and Loe Gingival-Index (GI), and bleeding on probing, were assessed.

The "passive drool method" was used to acquire unstimulated whole saliva (UWS), which is regarded as the ultimate benchmark because it effectively duplicates baseline salivary flow and content from both the major and minor salivary glands in a resting position. In order to avoid coming into contact with the ingredients in toothpaste, patients were told to

avoid tooth brushing in the morning of the sample collection. The patient was advised to avoid drinking, eating and chew gum 30 minutes before the collection procedure. Before starting the saliva collection process the patient was asked to sit quietly with their head bent downwards and mouth open to allow the saliva to passively drip into the saliva collecting kit Fig 3 until it reaches the 2ml graduation line on the collection tube. Then a preservative solution was poured into the collection tube. The lid on the collection tube was tightened and the collection tube was turned upside down 5-10 times to mix the saliva and the preservative solution. The sample was labeled and kept at  $-80^{\circ}\text{C}$  in the lab pending additional examination.

Saliva was collected at two intervals: at day 0 before the placement of the archwire at the start of treatment and four weeks after the placement of the archwire. Following collection, the sample was carefully collected and stored at  $-80^{\circ}\text{C}$  until further examination. It was then centrifuged for 20 minutes at 2000–3000 rpm. Sandwich-ELISA was the technique employed with this ELISA kit. An 8-OHdG-specific antibody was pre-coated on the microelisa stripplate that was included in the kit. The relevant microelisa stripplate wells were filled with saliva samples, which were then mixed with the particular antibody. Each micro-ELISA stripplate was then thoroughly coated with a Horseradish Peroxide (HRP) conjugated antibody specific for 8-OHdG, and the plates were then incubated. Free parts were removed by washing. To every well, the TMB substrate solution was applied. The only wells that showed blue coloration before becoming yellow with the addition of the stop solution were those that contained 8-OHdG and HRP conjugated 8-OHdG. The optical-density (OD) was determined spectrophotometrically at a wavelength of 450 m. The 'OD' value correlated with the 8-OHdG concentration. The concentration of 8-OHdG in the samples was calculated by comparing the 'OD' of the samples with the standard curve. The Multiskan-EX is an 'eight-channel' vertical light path filter microplate photometer designed to perform standard photometric measurements by measuring absorbance from suitable microplates and strips in 96-well plate format. It has internal software and can be controlled by a computer. The results in the form of optical density value 450m were generated

by this microplate reader.

Using SPSS 25, the measurements from each of the four groups were evaluated and compared. The age and 8-OHdG level numerical data were displayed as mean  $\pm$  standard deviation. Numerical variables were tested for normalcy using the Shapiro-Wilk test. The groups' means of age were compared using a one-way ANOVA test. To compare numerous pairs of data, the post hoc Tukey test was employed. The gender distribution was compared using the Chi-square test. The confounding variables were controlled through stratification. A p-value of less than 0.05 was deemed significant.

## **RESULT**

Table 1 displays the average age of the patients. The mean age of the patients in each group did not significantly differ, according to the findings. ( $p = 0.132$ ) Table 2 displays the distribution of male and female patients within the study groups. The gender distribution among the groups did not differ significantly, according to the results of the Chi-square test. ( $p$  is 0.977). On day zero, the salivary 8-OHdG level was assessed in each group. Group 1 had  $1.60 \pm 0.43$ , Group 2 had  $1.60 \pm 0.52$ , Group 3 had  $1.70 \pm 0.12$ , and Group 4 had  $1.63 \pm 0.40$ .

On day zero, the salivary 8-OHdG level was assessed in each group. Group 1 had  $1.60 \pm 0.43$ , Group 2 had  $1.60 \pm 0.52$ , Group 3 had  $1.70 \pm 0.12$ , and Group 4 had  $1.63 \pm 0.40$ . At four weeks, the salivary 8-OHdG level was also assessed in each group. Table 3 displays the values, which were  $3.68 \pm 0.47$  in the NiTi group,  $3.50 \pm 0.77$  in the epoxy resin group,  $3.50 \pm 0.77$ ,  $3.06 \pm 0.70$  in the rhodium group, and  $2.75 \pm 0.72$  in the PTFE group. It was discovered that there was a significant difference in the groups' mean salivary 8-OHdG levels at week four, while there was no significant difference in the groups' mean salivary 8-OHdG levels at day 0 ( $p = 0.812$ ). ( $p < 0.001$ ).

Post hoc Tukey test showed that mean salivary 8-OHdG level in groups 4 was significantly lower as compared to group 1 and 2 while there was no significant difference observed between group 3 and 4. The salivary 8-OHdG level was also significantly lower in group 3 as compared to groups 1 and 2. However, no significant difference was observed in the mean salivary 8-OHdG level between 1 and 2 groups

The salivary 8-OhdG level at day 0 and at 4 weeks in each group was also compared. As data was normally distributed, therefore, paired sample t-test was used as shown in table 5. It was found that there was significant increase in mean salivary

8-OhdG levels after 4 weeks compared to day 0 in each group. It was found that there was a significant increase in mean salivary 8-OhdG levels after 4 weeks compared to day 0 in each group.

### DISCUSSION

While earlier studies have assessed the levels of oxidative stress during traditional orthodontic treatment using a range of biomarkers, such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-), interleukin-1 beta (IL-1), malondialdehyde (MDA), and TNF<sup>12,16</sup>.

In order to look for oxidative stress during fixed orthodontic therapy brought on by the disintegration of coated arch-wire surface coatings, we examined the amounts of 8-OHDG in the current study.

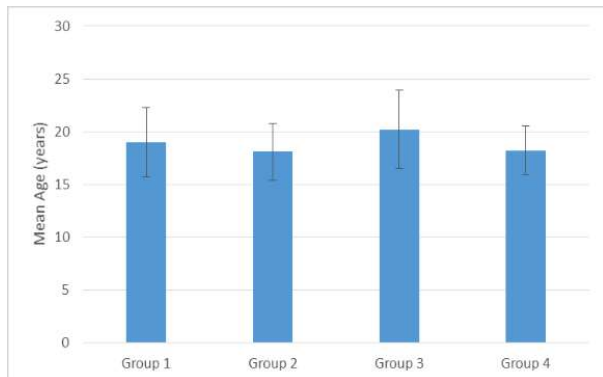


Fig 1: Bar chart showing the comparison of mean age of study participants among groups.

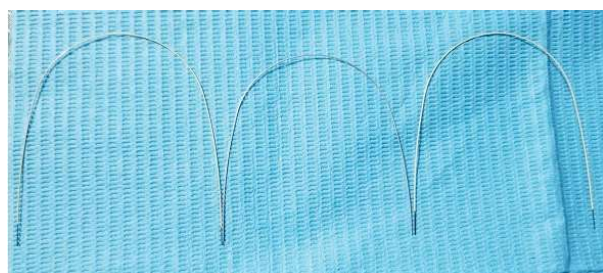


Fig 2: Different coated aesthetic archwires.

Table 4: Pairwise comparison of mean salivary 8-OhdG level among study groups

Groups	Groups	Mean Difference	p-value #
1	2	0.178	0.837
	3	0.620	0.024*
	4	0.928	<0.001*
2	3	0.442	0.172
	4	0.750	0.004*
3	4	0.308	0.475

\*p value significant # Tukey test

Table 1: Showing mean age of study participants among groups.

Parameters	Group 1	Group 2	Group 3	Group 4	p-value
Age (years)	19.0 ± 3.3	18.1 ± 2.7	20.2 ± 3.7	18.2 ± 2.3	0.132

#one way ANOVA

Table 2: Gender distribution among groups

Gender	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	p-value#
Male	8 (40.0%)	8 (40.0%)	9 (45.0%)	9 (45.0%)	0.977
Female	12 (60.0%)	12 (60.0%)	11 (55.0%)	11 (55.0%)	

# Chi-square test

Table 3: Showing mean salivary 8-OHDG Level among study groups at day 0 and week 4.

Salivary 8-OHDG Level	Group 1	Group 2	Group 3	Group 4	p-value#
Day 0	1.60 ± 0.43	1.60 ± 0.52	1.70 ± 0.12	1.63 ± 0.40	0.812
Week 4	3.68 ± 0.47	3.50 ± 0.77	3.06 ± 0.70	2.75 ± 0.72	<0.001*

#one way ANOVA significant

Table 5: Showing mean salivary 8-OhdG level between day 0 and 4 weeks in each group

Salivary 8-OhdG Level	Group 1	Group 2	Group 3	Group 4
Day 0	1.60 ± 0.43	1.60 ± 0.52	1.70 ± 0.12	1.63 ± 0.40
4 weeks	3.68 ± 0.47	3.50 ± 0.77	3.06 ± 0.70	2.75 ± 0.72
p-value #	<0.001*	<0.001*	<0.001*	<0.001*

# Paired sample t-test

8-OHdG is an indicator that is explicitly elevated during periodontal deterioration triggered by free radicals released from fixed orthodontic appliances.

These free radicals trigger oxidative stress by peroxidation of membrane phospholipids, resulting in a raise in ROS-induced periodontitis. Consequently, 8-OHdG is a valid indicator that represents the oxidative stress concentrations of the periodontium in individuals receiving conventional orthodontic therapy<sup>17</sup>.

In the present study, patients of both sexes between the ages of 15 and 30 were selected because patients in this age range seek orthodontic treatment more frequently and are more interested with aesthetic treatment alternatives. Several previous investigations demonstrate that coated wires incur partial or complete coating degradation after three to four weeks<sup>18,19</sup>.

The mechanical and physical qualities of these wires have been the subject of significant research and investigation. Matias et al. (2018) used ceramic brackets to measure deflection forces during unloading for various coated wires, finding that rhodium-coated wires performed best due to their ability to generate the largest forces during offloading while epoxy resin-coated wires generated the least forces<sup>20</sup>. Epoxy resin wires generated reduced loading and unloading forces relative to Teflon-coated wires, according to the findings of another investigation that was carried out<sup>21</sup>.

Epoxy resin-coated wire was found to have a greater frictional resistance and surface irregularities than teflon and polymer-coated wires, according to prior research that analyzed the surface roughness of these coated wires<sup>22</sup>. When epoxy, Teflon, and rhodium-coated wires are compared on the basis of surface roughness, epoxy resin-coated archwires showed the most roughness, and rhodium-coated wire was the least rough. This was determined by the studies that was determined independently by both<sup>23,24</sup>.

The study aimed to assess the oxidative stress marker 8-OHdG produced by various esthetic-coated archwires after four weeks of being in the oral cavity. While these coated archwires offer aesthetic and mechanical advantages compared to traditional metallic wires, their ability to withstand oral challenges without losing their coating is crucial for

validation. Considering the higher cost of coated wires, gathering clinical data becomes essential for informed treatment decisions and further research. This research specifically compared 8-OHdG production from three types of coated wires (PTFE, epoxy resin, rhodium) and conventional metallic NiTi wires over four weeks, focusing on coating loss and subsequent corrosion causing oxidative stress.

A meta-analysis of several articles done about 8-OHdG levels in healthy and periodontally involved patients revealed a mean 8-OHdG concentration of 1.67 ng/ml in healthy individuals and patients suffering from periodontal diseases on average have 8-OHdG levels 2.11ng/ml higher than healthy individuals<sup>25</sup>. All wires caused various amounts of oxidative stress in the oral cavity, with typical metallic NiTi wire producing the most oxidative stress at  $3.68 \pm 0.47$  ng/ml and epoxy resin-coated wire producing the second highest increase in 8-OHdG levels at  $3.50 \pm 0.75$  ng/ml. Rhodium-coated wires produced  $3.06 \pm 0.70$  ng/ml, whereas PTFE-coated wires produced the least at  $2.75 \pm 0.72$  ng/ml.

The results of this study are supported by the findings of Yang and Lee (2015)<sup>27</sup>, who conducted an immersion test lasting 21 days and discovered that epoxy resin wire is more prone to lose its coating than PTFE-coated wires. The findings of Elayyan et al. (2008), which further complement the findings of our investigation, demonstrated that recovered epoxy resin-coated wires exhibited a loss of 25 percent of their coating after being placed in the mouth cavity for 33 days. Yet, the results of the research conducted by Bradley et al. (2013)<sup>28</sup> attributing to their findings are as follows: Epoxy resin-coated wire has a lower coating loss of 26.44% compared to PTFE-coated wire, which has a coating loss of 44.3%, contrary to our findings. The findings that NiTi yields the highest levels of 8-OHdG as a result of corrosion-induced metal ion release are consistent with earlier research documenting the cytotoxic and genotoxic effects of this alloy. Nickel and its alloys produced oxidative injury by simultaneously increasing ROS generation and impeding the antioxidant mechanism<sup>28</sup>.

There are several limitation of this study such as small sample size, in-vivo nature and conducted at a single center, However within these limitations the results showed that epoxy resin-coated wires are the least biocompatible of the commercially avail-

able options for aesthetic coated wires, producing the most 8-OHdG, while PTFE-coated wires are the most biocompatible, producing the least amount of 8-OHdG and preserving their structural integrity in the oral cavity. The findings of our study on biocompatibility will provide crucial insight to the orthodontist when choosing among different coated archwires based on scientific knowledge rather than the manufacturer's claims which will ultimately affect the quality of treatment provided.

## CONCLUSION

It was concluded that the epoxy resin-coated wires are the least biocompatible of the commercially available options for aesthetic coated wires, producing the most 8-OHdG, while PTFE-coated wires are the most biocompatible, producing the least amount of 8-OHdG and preserving their structural integrity in the oral cavity.

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**CONFLICT OF INTEREST**  
Authors declare no conflict of interest.  
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#### **AUTHORS' CONTRIBUTION**

The following authors have made substantial contributions to the manuscript as under:

Conception or Design: IBS, SK, AA, ZF, HA, MA

Acquisition, Analysis or Interpretation of Data: IBS, SK, AA, ZF, HA, MA

Manuscript Writing & Approval: IBS, SK, AA, ZF, HA, MA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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