Cross linking is a procedure that can inhibit the progression of function [12]. Residual crystallites in the demineralized tissue or by treatments that incorporate the same ions from external sources [11]. Carious dentin can happen either by a inherent repair or remineralisation strategies that are more easily translatable as clinical interventions.

The course of action, intensity and content of the demineralisation processes of human dentine in vivo, will enable the scientists to create predictable study models for in-vitro researches of these processes and in this way look for effective repair or remineralisation strategies that are more easily translatable as clinical interventions [10]. Remineralization of carious dentin can happen either by a inherent including of ions (calcium, phosphate and fluoride) from the oral fluid onto residual crystallites in the demineralized tissue or by treatments that incorporate the same ions from external sources [11].

Dentin collagen has an important role in the remineralization of demineralized dentin [8]. Previous studies found that crosslinking treatment of demineralized dentin collagen with crosslinking agents depends on time [9]. Understanding the course of action, intensity and content of the demineralisation processes of human dentine in vivo, will enable the scientists to create predictable study models for in-vitro researches of these processes and in this way look for effective repair or remineralisation strategies that are more easily translatable as clinical interventions [10]. Remineralization of carious dentin can happen either by a inherent including of ions (calcium, phosphate and fluoride) from the oral fluid onto residual crystallites in the demineralized tissue or by treatments that incorporate the same ions from external sources [11].

Riboflavin is an antioksidant and water soluble vitamin B2 extract; involved in body cell growth and function [12].

Riboflavin is a crosslinker and activated by UVA. Riboflavin is a treatment method for keratokonus [13]. Riboflavin is an crosslinking antioksidant and water soluble vitamin B2 extract; involved in body cell growth and function [12].

Dental caries is a preventable disease. On the other hand it is stil remains most prevalent disease and are high-priced, currently compose an increased global financial burden, with significant differences between countries [1,2,3]. Dentin is a dental hard tissue and mainly composed of hydroxyapatite (HA), non-collagenous matrix proteins and collagen [4]. The dentin matrix is mainly be formed of type I collagen fibrils with related noncollagenous proteins, forming a three-dimensional matrix that is strengthen by minerals [5]. Dental caries is a dynamic process of rapidly interchangeable periods of pathologial remineralization which is a loss in mineral content will occur and physiological protective remineralization [4,6,7].

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activated by UV light induces covalent bonds, otherwise known as crosslinks, between collagen fibrils. Riboflavin, has proven to support collagen type I crosslinking [14,15]. These crosslinks eventually help patients restore visual acuity [16]. Riboflavin is biocompatible and it has ability to produce free radicals when photo-activated by light which has spectral range from uv to visible light [12,16]. The covalent crosslinks among the neighbouring collagen molecules forms by these free radicals, or reactive oxygen species like O$_2$ and O$_2^-$[17].

The aim of this in vitro study is to evaluate the effect of photoactivated 0.1% Riboflavin on primary molar dentin remineralization using surface microhardness measurements (SMH).

2. RESULTS

The mean and standard deviations (SD) of SMH of dentin values at baseline, demineralization and after different photoactivation time of photoactivated 0.1% RF treatment and level of significance for all groups were shown in Table 1. After photoactivated 0.1% RF, the mean SMH values of all test groups were significantly higher than those of the negative control group ($p=0.01$), and Group I (30 s. photoactivated 0.1% RF treatment) showed the highest mean SMH values among test groups.

Table 1. Surface microhardness test results of dentin specimens.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Baseline Mean (SD)</th>
<th>After Demineralization Mean (SD)</th>
<th>After Riboflavin Treatment Mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 30s photoactivated RF</td>
<td>75.68 ± 7.79</td>
<td>38.14 ± 4.02</td>
<td>42.34 ± 4.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Group II 60s photoactivated RF</td>
<td>55.3 ± 5.88</td>
<td>27.66 ± 3.56</td>
<td>32.39 ± 3.87</td>
<td>0.001</td>
</tr>
<tr>
<td>Group III Positive control</td>
<td>48.43 ± 6.22</td>
<td>25.9 ± 3.73</td>
<td>33.47 ± 3.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Group IV Negative control</td>
<td>59.14 ± 6.65</td>
<td>30.31 ± 4.32</td>
<td>32.29 ± 4.66</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The percentage recovery of SMH (%SMHR) among groups were shown in Table 2. There was statistically significant difference of %SMHR values between all groups at after photoactivated 0.1%RF treatment ($p=0.001$) (Table 2). The lowest percentage recovery of SMH in negatif control, whereas the highest mean value %SMHR was found in positive control which is 34.76 ± 20.62 (Table 2).

Table 2. The %SMHR results after photoactivated 0.1%RF treatment of dentin specimens.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>%SMHR Mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 30s photoactivated RF</td>
<td>11.49 ± 6.95</td>
<td></td>
</tr>
<tr>
<td>Group II 60s photoactivated RF</td>
<td>16.62 ± 11.93</td>
<td>0.001*</td>
</tr>
<tr>
<td>Group III Positive control</td>
<td>34.76 ± 20.62</td>
<td></td>
</tr>
<tr>
<td>Group IV Negative control</td>
<td>6.77 ± 9.35</td>
<td></td>
</tr>
</tbody>
</table>

*Significance difference $p <0.05$. SD: standard deviation.

Kruskal Wallis and Dunn test. (p < 0.05), SD = standard deviation.
Nevertheless, the comparison between groups showed no statistically significant differences in %SMHR among Groups I with Group II and IV (p> 0.05), as shown in Table 3. Meanwhile, on analysis for multiple comparisons between various groups, statistically significant difference was seen in values of %SMHR at the photoactivated 0.1% RF treatment stage between Group I with Group III; Group 2 with Group III and IV; and Group III with Group IV (Table 3).

Table 3. Analysis of intergroup comparisons of the %SMHR

<table>
<thead>
<tr>
<th>%SMHR</th>
<th>Group I 30s photoactivated RF</th>
<th>Group II 60s photoactivated RF</th>
<th>Group III Positive control</th>
<th>Group IV Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 30s photoactivated RF</td>
<td>0.151</td>
<td>0.001*</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>Group II 60s photoactivated RF</td>
<td>0.005*</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III Positive control</td>
<td>0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. DISCUSSION

Regarding in restorative dentistry towards a minimally invasive techniques has totally changed the manner in which dentinal caries is handled [21]. In this study, the demineralization and remineralization process was used to imitate caries formation that occur in oral cavity [19]. Dentin lesions has become after 7 days of demineralizing period in primary teeth, which is the most appropriate time for this study [19,20]. Demineralized dentin is mainly composed of collagen fibrils [22]. The organic matrix of dentin contains noncollagenous proteins (NCPs), which play a very important role in the biomineralization of dentin [23]. Dentin caries lesions results in reduced mineral contents, means that has been diminished mechanical properties [24].

In an attempt to evaluate the conditions found in artificial caries-affected dentin, several methods of producing caries-like lesions in vitro have been developed [25].

Riboflavin is used to improve the chemical, physical and antimicrobial properties of demineralized dentin [22]. Meanwhile, the clinical applicability of riboflavin activated using UVA is a concern [13]. The usage of riboflavin and UVA irradiation to crosslink corneal collagen in vivo has been approved by the U.S. Food and Drug Administration in 2016. The absorbance UV/VIS spectrums of riboflavin: two in the UV (230, 260 nm), one at 370 nm and one at 450 nm [24,25].

Therefore the tungsten/halogen lamp curing units which are frequently used in dental offices, the blue light can also be used for photoactivation [13]. The alternatives to the UVA light source, like conventional blue-light halogen-lamp curing units, can activated riboflavin, by means of its ready availability and its ease and using safely in dentistry [13]. The use of UVA has reported that it is been using effectively as a photactivator of riboflavin for crosslinking but for dental use security measures and its practicality should be noted [13,17].

While as its ideal curing functions, D-Light Pro also has a unique Detection mode (DT) that uses near-UV light only. Thus, UV light at 400 nm used in the present study would match the riboflavin 370 nm peak. The use of 30 s of blue light to activate riboflavin in the current study was identical to that used by Fawzy et al. [13] and Abunaveg [26]. As well as its ideal curing functions, D-Light Pro also has an unique Detection mode (DT) that uses near-UV light only.

RF has been shown to enhance the microhardness of demineralized dentin by promoting chemical modifications of the collagen. The photoactivated RF treatments induced chemical modifications in the dentin collagen and revealed remineralizing effects. In the present study, the microhardness of dentin was increased by 30 and 60 s photoactivated-RF treatment. The 60 s photoactivated-RF yielded the most pronounced remineralization under the in vitro conditions chosen. Therefore, the use of RF can be preferred within the concept of supporting the increase of dentin microhardness. Whereas, the 30 and 60 min UVA-activated riboflavin resulted in a significant increase in mechanical properties when compared to the untreated dentin samples. Additionally, the 30 and 60 min UVA-activated riboflavin treatment concluded in chemical modifications in the dentin collagen observed by spectroscopic analysis [27].
Nevertheless, as we had controlled all factors, that could affect the examination of SMH values, like sample preparation it was found that the difference of SMH values between control and all test groups at baseline and after riboflavin treatment was significantly different ($p > 0.05$) [19].

To improve the long-term strength of resin-dentin interfaces, strategies to reduce protease inhibitors activity on dentin, as collagen cross-linking agents, such as riboflavin, glutaraldehyde, proanthocyanidin, grape-seed extract, tannic acid, carbodiimide, have been suggested [28].

Recent studies suggest that the organic matrix degradation process starts with revealing of cleavage sites of collagen fibers and also have show that the proteolytic enzymes are inactivated when pH decreases [29,30]. Dentin matrices comprise proteolytic enzymes, the most well-known group being matrix metalloproteinases (MMPs) that were inactive in mineralized dentin [31]. Cross linking is nonspecific for the inactivation of proteolytic enzymes [26].

Moreover, in this study we suggested that the dental visible blue curing light could be regarded to be a efficient and safer substitute compared to the UVA activated RF [18].

4. CONCLUSION

In conclusion, within the limitations of the experiments performed, photoactivated-RF treatment increased the microhardness of demineralized dentin, possibly because of increased crosslinking of the dentin collagen matrix.

5. MATERIALS AND METHODS

5.1 Specimen preparation

The extracted human primary molars were selected for this study following approval by the Research Ethics Committee at the Dental School, University of Marmara (2018-235).

All experiments in this study involved caries free primary molars without any previous restorations that were extracted for surgical reasons. The specimens were kept in 0.1% thymol at 4°C prior to the experiment to prevent any microbial growth and were used within one month of extraction.

Each tooth was embedded in epoxy resin and the occlusal dentin surface was exposed (Struers® EpoFix Kit) for microhardness measurements. Dentine blocks (5x4x5 mm) were prepared from coronal dentine by sectioning with a slow speed precision blade saw (Discoplan-TS, Struers TS-Method™) with a diamond wafering blade. The blocks were placed in a polisher (Struers –Rotapol 35, 50-500 rpm, Copenhagen, Denmark), followed by ultrasonically cleaning in deionized water for 15 min to remove the residues. Each dentine sample was partitioned into 3 parts using nail varnish (Nail enamel, Flormar, Turkey).

5.2 Surface treatment and remineralization agents

From a total of 40 specimens of primary teeth randomly divided into four groups, Group I: photoactivated 0.1% RF treatment for 30 s 400 nm, Group II: photoactivated 0.1% RF treatment for 60 s 400 nm, Group III: positive control (remineralization solution), Group IV: negative control (deionized water). The demineralizing solution consisted of 1.5 mM of CaCl2, 0.9 mM of KH2PO4, 50 mM of acetic acid and 0.02% of NaN3 with the pH value adjusted at 4.5 using NaOH) at 37°C for 3 days. Following this, the samples were rinsed thoroughly with deionized water. The remineralizing solution consisted of 1.5 mM CaCl2, 0.9 mM NaH2PO4, 0.15 M KCl and adjusted to a pH of 7.0 with 1 M KOH. The demineralizing and remineralizing solutions were freshly prepared for each process and kept separately in the containers for each group throughout the study [19,20]. The other experimental groups were treated with the 0.1% Riboflavin solution irradiated by 400 nm photoactivation (D-Light® Pro-GC Europe).

5.3 Surface Microhardness (SMH) analysis

Surface Microhardness analysis (SMH) was measured at the baseline, after the demineralization and after remineralization for each group using a Vickers microhardness tester (EmcoTest, Duravision 20 G5, Vickers, DV250539, Mechanical Instrument Hardness Tester, Germany). Vickers microhardness (VHN) of the dentin was measured in a moist state immediately after removing from distilled water. The specimens were placed under the Vicker indenter of a microhardness tester and subjected to a load of 50 g and dwell time of 10 s at each test point. Three measurements were made for each sample and the mean value was established as the VHN and the mean of 3 measurements was calculated for each sample. The percentage recovery of the surface microhardness (%SMHR) was then calculated.

SMHR- Surface MicroHardness Recovery (S= initial microhardness value, S1= after demineralization, S2= after treatment)
5.4 Statistical analysis

The one-way analysis of variance (ANOVA) was used to compare the SMH values at baseline, before, and after pH-cycling. Data were analyzed with SPSS statistical software (SPSS 22 package program). Shapiro-Wilk was used to evaluate normality. It was determined that the groups had a normal distribution (p<0.05). The data analysis was set at 0.05 for significant level in all tests. Tukey’s multiple comparison tests were used to test the difference of mean SMH and percentage recovery of SMH among groups. In the subsequent stages of analyses Dunn test and Kruskal-Wallis test were used.

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**Conflict of interest statement:** The authors declared no conflict of interest.

**REFERENCES**


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