

EVALUATION OF THE EFFICACY OF REGENAMEL® IN THE TREATMENT OF WHITE SPOT LESIONS (AN IN VITRO STUDY)

Karim Hany¹, Nagwa M. Enany², Ahmed R. Elkalza³

ABSTRACT:

Objective: The aim of this study was to evaluate the efficacy of REGENAMEL® on white spot lesions (WSLs) in terms of the extent of color change and the degree of mineralization. **Materials and methods:** Artificial white spot lesions were induced in 40 extracted human premolars, divided into a test and a control groups. REGENAMEL® was applied to the test group according to manufacturer's instructions, while the control group received no treatment. Both groups were incubated in remineralizing solution for 90 days at 37 °C. Specimens were evaluated using VITA Easyshade advance spectrophotometer at baseline (E0), after WSLs induction (E1) and 90 days following treatment (E2). DIAGNOdent evaluation was done After WSLs induction (D1) and at the end of incubation period (D2). **Results:** The Data was collected and statistical analyses were done using Statistical Package for Social Sciences (SPSS). No significant difference between groups was found after formation of WSLs AE (T0 - T1). Meanwhile significant difference was present between the two groups when comparing the color change after 90 days (T2-T1). AE values were 8.2 ± 4.81 and 1.92 ± 1.21 for the test and control groups respectively. Despite

the final outcome showed marked improvement when compared to the baseline readings (T2-T0) 4.94 ± 3.66 , the final color of the lesions was still not matching the baseline color. For the DIAGNOdent readings, at T1 the mean value for the test group was 15.55 ± 3.81 while that of the control group was 14.95 ± 2.16 . At T2, for the test group, all specimens showed significantly decreased fluorescence values towards the values of sound dental tissues with mean value of 9.9 ± 1.94 . The fluorescence values for the control group at T2 showed minimal change with mean value of 13.65 ± 2.03 . **Conclusion:** REGENAMEL® is effective in improving the color of WSLs and remineralization of demineralized enamel surface.

Keywords: Orthodontics, White spot lesions, REGENAMEL®, Spectrophotometer, DIAGNOdent.

INTRODUCTION

Enamel is the hardest tissue in the human body comprising 96% inorganic minerals, with water and organic material composing the rest 4%. Enamel is completely formed before tooth eruption and unfortunately has a very limited capacity to repair itself. (Staines ,1981)

Enamel normally undergoes continuous cycling between demineralization and

- 1- Researcher, Department of Orthodontics, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.
- 2- Professor, Department of Orthodontics, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.
- 3- Assistant Professor, Department of Orthodontics, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.

®: Regenamel, Dr. Wild & Co. AG, Muttenz, Switzerland

remineralization according to the pH within the oral cavity. Both processes are in equilibrium in order to maintain its integrity. However, whenever the pH falls to certain limit demineralization process predominates leading to decalcification which if not intercepted at early stage will proceed to incipient caries. (González-Cabezas, 2010)

Bishara defined White spot lesions (WSLs) as the subsurface porosity of demineralized enamel that manifests itself as milky white opacities localized on smooth surfaces. (Bishara SE, 2008)

Enamel demineralization in the form of WSLs is among the most common discouraging disheartening sequel of orthodontic treatment. Incidence of post orthodontic WSLs in the literature varies from 0-97%.

Management of WSLs requires remineralization, or microinvasive or minimally invasive intervention according to the size and depth of each individual lesion. However the best treatment strategy for WSLs is prevention. (Iopatiene 2016)

Over the past decades, Many preventive and remineralizing procedures have been introduced in modern dentistry that are capable of managing WSLs. fluoride have been the gold standard for remineralization of WSLs for years, Despite the efficacy of different fluoride preparations has been thoroughly investigated, A Cochrane systematic review showed moderate evidence for different fluoride formulations in caries prevention, while being less effective in deep already manifested lesions. (Marinho VC, 2013).

Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) was found in some studies to penetrate through the

demineralized enamel surface and absorb the bioavailable calcium and phosphate ions in the saliva, thus arresting the demineralization process and promoting remineralization. (Reynolds, 2009)

However in the systematic review performed by Chen et al (2013), it was concluded that current clinical evidence is insufficient to support the effectiveness of either fluoride or CPP-ACP for the treatment of post-orthodontic WSLs.

Over the past two decades self-assembling peptides have emerged as potential candidates for the development of safe nanostructured scaffolds in the field of tissue engineering for enamel regeneration.

Scientists from the University of Leeds have developed a new technology "CUROLOX®" for the regeneration of enamel using the self-assembling peptide (SAP) P11-4, commercially known as REGENAMEL®. The peptide possesses a high affinity to bind with the hydroxyapatite of the tooth enamel, capture Calcium and Phosphorous from the saliva and limit their loss from the demineralized enamel. (Kirkham A, 2007)

A recent study by Schlee, Schad, Koch, Cattin, and Rathe (2017) also proved that when P11-4 is applied to the tooth the peptide diffuses into the subsurface micropores and forms a 3D scaffold which is made up of small fibers these scaffold mimics proteins found in teeth development and supports hydroxyl apatite crystallization around it to regenerate tooth enamel over a period of three months.

Managing post-orthodontic WSLs includes two components; a structural aspect and an aesthetic one. The former has been thoroughly investigated in many studies, whereas the latter hasn't been into studies up to current date. This study focuses on the

aesthetic effect of the remineralizing agent REGENAMEL® evaluating the color change of the WSLs treated with REGENAMEL® and those that received no treatment compared to the baseline colorimetric readings.

Dozić et al (2007) evaluated the accuracy of five of the commercially available tooth color-measuring devices both in laboratory conditions and clinically. The study showed that the Vita Easyshade spectrophotometer was the most reliable instrument in both in vitro and in vivo circumstances.

MATERIALS AND METHODS

Selection of the teeth

First, permission to conduct the study was obtained from the ethics committee - Faculty of Dentistry- Alexandria University.

Sample size:

Sample size calculations were done using computer software*. A sample size of 20 teeth per group (number of groups = 2) (total sample size = 40) (Killeen, 2005) is the enough required sample to detect a standardized effect size of 0.891 (minimum difference in the color $\Delta E = 3.7 \pm 3.5$) (Johnston, 1989) of the primary outcome, as statistically significant with 90% power and at a significance level of 95%.

Accordingly, forty extracted human premolars were collected from patients seeking orthodontic treatment at age ranging between 13 to 18 years from the Orthodontic department – Faculty of Dentistry – Alexandria University.

Inclusion Criteria:

All teeth were examined macroscopically using magnifying loupes to match the following criteria:

1. Sound teeth with intact buccal surface with no visible cracks or hypoplastic area.
2. No pretreatment with chemical agents.

Sample preparation

1) At the beginning of the study, all teeth roots were cut 2 mm apical to the cemento-enamel junction with a diamond disk.

2) In order to facilitate examination of the buccal surface, the lingual surfaces of the teeth were embedded in self-cured acrylic resin held by a standardized ready-made metal mold so that the buccal surface is aligned parallel with the base of the mold.

3) Each specimen was given a number with a permanent marker.

4) 3-mm x 3-mm patches of adhesive tape were prepared and attached to the buccal surface of teeth to identify the area to be tested.

5) The whole buccal surface was covered with a black acid-resistant nail varnish except for the window area covered by the adhesive tape.

6) All teeth were polished with rubber prophylaxis cup with a low-speed hand piece with non-fluoridated oil-free pumice and then rinsed with running water.

Grouping

Teeth were randomly allocated into 2 groups; 20 teeth each.

Group 1: with REGENAMEL® treatment, group 2: without REGENAMEL® treatment.

Spectrophotometric evaluation:

Baseline colorimetric readings were recorded at (E0) before any intervention using

digital spectrophotometer (VITA EasyshadeAdvance ,Vident, Brea, Calif).

Sample demineralization

Artificial WSLs were created in the enamel by immersing the specimens in a demineralizing solution(Montasser, 2015) at pH 4.4 and at 37°C for 21 days.During decalcification, pH was monitored daily and was adjusted with 10% "HCl" or 10 M "KOH" when necessary.

Pre-treatment measurements:

After induction of WSLs, pretreatment colorimetric readings (E1) and DIAGNOdent readings (D1) were assessed for all teeth of both groups.

REGENAMEL® application:

REGENAMEL® was applied according to the manufacturer's instructions:

- Samples were washed with distilled water.
- Areas to be tested were further cleaned by 2% sodium hypochlorite for (20 sec).

- Samples were etched using 35% phosphoric acid (20 sec).
- Samples were rinsed thoroughly with water and dried.
- REGENAMEL® applicator was activated by pushing the two cylinders together.
- REGENAMEL® application tip was gently pressed onto the tooth surface.
- REGENAMEL® was left on the tooth surface for 5 minutes till the surface appears dry.

Remineralization process

Both groups were kept in remineralizing solution with pH value maintained at 7.0 (Ionta, 2014) and the solution was replaced daily. All samples were stored in an incubator at 37 °C for 90 days (Bröseler, 2013).

Post-treatment measurements:

After incubation period, post-treatment colorimetric (E2) and DIAGNO-dent (D2) measurements were assessed for all teeth of both groups .

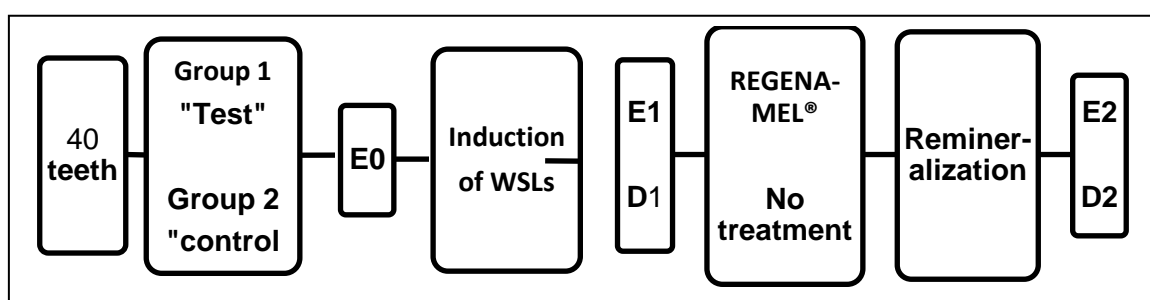


Fig1: Flow chart showing summarized procedures

RESULTS

Data were fed to the computer and analyzed using IBM SPSS software package. The color difference (ΔE^*) after completion of WSLs formation (T0 - T1) did not differ significantly between groups ($p=0.565$, Table 1). The mean color difference for the REGENAMEL® group was 11.34 ± 6.80 , while the corresponding value for control group was 10.19 ± 4.74

After the treatment of WSLs (T1 - T2) there were significant color differences between treatment group and control group. The REGENAMEL® group showed regression of all the values toward pre-treatment levels, with significant decrease in L^* , increase in a^* value and increase in b^* value after 90 days of REGENAMEL® application, while the control group showed slight changes. The ΔE^* for the REGENAMEL® group from T1- T2

was 8.20 ± 4.81 , while the ΔE^* for the control group was 2.13 ± 1.21 ($p<0.001^*$, Table 2). Significant color differences were also found between baseline and after treatment (T0- T2). The REGENAMEL® group had a ΔE^* of 4.94 ± 3.66 , while the control group had a ΔE^* of 10.30 ± 5.16 ($p<0.001^*$, Table 3).

Values at T1 for both groups after the demineralization process conforms with the criteria specified by the DIAGNOdent pen manufacturer (Staudt CB, 2004); The mean value for the test group was 15.55 ± 3.81 while that of the control group was 14.95 ± 2.16 . At T2, for the test group, all specimens showed significantly decreased fluorescence values towards the values of sound dental tissues with mean value of 9.9 ± 1.94 . The fluorescence values for the control group at T2 showed minimal change with mean value of 13.65 ± 2.03 . ($p<0.05$, Table 4).

Table 1: Comparison of color change (ΔE) from T0 to T1

ΔE 1-0	Test (n = 20)	Control (n = 20)	U	p
Min. – Max.	1.96 – 20.96	5.19 – 22.11		
Mean \pm SD.	11.34 ± 6.80	10.19 ± 4.74	178.50	0.565
Median	9.69	8.70		

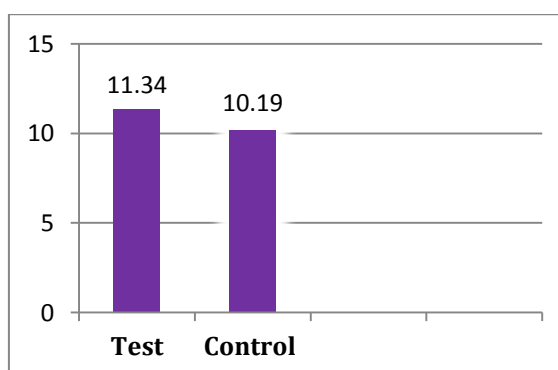


Fig. 2: Bar chart representing Changes in ΔE value between groups at T0 & T1.

Table 2: Comparison of color change (ΔE) from T1 to T2

ΔE T2-T1	Test (n = 20)	Control (n = 20)	U	p
Min. – Max.	2.75 – 18.37	0.81 – 4.61		
Mean \pm SD.	8.20 \pm 4.81	2.13 \pm 1.21	20.0*	<0.001*
Median	8.33	2.01		

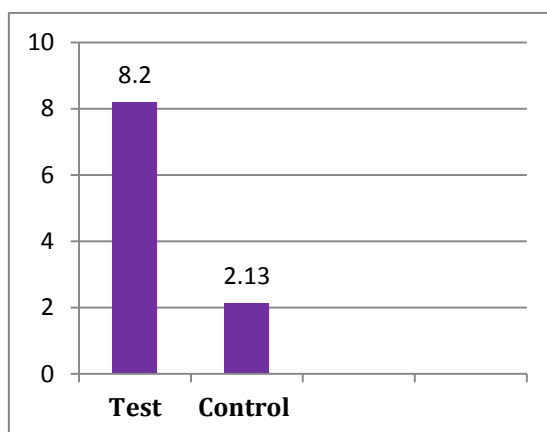


Fig. 3: Bar chart representing Changes in ΔE value between groups at T1 & T2.

Table 3: Comparison of color change (ΔE) from T0 to T2

ΔE 2-0	Test (n = 20)	Control (n = 20)	U	P
Min. – Max.	1.19 – 15.50	4.50 – 25.85		
Mean \pm SD.	4.94 \pm 3.66	10.30 \pm 5.16	71.0*	<0.001*
Median	4.67	10.09		

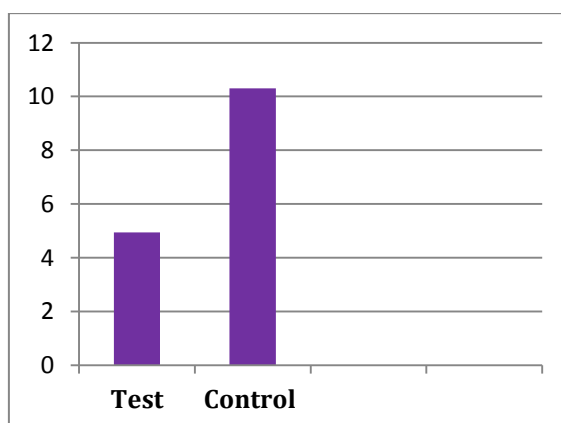


Fig. 4: Bar chart representing Changes in ΔE value between groups at T0 & T2.

Table 4: Comparison of laser fluorescence between T1 to T2 among the study groups.

		Test (n=20)	Control (n=20)	t ₁	p ₁
T1	Min. – Max.	11.0 – 23.0	11.0 – 21.0	0.613	0.544
	Mean ± SD.	15.55 ± 3.81	13.65 ± 2.16		
	Median	16.0	14.50		
T2	Min. – Max.	8.0 – 15.0	10.0 – 18.0	4.007*	<0.001*
	Mean ± SD.	9.90 ± 1.94	13.65 ± 2.03		
	Median	10.0	14.0		
t ₂ (p ₂)		8.692* (<0.001*)	2.024 (0.057)		

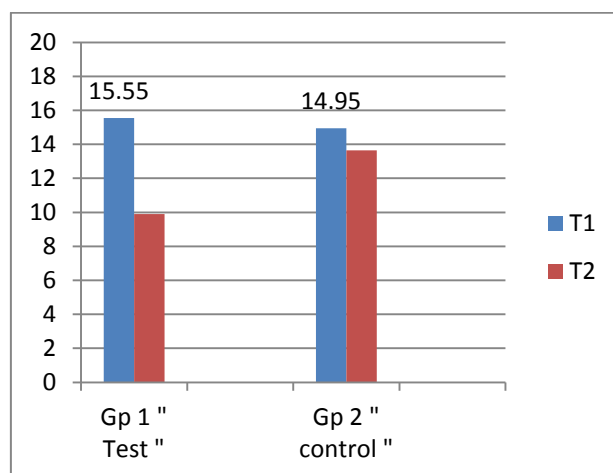


Fig.5: Bar chart representing changes in laser fluorescence between groups at T1 & T2.

DISCUSSION

The refractive index (RI) of sound enamel is 1.62, while that of porous demineralized enamel is 1.00 when dry and 1.33 when wet. This difference in RI between enamel crystals and the medium within the WSLs alters the light scattering pattern causing the demineralized areas to appear whiter than sound enamel (Khoroushi M, 2017).

The clinically acceptable color difference in this study was set for $\Delta E = 3.7$ (Johnston, 1989). When the WSLs were treated with P11-4 and incubated for 90 days the average ΔE was 8.2 indicating good color improvement. However, ΔE between the post treatment results and the baseline color was 4.94 which were still slightly clinically detectable. Unfortunately despite the good color improvement complete recovery to initial tooth color was not completely recovered.

These results confirm what was concluded by Brunton et al. (2013) where they tested the efficacy of P11-4 on early enamel lesions using Visual Analogue Scale (VAS). There was highly significant improvement in the scores after 30 days which was maintained after 180 days when compared to baseline scores regarding color and shape of the lesions.

A recent study was done in Al-Azhar University in 2018 to evaluate the remineralization potential of different agents. Three months following treatment of the lesions with P11-4, the ICDAS II scores showed significant improvement. The predominant score before treatment was 66.6% for score 3 and 33.3% for score 2. The post treatment scores were 53.3%, 33.3% and 13.3% for scores 2, 1 and 0 respectively. (Kamh, 2018)

CONCLUSION

Based on the results of the current study, it can be concluded that the self-assembling peptide P11-4 "REGENAMEL®" shows promising efficiency to improve the color of WSLs and promote remineralization of decalcified enamel surfaces.

RECOMMENDATIONS

According to the results obtained from this study, we recommend:

1- Long-term clinical studies are needed to confirm the results and identify the effect of time and aging factors on the obtained positive results.

2- Further studies are needed for thorough evaluation of the effect of different salivary parameters on the treatment outcome.

REFERENCES

- Brunton, P. A., Davies, R. P. W., Burke, J. L., Smith, A., Aggeli, A., Brookes, S. J., & Kirkham, J. (2013). Treatment of early caries lesions using biomimetic self-assembling peptides – a clinical safety trial. *British Dental Journal*, 215(4). doi: 10.1038/sj.bdj.2013.741
- Bishara, S. E., Ostby, A.W. (2008). White spot lesions: Formation, prevention, and treatment. *Seminar of Orthodontics*, 14(3), 174- 182.
- Chen, H., Liu, X., Dai, J., Jiang, Z., Guo, T., & Ding, Y. (2013). Effect of remineralizing agents on white spot lesions after orthodontic treatment: A systematic review. *American Journal of Orthodontics and Dentofacial Orthopedics*, 143(3). doi: 10.1016/j.ajodo.2012.10.013
- Dozić, A., Kleverlaan, C. J., El-Zohairy, A., Feilzer, A. J., & Khashayar, G. (2007). Performance of Five Commercially Available Tooth Color-Measuring

Devices. *Journal of Prosthodontics*, 16(2), 93–100. doi: 10.1111/j.1532-849x.2007.00163.x

▪ González-Cabezas, C. (2010). The Chemistry of Caries: Remineralization and Demineralization Events with Direct Clinical Relevance. *Dental Clinics of North America*, 54(3), 469–478. doi: 10.1016/j.cden.2010.03.004

▪ Ionta, F. Q., Mendonça, F. L., Oliveira, G. C. D., Alencar, C. R. B. D., Honório, H. M., Magalhães, A. C., & Rios, D. (2014). In vitro assessment of artificial saliva formulations on initial enamel erosion remineralization. *Journal of Dentistry*, 42(2), 175–179. doi: 10.1016/j.jdent.2013.11.009

▪ Johnston, W., & Kao, E. (1989). Assessment of Appearance Match by Visual Observation and Clinical Colorimetry. *Journal of Dental Research*, 68(5), 819–822. doi: 10.1177/00220345890680051301

▪ Khoroushi, M. & Kachuie, M. (2017). Prevention and treatment of white spot lesions in orthodontic patients. *Contemporary Clinical Dentistry*, 8(1), 11. doi: 10.4103/ccd.ccd_216_17

▪ Kamh, R. A., Niaz, M. A., & El-Yasaky, M. A. (2018). Clinical performance and remineralization potential of different biomimetic materials. *ADJD*, 5(4), 349–358.

▪ Kirkham, J., Firth, A., Vernals, D., Boden, N., Robinson, C., Shore, R., ... Aggeli, A. (2007). Self-assembling Peptide Scaffolds Promote Enamel Remineralization. *Journal of Dental Research*, 86(5), 426–430. doi: 10.1177/154405910708600507

▪ Killeen, P. R. (2005). An Alternative to Null-Hypothesis Significance Tests. *Psychological Science*, 16(5), 345–353. doi: 10.1111/j.0956-7976.2005.01538.x

▪ Lopatiene, K., Borisovaite, M., & Lapenaite, E. (2016). Prevention and Treatment of White Spot Lesions During and After Treatment with Fixed Orthodontic Appliances: a Systematic Literature Review. *Journal of Oral and Maxillofacial Research*, 7(2). doi: 10.5037/jomr.2016.7201

▪ Marinho, V. C., Worthington, H. V., Walsh, T., & Clarkson, J. E. (2013). Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews*. doi: 10.1002/14651858.cd002279.pub2

▪ Reynolds, E. (2009). Casein Phosphopeptide-Amorphous Calcium Phosphate: The Scientific Evidence. *Advances in Dental Research*, 21(1), 25–29. doi: 10.1177/0895937409335619

▪ Schlee, M., Schad, T., Koch, J. H., Cattin, P. C., & Rathe, F. (2017). Clinical performance of self-assembling peptide P11-4 in the treatment of initial proximal carious lesions: A practice-based case series. *Journal of Investigative and Clinical Dentistry*, 9(1). doi: 10.1111/jicd.12286

Staines, M., Robinson, W. H., & Hood, J. A. A. (1981). Spherical indentation of tooth enamel. *Journal of Materials Science*, 16(9), 2551–2556. doi: 10.1007/bf01113595

