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Original article

HISTOLOGICAL ANALYSIS OF PULP AFTER CHEMICAL AND LASER TEETH WHITENING

Stefanovic Dushanka[1], Shukov Atanas[2], Filipovska Micevska Vesna[1], Filipovski Vladimir [3,4]

[1]Department of Restorative Dentistry and Endodontics, University Dental Clinical Center "St. Pantelejmon", Skopje, Republic of North Macedonia
[2]Department of Removable Prostodontics, University Dental Clinical Center "St. Pantelejmon", Skopje, Republic of North Macedonia
[3]Department of Oral Surgery, University Dental Clinical Center "St. Pantelejmon", Skopje, Republic of North Macedonia Skopje
[4] Faculty of Dentistry, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia Skopje
e-mail: dusanka_s@yahoo.com

Abstract

Tooth discolorations are multifactorial dental diseases caused by external and internal factors.

Whitening/bleaching of vital teeth is carried out by different methods, thus the strength of the bleaching agent, hydrogen peroxide or carbamide peroxide, may range from 10%-40%. The basic reaction of bleaching is the chemical process of oxidation, which leads to penetration of oxygen molecules through the enamel and dentin. This reaction also raises the question of what happens to the dental pulp as the most important tissue in the teeth. Therefore, we performed histological analysis of the pulp, after applying 35% hydrogen peroxide for chemical and laser teeth whitening at different time intervals. The changes in the pulp were more pronounced with laser bleaching especially within 30 seconds of laser whitening. Among the alterations of the pulp tissue, dilatation of blood vessels was observed, as well as disruption in the sequence of the odontoblastic layer.

Key words: vital teeth whitening, oxidation, laser

Introduction

The American Dental Association (ADA) divides whitening agents into two large groups: whitening agents for vital teeth and whitening agents for non-vital endodontically treated teeth. Among these, the tetracycline teeth are the most complex one for whitening. Many authors in their studies have indicated the strong relationship between tetracycline and calcium in the hydroxy apatite in enamel and dentin. Consequently, great persistence is required by both, the patient and the dentist. Penetration of bleaching agents through enamel and dentin raises the question of what happens to the pulp tissue.

Similar type of analysis was also performed by Cintra LTA e*t al.*[1], showing the changes in the pulp that had led to hemorrhage. Additionally, Marin PD e*t al*.[2] observed hemolysis and hemorrhage in a similar experiences. Costa CA[3] detected an irreversible pulpal reaction after performed bleaching. On the contrary, Soares DG e*t al.*[4,] presented a reduced

pulp reaction after whitening. Later in 2015, the same author stated that after whitening, odontoblasts quickly restore the structure of the pulp. By examinating the pulp cells and the action of 37% hydrogen peroxide, Soares DG e*t al*,also showed a reduction of the odontoblasts and their differentiation in a period of 7 days after which the pulp returned to normal [5]. Contrary to this, Wu TT *e*t *al*., [6] believe that a reduced concentration of hydrogen peroxide is cytotoxic and causes dental pulp cells apoptosis.

We, therefore, made a histological analysis to accurately see the state of the pulp after using a bleaching agent and chemical or laser whitening.

The aim of the study was to see histological and cellular changes in the dental pulp during the chemical and laser bleaching of the tooth.

Material and methods

All teeth used for this study were extracted for orthodontic reasons, were supernumerary teeth, or permanent molars with indication for extraction as well as teeth required for extraction for prosthetic reasons.

Three (3) experimental groups of teeth were analyzed.

The first group of teeth, without any treatment, was the control group.

The teeth that were bleached chemically for 15 and 30 minutes, represented the second experimental group, while the third group of teeth consisted of teeth treated with bleaching agent and laser energy, exposed for a period of time of 15 and 30 seconds.

As a laser, the Fotona Fidelis 3 laser was used with a strength of 6 Hz and 8W in duration of 15 and 30 seconds.

Each of these teeth, except the control group, were treated with Opalescence Boost whitening agent, containing hydrogen peroxyde.

After the performed bleaching, the teeth were well cleaned and trepanned, preserving the coronal pulp in the crown of the tooth and apically, they were cut to the physiological apical foramen.

Histological analyses of possible changes in the pulp tissue were made on formalin fixed paraffin embedded tissue (FFPE).

The samples prepared in this way were placed in buffered 10% formalin, which would penetrate inside the pulp and harden (fix) it, so that we could remove the pulp more easily from the tooth. Formalin is the most widely used fixative in histological techniques because it has such an ability to preserve the shape and structure of the examined material, and at the same time ensures its good staining. The pulp had to remain in formalin for up to 72 hours.

After this, the teeth were well dried and ready for careful removal of the pulp tissue. Each specimen was carefully cut so as not to damage the pulp, and using a sharp excavator and a plastic instrument the pulp was removed from the tooth (Figure1) and placed back in formalin (Figure 2).

Prior to histological analysis of the dental pulp samples, was performed application of the paraffin technique - molding in paraffin, than cutting the samples with a thickness of 4 um, placing the samples on glass slides, deparaffinization and hydration.

Hematoxylin-eosin and Masson trichrome histological staining methods were used for all groups of teeth.

Fig. 1. Fixation of the pulp in formalin after chemical and laser bleaching at 15 and 30 min.

Fig. 2. Specimen with the extracted pulp

Results

Dental pulp - control group

The results of histological analysis of tooth preparations obtained from the dental pulp of the control group are as follows:

Pulp represented by dense connective tissue was observed in the root canal of the tooth, that is, the presence of bundles of parallel laid collagen fibers with a moderate presence of cellular elements. Fibroblasts and fibrocytes prevailed the cellular elements. In the central parts of the pulp of the root canal, axially placed larger blood vessels were observed.

The layer of densely laid odontoblasts was visible on the surface of the pulp, while immediately below it was the acellular (Weil) zone (Figure 3).

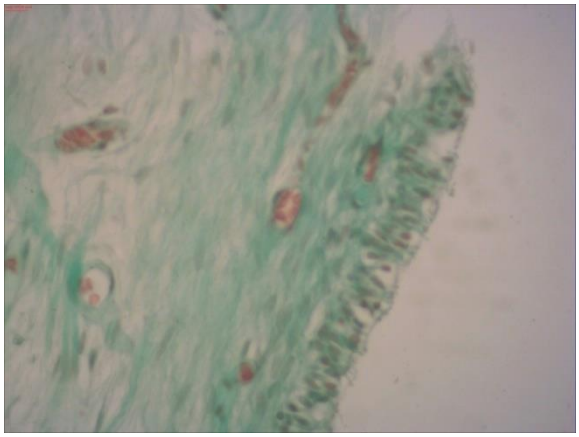

Fig. 3. Control group: dental pulp and odontoblasts layer, Masson,10 x 40

Dental pulp – chemical teeth bleaching group (for 15 and 30 minutes)

The morphological characteristics of the structural components of the dental pulp obtained after the chemical treatment of the teeth for 15 and 30 minutes were very similar, which is why the qualitative histological analysis for these two groups will be presented together.

Histological analysis of dental pulp in chemically treated teeth showed moderate changes in the ratio of representation of collagen fibers and amorphous matter from the intercellular matrix in favor of collagen fibers.

This phenomenon of increased collagen fiber density was observed in both root canal pulp and coronary pulp (increased collagen fiber density is commonly associated with accelerated tissue aging) (Figure 4 and 5).

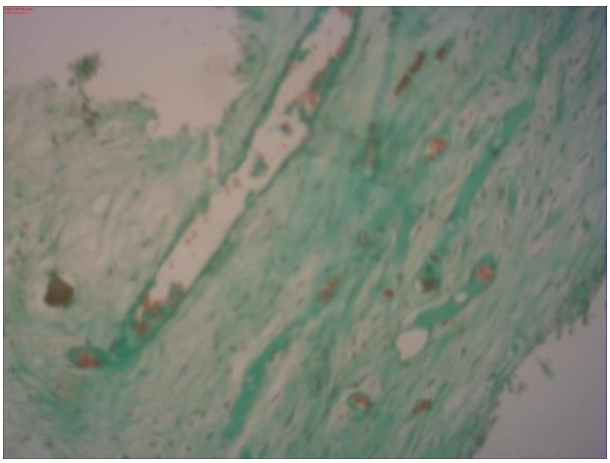

Fig. 4. Chemical teeth bleaching group: structure of the pulp in the root canal. Increased collagen fiber density, Masson 10 x 20

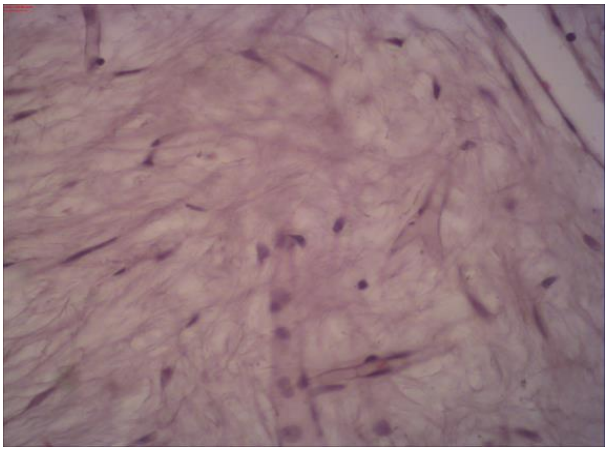

Fig. 5. Chemical teeth bleaching group: structure of the pulp in the coronary part, Hematoxylin-eosin, 10 X 40

The odontoblast layer was almost completely intact as seen in Figure 6.

The vascularization of the pulp both in the root canal and in the coronary part showed no particular changes in relation to the control group, meaning that the representation and structure of the blood vessels fully corresponded to the histological findings in the control group (figure 7).

The morphology of the odontoblasts also showed no significant differences compared to the odontoblasts in the control group.

The only difference is that after the chemical treatment of the tooth, small changes were observed in terms of the appearance of small defects in the continuity of the odontoblastic layer.

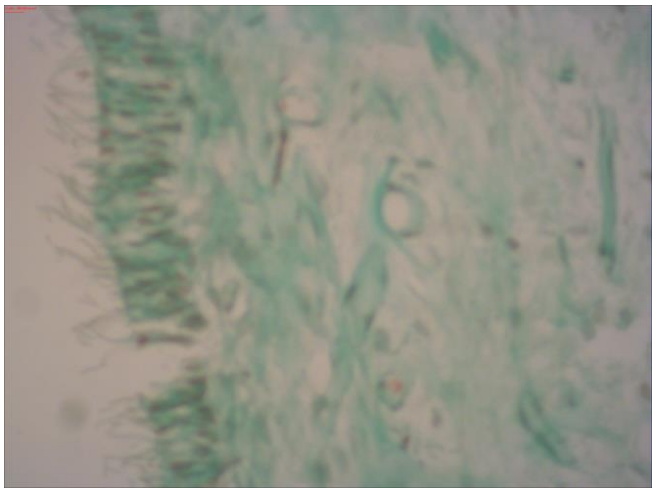

Fig. 6. Chemical teeth bleaching group: **o**dontoblasts layer after chemical whitening of the tooth for 30 minutes, Masson,10 x 40

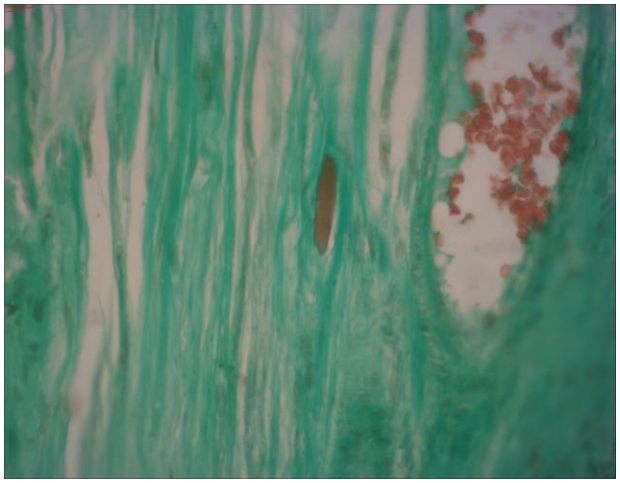

Fig. 7. Chemical teeth bleaching group: after chemical whitening of the tooth for 30 minutes, Masson,10 x 40

Dental pulp – laser teeth whitening group (in duration of 15 seconds)

Histological analysis of the structural components of the connective tissue from the dental pulp after laser teeth whitening for 15 seconds showed an increase in the density of the connective tissue in all parts of the dental pulp. A particular increase in the density of collagen fibers was observed in the pulp of the root canal and a markedly increased density of collagen fibers in the upper half of the coronary pulp (a phenomenon that was not registered in the pulp after chemical teeth whitening).

The vascular network of the dental pulp showed certain deviations from the morphological characteristics of the vascular network of the pulp in the control group. Namely, in the pulp of the root canal, dilatation was noticed of the wall of the larger blood vessels passing through it axially (Figure 8). Also, stressed capillary network in its peripheral zone located under the odontoblastic layer of cells could be noticed (Figure 9).

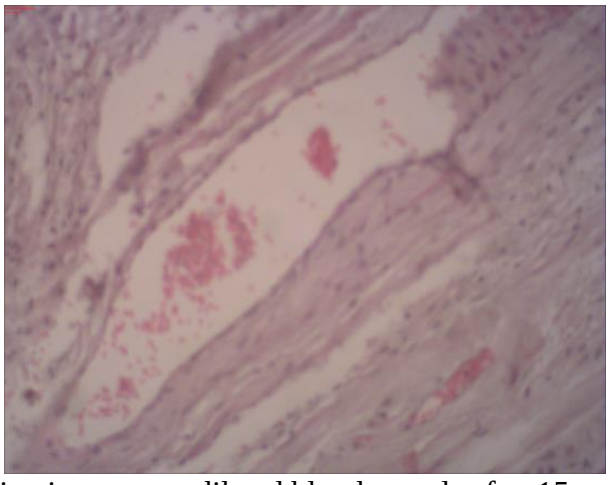

Fig.8. Laser teeth whitening group: dilated blood vessels after 15 seconds laser whitening in the root canal pulp, Hematoxylin-eosin, 10 X 20

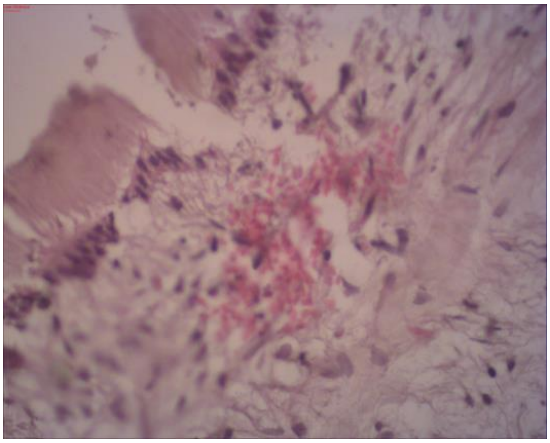

Fig. 9. Laser teeth whitening group: bleeding after 15 seconds of laser whitening. Hematoxylin-eosin, 10 X 40

Extravasation of blood elements could be observed in certain zones of the dental pulp. This phenomenon (as well as dilated capillary blood vessels) is especially often noticed in the superficial layers of the coronary pulp below the layer of odontoblasts.

The presence of blood cell elements could also be observed between the odontoblasts. In certain places, and in the superficial parts of the dental pulp, a break in the continuity of the blood vessel wall and the appearance of bleeding could be clearly observed.

The layer of odontoblasts along the surface did not show marked changes compared to that in the control group. The only changes that could be observed were in the odontoblasts located above the bleeding zones and consisted of a break in the continuity of the odontoblast layer, or appearance of moderate destructive changes in the odontoblasts.

Dental pulp – laser teeth whitening group (in duration of 30 seconds)

Histological analysis of the structural components of the dental pulp after laser teeth whitening for 30 seconds showed pulp tissue changes that were completely identical to the morphological changes of the pulp after laser teeth treatment for 15 seconds. Thus, only those histological changes that were not observed in the previously analyzed group would be mentioned here (Figure 10).

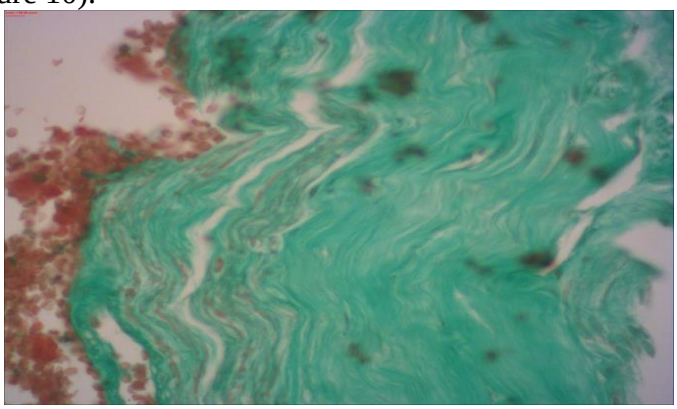

Fig. 10. Laser teeth whitening group: pulp after 30-second laser tooth whitening (structure of the pulp in the root canal), Masson,10 x 40

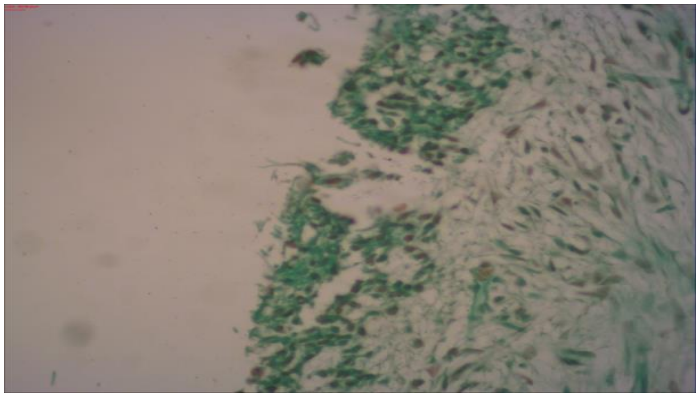

Fig. 11. Laser teeth whitening group: **se**paration of odontoblasts after 30-second laser tooth whitening. Masson,10 x 40

In addition to bleeding in the coronary pulp, after longer laser treatment bleeding in the deeper, axial parts of the root pulp was also observed.

Histological analysis of the odontoblastic layer showed separation of the odontoblasts from each other (with the loss of contacts between their lateral surfaces) or their separation from the basal surface (the underlying layer of the pulp) (Figure 11).

Discussion

According to histological analyses performed on extracted non-carious teeth after chemical or laser whitening, we can expect onset of pulp tissue changes like hyperemia, vasodilatation, changes in the odontoblasts.

The samples obtained from the control group of extracted healthy teeth clearly whitout any bleaching treatment, showed the presence of cellular elements, such as fibroblasts, fibrocytes, large blood vessels, as well as odontoblasts. Below them, the Weil's zone was clearly visible.

Compared to this group, the second group of teeth treated chemically for 15 and 30 minutes showed no difference, except for the density of collagen fibers which was higher in both, the coronal and radix part of the pulp.

The third group treated with bleach and laser for 15 and 30 seconds pointed out a larger difference in their pulp structure, mostly in the teeth treated for 30 seconds. We can say that the differences consisted in the separation of the odontoblasts and their separation from the basal surface, as well as heavy bleeding in the coronal part of the pulp. Dilatation of larger blood vessels, extravasation of blood elements and an increase in the density of collagen fibers were also observed.

Histological results similar to ours were also obtained by Soares DG *et al.* [5], Hanks CT *et al*[7], Cohen SC [8], Costa C *et al*.[9], Baik JW *et al*.[10], White JM[11], Sulieman M.[12], Sulieman M *et al.* [13], Michida CM e*t al*. [14], Nathanson D[15], Fugaro JO *et al.*[16], Gerlach RW and Zhou X[17], Kwon SR *et al*.[18], Llena C *et al.* [19].

These numerous studies are in agreement with our results, although some of them obtained their first results by examining only one part of the pulp – the root or coronary. They, however, arrived to the same results.

Conclusion

A histological analysis of the pulp tissue obtained from teeth treated with bleaching agent and chemical or laser method whitening has shown that changes-alterations including hyperemia, vasodilatation as well as partially disturbed odontoblast's layer were more

pronounced when using the bleaching agent and laser than only bleaching agent for chemical whitening.

Conflict of interest statement. None declared.

References

1. Cintra LTA, Benetti F, da Silva Facundo AC, Ferreira LL, Gomes-Filho JE, Ervolino E, *et al*. The number of bleaching sessions influences pulp tissue damage in rat teeth. J Endod 2013; 39(12): 1576-1580. doi: 10.1016/j.joen.2013.08.007.
2. Marin PD, Bartold PM, Heithersay GS. Tooth discoloration by blood: an in vitro histochemical study. *Endod Dent Traumatol* 1997; 13(3): 132-138. doi: 10.1111/j. 1600-9657.1997.tb00026.x.
3. Costa CA, Riehl H, Kina JF, Sacono NT, Hebling J. Human pulp responses to in-office tooth bleaching. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 109(4): e59-e64. doi: 10.1016/j.tripleo.2009.12.002.
4. Soares DG, Basso FG, Hebling J, de Souza Costa CA. Concentrations of and application protocols for hydrogen peroxide bleaching gels: effects on pulp cell viability and whitening efficacy. *J Dent* 2014; 42(2): 185-198. doi: 10.1016/j. jdent.2013.10.021.
5. Soares DG, Basso FG, Scheffel DS, Hebling J, de Souza Costa CA. Responses of human dental pulp cells after application of a low-concentration bleaching gel to enamel. *Arch Oral Biol* 2015; 60 (9): 1428-36. doi: 10.1016/j.archoralbio.2015.06.014.
6. Wu TT, Li LF, Du R, Jiang L, Zhu YQ. Hydrogen peroxide induces apoptosis in human dental pulp cells via caspase-9 dependent pathway. *J Endod* 2013; 39(9): 1151-1155. doi: 10.1016/j.joen.2013.06.006.
7. Hanks CT, Fat JC, Wataha JC, Corcoran JF. Cytotoxicity and Dentin Permeability of Carbamide Peroxide and Hydrogen Peroxide Vital Bleaching Materials, in vitro. *Journal of Dental Research* 1993; 72(5):931-938. doi: 10.1177/00220345930720051501C.
8. Cohen SC. Human pulpal response to bleaching procedures on vital teeth. *J Endod* 1979; 5(5): 134-138. doi: 10.1016/s0099-2399(79)80033-3.
9. da Costa C *et al* . Dental pulp vascular permeability changes induced by dental bleaching .Braz.A.of B.T.2012. doi.org/10.1590/S1516-89132012000100006
10. Baik JW, Rueggeberg FA, Liewehr FR. Effect of light-enhanced bleaching on in vitro surface and intrapulpal temperature rise. *J Esthet Restor Dent* 2001; 13(6): 370-378. doi: 10.1111/j.1708-8240.2001.tb01022.x.
11. White JM, Surface and pulpal temperature comparison of tooth whitening using laser and curing lights. *Proceedings of SPIE,n°3910,* 2000, doi:10.1117/12.380813
12. Sulieman M. An over view of tooth bleaching tehnigues: chemistry, safety and efficacy. *J.Periodontology* 155 ; 2000. doi.org/10.1111/j.1600-0757.2008.00258.x
13. Sulieman M e*t al*. Surface and pulp chamber temperature rises during tooth bleaching using a diode laser: a study *in vitro*. *British Dental J* 2006; vol.200: 631-634. PMID: 16767142; doi: 10.1038/sj.bdj.4813644
14. Michida SM, Passos SP, Marimoto AR, Garakis MC, de Araújo MA. Intrapulpal temperature variation during bleaching with various activation mechanisms. *J Appl Oral Sci* 2009; 17(5): 436-439. doi: 10.1590/s1678-77572009000500016.
15. Nathanson D. Vital tooth bleaching: sensitivity and pulpal considerations. *J Am Dent Assoc* 1997; 128 Suppl: 41S-44S. doi: 10.14219/jada.archive.1997.0423.
16. Fugaro JO, Nordahl I, Fugaro OJ, Matis BA, Mjör IA. Pulp reaction to vital bleaching. *Oper Dent* 2004; 29(4): 363-368. PMID: 15279473.

17. Gerlach RW, Zhou X. Vital bleaching with whitening strips: summary of clinical research on effectiveness and tolerability. *J Contemp Dent Pract* 2001; 2(3): 1-6. PMID: 12167923 DOI:10.5005/jcdp-2-3-28.
18. Kwon SR, Dawson DV, Wertz PW. Time Course of Potassium Nitrate Penetration into the Pulp Cavity and the Effect of Penetration Levels on Tooth Whitening Efficacy. *J Esthet Restor Dent* 2016; 28 Suppl 1: S14-S22. doi: 10.1111/jerd.12192.
19. Llena C, Collado-Gonzalez M, Garcia-Bernal D, Onate Sanchez RE, Martinez CM, Moraleda JM, *et al.* Comparison of diffusion, cytotoxicy and tissue inflammatory reactions of four commercial bleaching products against human dental pulp stem cells. *Sci Rep* 2019; 9(1): 7743. doi: 10.1038/s41598-019-44223-1.