Tissular response to polyuretan sponge implants coated with a commercial dental adhesive polymerized with two different light sources

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MONA IONAȘ 1, TIBERIU IONAȘ 2, ADA DELEAN 3, SEPTIMIU TOADER 4
1 Departament of Dentistry, Faculty of Medicine, University 'Lucian Blaga', Sibiu, Sibiu, Romania
2 Privat practice, Sibiu, Sibiu, Romania
3 Faculty of Dentistry, University of Medicine and Pharmacy 'Iuliu Hatieganul', Cluj-Napoca, Cluj, Romania
4 Faculty of Medicine, University of Medicine and Pharmacy 'Iuliu Hatieganul', Cluj-Napoca, Cluj, Romania

Corresponding author: Mona Ionas, home address: str. Rahova nr. 14 ap. 11, CP 550340 Sibiu, Sibiu, Romania, phone/fax 0040(0)269210524, mobile 0040(0)730210525, e-mail monaionas@yahoo.com.

Abstract

This study tries to determine whether the polymerization source, a halogen lamp or a LED lamp, has any influence on the biocompatibility of the dental adhesive Single Bond 2 (3M ESPE). The treated material was subcutaneously implanted in adult Wistar rats. Local effects were macro- and microscopically evaluated over a period of three weeks. The study was performed following the ISO 10993 standards, part 6, observing the local effects after implantation.

Conclusions

There was no difference in the biocompatibility of the dental adhesives, regardless of the curing lamp used. The degree of conversion of monomers of the dental adhesive layer depends very much on the parameters of the curing light but also on the physical-chemical characteristics of the adhesives.

Keywords: dental adhesives, curing light, LED, halogen, subcutaneous implant, biocompatibility.

Introduction

The cytotoxicity of dental adhesives is caused by monomers that are released during the application of the adhesive and subsequently during the light curing. (1, 2, 3, 4)

The degree of conversion of the monomers during the photopolymerization depends on several factors, such as the power density of the photopolymerization light, the time of exposure, the emission spectrum of the lamp, the distance to the adhesive layer, the contamination with water/dentinary fluid, etc. (5, 6, 7, 8, 9, 10).

A cell culture study indicates that there are clear differences between the cytotoxic effects generated by a dental adhesive during the curing with lamps of different light sources (11).

This study tries to determine whether the polymerization source, a halogen lamp or a LED lamp, has any influence on the biocompatibility of a dental adhesive.

Material and Method

The biologic analysis of the dental products was performed in compliance with the ISO 10993 norms, part 6, and evaluated the local effects of the implant over the general ones. All procedures involving animals were approved by the Ethic Committee of the University of Medicine and Pharmacy ‘Iuliu Hatieganu’, Cluj-Napoca.

The products were tested in subcutaneous implants in male and female adult Wistar rats.
The goal of the experiment was to analyze the reaction of the subcutaneous connective tissue to the sponge fragments impregnated with dental adhesives over a period of three weeks after inoculation. Throughout the entire period of the experiment, the following aspects were observed:

- How the subjects (Wistar rats) withstood the operation;
- The local and general reaction of the implants;
- The clinical state of the subjects;
- At the end of the experiment, the local reaction of the implant, the relationship with the host tissue, and the histological examination were evaluated.

As dental adhesive came in liquid form, it was impregnated on an absorbent polyurethane sponge material of the size 5 x 5 x 2 mm in order to be easily subcutaneously inoculated. Before inoculation, the sponge fragments were sterilized through autoclaves and impregnated with the implant product.

The experimental protocol was as follows:

An overall of three groups was formed with five experiments per group:

- group I –control group to which a small piece of sponge impregnated in saline solution was subcutaneously implanted;
- group II –were inoculated with a sponge impregnated with Single Bond 2 (3M ESPE), polymerized with the LED light Elipar Freelight 2, (3M ESPE), 1000 mW/cm² for 5 sec;
- group III –inoculated subcutaneously with a sponge impregnated with Single Bond 2 (3M ESPE), light cured with the halogen light Elipar 2500 (3M ESPE), 600 mW/cm² for 10 sec;

At the end of the experiment (after 21 days) the subjects were euthanized and the local reaction was studied; fragments of the peri-implant tissues were taken and processed through the classic histological technique, the sections were marked out through the hematoxylin-eozin staining technique and morpho-pathologically examined at a magnification of 200 x.

The groups were made up of adult male and female rats, with a body weight of 180-220 g, male and female, healthy, nourished and bred according to the standards for research animals.

After the homogenization and adaptation of the groups, the products, impregnated into sponge fragments, were inoculated by using the methods mentioned above.

The surgical act was performed according to the following protocol:

- Each animal was anesthetized and the selected implant region was mechanically cleaned and aseptically prepared.
- The animals were restrained in sterno-abdominal position, and an incision of 2-4 cm was performed in the center of the selected region.
- The resulting flap allowed the sectioning the subcutaneous connective tissue forming a compartment (pocket) for the implant.
- The sectioning of the connective tissue was performed very carefully to prevent tissue damage. After this, the sponge fragments were inserted and stabilized.
- After the stabilization of the implant, the flap was sutured with non-resorbable suture material.
Results

The material which was subjected to the biocompatibility test using the technique and the protocol mentioned above for the inoculation showed no negative effects, the subjects of all groups easily surpassing the healing period from the implant wound.

All animals proved per primam healing, without any vicious scarring or rejection process of the implants.

Twenty-one days after the inoculation, the implant area was totally exposed; only some scarring from stitches could be observed. The implant area showed no sensitivity, and when palpated a painless and immobile subcutaneous nodular formation in the subcutaneous connective tissue could be detected.

The macro- and microscopic modifications for every group are as follows:

Group I – Control Group.

Fig.1. Control group. Subcutaneous implant fixed through the proliferation of connective tissue of neoformation and formation of foreign body granulome. Group 1 – control group 21 days after inoculation.

Fig.2. Control group, 21 days after the inoculation. The histopathologic exam reveals that around and within the sponge body young connective tissue developed with high capillary formation, fibroblasts but also epithelial macrophage cells and giant foreign body cells. The monocyte macrophage and giant cell reaction developed around the material particles of the sponge structure, which is reabsorbed in several areas and allows for the connective tissue to become a fibrous scar. Hematoxylin Eosin stain.
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**Group II – Sponge impregnated with the product Single Bond 2 cured with the LED light.**

![Fig.3](image1.png)

**Fig.3.** Sponge impregnated with the product Single Bond 2 cured with the LED lamp. In the subcutaneous connective tissue the sponge fragment was fixed through a proliferation of well-vascularized tissue formation 21 days post inoculation, group II.

![Fig.4](image2.png)

**Fig.4.** Sponge impregnated with the product Single Bond 2 polymerized with the LED lamp. The histopathologic examination showed the same reaction as group I, characterized by the proliferation of new formation tissue covering and invading the structure of the sponge. The same monocyte macrophage proliferation and the same proliferation of foreign body cells occurred in the structure of the new tissue created. Hematoxylin-Eosin staining.

**Group III – Sponge impregnated with the product Single Bond 2 polymerized with the halogen light.**

![Fig.5](image3.png)

**Fig.5.** Sponge impregnated with the product Single Bond 2 polymerized with the halogen light. After 21 days, the sponge fragment is immobilized through the formation of a connective capsule around it, with a stronger vascularization at the contact area with the host tissue.
Fig. 6. Sponge impregnated with the product Single Bond 2 polymerized with the halogen light. Newly formed connective tissue; also within the structure of the implant, 21 days post inoculation. Vague lymphohistiocytic reaction, monocyte macrophage and giant foreign body cells. HE staining.

A general feature of the implantation technique and of all the materials used is the fact that all subjects recovered from the surgical procedure without complications, and regardless of the material inoculated no implant was rejected and the inoculation flap healed without vicious scarring or fibrotic reaction.

In all variations, the sponge fragments, regardless of the product impregnated, were fixed into the host tissue through capsulation and newly formed connective tissue.

The histopathologic examination did not reveal any differences between the two polymerization techniques of the dental adhesive.

Discussion

The local reaction of the host tissue takes place both towards the absorbent layer (sponge fragments) and towards the absorbed substance. The biocompatibility and bioresorbability of the polyurethane sponge is well established, and it is confirmed by the results for the control group.

The study is deliberately based on dental adhesives and photopolymerization lights of the same manufacturer because we presume that this way the absorbing spectrum of photoinitiators from adhesives will closely correspond to the emission spectrum of curing lamps. The possibility of incomplete curing caused by the differences between the producers of equipment and materials will thus be reduced (10).

Other studies investigating the biocompatibility of dental adhesives showed similar histopathological reactions (12).

A comparison between the tissue response to the contact with dental adhesives and to the contact with calcium hydroxide shows that the inflammatory response is more powerful in the case of dental adhesives (13, 14, 15).

The power of the lamps was tested with the integrated spectrometer of the charging bay of the Elipar Freelight 2. The Elipar Freelight 2 showed density strength of 100%, while the density strength of the Elipar 2500 was at 60% at the moment of the test. Knowing that the density strength of the LED lamp is at 1000 mW/cm², according to the manufacturer’s information, we estimate density strength of 600 mW/cm² for the halogen lamp. The spectral measurement properties of the integrated spectrometer, which are not released by the manufacturer, remain under discussion.
Our research study identifies no differences between the two types of curing techniques, which could have the following cause as main factor: the light curing lamps achieved a similar degree of polymerization of the adhesive, which further led to the release of a similar quantity of toxic monomers.

Conclusions

Correlating the macroscopic and microscopic aspects observed at the end of the experiment (after 21 days), it can be concluded that:
1. The dental adhesive proved to be biocompatible.
2. The type of photopolymerization source had no influence on the biocompatibility of the dental adhesive used during this experiment.
3. The conversion degree of monomers very much depends on the parameters of the curing light but also on the physical-chemical characteristics of the adhesives.

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References