

# THE INFLUENCE OF BIOPTRON LAMP ON THE ORGANISATION AND EPITHELIZATION WOUNDS CAUSED BY TEETH EXTRACTION - EXPERIMENTAL STUDY

Kosta Todorović,  
Vuka Katić\*

Clinic of Dentistry, Faculty of Medicine, Department of oral surgery, University of Niš

\*The Institute for pathology, Clinical center in Niš, Serbia

## Introduction

The wound presents an interruption in tissue continuity. The healing of wounds is a significant process of preservation and reintegration of damage tissue and organs integrity. It can be primary (per primam intentionem) and secondary (per secundum intentionem).<sup>1</sup> The wounds in mouth cavity caused not only due to teeth extraction, but also due to multiple surgery interventions or injuries represents a unique localization because of the anatomic position and physiological activity in that place, rich vascularization, the influence of saliva or substance derived from it<sup>2</sup>, as well as due to specific innervations. If we are add to this the presence of different microorganisms, possible chronic inflammation of gingival mucous membrane and other oral tissue<sup>3</sup>, it is clear that mouth cavity presents a specific anatomic physiological entity with particularities which can influence wound healing. Teeth extraction, due to substantial mechanic tissue damage leads to the reaction of opened wound with bone defect and considerable damage of the surrounding tissue. Healing of such a wound goes through the formation of coagulum, its organizing into granulation tissue, epithelium restitution and bone reparation.<sup>4</sup>

So far, the therapeutic effect of laser x-ray<sup>5,6</sup> has been proved by many experimental clinical studies on the healing of extraction wounds. Lately however, there was an increasing interest in research in the field of polarized light effect on various physiological processes.<sup>7</sup> Polarized light encompasses the spectrum ranging from visible to easily heated infrared, it has wavelength between 400 -2000 µm, encompasses a large spectrum width and has small energy.<sup>8</sup>

## The aim of the work

The aim of this work was to determine the effect of Bioptron Lamp, i.e. of the influence the polarized light has on the stimulation of epithelization, i.e. creating of young granulating tissue as well as its maturing after tooth extraction.

## Material and the methods

The researches were conducted at the Institute for Pathology at the Clinical Center in Niš and the Institute for Biomedical Research at the Faculty of Medicine in Niš. As the source of polarized light in the experiments we used the biolamp "Zepter-Bioptron", which polarizes the light in wave spectrum of 400-2000µm. Light derived in such manner can penetrate the depth of tissue from 2 to 2,5 cm, depending on the length of the treatment. Bioptron compact lamp consists of the halogen part of 20W strength with adjoined cooling device. The lamp has a desk net-like device with the adapter and a time measurer (timer) that produce sound signal in two-minute intervals. Biopton light therapy creates the x-ray temperature of approximately 37° degrees, i.e. insignificant higher than normal temperature of the human body. All experiments were performed on heterozigoutos male rats of Wister breed, weighing between 300-350 g and around 3 months old. The animals were before and during the experiment kept in standard lab conditions, in a room where the temperature was kept between 18-20 degrees and with the moist in the air from 75 to 80%. Before the experiment the animals were on a strict food regime fed with pelleted food manu-factured in a Veterinary Institute in Subotica. Due to teeth extraction and the inability to intake solid food, the possible influence of food ingredients and the mechanical pressure on the healing of extraction wounds, the rats were fed in the course of experimental through application of infusions devices for parenteral nutrition. Because of the relatively short period of experiments the body weight of animals didn't go through drastic changes.

Experimental animals were divided in two groups: I control and II experimental. First group (control) was made up of 6 rats. The second group (experimental) of 18 rats was exposed to polarized light influence. Listed animals were divided in three sub-groups of 6 animals each, depending on the length of the treatment:

II-A group six animals treated for 3 days

II-B group six animals treated for 6 days

II-C group six animals treated for 9 days

From both groups two teeth were extracted from each animal from the upper jaw at the same time. The teeth extraction was performed in the facility for experimental surgery with sterile pliers and other surgical instruments. Prior to the extraction the animals received an anesthetic through intraperitoneal application of Ketaminhidrohloride (Ketalar, Rotexme-dicin, GmbH Trittan- Germany) in the dosage of 0,1ml per 100gr of body weight in the form of insoluble resolution. With rats from the control group they monitored the spontaneous wound healing. In period after 3,6 and 9 days were taken from 2 to 3 biopsies for the patohistological and histo-chemical analyses. Experimental animals were treated through application of polarized light (Bioptron Lamp) (Figure 1) after tooth extraction, and on each day lasting to three minutes. After the polarized light treatment, the procedure of taking biopsies for histological analysis was repeated in the same manner as it was already described for rats from

the control group. Animals from control and experimental group were selective sacrificed on third, sixth and ninth day.



Figure 1. Bioptrom Lamp

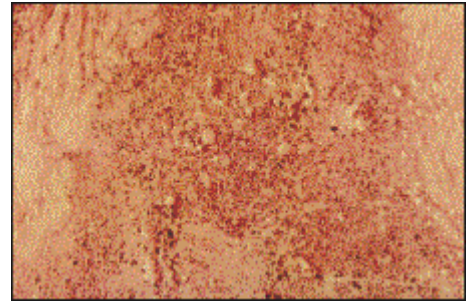


Figure 2. Necrotic epithelium (HE 10x20)

The specimens for biopsies were taken from the extraction wound. The size of the specimen is around 5mm. The specimens are fixated in the 10 percent buffered solution of formaldehyde whose pH was kept at 7,2. Five to six days from the fixation of the device, a dehydration through different alcohol concentrations of 70%, 80%, 90% performed, in the length of 120 min each, and in the absolute alcohol, in the total duration of 17 hours. Following that process the preparations were enlightened in xilol, for the period of 4 hours with two changes in the meantime and gradually the specimens were shifted into paraffin. The specimens were cut on a microtom with the diamond knife. The thickness of these cut pieces was 3 mm. Preparations were colored: HE (chematoxilín eosin); AB-PAS (for basic substance); Gomors (for reticulate fibers); Van Gieson (for collagen fibers).

## Results

All results are exhibited on photographs colored in each names method. We have decided to present in this work only the photographs that most explicitly document reached results.

The results of our research demonstrated that with the animals from the control group sacrificed after three days the necrotic epithelium and subepithel connective issue and covered with thick purulent exudative and fibrin. The contours of neutrophilic leukocyte are not clear, the cytoplasm is extremely eosinophil and granular while the nucleuses are picnotic, i.e. granulocyte leukocytes are in a state of necrobiosis. Between these cells, and first of all towards the healthy tissue, extend the homogenous vaguely pink strings of fibrin. In the necrotic tissue are located multiple small irregularly shaped homogenous dark blue fields of the dystrophic classification type (Fig. 2). With histochemical colority, which reacts on the presence of reticuline and cologne fibers, we managed to find their particular fragmentation. In the surrounding preserved epithelium strong PAS+ reaction is in the medium intermediary layer, however, the focuses of the necrosis with fibroticleukocyte infiltrate are found in the superficial part of the subepithel granular tissue. With animals treated by polarized light for three days it is detectable above necrotic, but also preserved epithelium, an escudo that contains very thick granulocytes. Around the multiplied granulocytes, there is an extremely thick and mononuclear infiltrat composed of lymphocytes, plasmocytes, hystocytes (Fig. 3). On the surface of the wound, with animals from control group sacrificed after 6 days, a narrower defect of epithelium is present. In the present epithelium the dominant is basal cell hyperplasiam without differentiation in inter-mediary layer. In the area of epithel defect notable is granulating issue in maturation. The basic matter is light pink in color, i.e. edematous. Reticular and collagen fibers are fragmented, wave-live and very slim (Fig. 4). With animals treated by bioptrom lamp for 6 days the epithelized wound area is built from double-file cubic epithelium, which contains large and hiperchromans nucleus. Sube-pithelously, the angioblasts are reduced, the basic matter loses its edema and blood cells. Maturing of the granulating tissue into mature granular tissue can be seen through coloring of the cologne, present in the form of wide anastomosis red ribbons (Fig. 5). Reticular fibers in subepithelium granular issue are as well multiplied. Micro morphological finding with animals sacrificed after 9 days from the control group shows that the majority of angioblasts are without luminary, onion-like appearance. Fybrcits extend as rays in horizontal and aslant direction. Basic matter is light pink, infiltrated with rare mononuclear cells of type lympho-plasmocytes, as well as with macrophage (Fig. 6). The fibrosis process is best seen through collagen coloring, when the fibers of collagen are tick, red in color.

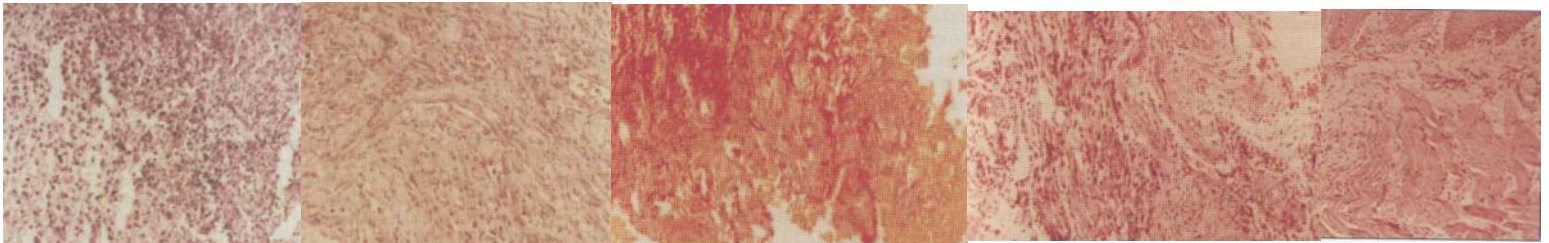


Figure 3. Mononuclear infiltrat of the lymphoplasmocytes type (AB-PAS, 10x40)

Figure 4. Granulation tissue in maturation (HE 10x40)

Figure 5. Maturing of collagen fibers (Van Gieson 10x20)

Figure 6. Notable granulation tissue (HE 10x20)

Figure 7. Notable reduction of subepithel fibrous tissue (HE 10x20)

Micro morphological finding with animals treated for 9 days shows that the regenerated epithelium without an epithelium with basalt cells' hyperplasiom and with an increased mitotic index and hiperchromization of nucleus in its lower third. Evident find is also the epithelium differentiation in PAS+ granular intermediary layer (Fig. 7). Epithelium on the edge of the wound is hyperplasic with visible pa-pilla. Mitotic index in the area of papilla is also increased. Subepithelious granular issue contains very multiplied collagen, as well as reticular fibers. From the cells elements, multiplied are macrophages in the form of small islands, and with extremely foamy cytoplasm thus reminding of pseudoxantomtic cells. The colagenization of fiber in subepithel region is extremely notable and in the form of tongue or wide ribbons divided even the deeply rooted muscular fascicle.

## Discussion

Biopton polarized light in contact with cells increases the energetic activity of the cells membrane, encourages the regenerative pro-cesses and increases the energetic potential of the cell. In that way the cell metabolism is encouraged, the exchange of matter and energy, the cell is brought into the state of energetic balance and also the oxidoreduction processes in the organism are activated.<sup>9</sup> A large number of experiments performed on this model<sup>10</sup> show that it creates a good founda-tion for studying of this clinical phenomenon. In our researches in the first experimental group of animals treated with biolamp for three days, micro morphological cell substrate of acute inflammation largely differed from inflamed cells of animals from the control group. Dominant were the lymphocytes, plasmocytes and macrophages, while in the control group they were present in trail. From the specific significance is gathering of macro-phages inside the wound (due to exudation) armed with hydrolytic enzymes, cytokines and growth factors. Considering that for their exudation from luminary of the capillary, essential is the increased synthesis of Tumor Necrosis Factor (TNF) from helper - T lymphocytes, the presence of macrophages proves the increased capillary leaks as well as the increased cytokine synthesis, first of all of TNF and g interferons. In support to the last suggestions tells us also gathering of small lymphocytes in the area of extraction wound.<sup>11</sup> In the second group of experimental animals treated with biolamp for six days, increased regenerative ability of epithelium can be seen in stratification, large hiperchromic nucleus and increased mitotic index of basal cells of epidermis. Faster maturing of granulating tissue was quite evident and manifested in the reduction angioblast interstitial edema and blood cells. Piling up of fibrocites and hyperplasic of reticular and collagen fibers can be explained through increased number and activity of macrophages. As the highlight of research is the significance of macrophage in the synthesis of Fibroblastic Growth Factor (FGF), the most important factor in fibro genesis. Accordingly the biolamp most lively increases not only the cytokine synthesis in lymphocytes, but also the synthesis of fibroblastic growth factor in macrofages, thus reducing the time required for wound healing. In the third experimental group (after nine days of biolamp treatment) epithelization was complete with differentiation in intermediary and granulating layer, which is not noticeable in the control group. The angioblasts are fibrolized in the granulating tissue, macrophages are transformed in pseduoxantomtic cells, while the cologenization reaches the entire thickness of the wound. Having in mind that changes such as these on wounds that heal spontaneously (even in case of surgical incisions wounds) are seen after thirty days, the complete indicates the positive effects of biolamp in this highly frequent pathological process, known under the name of "reparation".

## Conclusion

Based on histopathological results reached through treatment of extraction wounds by Biopton Lamp, it can be concluded that:

1. The polarized light speed phase of organi-zation and epithelization of extraction wounds,
2. Stated effects it accomplishes through faster metabolism and activation of mononuclear fagocit system.

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#### **Authors and Reprint Information**

**Kosta Todorović, D.D.S.,MSD,  
Clinic of Dentistry,  
52 Braće Tasković Street ,  
18000 Niš, Serbia,  
Serbia and Montenegro  
Telephone: +381 (0)18 326 216**