Biocompatibility of Tissue Conditioners—Histological Study in Rats—

by

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Abstract

The purpose of this study was to verify histologically the biocompatibility of the tissue conditioners, F.I.T.T., Lynal, Visco-gel, and a zinc oxide and eugenol impression paste, when placed in intraoral wounds and surgical osseous cavities in rats. It was possible to conclude that the paste was the most irritating material. Among the tissue conditioners, Lynal and Visco-gel presented similar results and were more biocompatible.

Keywords: tissue conditioners, F.I.T.T., lynal, visco-gel, zinc oxide and eugenol impression paste, biocompatibility.

Introduction

The most commonly used tissue conditioners are composed of powder and liquid; the powder is a mixture of copolymers in a polyethyl methacrylate base and the liquid is a mixture of an aromatic ester, butyl-phthalate-butyl-glycolate, with 6 to 30 % ethanol[1]. The tissue conditioners have been used as temporary soft liners[2–5], in taking functional impressions[6,7], as dressings after surgical treatment[8,9] and in immediate dentures[10–12]. In those applications, the material intimately contacts bloody surfaces and osseous tissues, used as a substitute for zinc oxide and eugenol impression paste.

The purpose of this histological study was to verify the biocompatibility of three tissue conditioners and a zinc oxide and eugenol impression paste when placed on intraoral wounds and in surgical osseous cavities in rats.

Materials and Methods

One hundred and twenty male albino rats (Rattus norvegicus, albinus, Wistar), weighing between 300/350 g, were selected and maintained on a balanced, solid diet during the whole experiment, and water was given “ad libitum.”

Three tissue conditioners: “F.I.T.T.” (Kerr Manufacturing Co.), “Lynal”
(The L.D. Caulk Co.) and Visco-gel (Amalgamated Dental); and a zinc oxide and eugenol impression paste: Pasta Lysanda (Lab. Lysanda Ltda.) were appraised.

For the experimental surgical operations, the animals received general anaesthesia through intraperitoneal infiltration of sodium pentobarbital.

In sixty animals, a bloody area was created on the palate, measuring approximately 1 x 5 mm, near the left molars. In this area a polyethylene tube, with a 3 mm inner diameter and a height of approximately 1.5 mm, cut transversally, was put on the wound area and maintained in position by polypropylene 4-0 sutures. With the exception of twelve animals that constituted the control group, the tubes were supplied with the material to be tested.

In the other sixty rats, an incision of approximately 10 mm in length was made in the right masseteric region which was previously depilated. This incision was perpendicular to the inferior border of the mandible and followed the direction of the ramus and included the periosteum. The tissues were divulsed and the bone was exposed. A cavity was prepared with a number 34 lowspeed inverted-cone bur which measured approximately 1 mm in diameter. The cavity was supplied with the test materials, and the control group received no material. The borders of the surgical wounds were approximated and sutured with cotton thread.

Three animals from each group were sacrificed by sulfuric ether inhalation, at the postoperative periods of 2, 5, 10, and 15 days for the animals with palatal wounds and 4, 8, 16 and 30 days for the rats with mandibular cavities. The left maxilla and the right mandible were obtained.

The pieces were fixed in a 10 % formalin solution, decalcified in a formic acid-sodium citrate solution and embedded in paraffin. The blocks thus obtained were sectioned semiserially to be 6 micrometers thick. The sections were stained with hematoxylin and eosin for histological study.

Results

Palatal Mucosa

On the 2nd day, the wound had a fibrin network and was infiltrated with polymorphonuclear neutrophils, more intensely in the F.I.T.T. group, and mildly in the Visco-gel group. The adjacent connective tissue presented infiltration by neutrophils and lymphocytes, also, mildly in the Visco-gel group.

By the 5th postoperative day, principally in the zinc oxide and eugenol impression paste group, there was neutrophil and lymphocyte infiltration in the fibrin network that supplies the wound (Fig. 1); the epithelium is in the direction of the cortical bone, and capillaries and fibroblasts were observed, principally in the Lynal and Visco-gel groups (Figs. 2 and 3).

By the 10th postoperative day, in the Visco-gel group there was epithelial proliferation and the margins of the wound juxtaposed one another. The connective tissue is infiltrated by neutrophils and lymphocytes, and in the zinc oxide and eugenol impression paste group it was most evident. In all the groups the presence of acellular bone fragments surrounded by osteoclasts and/or multinuclear cells was observed.

On the 15th day, with the exception of the zinc oxide and eugenol impression paste group, the margins of the wounds juxtaposed one another and presented
parakeratin. Neutrophil and lymphocyte infiltration involving the acellular bone fragments (Fig. 4) were observed in all the tissue conditioner groups.

*Mandibular Cavity*

By the 4th postoperative day, in the limits of the bone cavity basophiles, acellular points and some osteoclasts can be observed. In the interior of the cavity, bone fragments were seen adjacent to hemorrhagic points. In the controls, F.I.T.T., Lynal and Visco-gel groups the connective tissue was rich in fibroblasts. In the osteoid tissue, in contact with the zinc oxide and eugenol impression paste and F.I.T.T., a moderate neutrophilic infiltration was seen with some degenerate cells.
Fig. 5 Control group exhibits thick osseous trabeculae (Mandibular cavity, 30 days, Hematoxylin and eosin stain, Original magnification ×63).

Fig. 6 Fragments of zinc oxide and eugenol impression paste involved by granulation tissue. At cavity border there are fibroblasts and collagen fibers (Mandibular cavity, 30 days, Hematoxylin and eosin stain, Original magnification ×160).

Fig. 7 Connective tissue between F.I.T.T. and some areas of newly formed osseous trabeculae. (Mandibular cavity, 30 days, Hematoxylin and eosin stain, Original magnification ×160).

Fig. 8

Fig. 9

Figs. 8 and 9  The same aspect of the anterior figure can be seen in the Lynal and Visco-gel groups, respectively. (Mandibular cavity, 30 days, Hematoxylin and eosin stain, Original magnification ×160).
In the Lynal and Visco-gell groups, the inflammatory infiltration was more discreet.

On the 8th postoperative day, the control group was almost completely supplied by newly formed osseous trabeculae with osteoblasts. In the zinc oxide and eugenol impression paste group, the connective tissue had a few fibroblasts, and was mildly infiltrated by polymorphonuclear neutrophils, degenerated tissue and fragmented material with the presence of multinuclear cells. In the tissue conditioner groups, the connective tissue was rich in fibroblasts and discreetly infiltrated by neutrophils. In the Visco-gel group, newly formed osseous trabeculae accompanied by osteoblasts were seen.

By the 16th day, in the control group, the surgical cavity was filled by mature osseous trabeculae. In the zinc oxide and eugenol impression paste group, the material is reduced to fragments and the connective tissue is moderately infiltrated by neutrophils, lymphocytes, histiocytes and some multinuclear cells. In the layer beneath the material newly formed osseous trabeculae could be observed. In the tissue conditioner groups newly formed osseous trabeculae and discreet neutrophilic infiltration can be observed around the material.

By the 30th day, the control group presented thick osseous trabeculae (Fig. 5). In the zinc oxide and eugenol impression paste group, the fragments of this material were involved with connective tissue and exhibited some neutrophils, lymphocytes and histiocytes. In the direction of the cavity border fibroblasts, collagen fibers (Fig. 6), and some multinuclear cells were seen. In the F.I.T.T., Lynal and Visco-gel groups, the surgical cavities were filled by the material and were surrounded by fibrous connective tissue and thick osseous trabeculae (Figs. 7, 8 and 9).

**Discussion**

The tests recommended by the American Dental Association include the evaluation of the material in laboratory animals, with the aim of reproducing the conditions of application\(^{13}\). For this reason, the material was placed in contact with the palatal wound area using a polyethylene tube\(^{14}\), and polypropylene sutures\(^{15}\), recognized as innocuous materials.

Epithelial proliferation was observed after 5 postoperative days, indicating the beginning of healing. All the groups presented acellular bone fragments, indicating necrosis, which we believe to be a consequence of bone exposition, with the removal of the periosteum.

In the initial period, the results are similar to those obtained by SATO et al\(^{16}\). But these authors used dogs and described the completed repair after 8 postoperative days. They probably used a proportionally smaller wound which was not deep enough to affect the periosteum.

The zinc oxide and eugenol impression paste was an irritating material, mainly because of its high concentration of eugenol and its hard consistency. We agree with the authors\(^{12,16}\) who indicated the use of tissue conditioners, that the results show an improved biocompatibility and these materials are more biofunctional, due to their plasticity.

The F.I.T.T. group presented an inflammatory infiltration, slightly more intense and persistent to 15 postoperative days. The Lynal and Visco-gel groups presented
similar results, but after 5 days, displayed connective neoformation which was more evident in the Visco-gel group.

With the introduction of the materials in the mandibular bone cavities, we aimed to reproduce the penetration of this material into bone spaces, when used as wound dressings such as immediate denture insertions.

In the control group, wound healing was noted after 4 postoperative days and it was completed at 30 days, with the cavity filled by thick osseous trabeculae.

In all groups, after 4 postoperative days, the borders of the wound presented a necrotic process and bone resorption. Nevertheless, this occurred in an intense manner in the zinc oxide and eugenol impression paste group. In this group, the number of neutrophils was higher, and fibroblast proliferation was smaller. The F.I.T.T. group exhibited multinuclear cells, probably as an attempt to eliminate the material.

The wound healing presented more accentuated osteogenesis in the tissue conditioner groups. In the zinc oxide and eugenol impression paste group wound healing was delayed until 30 days, when multinuclear cells were still present. Our results with the zinc oxide and eugenol impression paste are compatible with those of ABREU et al.[17], using dental sockets of dogs. This material presented irritant characteristics.

The tissue conditioners were isolated by a fibrous capsule which is a natural response to the implant. The capsule thickness and its cellular population are related to the degree of irritation of the implanted materials. Thus, a thin capsule, free of inflammatory cells, suggests an inert material[14].

In the manner of GARCIA et al.[18], we observed different reactions from one tissue conditioner to another, which may be justified by the formula or final reaction of the material. However, those authors noted the best results with the "Visco-gel" and found "Lynal" the most irritating conditioner. Our results showed that they behaved similarly. F.I.T.T. was the least biocompatible, and for those authors, this material was an intermediary. This can be justified by SATO et al.[19] who found different results with tissue conditioners in rat connective tissue and dog palatal mucosa.

Based on the results from intraoral wounds and mandibular cavities, we can classify zinc oxide and eugenol impression paste as a cytotoxic material. Although the tested tissue conditioners presented varying degrees of biocompatibility, these materials are useful as protectors of intraoral surgical wounds.

**Summary and Conclusions**

The biocompatibility of tissue conditioners was verified histologically using 120 albino rats. The animals operated on in the palatal mucosa were sacrificed after 2, 5, 10, and 15 postoperative days, and those operated on in the mandible were sacrificed after 4, 8, 16, and 30 postoperative days. Based on histological sections, it was possible to conclude that zinc oxide and eugenol impression paste is the most irritating material to the tissues; the tissue conditioners presented varying degrees of biocompatibility and the best results were provided by "Lynal" and "Visco-gel."
References