ABSTRACT:

The purpose of this work was to study microscopically the behavior of the organic matrix compound from dried-bovine bone (MOOBL), absorbable hydroxyapatite and agglutinate into dental sockets from rats. For this, we used 40 rats (Rattus novegarius, albinus, wistar) divided into two groups: G1, the control group, whose alveolus was filled with blood clot and in the experimental group G2, which received the implant of the compound MOOBL+HAA+AG. The animals were sacrificed in the intervals of postoperative 3, 7, 15, 21 and 30 days and the samples obtained, after routine laboratory procedure, were embedded in paraffin to be cut in the microtome. The 6 micrometers slices were processed through hematoxylin and eosin technique for microscopic study. The results allowed to conclude that: (1) there was delay in the alveolar repair chronology in the group G2; (2) the compound MOOBL+HAA+AG showed itself as biocompatible; in group G2 there was delay in the gingival mucosa epithelialization; (3) the compound MOOBL+HAA+AG underwent the process of alveolar crest reabsorption.

Key Words: implant bovine bone, hydroxyapatite, dental socket.

INTRODUCTION

The adoption of alloplastic and bone implant, after some surgical procedures in the oral cavity, has been suggested aiming to restore function.
and shape of the areas after surgical interventions. Among them, it is noted the increase of the atrophic bone rim, surgical sinus due to periapical lesions, bone loss following periodontal disease associated to metallic implants, bone loss due to trauma or extensive lesions and as an homeostatic in a variety of surgical procedures in the oral cavity (Hayward et al., 1958).

Presently, biomaterials have improved their physical and biological properties, allowing the substitution of bone tissue with the same or greater performance than that of autogenous bone.

Biomaterials should be inert, showing no side effects to the organism. This condition can be assessed by biocompatibility tests in laboratory animals, which are mandatory previous to the use of these materials in humans.

Biomaterials can be classified in two main groups: bone promoters and bone conductors (Taga, 1996).

Bone promoters biomaterials are those that aid in the restoration of a bone wound once applied over the wound, inducing the formation of new bone tissue from undifferentiated mesenchimal cells that are transformed in osteoblasts (Taga 1996).

Bone conductor biomaterials are those that once placed in the bone wound fill up the cavity and guide the bone tissue in its neoforation. The bone conductor material may be incorporated by the new bone tissue, remaining as part of the new tissue. The main difference among both biomaterials is that the former is biologically active and the latter is inert (Taga 1996).

In regard to the host site, the dental socket has been used as an experimental model to evaluate the compatibility of different materials (Okamoto et al., 1973, 1974, 1983, 1994, 1998; Sanches et al., 1972, 1982).

This study aims to evaluate an matrix organic compound from dried bovine bone (MOOBL) in dental, absorbable hydroxyapatite (HAA) and a dextran agglutinate (AG) applied in dental socket.

**MATERIAL AND METHOD**

Forty male rats (*Rattus norvegicus*, albinus, wistar) were used in this study with weight ranging from 200 to 250g. Before and during the experiment the animals were fed with balanced ration, except for the initial 24 hours after surgical intervention.

Animals were anesthetized with an intraperitoneal injection of Sodium pentobarbital (40 mg/kg).

After antisepsis with iodine poli vinilpirrolidona, the right superior incisive was removed with adequate surgical instrument (Okamoto & Russo, 1973).
Animals were separated in two groups: Group I (control) and Group II (experimental).

**Group I** (control), received compressive hemostasis with gauze after the exodonty. Afterwards the mucosa wound was closed with silk 4-0 m.

In **Group II** (experimental), the hemorrhage, after the exodonty, was carefully controlled with gauze compression and applying an absorbent paper cone in the dental socket, which was later removed. After, it was implanted the organic matrix compound from drie-freezed bovine bone, absorbable hydroxyapatite and agglutinate in the dental socket with an amalgm carrier for retrograde obturation. To prepare the composite, it was used two parts of the organic matrix of bovine bone to one part of absorbable microgranular hydroxyapatite, with proportions among weight assessed with a precision scale.

The lyophilized bovine bone matrix was mixed to hydroxyapatite and the mixture was homogenized adding the agglutinate until the mixture had a consistence of dental cement. (Taga, 1996). After applying the material in the socket the gingival mucosa was closed as in the control group.

In the 3rd, 7th, 15th, 21st and 30th days four animals of each group were euthanized under excess of sulfuric ether. The specimens containing the dental socket were removed after a median incision separating both sides of the maxilla and a net cut with straight scissors just behind the distal face of the molars.

The specimens were fixed in formaldehyde 10% and then decalcified in a solution of formic acid and sodium citrate a.a (Morse, 1945). Thus, the specimens were processed in routine laboratory and then included in paraffin to allow cuts of the dental socket in longitudinal direction.

Thus, a series of 6 micrometer thick preparations were obtained and stained by hematoxilyn-eosin for optical microscopy.

**RESULTS**

**3rd Day**

**Group I** (Control). The dental socket was occupied by a blood clot with margraphages. The remains of the periodontal ligament, with good vascularization, show a considerable number of fibroblasts, mainly in the mean third. Close to the periodontal ligament there was scarce number of fibroblasts and neoformed capillaries close to the mean third.
(FIGURE 1). The alveolar bone crest showed slight reabsorption mainly in the lingual aspect.

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FIGURE 1 – GROUP II (MOOBL+ H.A.A.+AG.)- 3rd day. Medium third of the dental socket with mild fibroblastic and capillary proliferation (arrow) close to the remains of the periodontal ligament. HE. Original. 63X.

The epithelium of the gingival mucosa, with interruption of continuity, shows discrete proliferation and there is subjacent presence of some lymphocytes and macrophages, besides a few polymorphonuclear neutrophiles.

**Group II** (MOOBL+H.A.A+AG). In all specimens, the implanted material was close to the cervical third with some non-organized blood clots nearby (FIGURE 2). It was also noted an inflammatory exudate contouring the blood clot.

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FIGURE 2 – GROUP II (MOOBL+ H.A.A.+AG.)- 3rd day. Cervical third of the dental socket showing implant material and some non-organized blood clots. HE. Original. 25X.

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At the level of the mean and apical third, close to the lingual bone wall, it was seen the remainings of the periodontal ligament with a moderate number of fibroblasts and blood vessels. It is also observed a mild capillary and fibroblastic proliferation in the area of the mean third.

The epithelium of the gingival mucosa, with interruption of continuity, shows an elevated number of polymorphonuclear neutrophiles.

7th Day

Group I (Control). In the cervical third and close to the lingual bone wall it is observed small and thin bone trabecula with osteoblasts in the border. The main part of this third is filled by conjunctive tissue with moderate number of fibroblasts and a small number of blood vessels, besides the non-organized blood clot. At the level of the apical and mean third, the remains of blood clot are smaller and it is noted thin bone trabecula filling a greater space mainly in the mean third (FIGURE 3).

The alveolar bone crest shows mild absorption in the lingual aspect.

The epithelium of the gingival mucosa almost covers the dental socket in all specimens, showing a subjacent conjunctive tissue with a mild number of lymphocytes and macrophages.

Group II (MOOBL+H.A.A+AG). The implant material is found in the cervical third of the dental socket, showing, in most cases, a reduction in the volume while compared to the previous stage. In the vicinity there is conjunctive tissue with low organization (FIGURE 4) with a great number of fibroblasts together with some lymphocytes and macrophages.
At the level of the apical and mean thirds, the dental socket is partially filled with newly formed conjunctive tissue. In some specimens there is a small newly formed bone trabecula in the mean third.

The epithelium of the gingival mucosa shows, in all specimens, interruption of continuity, with the presence of a moderate number of polymorphonuclear neutrophiles next to some lymphocytes and macrophages.

**15th Day**

**Group I (Control).** The dental socket is filled with neoformed conjunctive tissue, with the exception of blood clot in one area. At the level of the cervical third, it is observed thin bone trabecula with a wide intertrabecular space (FIGURE 5). In the apical and mean third, the newly formed bone trabecula is irregular and it is observed some conjunctive tissue with no bone differentiation.
The epithelium of the gingival mucosa recovers the entire dental socket and the conjunctive tissue shows a few lymphocytes.

**Group II** (MOOBL+H.A.A+AG). In some instances, the implant is absent and, in others, it is possible to find small particles close to the cervical third (FIGURE 6). In the vicinity it is seen conjunctive tissue loosely organized with a moderate number of macrophages and lymphocytes. Some of the particles undergo absorption and some foreign-body giant cells are observed close to the material. In one of the specimens there is mild ossification close to the cervical third.

**FIGURE 5** – **GROUP I (Control)**- 15th day. Cervical third of the dental socket showing bone trabecula with a wide inter trabecular space. HE. Original. 63X.

**FIGURE 6** – **GROUP II (MOOBL+H.A.A+AG)**- 15th day. Small implant particles close to the cervical third. HE. Original. 63X.
At the level of the apical and mean third it is observed thin, although more regular bone trabecula.

The epithelium of the gingival mucosa recovers the dental socket in two cases and the remaining, with interruption of continuity, shows conjunctive tissue with a moderate number of lymphocytes and macrophages besides some polymorphonuclear neutrophiles.

21st day

Group I (Control). Aside from areas close to the bone wall, which shows thick bone trabecula, the remaining areas of the cervical third of the dental socket shows a greater amount of conjunctive tissue without bone differentiation (FIGURE 7). The apical and mean third of the dental socket are filled with more regular and neatly developed bone trabecula.

Group II (MOOBL+H.A.A+AG). In one of the specimens it is observed, close to the cervical third, small particles of the implanted material being absorbed (FIGURE 8). Furthermore, there are small newly formed bone spicules close to the implant. In the remaining cases, the cervical third is filled with thin bone trabecula. Close to the mean and apical third the bone tissue is well established if compared to the cervical third. However, there are still some areas with conjunctive tissue with no bone differentiation.
30th day

Group I (Control). In all specimens the dental socket is filled with well-established bone trabecula (FIGURE 9). The alveolar bone crest is reshaped.

Group II (MOOBL+H.A.A+AG). In all specimens the implanted material is absent. In the cervical third it is observed newly formed bone trabecula, showing also areas with conjunctive tissue without bone dif-
differentiation (FIGURE 10). At the level of the mean third the trabecula are thicker. The alveolar bone crest is reshaped.

The structures of the oral cavity have a more prominent blood irrigation, which implies in a better local defense against infections, although favoring severe reaction against foreign bodies. In addition, there is a possibility of contamination since the oral structures are washed by saliva and that it is the habitat of several microorganisms. This way, the different behavior of the oral cavity in comparison to other body sites after implanted and grafted materials is understandable.

In the present study the use of rats dental sockets was made taking into consideration the possibility of standardizing the results and also because it is an area in which the biological phenomena dealing with repair are quite known. Thus, any interference in the standard evolution of the alveolar repair may be histologically detected and recognized (Sasaki & Okamoto, 1968; Andrade, 1989; Rosa, 1994). As a result, the dental socket has been considered as an adequate experimental model to the study of the biological compatibility of different materials.

The evaluation of the results of the present study showed that the intralveolar implant of MOOBL+HAA+AG produced qualitative modification in the alveolar repair in its different stages.

The implanted material had a compatible biological behavior although producing some delay in the chronology of the repair process. In all studied periods the material was located at the level of the cervical third and bleeding could be the reason for this displacement of the implanted material out of the mean and apical thirds.

In this study, the implanted material undergoes diminution of volume and totally disappears in all specimens in the 30th day. This finding could

FIGURE 10 – GROUP II (MOOBL+H.A.A.+AG.)- 30th day. Cervical third of dental socket with bone trabecula showing areas without bone differentiation. HE. Original. 63X.
be due to the partial extrusion of the material through the alveolar opening since in the 15th day only two specimens showed the dental socket being lined with gingival mucosa. In the remaining, the interruption of continuity of the epithelium was present.

On the other hand, the results show that part of the material undergo gradual absorption and that associated with the partial elimination through the alveolar opening, leads to complete absence of the implant in the last period analyzed (30th day). One should also consider that the interruption of continuity in the epithelium might have induced contamination of the implanted material and thus contributing to its elimination. Further studies, associating absorbable or non-absorbable membranes and biological barriers, with or without addition of antibiotics to the homogenized material, may lead to better results.

According to the present study, the implanted material in the dental socket showed to be biologically compatible, which was proved by the presence of mild inflammatory reaction between the implant and the newly formed bone. It was not observed any cellular death along the studied periods. These findings are in accordance with other studies conducted in animals and humans beings (Xavier, 1996; Jensen, 1996; Skoglund, 1997).

According to clinical findings in patients (Mulatinho & Taga, 1996; Marzola, 1996; Zenôbio 1997; Zenôbio 1998), Biohapatita® and Osseobond® are biological compatible materials, bone conductors and probably bone inductors. Regarding to the present experimental results, the material showed to be bioincompatible. However, as far as bone conduction is concerned the results are not compatible with the ones mentioned by the above authors. The difference may be due to the use of biological membranes to protect the implanted material, since the above mentioned authors claim to have used such membrane.

In the control group, in the 21st day, the dental socket was mostly filled with regular and well-developed bone trabeculae, which are findings similar to those of Carvalho & Okamoto (1987).

In the 30th day, in the control group, the socket was filled by well-developed bone trabeculae and the alveolar bone crest was reshaped. However, in the group with implanted material it was observed some delay in the chronology of the alveolar repair, being more evident in the cervical third. On the other hand, Carvalho & Okamoto (1987) confirm this delay in the repair process. According to these authors, the delay may be due to the disturbance caused by the material in the or ganization of the blood clot and damage to the periodontal ligaments.

According to the methodology used, it was not possible to observe bone induction by the material. This could be achieved only, for example, implanting the material in the musculature of the abdominal wall of the rat (Reddi et al., 1989; Pinholt, 1991). Therefore, new studies should be conducted to prevent or reduce the contamination of the biomaterials while implanted in dental socket. This fact supports the divergences
found in the results by Sanches (1972) and Okamoto (1994) that had different results with the same material. The only difference was the site of implantation: the dental socket and the tibia of the rat, respectively.

**CONCLUSION**

The results allow us to conclude that:

1) the implanted material MOOBL+HAA+AG has caused delay in the chronology of the alveolar repair and epithelization of the gingival mucosa;

2) the material showed to be biocompatible and absorbable;

3) the composite MOOBL+HAA+AG did not prevent the process of alveolar crest reabsorption from taking place.

**BIBLIOGRAPHICAL REFERENCES**


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