Histomorphological evaluation of Compound bone of Granulated Ricinus in bone regeneration in rabbits

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Abstract. Histological evaluation is an effective method in the behavioral description of the qualitative and quantitative implanted materials. The research validated the performance of Compound bone of Granulated Ricinus on bone regeneration with the histomorphological analysis results. Were selected 30 rabbits, females, divided into 3 groups of 10 animals (G1, G2, G3) with a postoperative time of 45, 70 and 120 days respectively. Each animal is undergone 2 bone lesions in the ilium, one implemented in the material: Compound bone of Granulated Ricinus and the other for control. After the euthanasia, the iliac bone was removed, identified and subjected to histological procedure. The evaluation histological, histomorphological results were interpreted and described by quantitative and qualitative analysis based facts verified in the three experimental groups evaluating the rate of absorption of the material in the tissue regeneration, based on the neo-bone formation. The histomorphologic results classified as a material biocompatible and biologically active. Action in regeneration by bone resorption occurs slowly and gradually. Knowing the time and rate of absorption and neo-formation bone biomaterial, which can be determined in the bone segment applicable in the clinical surgical area.

1. Introduction

Currently, the amount of materials used in the repair of bone discontinuity has increased.

In search of a material to be used in the correction of a bone discontinuity, ie to promote osteoconduction and / or osteoinduction and therefore reparative osteogenesis, autologous bone is the most appropriate indication [1].

Parallel to its availability, a replacement or an ideal bone graft needs to be found in the amount and form required to be deposited in the bone defect [2].

Osteoconductive biomaterials possess properties that serves as a skeleton or frame for the arrival of cells, where they will host. Leading to cell proliferation and osteoblastic activity [3],[4].

The biomaterials are used in medical devices, especially those who are temporarily or permanently implanted in the human body. The term biomaterial was defined at the conference of the National Institute for Health Development in 1982 as "any substance (no drug) or combination of substances, natural or synthetic origin, that can be used for a period of time, completely or partially as part of a system that treats, augment or replace any tissue, organ or body function"[5].

The application of biomaterials in surgical field – dental and orthopedic treatment, is focused on generation properties of a material with perfect reproducibility of bone, with the biocompatibility,
biodegradability, the ability to initiate osteogenesis, composition and mechanical properties similar to those bone [6].

The biomaterial addition to their specific mechanical function once implanted, is a needed for compatibility with the properties of living tissue. These materials are divided into four groups: metals, ceramics, polymers and composites, taking into account that according to the induced biological response can be classified as: biotolerables, bioinert, bioactive and biodegradable [7].

The biomaterial contact with living tissues through the interface will influence a cellular response / tissue, thus activating the desired mechanism and obtaining accurate results [8].

Bioinert and biotolerables materials and leagues such as titanium and carbon, alumina (alpha-Al2O3) and zirconium oxide (ZrO2) are in response of bone tissue encapsulation of the implant by a layer of fibrous tissue [9].

Biodegradable materials: calcium sulfate and calcium triphosphate are designed to degrade or be engulfed by the body gradually, allowing a complete replacement of the implant to new tissue growth [9].

The material to induce a specific biological response in living tissue, forming a bond between the tissue and the implanted material (osteointegration), without the intervention of a layer of fibrous tissue, taken for the bioactivity [9].

The use of chemicals to facilitate bone regeneration through osteogenesis, osteoinduction and osteoconduction, is the target of ongoing research [10], [11].

And according to [12], it includes the factors of biocompatibility, absorption, bioactivity, biodegradation, depending on the physical and chemical properties of the material, which must be compatible with the physiological reactions of bone.

The compound bone of granulated Ricinus (Ricinus communis) when implanted in biological tissue has chemical stability, mechanical strength and biocompatibility [13], increasingly in the field of bone tissue restoration.

The study monitored by the bone regeneration biomaterial, based on new bone formation by histological evaluation.

2. Materials and methods
The experiments of this work were conducted with 30 female rabbits (Oryctolagus cuniculus), adult age of 90 days with weights between 2.5 and 3.0 kg.

The rabbits were divided randomly into 3 experimental groups of 10 animals (G1, G2 and G3), establishing post-operative periods of 45 days for G1, 70 days for G2 and 120 days for G3. Each animal received 2 bone defects in the ilium, one implemented em material defects and the other for control.

The implanted material was:

   Compound bone of Granulated Ricinus (COR)* in presentation granulated with 450μm grain size, being a polymer derived from castor oil (Ricinus communis).

* Poliquil Araraquara Polímeros Químicos Ltda.

In the pre-operative procedures, the animals are exposed to fast for 8 hours and is performed trichotomy in the region to be operated.

It applies preemptive analgesia with morphine sulfate in doses of 1mg. kg every 6 hours for 24 hours (intramuscularly) and cloridrate Tramadol in doses of 2 mg.kg-1 every 8 hours for 72 hours (intramuscularly).

Like antibiotic, enrofloxacin is supplied by 1 mg.kg-1 every 24 hours for 7 days (intramuscularly). Protocol dissociative anesthetic agents with Xylazine and tiletamine-zolazepam at a dose of 5 mg.kg-1 and 15mg.kg-1, respectively (intramuscularly).

Then, antisepsis with chlorhexidine digluconate to 4%. The surgical procedure of the study region, the iliac bone is made by cranio-dorsal approach. An incision in the skin and subcutaneous tissue, and
divided the origin of the fascia of the gluteus medius muscle to provide access and elevation separation subperiostal.

With a trephine of 4 mm external diameter attached to a micro motor for low speed, we made two bone defects in iliac bone under constant irrigation with saline solution 0.9%. A bone defect was filled with Compound bone of Granulated Ricinus and the other for control.

After filling the bone defects, the starts in continuous pattern suture to the gluteus medius muscle with absorbable thread and suturing of skin and subcutaneous tissue with thread no absorbable in simple interrupted pattern.

Surgical times are recorded in photographs.

After the procedures, the rabbits were brought to their cages, fully recovered, not causing weight loss and keeping intact surgical wounds.

After the experimental period of the 3 groups, practiced euthanasia of animals under ethical criteria.

The surgical specimens were removed, identified by a separate group and placed in containers with 10% neutral formalin.

The pieces were removed for histology laboratory for histological processing stages. Were made semi-serial cuts of 6 µm in thickness longitudinally on a rotary microtome. Mounted on slides and stained using the technique of hematoxylin-eosin (HE).

Slides were analyzed and photographed by Zeiss photomicroscope.

The selection of slides for the histological evaluation, is attributed to interactions of the interfaces bone / implant.

Histomorphologic results are interpreted and presented by descriptive analysis based on the interplay of material in the three postoperative periods, evaluating the rate of resorption in tissue regeneration based on the newly bone formation.

The study monitored by the bone regeneration biomaterial, based on new bone formation by histological evaluation.

3. Results

In the experimental group G1, surveying large areas occupied by compound bone of granulated Ricinus and the formation of small bone spicules between them (Fig. 1A). In other slides, most notably the amount of newly formed bone tissue filling the spaces between fragments of the material (Fig. 1B).

Besides newly formed bone tissue, is evident in some areas the presence of connective tissue without bone differentiation (Fig.1C).

![Fig.1. A- Small bone spicules; B- Greater amount of newly formed bone tissue; C- Connective tissue without bone differentiation. H.E., 25x.](image-url)
In the control group G1, the surgical area is occupied by thin bone trabecula and wide space intertrabecular (Fig. 2A). In other slides, the bone trabeculae are more crowded and occupied by connective tissue between the bone trabeculae (Fig. 2B).

Fig.2. A- Surgical areal occupied by thin trabeculae; B- Connective tissue between the bone trabeculae. H.E., 25x.

Can be observed in G2, an extensive surgical area occupied by the compound bone of granulated Ricinus (Fig.3A), and note on the extremities, connective tissue without bone differentiation with a moderate number of fibroblastos (Fig. 3B). The other slides, there is presence of trabecular bone more developed and less of the material (Fig. 3C).

Fig.3. A- Specimen showing large area of the surgical space, occupied by the material, B- same specimen in Fig. 3A, showing areas with connective tissue without bone differentiation, C- bone trabeculae more developed and less material. HE., 25x, 63x, 25x.

All surgical areas in the control group G2 are occupied by thin bone trabeculae and found large areas with connective tissue without bone differentiation (Fig. 4).

Fig.4. Thin bone trabeculae filling the surgical area. HE., 25x.
The observation period in G3, the bone defect where the compound bone of granulated Ricinus was deposited, there was a greater quantity of material and note discrete areas occupied by bone tissue (Fig. 5A). In one of the slides, is shown moderate amount of material next to trabeculae of crowded (Fig. 5B).

In other cases, there is the presence of lower quantity of material and the presence of newly formed bone trabeculae in contact with the polymer (Fig. 5C).

![Fig. 5. A- Specimen showing greater amount of material with discrete areas occupied by bone tissue; B- Moderate amount of material next to thicker trabeculae; C- Presence of newly formed bone trabeculae in contact with the polymer. H.E., 25x.](image)

In the control group G3, in all slides, the surgical area is fully proven by well developed bone tissue (Fig. 6).

![Fig. 6. In all slides, the surgical area is occupied by trabecular bone well developed. H.E., 25x.](image)

4. Discussion
The histological evaluation determined that for longer periods, the implanted materials suffered a slow and gradual resorption.

It also coincides with the position taken by [14] on slow resorption, defining that the use of um material that is slowly absorbed, with strength and elasticity close to the bone and capable of stimulating bone regeneration, would be revolutionary in the filling of bone defects and in the manufacture of implants.

This would also eliminate the disadvantages of autologous graft and metal implants.

The histological evaluation validates the its application to be used to evaluate the biocompatibility of implanted material, as cited by [15], where most of the protocols are based on the qualitative
determination of relative number of cell types and quantity components of the extracellular matrix around the implants.

The performance of compound bone of granulated Ricinus in the three groups is shown surrounded by a developing trabecular bone, which achieves its physical form having an area with a high number of granules, promoting cell migration and obtaining a matrix bone.

The findings come from meeting with [16], reporting that osteoblasts prefer pore size ranging from 200 to 400 μm in diameter to facilitate migration, adhesion and proliferation.

The Compound bone of Granulated Ricinus has the approval from the Food and Drug Administration (FDA), U.S. government agency responsible for authorizing the circulation of new foods and medicines, in June 2003.

5. Conclusions
The new bone formation was characterized by a slow and gradual resorption.

The Compound bone of granulated Ricinus proved to be biocompatible and biologically active.

The days of G1, G2 and G3 will be the basis for understanding the rate of resorption and new bone formation of Compound bone of granulated Ricinus, can determine which (is) segment (s) of bone (s) is applicable within the clinical area -surgical.

6. Future work
Put under study the evaluation by computer analysis of qualitative and quantitative bone mineral density, wich can be monitored the bone regeneration of the material.

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