HISTOMORPHOMETRIC EVALUATION OF THE EFFECT OF BOVINE COLLAGEN GRANULES ON BONE HEALING. AN EXPERIMENTAL STUDY IN RATS

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ABSTRACT

Collagen materials have been utilized in medicine and dentistry because of their proven biocompatibility and capability of promoting wound healing. The aim of the present experimental study was to perform a histomorphometric evaluation of the effect of bovine collagen granules on post-extraction alveolar wound healing in rats. Twenty male Wistar rats were submitted to bilateral extraction of the first lower molars under ketamine/xylazine anesthesia according to the technique previously described by Guglielmotti and Cabrini. Sterile Bovine collagen granules of approximately 80 ± 10 µm (Membracel G. Lab. Celina, Buenos Aires) were hydrated with saline solution and placed into the right mesial socket (experimental side) with gentle pressure, completely filling the site. The contralateral sockets were considered as the control side. Sutures were not performed. After surgery neither special diet nor antibiotics were given. The rats were fed rat chow and water ad libitum. All the animals were killed on the 30th day following surgery by ether overdose. The jaws were dissected, radiographed, decalcified, and embedded in paraffin. Sections were obtained at the level of the first molar mesial socket in a buccolingual orientation and stained with hematoxylin-eosin. The trabecular area and volume density of trabecular bone were measured histomorphometrically. The trabecular area was greater in alveoli treated with collagen granules than in control alveoli ($P<0.05$). Values of volume density of trabecular bone were greater in experimental than in control sockets ($P<0.05$). This experimental study provides evidence for the use of bovine collagen granules as bone grafting material, as a therapeutic alternative to fill postextraction sockets.

Key words: *bovine collagen, bone-healing, histomorphometry, rats.*

EVALUACIÓN HISTOMORFOMÉTRICA DEL EFECTO DE GRÁNULOS DE COLÁGENO BOVINO EN LA REPARACIÓN ÓSEA. ESTUDIO EXPERIMENTAL EN RATAS

RESUMEN

La biocompatibilidad del colágeno determinó la utilización del mismo como material de implante. El objetivo del presente estudio fue evaluar histomorfométricamente los efectos de gránulos de colágeno bovino en un modelo experimental de cicatrización ósea post-extracción dentaria. Se utilizaron 20 ratas Wistar macho, a las que bajo anestesia, se procedió a la exodoncia de los primeros molares inferiores siguiendo la metodología descripta por Guglielmotti y Cabrini. En el alvéolo mesial derecho se implantaron gránulos de colágeno bovino de aproximadamente 80 ± 10 µm (Membracel G. Lab. Celina, Buenos Aires) vehiculizados con solución fisiológica estéril. Los alvéolos contralaterales se utilizaron como control. No se realizó sutura ni se administró antibioticoterapia. Los animales fueron sacrificados por sobredosis de éter a los 30 días post-implantación, se resecaron las mandíbulas, se fijaron en formol al 10%, radiografiaron, decalcificaron y procesaron para su posterior inclusión en parafina. Se realizaron cortes orientados en sentido vestíbulo-lingual a nivel del alvéolo mesial y se colorearon con hematoxilina-eosina. Se evaluó histomorfométricamente el volumen óseo total y la densidad ósea en el tercio apical del alvéolo. Los alvéolos donde se utilizó gránulos de colágeno como material de implante evidenciaron mayor volumen de tejido óseo neoformado y mayor densidad ósea con respecto a los alvéolos control ($P<0,05$). Los resultados obtenidos en este modelo experimental permitirían establecer que la utilización de gránulos de colágeno bovino no interfiere el proceso de cicatrización ósea post-extracción dentaria.

Palabras clave: *colágeno bovino, cicatrización ósea, histomorfometría, ratas.*

INTRODUCTION

Collagen is the most abundant protein (by weight) in animals, accounting for 30% of all proteins in mammals. In humans, 21 types of collagen (I-XXI) have been described (1-3). Collagens can be divided into several subfamilies according to their primary structures and/or forms of supramolecular organization: fibrillar collagens (types I-III, V, and XI), and nonfibrillar collagens (1-3).

Among the vertebrate fibrillar collagens, type I represents the most abundant protein of the body. It has important functions, for example, in the mechanical properties of bones, tendons and skin.

Biomaterials made from collagen are marketed in two forms: the first is derived from native collagen where the original structure of collagen, consisting of interwoven wavy fibre bundles, is retained. The second type of biomaterial is prepared from solubilized collagen, the fibrous structure of the collagen is not retained and the biomaterial has a dense appearance under the microscope (4).

Collagen materials have been utilized in medicine and dentistry because of their proven biocompatibility and capability of promoting wound healing (2-7). Various collagen-based materials have been used in the biomedical field for applications such as abdominal wall repair, and tendon, ligament, blood vessel replacement, peripheral nerve growth support, soft tissue augmentation, burn and wound dressing. Collagen membranes have also been applied in both guided tissue and bone regeneration procedures (2-7). However, to the best of our knowledge, the role of collagen granules as bone grafting material has not been addressed to date.

The aim of the present experimental study was to perform a histomorphometric evaluation of the effect of bovine collagen granules on post-extraction alveolar wound healing in rats.

MATERIALS AND METHODS

Twenty male Wistar rats (80 ± 5 g body weight) were submitted to bilateral extraction of the first lower molars under ketamine/xylazine anesthesia (8/1.28 mg per 100 g of body weight) according to the technique previously described by Guglielmotti and Cabrini (8). Sterile Bovine collagen granules of approximately 80 ± 10 µm (Membracel *G*, batch #80, Lab. Celina, Buenos Aires) were hydrated with saline solution and placed into the right mesial socket (experimental side) with gentle pressure, completely filling the site. The contralateral sockets were considered as the control side.

Sutures were not performed. After surgery neither special diet nor antibiotics were given. The rats were fed rat chow and water *ad libitum*. National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication N° 85-23, Rev. 1985) were observed.

All the animals were killed on the 30th day following surgery by ether overdose. The jaws were dissected, radiographed, decalcified in 7.5% nitric acid, and embedded in paraffin. Sections were obtained at the level of the first molar mesial socket in a bucco-lingual orientation and stained with hematoxylin-eosin.

A histomorphometric analysis based on standard stereologic methods was performed using an image analysis system (Kontron MOP AM 03, Carl Zeiss) to measure tracings obtained from projections of the sections. The trabecular area, and volume density of trabecular bone, considered as the ratio between trabecular area and total bone area, were measured in a rectangle outlined on the apical third of the socket, as previously described (9). Results are presented as mean ± S.D. Statistical significance was determined by Student's *t* test ($P<0.05$).

RESULTS

Uncomplicated healing after surgery in all rats was observed. The mucosa completely covered the wound surface.

Radiographic Findings

Thirty days after surgery the experimental and control sockets were occupied by radiopaque tissue, making the socket walls indistinguishable.

Histologic Findings

The alveolus of the control side was almost completely occupied by lamellar bone, its trabecular surfaces were lined by few cuboidal osteoblasts, predominantly bone lining cells (Fig. 1A,C). The experimental side showed the socket filled by lamellar bone and covered by cuboidal osteoblasts (Fig. 1 B,D). The implanted material was not evident.

The epithelium completely covered the wound surface in all the animals. In some cases, we observed giant cells associated to residual implant material in the underlying connective tissue (Fig. 2).

Histomorphometric Analysis

The trabecular area was greater in alveoli treated with collagen granules (552 ± 48 mm^2)* than in control alveoli (320 ± 28 mm^2) ($P<0.05$). Values of volume density of trabecular bone were greater in experimental (0.53 ± 0.05) than in control sockets (0.44 ± 0.07) ($P<0.05$).

*mm^2 of projection

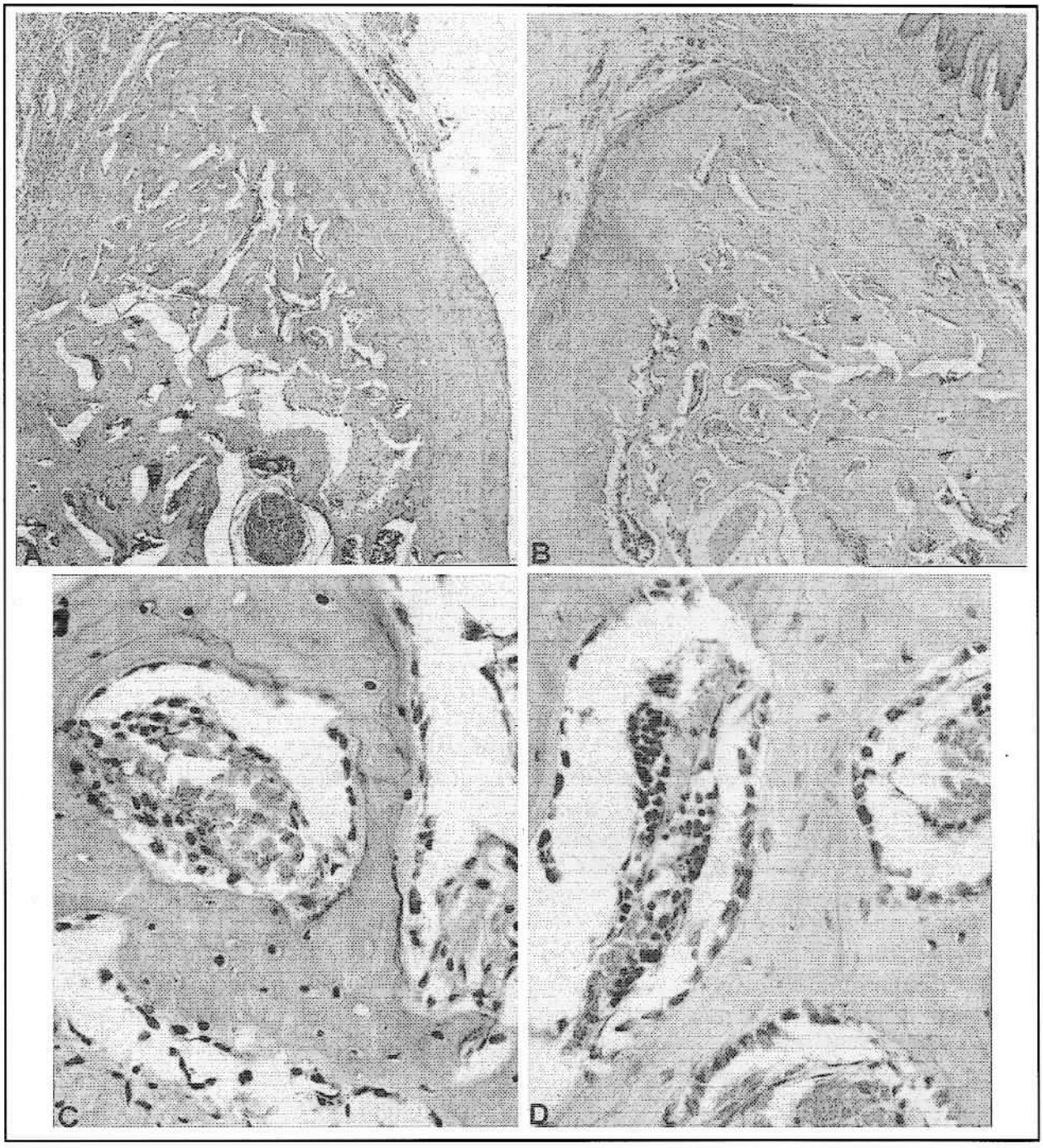

Fig. 1: Light microscopy section of the mesial socket of first molars, at 30 days of healing without implant material (A,C) and with implantation of collagen granules (B,D). Both sockets were filled with lamellar bone. A higher bone density is observed in B (H-E, X50). Notice the trabecular surfaces lined by a higher proportion of active osteoblasts in the experimental socket (H-E, X400).

DISCUSSION

This experimental study provides evidence that the treatment of extraction socket with bovine collagen granules produced a rise in osteogenesis.

The accuracy of the methodology used in this experiment has been repeatedly verified both in terms of the procedure of tooth extraction, and the histometric methods (8-9). The apical third of the alveolus is a representative area exclusively occupied by new bone tissue formed during the healing process. This methodology was employed in the study of alveolar wound healing under local or sys-

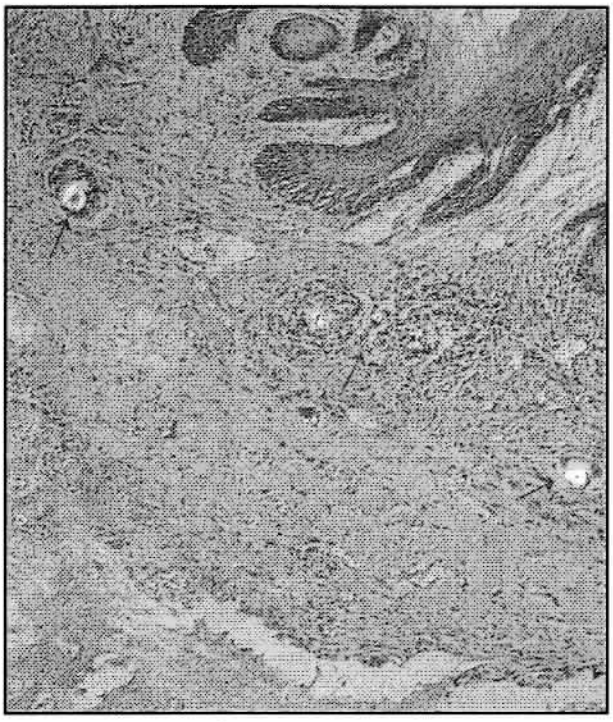

Fig. 2: Remnants of implanted collagen (arrows) surrounded by giant cells were observed in the subepithelial connective tissue (polarized light microscopy, X50).

temic conditions, affording a quantitative characterization of the process (8-14).

Collagen has a number of biological properties, which makes it a widely employed biomaterial (4-7). It is a natural substrate for the support and growth of a variety of cells and tissues in the body. It works as a framework in conjunction with other extracellular molecules such as glycosaminoglycans and fibronectin. In addition, it is thought that collagen may promote wound healing through clot stabilization, hemostasis, and chemotactic properties (2,6,7).

Collagen has low immunogenicity, particularly when it is in a purified, undenatured form (3). Absence of a systemic immune reaction after implantation of bovine and porcine collagen materials was reported (5,7). *In vivo* and *in vitro* studies have shown that collagenous material is degraded by the action of a complex system of collagenases or colagenolytic enzymes, present mainly in granulocytes and macrophages (5). Several animal studies have been conducted to evaluate the biocompatibility and biodegradation rate of collagen implants (2,15,16). A great variability of results was reported. That variation may be due to variations in the animal model used, the anatomical localization, the critical healing period considered, and chemical and physical properties of collagen implants (2-7). In the present experimental study we used granules of young native bovine collagen, extracted, purified, rebuilt, and unmodified, with no other added component. The implanted granules cannot be detected at 30 days post-implantation. However, we observed giant cells associated to residual implant material in the surface area of the wound. In clinical practice, this could be avoided by covering the alveolus with a membrane so that the biomaterial is withheld.

The bovine collagen granules used in this study were biocompatible, and could be used as temporary scaffolds for bone tissue regeneration.

CONCLUSION

This experimental study provides evidence for the use of bovine collagen granules as bone grafting material, as a therapeutic alternative to fill postextraction sockets.

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REFERENCES

1. Exposito JY, Cluzel C, Garrone R, Lethias C. Evolution of collagens. Anat Rec 2002; 268: 302-316.
2. Bunyaratavej P, Wang HL. Collagen membranes: a review. J Periodontol 2001; 72: 215-229.
3. Patino MG, Neiders M, Andreana S, Noble B, Cohen RE. Collagen: an overview. Implant Dent 2002; 11: 280-285.
4. Boon ME, Ruijgrok JM, Vardaxis MJ. Collagen implants remain supple not allowing fibroblast ingrowth. Biomaterials 1995; 16: 1089-1093.

5. Goissis G, Marcantonio E, Chierici Marcantonio RA, Comelli Lia RC, Cancian DCJ, Carvalho WM. Biocompatibility studies of anionic collagen membranes with different degree of glutaraldehyde cross-linking. Biomaterials 1999; 20: 27-34.
6. Vardaxis NJ, Ruijgrok JM, Rietveld DC, Marres EM, Boon ME. Chemical and physical properties of collagen implants influence their fate *in vivo* as evaluated by light and confocal microscopy. J Biomed Mater Res 1994; 28: 1013-1025.
7. Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE. Cellular inflammatory response to porcine collagen membranes. J Periodont Res 2003; 38: 458-464.
8. Guglielmotti MB, Cabrini RL. Alveolar wound healing and ridge remodeling after tooth extraction in the rat: a histologic, radiographic and histometric study. J Oral Maxillofac Surg 1985; 43: 359-364.
9. Guglielmotti MB, Ubios AM, Cabrini RL. Morphometric study of the effect of low dose of uranium on bone healing. Acta Stereol 1987; 6: 357-368.
10. Guglielmotti MB, Ubios AM, Cabrini RL. Alveolar wound healing alteration under uranyl nitrate intoxication. J Oral Pathol 1985; 14: 565-572.
11. Guglielmotti MB, Ubios AM, Cabrini RL. Aveolar wound healing after x irradiation: a histologic, radiographic, and histometric study. J Oral Maxillofac Surg 1986; 44: 972- 976.
12. Ubios AM, Guglielmotti MB, Cabrini RL. Effect of diphosphonate on the prevention of X-radiation-induced inhibition of bone formation in rats. J Oral Pathol 1986; 15: 500-505.
13. Guglielmotti MB, Alonso C, Itoiz ME, Cabrini RL. Increased osteogenesis in alveolar wound healing elicited by demineralized bone powder. J Oral Maxillofac Surg 1990; 48: 487-490.
14. Giglio MJ, Gorustovich A, Guglielmotti MB. Bone healing under experimental anemia in rats. Acta Odont Latinoamer 2000; 13: 63-72.
15. Anselme K, Bacques C, Charriere G, Hartmann DJ, Herbage D, Garrone R, tissue reaction to subcutaneous implantation of collagen sponge. A histological, ultrastructural, and immunological study. J Biomed Mater Res 1990; 24: 689-703.
16. Schlegel AK, Mohler H, Busch F, Mehl A. Preclinical and clinical studies of a collagen membrane (Bio-Gide). Biomaterials 1997; 18: 535-538.