REVISTA ESPAÑOLA DE INVESTIGACIONES QUIRURGICAS
Spanish Journal of Surgical Research
Vol. VII n°:3 (107-110) 2004

EFFECT OF COLLAGEN MEMBRANES AS A BARRIER METHOD ON HETEROTOPIC BONE INDUCTION. EXPERIMENTAL STUDY IN RATS

Alonso Ovies A [1], Verrier A [2], Redondo LM [2], Vaquero C [3]

[1] Department of Oral and Maxillofacial Surgery, Hospital Clínico "San Carlos". Madrid, Spain.
[2] Department of Oral and Maxillofacial Surgery, Hospital Universitario "Del Rio Hortega". Valladolid, Spain.
[3] Experimental Surgery Laboratory. Faculty of Medicine. Valladolid, Spain

KEY WORDS
Osteogenesis, collagen membranes, guided tissue regeneration.

Correspondence:
DRA. A. ALONSO OVIES
Servicio de Cirugía Oral y Maxilofacial
Hospital Clínico "San Carlos"
C/Prof Martín Lagos s/n,
28040 Madrid.
E-mail: almalovi@telefonica.net.

SUMMARY
Collagen membranes are proven barrier methods, used in guided tissular bone regeneration. We have implanted cubic molds of collagen membrane stuffed with demineralized bone in the area of the superficial epigastric artery of the rat. In this experimental study what we are trying to find out is if these membranes will allow the heterotopic osteoinduction of the demineralized bone contained in the molds made out of said membranes. These collagen molds would be able to maintain the desired shape and the barrier effect of the membrane could prevent the undesired cellular migration from the surrounding tissues. We have observed that the collagen membranes allow osteoinduction in the interior of the mold, although in a delayed way and that they maintain their shape in the osteoinducted bone obtained.

INTRODUCTION

In recent years great progress has been made in the search for alternatives for bone reconstruction, including the generation of bone matrix, the obtaining of osteogenic cells and the manufacturing of artificial materials similar to bone and that can stimulate the formation of the same. The demineralized bone matrix and the different osseous morphogenetic proteins are part of this line of research.

The use of demineralized bone matrix was considered appropriate primarily for craneomaxilofacial reconstruction [4] and, since then, many studies have been carried out to investigate its bone inductive properties [17,8].

Zellin and coworkers developed the theory of osteopromotion as an aid in the cure and regeneration of bone [10, 19]. This theory implies that to promote bone cure and bone neogenesis, the compartment that has to be filled should be sealed by physical means, thus preventing any interference with osteogenesis from the surrounding soft tissue cells.

Barrier membranes were first tested in the 1950¥s and the early part of the 1960's for the cure of bone defects in orthopedic applications [7] and later they were also used in oral surgery.

The aim of this study is to evaluate the influence of the barrier efect of collagen membranes in the heterotopic osteoinductive process. To that end, we will implant the mold stuffed with demineralized bone in the area irrigated by the superficial epigastric artery of the rat. Our hypothesis is that after a period of time the implant of demineralized bone will have turned into living bone its vascularization being dependent on the surrounding tissues.

MATERIAL AND METHOD

A total of twenty adult Wistar rats were used in this study. All the animals were implanted with a mold of collagen membrane fillled with demineralized bone.

The molds were made under sterile conditions with membranes of reabsorbable BioMendTM collagen (Sulzer Calcitek, Carlsbad, CA 92008 USA). The membranes were cut to form cubic structures of 4 x 4 x 2 mm, with one of the 4x2 mm faces missing.

The molds were filled with demineralized bone powder (particle size of between 72-420 µm). This bone was obtained from the femur and tibia of donor rats.

The area chosen for the implant was the tributary of the superficial epigastric vessels.

Two groups of animals were considered, each of them composed of ten rats. They were put to sleep for data collection purposes at 40 days (group G-1) and at 80 days (group G-2).

The implants were subjected to histological analysis under the following headings:

1. Persistence of the collagen membrane

2. Cellularity

3. Presence of osteoid material

4. Existence of bone marrow

5. Quantity of blood vessels.

For the study, the molds were divided in three areas: S (upper), C (central) and I (area nearest to the mold face without collagen membrane).

RESULTS

The collagen membrane was identified as a series of superimposed and intertwined fibres that surrounded the implant.

In all the examples of the G-1 group the histological persistence of the collagen membrane was observed, although with different degrees of reabsorption (**Fig. 1**). In the G-2 group, in 19 cases the membrane had totally disappeared, the only sign of its presence being an inflammatory cellular reaction in its place. The disappearance of the collagen membrane was not accompanied by any loss in the configuration of the implant.

The cells existing among the particles of demineralized bone were characterized by their fusiform morphology, similar to that of fibroblasts.

A certain prevalence of the cellularity was observed in the area of the face of the mold without any membrane. In the G-2 group the cells were more abundant, a little more rounded, with a more circular nucleus and basophil, with a direct evolution towards osteocytes. It is for this reason that we consider these cells as precursors of osseous tissue (**Fig. 2**).

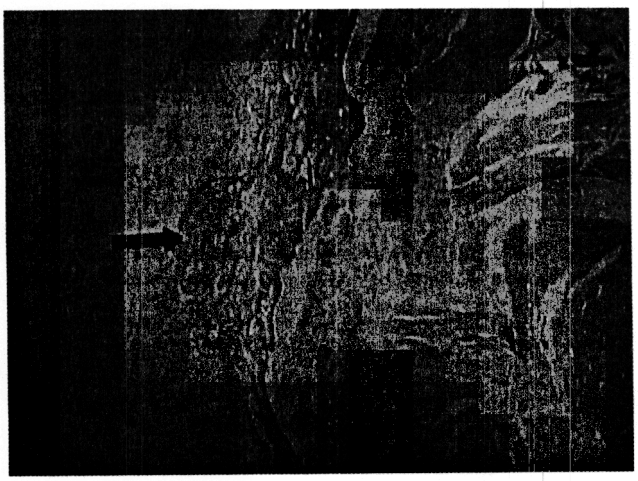

Figure 1.- *Persistence of the collagen membrane in G-1 group, whit a high reabsortion degree (arrow). Hematoxilin-eosin x100*

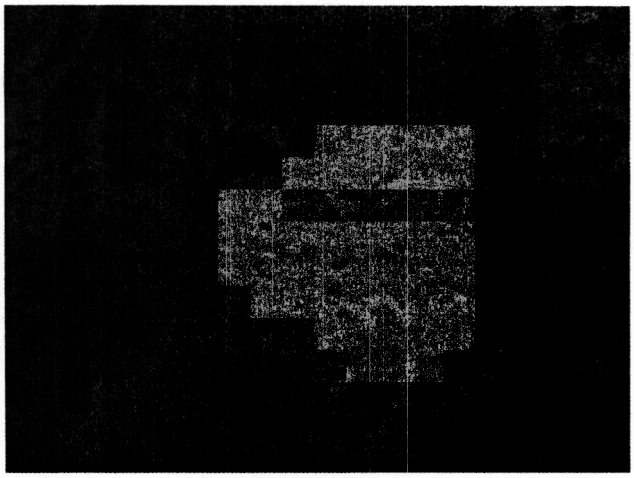

Figure 2.- *In G-2 group the cells have a little more rounded morphology and a nuclei more basophil and rounded. Hematoxilin-eosin x100*

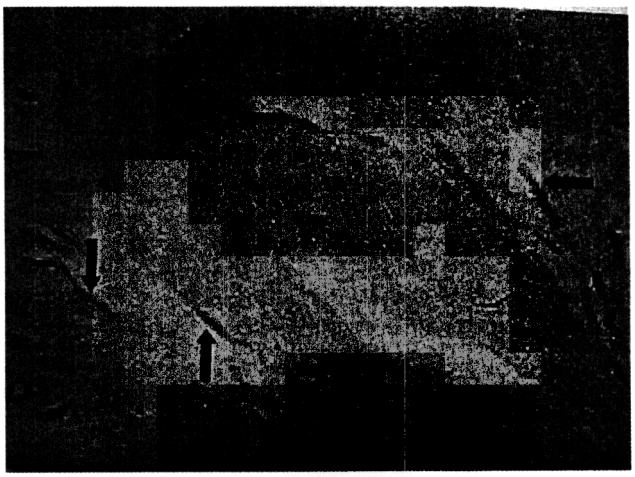

Figure 3.- *The osteoide, of fibrilar characteristics, is situated in the space among the particles of demineralized bone (arrows). Hematoxilin-eosin x100.*

The osteoid material is young osseous tissue before calcification, with fibrilar characteristics and situated in the spaces among the particles of demineralized bone (**Fig. 3**). It was most abundant in the G-2 group having a greater density in its intertwining and sometimes it was difficult to differentiate it from the particles of demineralized bone proper, with which it is intimately joined.

Bone marrow was observed in 65% of the cases of group G-1 and in 100% of group G-2. This difference is very significant statistically, with p=0.00416. Besides, the area occupied by the foci of hematopoietic tissue was bigger in the molds of the G-2 group.

The foci of hematopoietic tissue were located more frequently in areas C and I in both groups, springing up both in the interior of a particle of demineralized bone as well as in osteoid tissue. These foci expand centrifugally. As expansion takes place, the center empties of fundamental substance and filles in with spongy tissue (**Fig. 4**). Only in some cross sections could isolated chondrocytes be observed.

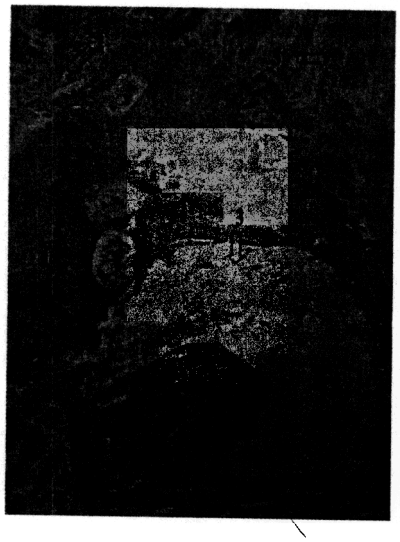

Figure 4.-
The foci of hematopoietic tissue is expanded centrifugalment. As the expansión takes place, the center empties and being filled in with a fat tissue. Hematoxilin-eosin x100

Bone trabeculae were not evident either in the newly formed osseous tissue.

In both groups the presence of the formation of new vessels was observed, with a progressive increase of the same the closer we get to the area lacking the collagen membrane. When comparing both groups a greater vascular presence was appreciated in the implants corresponding to group G-1.

DISCUSSION

Given the use of osteoinductive factors without arquitectural limit in the substratum it would be difficult to obtain a piece of bone with the exact three-dimensional size necessary for the reconstruction of complex bone defects.

This is why it is essential to look for a way of containing the osteoinductive compounds.

In recent years various ways of containing said compunds have been tried, such as titaniun [14], polyethylene [12], or hydroxyapatite [13] .We have used molds made with collagen membrane.

Collagen membranes as a barrier system allow a guided regeneration of tissue[10], its main advantage compared to other types of membranes being the fact that they are reabsorbable, thus avoiding the need for removal[20], which means we do not alter the vascularización dependent on adjacent tissues. In addition, they offer excellent biocompatibility [3,1], with colonization in the process of degradation by cells of connective tissue, which suggests that they can be incorporated in curative connective tissue and be replaced by new collagen [15].

Regarding the influence of the inflammatory and foreign body reaction to the membrane in the osteogenetic process, in our experimental study, this reaction practically does not exist. The membrane was replaced by connective tissue and bone formation took place and seems stable.

The barriers used for orthotopic guided osseous tissue regeneration do not need to remain intact for more than seventy days after insertion [5]. In spite of that, at present slow reabsorption membranes are still being investigated to clarify whether the delay in the reabsorption process is advantageous [1].

In our study, the collagen membranes chosen have kept the medium isolated for the time necessary as well as keeping the desired shape. In our study, the demineralized (not yet living) bone implanted does not present a remarkable reabsorption rate either at 40 or 80 days, possibly due to the barrier effect of the collagen membrane. Some authors [2] have already noted the beneficial effects of barrier membranes on bone reabsorption when they are used to cover and isolate bone autografts from the surrounding soft tissues , protecting the former from reabsorption during the recovery period.

Our data are similar to the findings of Kusumoto [17,1], who reported a combination of cancellous and lamellar bone, which would mean ossification of a membranous type had taken place.

In the whole process of osteoinduction, the vascularización of the implant of demineralized bone is a very important factor [16] and the incorporation of the material implanted into the surrounding normal tissue depends a lot on the capacity of the new vessels to invade it. Is probable that the invasion of the blood vessels and the components associated with the basal membrane including the type IV collagen, attract the morphogenetic proteins and the members of the TGF-fl superfamily and perhaps orients them towards an optimum conformation for the localised inititation of bone differentiation. The blood stem cells and the endothelial cells synthesize a variety of growth factors that play a role in the phenotypical expression of osteoblasts [11]. In addition, the cells of the vascular neighborhood are potential sources of osteoprogenitor cells [18].

In our study the quantity of vessels decreases notably at 80 days compared to at 40 days, perhaps because of a greater osteoid density or perhaps because of the already generalized appearance of marrow bone one, whose characteristically high level of vascularización can make up for the functions previously carried out by the vessels situated among the particles of demineralized bone.

It has been confirmed in the majority of publications that this bone marrow formation already exists two weeks after the implant [6]. In our study cancellous bone was observed in 100% of cases at 80 days, whereas at 40 days it was present in only 60%. We have to suppose that the barrier effect of the membrane delays the whole process again. The blood marrow present is very rich in fatty tissue and seems to advance centrifugally, so that it is to be expected that if more time elapsed we would have the same findings as Hagen [6], who at 40 weeks after subcutaneous implantation of demineralized bone matrix describes how the implant consists of new bone with particles of remineralized bone incorporated and large spaces of cancellous bone.

Hagen [6] blames the lack of remineralization of the most peripheral part on the dissolution of the osteoinductive factors in the surrounding tissue. In our case this would not be the reason or if it were, it it would be very mitigated, since up to a certain point the membrane would prevent this diffusion. In addition, we have observed that the hematopoietic tissue t as it begins to form is located more frequently in the area of the face with no collagen membrane, which would be where a lesser concentration of factors would exist in the event of diffusion of the same taking place.

The prevalence of fat in the hematopoietic tissue could be related to the place of implantation and to the functional and mechanical demand to which the implants are submitted. The

lack of mechanical forces would lead to to the rapid conversion of the bone into ossicles consisting of marrow tissue surrounded by a thin layer of bone. This would explain equally well the absence of bone trabeculae in the implanted material

CONCLUSIONS

1. Collagen membrane as a barrier element preventing the passage of surrounding cells is a biocompatible and biodegradable material, with which molds can be obtained easily.

2. Heterotopic osteoinduction from powdered demineralized bone matrix is maintained in the interior of the collagen mold in spite of the barrier effect with the passage of stem cells probably taking place through the face of the mold with no membrane.

3. The process of membrane degradation allows for vascular nutrition of the implant from the surrounding tissues.

4. Osteoinduction is initiated in the area in contact with the medium, with formation of bone marrow very rich in fat. Trabecular arrangement of the bone was not observed.

5. Apparently all the phases of heterotopic osteoinduction are delayed, because of the barrier effect of the collagen membrane.

ACKNOWLEDGMENTS

This investigation was financed by the Health Investigation Fund Health Institute of the Carlos III Institute of Health (Reference 99/0918). (Fondo de Investigación Sanitaria del Instituto de la Salud Carlos III)

REFERENCES

1. *Brunel. G, Piantoni. P, Elharar F, Benqué E, Marin P, Zahedi S. Regeneration of rats calvarian defects using a bioabsorbable membrane technique: Influence of collagen cross-linking. J Periodontol: 1996; 67: 1342-8.*
2. *Buser D, Dula K, Hirt HP, Schenk RK. Lateral ridge augmentatiooon using autografts and barrier membranes: A clinical study with 40 partially edentulous patients. J Oral Maxillofac Surg: 1996; 54: 4220-4432.*
3. *Galgut P, Pitrola R, Waite I, Doyle C, Smith R. Histologic evaluation of biodegradable and nondegradable membranes placed transcutaneously in rats. J Clin Periodontol: 1991; 18: 581-6.*
4. *Glowacki J, Kaban LB, Murray JE, Folkman J, Mulliken JB. Application of the biological principle of induced osteogenesis for craniofacial defects. Lancet 1981; 1: 959-62.*
5. *Greenstein G, Caton JG. Biodegradable barriers and guided tissue regeneration. Periodontol 2000: 1993; 1: 36-45.*
6. *Hagen JW, Semmelink JM, Klein CPAT, Prahl-Andersen B, Burger EH. Bone induction by demineralized bone particles: long-term observations of the implant-connective tissue interface. J Biomed Mater Res: 1992; 26: 897-913.*
7. *Hurley L.A, Stinchfield FE, Bassett ACL. The role of soft tissues in osteogenesis. J Bone Joint Surg Am: 1959; 41: 1243.*
8. *Inoue T, Deporter DA, Melcher AH. Induction of chondrogenesis in muscle, skin, bone marrow, and periodontal ligament by demineralized dentin and bone matrix in vivo and in vitro. J Dent Res: 1986; 65: 12-22.*
9. *Kusumoto K, Bessho K, Fujimura K, Akioka J, Ogawa Y, Iizuka T. Prefabricated muscle flap including bone induced by recombinant human bone morphogenetic protein-2: an experimental study of ectopic osteoinduction in a rat latissimus dorsi muscle flap. Br J Plastic Surg: 1998; 51: 275-80.*
10. *Linde A, Alberius P, Dahlin C. Osteopromotion. A soft-tissue exclusion principle using a membrane for bone healing and bone neogenesis. J Periodontol: 1993; 64: 1116-28.*
11. *McCarthy TL, Centrella M, Canalis E. Effects of fibroblast growth factors on deoxyribonucleid acid and collagen synthesis in rat parietal bone cells. Endocrinol: 1989; 125: 2118-26.*
12. *Minkin C, Blackman, Newbrey J, Pokress S, Posek R, Walling M. Effects of parathyroid hormone and calcitonin on adenilate cyclase in murine mononuclear phagocytes. Biochem Biophys Res Commun 1977; 76: 875-8.*
13. *Mizumoto S, Inada Y, Weiland AJ. Fabrication of vascularized bone grafts using ceramic chambers. J Reconst Microsurg 1993; 9: 441-9.*
14. *Nettelblad H, Randolph MA, Östrup LT, Weiland AJ. Molded vascularized osteoneogenesis: A preliminar study in rabits. Plast Reconstr Surg: 1985; 76: 851-8.*
15. *Pitaru S, Tal H, Soldinger M, Azar-Avidan A, Noff M. Collagen membranes prevent the apical migration of ephitelium during periodontal wound healing. J Periodont Res: 1987; 22: 331-3.*
16. *Reddi AH, Cunningham NS. Recent progress in bone induction by osteogenin and bone morphogenetic proteins: challenges for biomechanical and tissue engineering. Journal of Biomechanical Engineering: 1991; 113: 189-90.*
17. *Reddi AH, Huggins CB. Biochemical sequences in the transformation of normal fibroblast in adolescent rats. Proc Natl Acad Sci USA: 1972; 69: 1601-5.*
18. *Schweigerer L, Neufeld G, Friedman J, Abraham JA, Fiddes JC, Gospodarowicz D. Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. Nature 1987; 325: 257-9.*
19. *Zelling G, Gritli-Linde A, Linde A. Healing of mandibular defects with different biodegradable and non-biodegradable membranes an experimental study in rats. Biomaterials: 1995; 16: 601-9.*
20. *Zelling G, Linde A. Treatment of segmental defects in long bones using osteopromotive membranes and recombinant human bone morphogenetic protein-2. Scand J Plast Reconstr Hand Surg: 1987; 31: 97-104.*