AUTOGENOUS GRAFTS OF COSTAL CARTILAGE PRESERVED IN 98% GLYCEROL TO DENTAL SOCKETS: HISTOLOGICAL STUDY IN RATS*

Tetuo OKAMOTO**
Mario Francisco R. GABRIELLI***
Marisa A. Cabrini GABRIELLI***
Eduardo Hochuli VIEIRA***

■ ABSTRACT: Sixty male albino rats (*Rattus norvegicus, albinus*, Wistar) were used in the present study. The animals had the right upper incisor extracted and received either fresh autogenous costal cartilage grafts or autogenous cartilage grafts preserved in 98% glycerol, positioned at the medium third of the dental alveolus. The animals were sacrificed after 6, 9, 15, 21 and 40 days postoperatively. The results showed that the fresh cartilage have maintained its vitality and growth during the whole experiment, and the perichondrium was integrated to the newly formed connective tissue of the host bed. The preserved material results in inflammatory reaction similar to the observed with the fresh grafts. However, the glycerol preserved cartilage undergoes faster resorption and substitution by newly formed bone.

■ KEYWORDS: Transplantation, autogenous; cartilage; glycerol.

Introduction

Cartilage is a suitable tissue for transplantation and survives for long hypoxic periods due to its high levels of anaerobic metabolism and low cellularity.[5,8] Moreover, cartilage grafts are less dependent of functional stimuli to maintain their volume and shape. The firm consistence of that tissue allows easy contouring, suturing of small fragments and compression into bone defects. Fresh cartilage grafts are gradually replaced by bone over long periods, though typical endochondral ossification and osteoinduction may occur.[12,17,22]

* Trabalho desenvolvido com o auxílio do CNPq.
** Departamento de Diagnóstico e Cirurgia – Faculdade de Odontologia – UNESP – 16015-050 – Araçatuba – SP.
*** Departamento de Diagnóstico e Cirurgia – Faculdade de Odontologia – UNESP – 14801-903 – Araraquara – SP.

Due to the low immunogenic properties of cartilage, methods of tissue preservation for cartilage grafts do not need to include overly aggressive chemical treatment. Thus, lyophilization, freeze-drying and freezing have presented good results. Those methods usually require specific equipament for processing, maintenance and transportation.[1]

Glycerol seems to be a suitable alternative method of tissue preservation. It has been used for preservation of corneas[7] and duramater for confection of cardiac valves.[13,14,15] It dehytrates tissue, removing most intracelular water, altering the ionic concentration of the cells and functions also as an antisseptic without altering the texture of the preserved material.[14,15,18] Moreover, 98% glycerol reduces the immunogenic properties of transplanted duramater.[6,19,23] Gabrielli et al.[4] transplanted 98% glycerol preserved cartilage to the malar process of rats, and found mild inflammatory reactions and intense bone substitution of the grafts.

Carvalho & Okamoto[2] transplanted fresh autogenous and homogenous grafts of cartilage to dental sockets of rats and have observed integration and growth of the grafts, without resorption. Those grafts presented very small amounts of ossification. Devitalization of the tissue will enhance bone substitution.[3, 11]

The present study aims to evaluate the behavior of 98% glycerol preserved autogenous costal cartilage grafts to the dental sockets of rats.

Material and method

For this study, 60 male albino rats (*Rattus norvegicus, albinus*, Wistar) were used. The animals weighted 150 to 200 g and were fed, before and during the experiment, with a solid diet (Ração Ativada Produtor, Anderson Clayton, S. A.), except for the first 24 hours postoperatively and water *ad libitum*.

1 *Removal of the grafts*: Under general anesthesia induced with intraperitoneal infiltration of sodic penthobarbital (Abbot Laboratories) in an aproximate doses of 50 mg/kg of body weight, the left thoracic region was depilated. Antisepsis was performed with merthiolate solution (Lilly).

The last rib (13th) was exposed through a skin cut and divulsion of deeper planes with straight scissors. A 7 mm segment was obtained from the free end of the last rib. To remove the segment, the rib was isolated with a small skin hook, which allowed to remove fascia and muscle attached to it, before its separation. Interrupted sutures of the deep and superficial planes were performed with 5-0 polyvicryl. Remaining debris of fascia and muscle were removed from the costal segment with a number 11 scalpel blade under magnification (x25).

2 *Classification of experimental groups and grafting procedures*: The animals were divided into two groups of 30 each, which received the following procedures: a) *Group I*: Each section of cartilage was divided into two fragments of approximately 3 mm in

length. All cartilage fragments were rinsed in physiologic solution for 15 minutes and placed in individually labeled flasks containing 98% glycerol for 20 days. Before autogenous transplantation to the previously identified donors, each fragment was hidrated in physiologic solution for 15 minutes.

After 20 days, the animals of this group were anesthetized as the same way and the right upper incisor was extracted with specially adapted instruments.[10] Immediately after, each socket received one preserved cartilage fragment placed between the middle and apical thirds with iridectomy forceps. The gingival mucosa was sutured with 4-0 polyvicryl. b) *Group II*: The 30 animals of this group received fresh autogenous grafts of the same size, following the same surgical procedures as described for the previous group.

3 *Histological procedure*: Six animals of each group were sacrificed by excessive sulphuric ether inhalation after 6, 9, 15, 21 and 40 days postoperatively. The right maxilla was separated from the left in the medium sagital plane, following the intermaxillary suture, with a lancet blade and a cut with straight blade tangent to the distal surface of the last molar. The whole dental alveolus was contained within the removed maxilla.

The specimens were fixed in 10% formalin solution for 24 hours and decalcified for approximately 40 days in a sodium citrate and formic solution,[9] following then routine laboratory procedures for 6 micrometer sectioning and staining with hematoxylin and eosin. The sections were cut in a longitudinal direction, presenting the whole dental socket. For description of results, the dental alveolus was divided into 3 thirds (apical, medium and cervical).

Result

6 days

Group I: The glycerol preserved graft is found at the medium third of the socket. Cartilage cells are well preserved morphologically. Adjacent to the grafts, connective tissue can be found with fibroblasts parallel to the surface of the cartilage (Figure 1). Moderate number of macrophages and lymphocytes can be noted at several sites.

At the remaining areas, the dental socket presents newly formed connective tissue particularly developed at the medium third. Blood clot appears at other areas and the epithelium partially covers the socket.

Group II: The graft is found at the medium third of the socket and it is vital in all cases. Connective tissue rich in fibroblasts and newly formed capillaries involves the cartilage. At some sites there is no visible limits between that tissue and the

perichondrium (Figure 2). Numerous macrophages and moderate number of lymphocytes are found.

At the remaining areas, the dental alveolus shows more differentiated connective tissue close to the lingual wall of the medium and apical thirds. Disorganized blood clot can be seen, specially at the cervical third. The epithelium partially covers the socket.

9 days

Group I: The grafts are positioned at the medium third and show small resorption areas. Close by, fibrous connective tissue and newly formed bone are observed. The remaining areas of the socket is filled with newly formed connective tissue similar as was found for the previous period. At some regions, clotted blood and more discreet ossification are seen. The epithelium totally covers the dental alveolus.

Group II: The cartilage is positioned at the medium third and appears vital in all specimens. Adjacent to the graft, cellular connective tissue and some collagen fibers parallel to the cartilage are found. Moderate number of macrophages and some lymphocytes are seen within the connective tissue. The remaining areas of the sockets is filled upwith connective tissue showing different characteristics according to the considered area. At the medium and apical thirds, bone trabeculae occupy approximately 1/3 of the total area. At the cervical third, ossification is more discreet. The epithelium totally covers the dental socket.

15 days

Group I: The grafts are positioned at the medium third. The cartilage is being resorbed at several sites, where it is replaced by fibrous connective tissue (Figure 3) or by small bone trabeculae. At the remaining areas, the sockets are filled up with newly formed trabecula occupying approximately 1/2 of the medium and apical thirds. At the cervical third, ossification is more discreet.

Group II: The cartilage is vital and presents moderate growth at several areas (Figure 4). The adjacent connective tissue is well developed with fibroblasts parallel to the surface of the graft. Discreet numbers of lymphocytes are still found. At some areas, small newly formed trabecula are observed close to the grafts. However, between cartilage and bone, considerable amount of connective tissue still remains and it is mildly infiltrated by lymphocytes. The remainer of the dental alveolus is filled up with thin disorganized bone trabecula. Disorganized blood clot can be found at some areas.

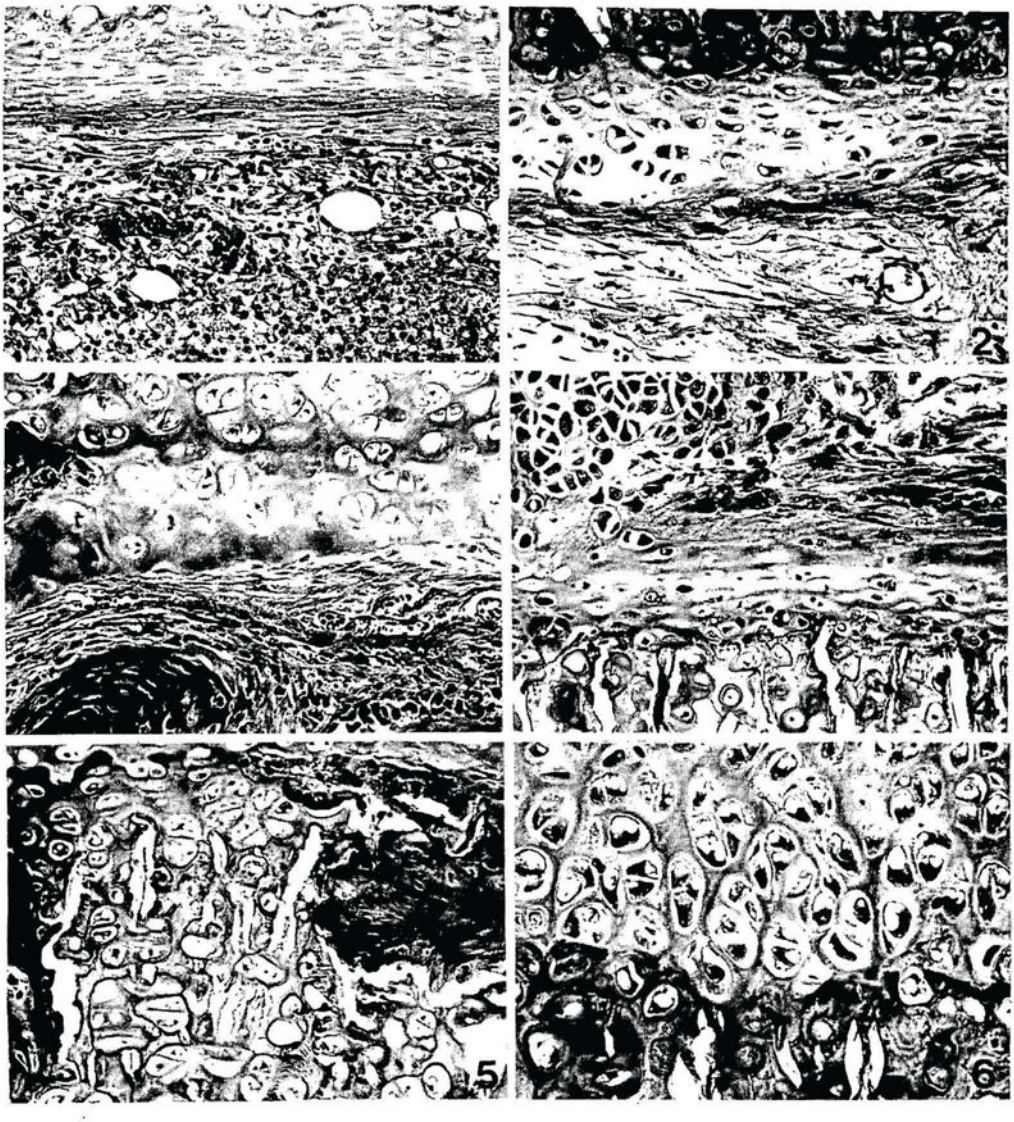

FIGURE 1 – Group I – 6 days. Adjacent to the grafts, connective tissue can be found with fibroblasts parallel to the surface of the cartilage. HE. 160x.

FIGURE 2 – Group II – 6 days. Sites where there is no visible limits between that tissue and the perichondrium. HE. 160x.

FIGURE 3 – Group I – 15 days. The cartilage is being resorbed at several sites where it is replaced by fibrous connective tissue. HE. 250x.

FIGURE 4 – Group II – 15 days. The cartilage is vital and presents moderate growth at several areas. HE. 250x.

FIGURE 5 – Group I – 40 days. Grafted cartilage can still be found, usually involved by newly formed bone. HE. 250x.

FIGURE 6 – Group II – Intense growth of the cartilage. HE. 250x.

21 days

Group I: In all specimes the grafts are reduced in size by resorption. Bone partially replaces the transplanted grafts. The other areas of the socket are filled up with thick trabeculae expept for the cervical third wich shows less differentiated bone.

Group II: Growth of the grafts has increased in comparison to the previous period. The amount of connective tissue between cartilage and alveolar bone has decreased. The amount of newly formed bone has increased. The connective tissue of the interface is very fibrous. The remainder 2/3 of the dental alveolus are filled up with regular thick bone trabecula.

40 days

Group I: In all specimens, small amount of grafted cartilage can still be found, usually involved by newly formed bone (Figure 5). At some points, a thin band of connective tissue can be found, separating the graft from alveolar bone. At the remaining areas, the socket is filled with thick well defined trabecula.

Group II: After 40 days, intense growth of the cartilage is seen in all specimens (Figure 6). This growth occurs all around the grafted material. At some points, newly formed bone is very close to the cartilage. At other sites, a thin fibrous band separates the structures. All other areas of the socket are repaired with thick well defined trabecula.

Discussion

Fresh autogenous cartilage grafts usually are well integrated and present significant growth. The resorption rates of those grafts are either slow or resorption does not occur.[2,20,21] Glycerol preserved cartilage, however, undergoes gradual resorption and substitution by newly formed bone, when transplanted to surgically prepared not contaminated sites.[11]

The dental socket presents different characteristics when compared to surgically prepared bone cavities, because of the presence of periodontal ligament and contamination by oral fluids.

The fresh grafts in the present study started their growth after 15 days. The perichondrium was vital and promoted appositional growth of the cartilage. A growth was seen in the periodontal related areas that are highly vascularized and fibroblasts riches in the rat.

After 6 days the inflammatory infiltrate was moderate and comparable for both groups, showing that the treatment by 98% glycerol does not remarkably alters the structure material.[4,13]

Bone formation close to the grafts is delayed for fresh material when compared to the preserved cartilage. The fresh material presents a major tendency to be isolated by fibrous connective tissue at the interface with alveolar bone. Perichondrium did not play a role in new bone formation, as previously observed.[4,20]

The preserved cartilage grafts resulted in earlier new bone formation in direct contact with the transplanted cartilage, which could be seen since 9 days postoperatively. Devitalized cartilage is usually, more readily resorbed than fresh material.[16,22]

It is probable that the perichondrium removal of the glycerol preserved material would result in even faster resorption and substitution, as it happened to the cut extremities of the ribs, where perichondrium was absent. Probably, homogenous grafts of 98% glycerol preserved material will resorb even faster than the autogenous glycerol preserved grafts.

Conclusion

- The fresh autogenous cartilage grafts have maintained their vitality the whole experiment and the perichondrium was integrated to the newly formed connective tissue of the host bed.
- The 98% glycerol preserved cartilage undergoes faster resorption and substitution by newly formed bone.
- The preserved cartilage promoted an inflammatory reaction comparable to the fresh material.

OKAMOTO, T. et al. Enxerto autógeno de cartilagem de costela preservada em glicerina em alvéolos dentais. Estudo histológico em ratos. *Rev. Odontol. UNESP (São Paulo)*, v.24, n.1, p.9-17, 1995.

■ *RESUMO: Para o presente estudo foram empregados 60 ratos (*Rattus norvegicus, albinus,*Wistar). Os animais, após extração do incisivo superior direito, receberam em seus alvéolos implantes autógenos de cartilagem hialina fresca ou conservada em glicerina a 98%. Os implantes foram posicionados em nível do terço médio do alvéolo. Os animais foram sacrificados 6, 9, 15, 21 e 40 dias após o ato operatório. Os resultados obtidos mostram que a cartilagem fresca mantém a vitalidade, notando-se o crescimento durante o período experimental e a integração do pericôndrio com o tecido conjuntivo neoformado. Por outro lado, a reação inflamatória nos estágios iniciais foi comparável entre os dois grupos. No entanto, a cartilagem preservada em glicerina sofre reabsorção e substituição por tecido ósseo neoformado.*

■ *PALAVRAS-CHAVE: Enxerto autógeno; cartilagem; glicerina.*

References

1 BOYNE, P. J. Transplantation, implantation and grafts. *Dent. Clin. North Am.*, v.15, p.433-53, 1971.

2 CARVALHO, A. C. P., OKAMOTO, T. A histological study of cartilage autografts and allografts placed in dental sockets of rats. *J. Oral Surg.*, v.37, p.11-5, 1979.

3 FONSECA, R. J., DAVIS,W. H. *Reconstructive preprosthetic oral and maxillofacial surgery.* Philadelphia: Saunders, 1986. p.19-39.

4 GABRIELLI, M. F. R. et al. Autogenous transplantation of rib cartilage preserved in glycerol to the malar process of rats: a histological study. *J. Nihon Univ. Sch. Dent.*, v.28, p.87-99, 1986.

5 GOODE, R. L. Bone and cartilage grafts: current concepts. *Otolaryngol. Clin. North Am.*, v.5, p.447-51, 1973.

6 JATENE, F. B. et al. Estudo experimental da dura-máter quando interposta em traquéia de cães. *Ars Curandi Odontol.*, v.12, p.23-39, 1977.

7 KING JUNIOR, J. H., McTIGUE, J. W., MERYMAN, H. T. A simple method of preservation of corneas for lamellar Keratoplasty. *Am. J. Oefthal.*, v.53, p.445-9, 1962.

8 LASKIN, D. M., SARNAT, B. G. The metabolism of fresh, transplanted, and preserved cartilage. *Surg. Glycerol. Obstet.*, v.96, p.493-9, 1953.

9 MORSE, A. Formic acid-sodium citrate decalcification and butyl alcohol dehydratation of teeth and bone for sectioning in paraffin. *J. Dent. Res.*, v.24, p.143, 1945.

10 OKAMOTO, T., RUSSO, M. C. Wound healing following tooth extraction. Histochemical study in rats. *Rev. Fac. Odontol. (Araçatuba)*, v.2, p.153-70, 1973.

11 OKAMOTO, T. et al. Autogenous transplantation of rib cartilage preserved in glycerol, after removal of the perichondrium, to the malar process of rats. A histological study (I). *J. Nihon Univ. Sch. Dent.*, v.32, p.116-26, 1990.

12 PEER, L. A. *Transplantation of tissues, cartilage, bone, fascia, tendon and muscle.* Baltimore: Williams & Wilkins, 1955. 421p.

13 PIGOSSI, N. Implantação de dura-máter homógena conservada em glicerina. Estudo experimental em cães. *Arch. Chirurg. Clin. Exp.*, v.27, p.213-47, 1964.

14 PUIG, L. B. et al. Valva cardíaca de dura-máter homóloga. Método de preparação da valva. *Rev. Hosp. Clin. Fac. Med. Univ. S. Paulo*, v.29, p.85-9, 1974.

15 PUIG, L. B. et al. Four years experience with duramater cardiac valves. *J. Cardiovasc. Surg.*, v.18, p.247-55, 1976.

16 SCHAFFER, E. M. Cartilage transplants into the periodontium of rhesus monkeys. *Oral Surg.*, v.9, p.1233-46, 1956.

17 SCHAFFER, E. M., PACKER, M. W. Bone regeneration, cartilage and tooth grafts in periodontal pockets. *Dent. Clin. North Am.*, v.6, p.459-69, 1962.

18 STOLF, N. A. G., ZERBINI, E. J. Correção de hérnia diafragmática utilizando dura-máter homógena conservada em glicerina. *Rev. Paul. Med.*, v.83, p.21-4, 1974.

19 TANNURI, V. et al. Utilização de dura-máter em cirurgia pediátrica. *Rev. Paul. Med.*, v.92, p.27-30, 1978.

20 TULIO, S., OKAMOTO, T., GABRIELLI, M. F. R. Free autogenous costal cartilage grafts to the mandible of rats. Histological study. *Braz. Dent. J.*, 1993. (In press).

21 _____. Fresh autogenous rib cartilage graft to the malar process of rats with and without removal of the perichondrium. Histological study. *J. Nihon Univ. Sch. Dent.*, v.32, p.116-26, 1992.

22 URIST, M. R., McLEAN, F. C. Osteogenic potency and new bone formation by induction in transplants to the anterior chamber of the eye. *J. Bone Joint Surg.*, v.34, p.443-76, 1952.

23 ZERBINI, E. J., PUIG, L. B. Valvas cardíacas e biológicas com especial referência à valva de dura-máter. *Arch. Inst. Cardiol. Mex.*, v.47, p.650-7,1977.