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Reprint from

IMMUNOBIOLOGY, AUTOIMMUNITY AND TRANSPLANTATION IN OTORHINOLARYNGOLOGY

Proceedings of the First International Academic Conference in Immunology and Immunopathology as applied to Otology and Rhinology, Utrecht, The Netherlands, April 11-12, 1984

edited by Jan E. Veldman, Brian F. McCabe, E.H. Huizing & N. Mygind

Kugler Publications/Amsterdam/Berkeley

MORPHOLOGICAL AND IMMUNOLOGICAL FEATURES OF ALLO- AND XENOGENEIC LYO-CARTILAGE

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The literature reveals that lyophilized tissues, mainly lyophilized bone, dura and vessels, have widely been used for experimental and clinical purposes. Surprisingly, there is only a limited knowledge of the morphological and immunological features of lyophilized cartilage (lyo-cartilage). This paper presents some of our results of an

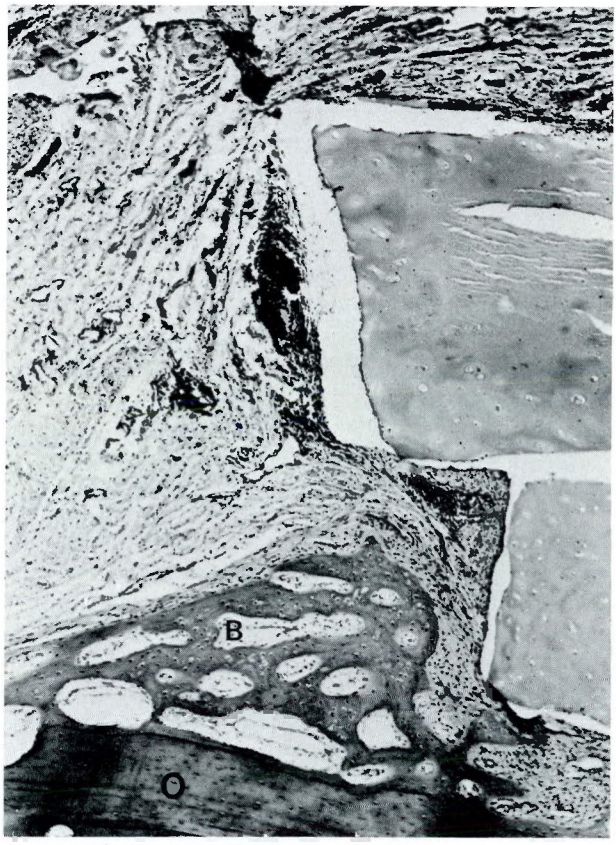


Fig. 1. Ossification on a connective tissue basis after subperiosteal grafting of xenogeneic lyo-cartilage blocks. B = New bone formation; O = Bone surface; H.E. × 40. (from Sailer[1]).

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Immunobiology, Autoimmunity and Transplantation in Otorhinolaryngology
Proceedings 1st International Conference
Utrecht, The Netherlands, April 11–12, 1984, pp. 213–218
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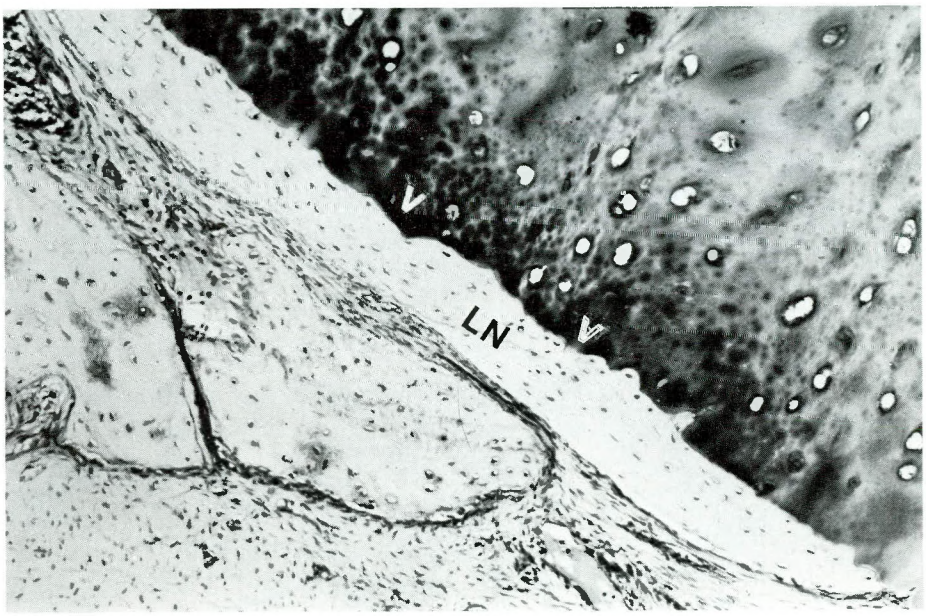


Fig. 2. Subperiosteal implantation of lyo-cartilage 260 days after operation. Irregular calcification spots (V) are transformed into lamellar bone (LN); Toluidine blue. H.E. × 100. (from Sailer[2]).

animal study which was performed at the Royal College of Surgeons of England. The results are extensively described in our Monograph.[1]

Morphological investigations

Twelve female macaques (*Macaca irus*) received 14 to 20 allo- or xenogeneic lyo-cartilage grafts in the form of blocks or slices. The description of the lyophilization process and the way of rehydration has been published elsewhere.[1] The grafts that were implanted in the facial region either subperiosteally or subcutaneously were observed for up to 260 days after the operation. Of the 207 implants of lyo-cartilage, 206 could be investigated, one graft was not found. No infection whatsoever was observed in the graft bed areas.

The animal experiments revealed a different morphological behaviour after subperiosteal and subcutaneous transplantation. Already after 23 days most of the subperiosteal transplants were fixed to the bony surface and no longer movable. This is caused by early progressive replacement of the lyo-cartilage by bony substance which commences where cartilage is in contact with bone. Two types of ossification could be observed after subperiosteal implantation:

(1) Ossification of the connective tissue all around the graft where the periosteum extends between the surfaces of the cartilage and the bone (Fig. 1).
(2) Ossification on the basis of irregular calcification which merges smoothly into lamellar bone (Fig. 2).

This last type of ossification is visible also after subcutaneous grafting of lyo-cartilage but not before 240 days after implantation (Fig. 3).

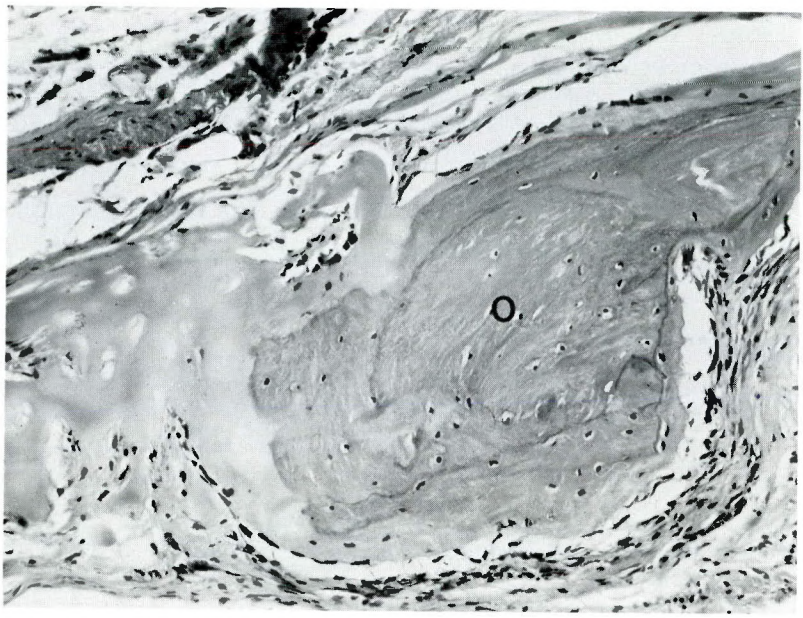


Fig. 3. 260 days after subcutaneous grafting into the forehead area of an animal. Ossification (O) of allogeneic lyo-cartilage is visible. H.E. × 130. (from Sailer[1]).

Macroscopic resorption was rarely seen in allo- and xenogeneic grafts during the observation period, but microscopically xenogeneic cartilage showed much greater resorption than allogeneic material after 240–260 days. In addition, polynuclear giant cells and lymph follicles were seen in the direct neighbourhood of xenogeneic lyo-cartilage implants (Fig. 4).

Immunological investigations

In ten of the animals immunological investigations using the leucocyte migration inhibition test[2] (LMI test) were performed. The test had to be adapted to the special conditions of macaque blood.[1] It differs from methods described by others.[2–4] With these methods we found that we were unable to achieve sufficiently high leucocyte counts in monkeys. The LMI test was carried out in ten animals at intervals varying from one week to nine months. In one animal fresh allogeneic cartilage removed from a donor animal was grafted into the forehead of the test animal in order to establish any differences in antigenicity between fresh and lyophilized cartilage. To eliminate any possibility of cross-reaction and cytotoxicity the LMI test was carried out under the following conditions:

(1) Leucocytes from animals without cartilage grafts were tested with allogeneic and xenogeneic lyo-cartilage antigen.
(2) Leucocytes from animals which had received allogeneic lyo-cartilage transplants were tested with non-grafted allogeneic lyo-cartilage antigen.
(3) All animals were tested with the antigen of the grafted lyo-cartilage type.

The immunological experiments showed that the total quantity of lyo-cartilage

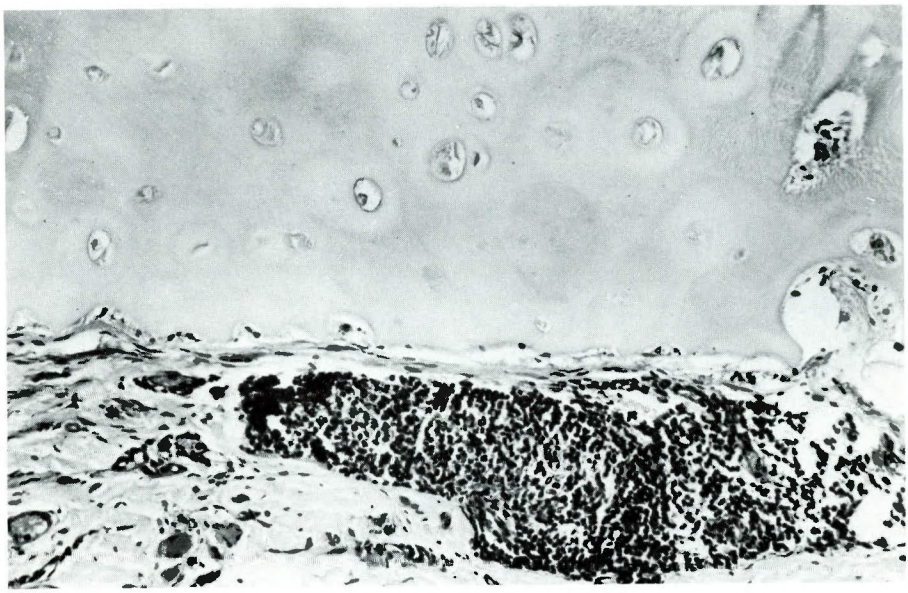


Fig. 4. Lymph follicle in the direct neighbourhood of a xenogeneic lyo-cartilage graft 240 days after operation. Resorption lacuna on the right side. H.E. × 160. (from Sailer[1]).

implanted had no influence on sensitization. On the contrary, a very small quantity of fresh allogeneic cartilage grafted resulted in sensitization, *i.e.*, the degree of antigenicity, not the quantity of the implants influenced sensitization of the animals. It could be calculated that fresh allogeneic cartilage is 500–1000 times more antigenic than allogeneic and xenogeneic lyophilized cartilage. There was only a slight difference between allogeneic and xenogeneic lyophilized cartilage. The migration indices for xenogeneic lyo-cartilage are slightly lower than for allogeneic lyo-cartilage. This slightly different degree of antigenicity is already sufficient to cause the appearance of lymph follicles, giant cells and more resorption. No cytotoxicity and no cross-reaction was revealed.

Discussion

Modern reconstructive surgery of the facial area is no longer imaginable without the use of allogeneic or alloplastic material. Whenever possible, we prefer materials of biological origin. Common with alloplastic materials, lyophilized tissues are preserved almost indefinitely, regardless of the surrounding temperature. They need no care at all when stored in the dried form under vacuum; this is in contrast to most other preservation methods, for instance preservation by Cialit® ; because of the risk of infection the Cialit® solution has to be changed every third to fourth week and continuous bacteriological examinations are necessary. Lyophilized allogeneic cartilage is not only incorporated like any other allogeneic or alloplastic material, it is transformed into the patients' own bone to a high degree by creeping substitution. Ossification takes place after calcification of the lyo-cartilage matrix.

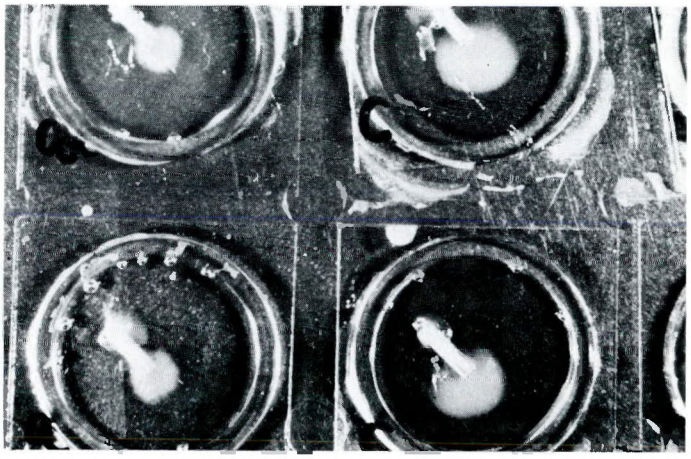


Fig. 5 Cell culture dish with capillary tubes which contain the isolated leucocyte population; the culture media in the two dishes on the left contain antigens (a), whereas the media on the right are controls, *i.e.*, antigen-free. The clearly visible inhibition of migration on the left suggests sensitization. (from Sailer[1]).

Undoubtedly, resorption occurs but is clinically not measurable and does not question the clinical result.[5] Concerning the true incorporation of the material by calcification and ossification, our hypothesis is that the amount of residual antigenicity of lyo-cartilage is small enough so that no rejection or resorption occurs, and great enough so that an interaction between graft and host takes place which leads to calcification and ossification.

As our animal experiments demonstrated, there was no infection around the lyo-cartilage grafts. This corresponds to our long-term clinical investigation of more than 2000 transplantations of lyo-cartilage up to the end of 1983; no difference could be found between the extra- and transoral surgical approach. The infection rate was in the beginning 2.6%,[5] then 1.7%[6] and is presently below 1%. This very low infection rate makes lyo-cartilage superior to other cartilage preservations and is based on the remarkable property of lyophilized tissues to absorb high doses of antibiotics during the rehydration of the tissues in antibiotic solution; this could be demonstrated in an *in-vitro* investigation.[1]

In comparison with autogeneic cartilage lyo-cartilage presents some important advantages:

(1) According to the literature the infection rate of autogeneic cartilage is up to 10%.[7]

(2) After subperiosteal implantation there is no bony fixation of autogeneic cartilage to the bone surface; the graft remains movable, and the patient considers it as a foreign body.

(3) Lyo-cartilage shows some, but less tendency of curling or warping than with autogeneic cartilage. The 'balanced cross-section' advocated by Gibson and Davis[8] for autogeneic cartilage should not be done with lyo-cartilage because the peripheral parts of lyo-cartilage show less resorption than the central zones.[1] In lyo-cartilage the whole cartilage material can be used whereas in autogeneic material usually the peripheral zone has to be discarded because of

severe warping. Fixation of lyo-cartilage is dependent on the location, the kind of reconstruction and the pressure of the soft tissues.

(4) Lyo-cartilage is available in unlimited quantities for reconstructive tasks of any size.

(5) Lyo-cartilage can be implanted in infected or irradiated tissues. It needs no supply from body fluids.

(6) Using lyo-cartilage the patient is not exposed to a second, usually painful, operation site including possible complications. This is also true for minor reconstructive work where the removal of autogeneic cartilage is not justified with the consequence that the correction is abandoned completely or alloplastic material with all its possible hazards is used.

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