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Reaction of connective tissue to cartilaginous and synthetic implants in rabbits

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ABSTRACT

In rabbits, the reaction of the surrounding tissue on various implanted biological materials (different types of cartilage), as well as on the synthetic material Silastic®, was examined and evaluated by macroscopic, morphologic and morphometric analysis of the tissue. The implanted materials were studied in order to investigate their potential practical use in reconstructive nasal surgery. The results of macroscopic, histomorphologic and morphometric studies indicate that, for practical use, preference should be given to biological materials rather than to the artificial material, Silastic®.

Key words: morphometry, rabbits, plastic surgery, cartilage implants, synthetic implants

Introduction

In reconstructive nasal surgery, cartilage has been used as a biological material for a long period of time, and probably most frequently (ADAMS, 1987). Due to the small number of cells located in the bulky mass of an extra-cellular matrix, it is slightly antigenic and immune privileged. Since it is nourished from its surroundings by diffusion it can be considered a good implant (ERSEK et al., 1984; GIBSON and DAVIS, 1958; GIBSON et al., 1958). The cartilage is easily moulded, it is sufficiently strong, elastic

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and accessible, and so it may be sterilised and kept for a considerable time (MERCHANT and NADOL, 1994). Moreover, it can be used fresh or preserved, autologous or autogenous, homologous allogeneic, and also heterologous (ADAMS, 1987).

Nowadays, in addition to cartilage, various synthetic materials such as Silastic® (Midland, Michigan, U.S.A.) are also employed in reconstructive surgery of the head and neck. Silastic® is a kind of silicone rubber which, according to some authors, is a convenient material for the practical use due to a weaker tissue reactivity and a better tissue tolerance (BEEKHUIS, 1967; BINDER et al., 1981; MACKAY and BULL, 1983). Other investigators implanted different types of materials such as a rib cartilage, bone or Silastic to patients with injured orbits with encouraging results, particularly for improvement of enophthalmos and diplopia (HEMPRICH and BREIER, 1993). According to these authors, if the rib cartilage could not be used, Silastic® is a very good alloplastic implant material due to its stability and availability. However, there are opinions that Silastic® may be an unsuitable implant, particularly for the application in reconstructive nasal surgery, due to a greater possibility of extrusion or infection (DAVIS and JONES, 1971).

Although the transplantation of cartilage is widely accepted in nasal surgery, there is still no uniform opinion on donor or type of cartilage, nor on the method of its preservation (DONALD, 1986). Furthermore, there exists no definite concept with regard to giving preference to biological or synthetic material for practical use.

Therefore, we decided to investigate the application of different kinds of cartilage as reconstructive material in surgery on the one hand, and the synthetic material Silastic® on the other. Macroscopic, histomorphologic and morphometric data on transplant tolerance were determined.

Materials and methods

Animals

New Zealand white rabbits were used. They were held and bred in a healthy separated colony. There were altogether 12 animals (6 males and 6 females), whose body masses ranged from 2100 to 2400 grams. All animals were healthy, as confirmed by veterinary examination carried out prior to surgery. The animals were separated and placed in wire cages in a bright, airy and warm room, being fed throughout the duration of the experiment with the same concentrated feed, and with free access to water.

Preparation of implants

The human thyroid and rib cartilage were taken from cadavers (aged from 20 to 29 years), who had died in a car crash. All cartilage material (from cadavers and from rabbits) was fixed in 9% watery formalin solution (for 6 days) and then washed with physiological saline. The perichondrium round cartilage was completely removed. After washing the cartilage was kept in 96% alcohol, which was changed three times, and until use was kept at a temperature of up to 4 °C. Immediately prior to implantation the cartilage was again washed in physiological saline. All implants were cut into identical-sized blocks (10×10×2 mm).

Inserting of implants

In all animals the same four kinds of implant were placed under the skin. After mild ether anaesthesia, on the back of all rabbits an area of skin (80×80 mm) was depilated paravertebrally, disinfected with Hibisept® (Pliva, Zagreb, Croatia) and covered with a sterile compress. The area was divided into 4 symmetrical quadrants. In each, an epidermal incision of 15 mm was made and 4 subcutaneous tunnels were prepared. In each of them, and in the same order in all rabbits, one block of the following implants was placed: a) in the cranial right tunnel a preserved human thyroid cartilage; b) in the cranial left tunnel a preserved human rib cartilage; c) in the caudal right tunnel the preserved rib cartilage of a rabbit; d) in the caudal left tunnel, medical grade silicone rubber, Silastic® (Midland, Michigan, U.S.A.).

The skin incision was closed with three silk stitches and covered with sterile gauze. The animals were re-dressed three times and the stitches were removed on the seventh day. All the wounds healed without either infection or haematoma (Fig. 1).

Sacrifice of animals

The animals were divided into two groups, (A and B, according to time of sacrifice), 6 animals in each group. Group A was sacrificed 6 weeks, and group B 12 weeks after the implantation.

At the sacrifice of the animals skin incisions were performed again at the same sites. The implants were removed together with their newly formed fibrous capsule of connective tissue and were fixed in a 9% watery formalin solution.

Tissue processing and morphometric analysis

After the usual histological procedure of fixation and embedding of implants in paraffin, serial sections were cut to thickness of 5-7 μm and stained with haematoxylin and eosin, PAS, after Mallory, PAF Halny and Berlin Blue and then analysed by a light microscope (VACCA, 1985).

The thickness of the connective tissue capsule around each implant was morphometrically measured on 30 slides. (Hence, for each animal with four implants inserted, 120 slides were measured.) The number of measurements needed for the investigation was determined by an orientation measurement of a sample, with the presumption that the aberration of the resulting values from the arithmetic mean would not be greater than 10%, according to the formula (KALIŠNIK, 1982):

$$n \quad \frac{200}{y} \quad \frac{s}{\bar{x}}^{2}$$

where n = the number of measurements to be analysed, $\bar{x}$ = arithmetic mean of orientation measurement, s = standard deviation, y = permitted aberration of results from the arithmetic mean.

The thickness of the connective tissue capsule was measured by an ocular micrometer at a magnification of the ocular (eyepiece) of 8×, and an objective of 10/0.25 and 40/0.65. All values were expressed in micrometers (μm).

Student's t-test was used to test the differences between the two groups.

In addition to morphometric, macroscopic and histomorphologic investigations of the mentioned materials were also carried out.

Results

Macroscopic analysis

It was established macroscopically that all surgical wounds healed "*per primam*". No haematoma, exudate or infection was present in implanted pockets. All implants retained their original shape as before implantation (Fig. 1).

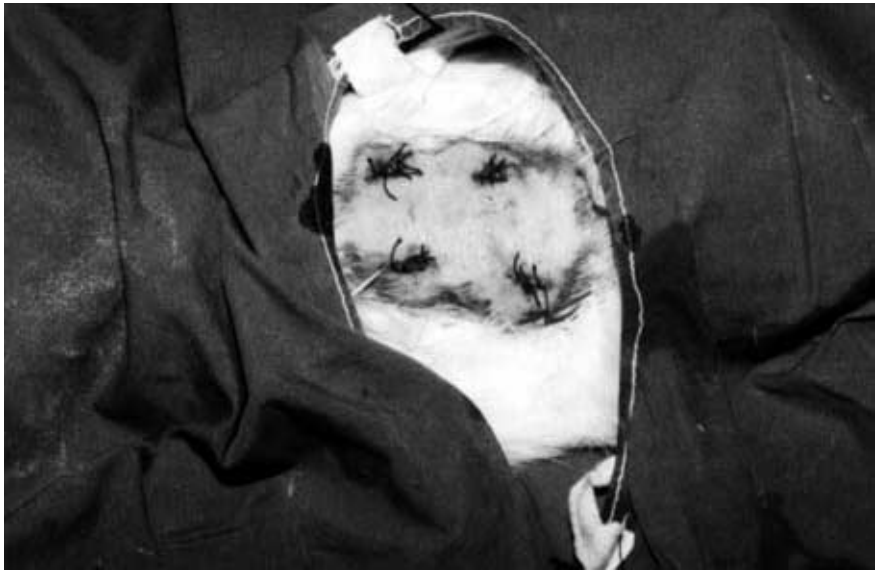

Fig. 1. Implantation of four kinds of implant under the skin of the New Zealand white rabbit

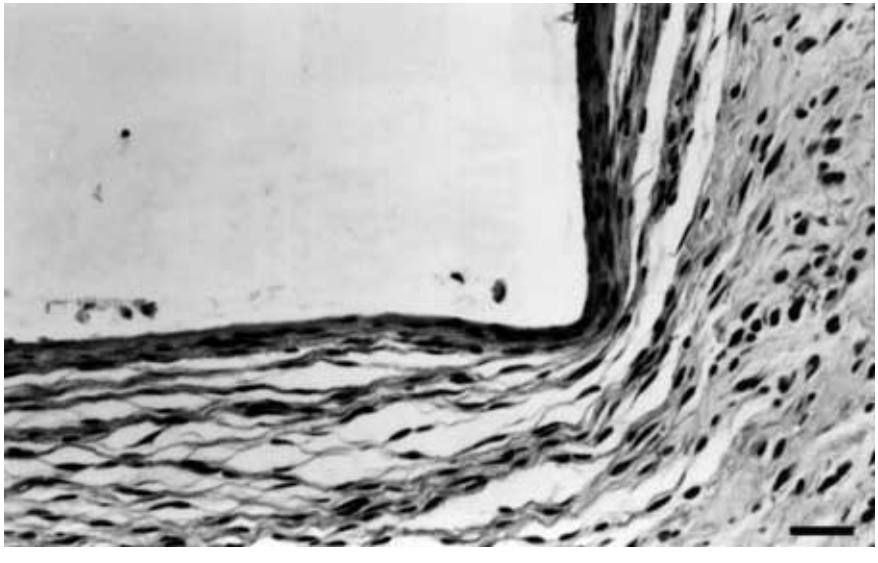

Fig. 2. Connective tissue capsule around implanted Silastic® after 6 weeks (group A). The connective tissue capsule did not infiltrate the implant. HE; 10×10; scale bar = 100 μm.

Histomorphologic analysis

Connective tissue capsules were formed around all implants. Each capsule was firmly bound to the surrounding tissue and implant material, except in the case of Silastic®, where the surrounding tissue did not infiltrate the implant (Fig. 2).

Analysis of the connective tissue capsule around implant showed the following:

1. In the connective tissue capsules around implants of group A, cells prevailed over connective tissue fibres (Table 1).

2. In the connective tissue capsules around implants of group B, connective tissue fibres were more prominent, with a small number of cells (Table 1).

Table 1. The relationship between cells and fibres in connective tissue formed around implants in New Zealand white rabbits.

A group (6 weeks after implantation)		B group (12 weeks after implantation)	
Connective tissue		Connective tissue	
Cells	Fibers	Cells	Fibers
+++	+	+	+++

+ = present; +++ = abudant

Concerning cell types present in group A, fibroblasts, fibrocytes and lymphocytes were noted. In group B, in addition to the mentioned cells, giant cells were also discovered. The location of these cells was limited around the cartilage implants. Giant cells were never present in the neighbourhood of Silastic® implants (Figs. 2 and 3).

The absorption of cartilage implants was observed sporadically, appearing in all kinds of cartilage implants, and the places of absorption were substituted by connective tissue (Fig. 4).

Quantitative analysis

Results of the morphometric investigation are shown in Fig. 5. Morphometric results showed a statistically significant difference ($P < 0.01$):

1. between groups A and B only for Silastic®, as the connective tissue capsule was thinner in group B for Silastic®.

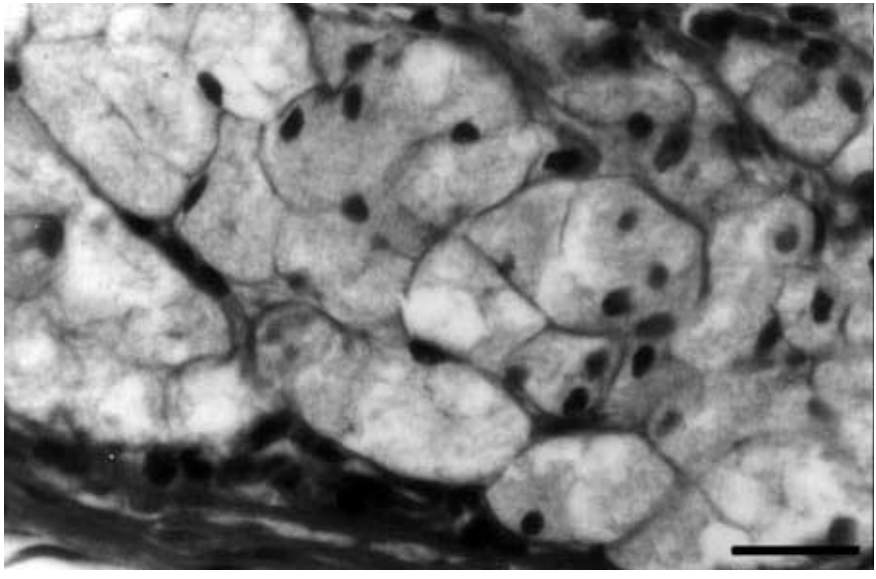

Fig. 3. Giant cells around implanted human thyroid cartilage after 12 weeks (group B). HE; 10×40; scale bar = 20 μm.

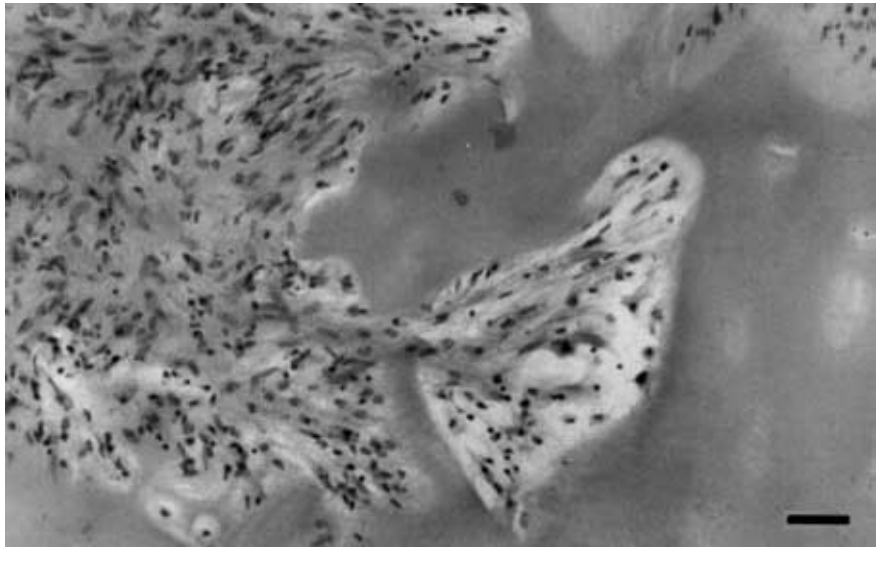

Fig. 4. Human rib cartilage implant after 12 weeks (group B). Invasion of the connective tissue of the capsule into cartilaginous implant at the site of its absorption. PAS; 10×10; scale bar = 100 μm.

2. between Silastic® and all other biological cartilage implants in groups A and B, with the thinner capsule around Silastic® in both groups.

3. between the preserved human thyroid and rib cartilage in groups A and B, with the thicker capsule around the human thyroid cartilage.

There was no statistically significant difference ($P<0.05$) when comparing the thickness of the connective tissue capsule around particular types of cartilage implants between groups A and B (Fig. 5).

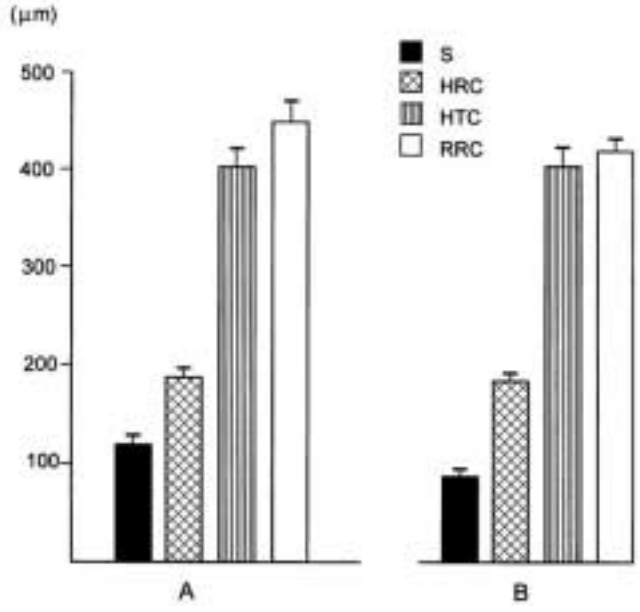

Fig. 5. Thickness of connective tissue capsule (mean ± SEM), around implants: Silastic® (S), human rib cartilage (HRC), human thyroid cartilage (HTC), rabbit rib cartilage (RRC), in New Zealand white rabbits of groups A and B (i.e. 6 and 12 weeks after implantation).

Discussion

For some time, the cartilage was considered to be a weak implant material because of its tendency to absorption. However, in recent decades, owing to various new methods of preservation that ensure sterility and reduce absorption (DONALD and COL, 1982), it has once again become an important implant material. Until now, the preserved homologous (DONALD, 1986; DONALD and COL, 1982; ERSEK et al., 1984; SCHULLER et al., 1977; SWENSON and KOOPMANN, 1984), as well

as fresh analogous cartilage, has been used in practice (DONALD, 1986; JAKSE and WOLFGRUBER, 1986; LENZ and PREUSSLER, 1986).

Moreover, cartilage possesses many advantages in relation to other types of implant material. This is due not only to its anatomic shape, thickness and elasticity, but also to its structure, which prevents stronger immunologic reactions. Namely, cartilage cells (chondrocytes) are located in special cavities (lacunae) in the bulky mass of the extracellular matrix, which is a barrier for immune active cells and also a filter for antibodies (GIBSON et al., 1958; GIBSON and DAVIS, 1959). Additionally, cartilage does not possess its own blood vessels, being nourished by diffusion from its surroundings. All the above-mentioned facts give it the attribute of a good implant, especially because a connective tissue capsule is formed around cartilaginous implants in all living beings.

The presented morphometric results clearly showed that the connective tissue capsule around Silastic® became thinner after certain period of time. This could explain the extrusion of Silastic® implants frequently seen in patients after reconstructive nasal surgery. In contrast, the thickness of the capsule around all types of cartilaginous implants was significantly larger in comparison with Silastic® implants. A thick capsule around cartilaginous implants would permit better fixation of these implants to the surrounding tissue.

Such morphometric, experimental results of the current study suggest that, for practical use, biological materials obviously may be better than artificial materials (in this case, compared with Silastic®), although even in the new literature contradictory opinions still exist.

Some authors stated that cartilage implants developed chondromalacia, lost stiffness and were resorbed after some time (MERCHANT and NADOL, 1994). They also described that after the application of synthetic materials, giant cells appeared as a response to a foreign body, with the microscopic degradation of the implant. This is in accordance with the results of some authors, who also noticed giant cells around implanted polyvinyl alcohol sponge (BAKER and KLAPPER, 1961). The giant cells possessed exceedingly active oxidative enzymes, probably occurring as a response to the implant. It is interesting to note that in our experimental material, giant cells were never present around implanted Silastic®.

Our experimental results for the first time demonstrate a lack of infiltration of the Silastic® implant by connective tissue. This fact, together with the thinnest connective tissue capsule formed around synthetic implants, could be the cause of the implant instability of this kind noticed in clinical practice (DAVIS and JONES, 1971).

In our study, a statistically significant difference was discerned between the preserved human thyroid and the rib cartilage in groups A and B. The connective tissue capsule was thicker around the implanted human thyroid cartilage than around the rib cartilage in both groups. The thicker connective tissue capsule around the implanted human thyroid cartilage suggests that this type of cartilage may contribute to a more effective binding of the implant with its surroundings, and, thus, to its better fastening.

The thickness of the connective tissue capsule round implants for particular types of cartilage implants in groups A and B was not statistically significant. This would suggest an early onset of formation of the connective tissue capsule around all cartilage implants after treatment, which was also confirmed by our macroscopic and histomorphologic results. At the same time, our macroscopic findings demonstrated that all implants were well accepted in the subcutaneous rabbit tissue, with no cases of rejection or infection being noticed.

The connective tissue capsule around Silastic® was the thinnest and could be removed from it considerably easily, when compared to the human thyroid or rib cartilage. This would speak in favour of applying cartilage implants in reconstructive surgery (DAVIS and JONES, 1971; SWENSON and KOOPMANN, 1984). The formation of the connective tissue capsule around the implant is a general phenomenon in all living beings. Consequently, although great evolutionary differences do exist between particular species, the forming of the connective tissue capsule around a foreign body is essentially always the same. The speed of this process may vary, and depends on the reactivity of the particular organism as well as on physical and chemical characteristics of implants. Reaction by the organism to a foreign body always has the same purpose, i.e. to confine it to the smallest possible volume.

On the basis of our histomorphologic findings it follows that the capsule of group B consists predominantly of connective tissue fibres, while a small number of cells were present (mainly giant cells). Giant cells were not discovered around any Silastic® implants.

However, in group A, cells prevailed, with a small quantity of connective tissue fibres. Among the cells fibroblasts, fibrocytes and a few lymphocytes were recognised. The finding of numerous cells in group A supports the opinion of some authors that an organism attempts to reject a foreign body immediately after implantation (VISTNES et al., 1978). When it does not succeed (because of the implant volume or its structure) fibroblasts become activated. These produce collagen (which is important in the forming of collagen fibres), as well as a series of other components,

parts of ground substance. The result of this is the formation of the connective tissue capsule around implants.

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PEZEROVIĆ-PANIJAN, R., Lj. BANEK, M. VUKOJA, D. JEŽEK, Ž. BUMBER: Reakcija vezivnog tkiva na hrskavične i sintetske implantate u kunića. Vet. arhiv 70, 1-12, 2000.

SAŽETAK

Istraživana je reakcija okolnog tkiva na različite implantirane biološke materijale (različite vrste hrskavice) kao i na sintetički implantat Silastic® u kunića. Ova reakcija je procjenjivana analizom makroskopskih i mikroskopskih promjena te morfometrijskom analizom tkiva. Implantirani materijali su istraživani da bi se utvrdila njihova možebitna praktična primjena u rekonstruktivnoj kirurgiji nosa. Rezultati ovih makroskopskih, histomorfoloških i morfometrijskih istraživanja pokazuju da bi u praktičnoj primjeni trebalo dati prednost biološkim materijalima pred umjetnim Silasticom®.

Ključne riječi: morfometrija, kunići, plastična kirurgija, hrskavični implantati, sintetički implantati