Pilot preliminary study on the morpho-functional integration level of the auricle elastic cartilage transplanted in different tissue structures

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Abstract

Background: The nose is a complex and defining organ not only for its respiratory, olfactory and phonatory function but also for facial esthetics. It is plastic and reconstructive techniques are at the same time an otorhinolaryngological issue and an interdisciplinary one.

Materials and Methods: Among the materials used for reconstructive-reparatory surgery of the nose, we can distinguish the elastic ear cartilage transplant from patients own auricle. By analogy, we used in our experiment the rabbit ear as donor site and three radically different types of tissue as integrating structures. The modifications of the cartilages transplanted into abdominal subcutaneous tissue, muscles of the hip and under the skull periosteum were monitored through monthly sacrificing of the experimental animals.

Results: No matter of the presence or lack of own perichondrium coverage the cartilage pieces showed radical transformation to total changes in structure from perichondral fibrosis to endochondral ossification and even complete resorption.

Conclusions: We consider that at least a part of the reconstructive and esthetic plastic surgery failure comes from not knowing these significant changes that take place on the insertion site of own transplanted cartilage. The future surgical guidelines should remember the phenomena described for the first time in our research.

Keywords: auricle, autotransplant, cartilaginous nose reconstruction, endochondral ossification.

Introduction

The complex roles of the nose are carried out on one hand by the respiratory function – a vital one, phonatory respectively olfactory function and on the other hand by great esthetic value as a defining part of the individuals’ physiognomy. This is why the studies concerning the midface are extremely useful also towards the final purpose of achieving a facial transplant. The nasal pyramid reconstructive techniques, as it is considered the midface tampon in traumatology, are an important research direction as preceding steps. These given, the esthetic and functional surgery of the nose are an important part of the plastic and reconstructive surgery. They are a main theme both for professional debating and for malpraxis linked debates, with major influences on the doctors that dedicated their careers to this chapter of radical treatments [1, 2]. The persistence of partial postoperative results is mainly due to surgeon’s lack of experience. They appear more rarely because of not applying correctly the techniques and are oftenly generated by the impossibility of foreseeing the evolution and interaction of the used materials. Whether we are talking about the transplanted tissue or the receiving one, they all have influences over the patients’ quality of life [3, 4]. This is why we considered necessary an experimental study on one of the basic components of these techniques [5]. This is the case of the organic and functional integration of the transplanted elastic auricular cartilage in the upper respiratory airways [6]. The purpose of our work is to enhance the predictability of the level and type of transformation of the cartilage pieces harvested from the auricle with or without perichondrium and transplanted at the level of different tissue structures of the upper respiratory pathways. All these are for better rehabilitation of these cases and subsequently reducing the number of failures.

Materials and Methods

The material for the experiment was represented by six mature rabbits, Mėtis race, one-year-old, weighing 4 kg, originated from the Animal Facility of the University of Medicine and Pharmacy of Tîrgu Mureș, Romania, selected after acceptance from the Experiments on Animals Ethics Committee No. 24 since 11/3/2011.

The surgical interventions meant resection of the right auricle and careful decollation of the skin on both sides for intact preserving of the cartilaginous membrane. The obtained piece was cut in half in order to obtain further two sets of three pieces (with a length of 15 mm and a width of 10 mm) that were implanted afterwards in symmetrical and identical sites of the two halves of the animal body – left and right – as it is described below:

• The ones with perichondrium covering on the right side and the ones without on the left side [7];
• One piece inside the subcutaneous abdominal connective tissue, another one inside the muscles of the hip and the third one under the skull periosteum.

These surgical interventions are classified as moderate impact procedures (pain, suffering, stress) according to the Severity Classification of Procedures appendix from EU Directive 63/2010.

General anesthesia of short duration was achieved by intramuscular administration of a mixture of Xylazine (10 mg/kg body-weight) and Ketamine (40 mg/kg body-weight) as the prescription indicates.

The pieces were extracted afterwards through euthanasia of the rabbits with overdose of the anesthetics (in accordance with the present EU legislation regarding animal protection, respectively The Ethics and Deontology Code for Research, without causing any unnecessary suffering to the animals included in the study) for complex and complete removal of the transplanted tissue and the receiving surrounding tissue.

The tissue fragments were processed through standard histological methods: formalin fixation, sampling, paraffin inclusion, sectioning and Hematoxylin–Eosin stain. Fragments of elastic cartilage, with or without perichondrium, implanted subperiosteal, subcutaneous and intramuscular were examined. Implantation was done simultaneously at all sites and later harvesting of the probes was done at fixed 30 days intervals.

For quantification of the histopathological lesions, we proposed a protocol in which we included the next parameters:

1. Presence or absence of perichondrium covering;
2. Cartilaginous cells – chondroblasts (CB) – younger cells arranged more on the peripheral sites of the cartilage, near perichondrium and chondrocytes (CC) – mature cells disposed all over the thickness of the cartilage which we observed:
   a. Their presence or absence – percentage of quantification;
   b. Number of cells per cavity;
   c. Morphologic transformations: cytoplasm and nucleus aspects.
3. The cavities – chondroplasts (CP) – for which we followed:
   a. Dimensions in relation to normal cartilage, considered element of comparison and harvested from the auricle and immediately examined without implantation;
   b. Morphologic aspects – the form;
   c. Walls discontinuities or confluences with surrounding cavities.
4. Extracellular matrix (ECM):
   a. Basophilia;
   b. Eosinophilia;
   c. Calcifications;
   d. Osteoid tissue formation;
   e. Destruction.
5. Inflammatory reaction:
   a. Present;
   b. Absent.
6. Vascular reaction:
   a. Present;
   b. Absent.
7. Other implant modifications:
   a. Present – which are those;
   b. Absent.
8. Other host tissue modifications:
   a. Present – which are those;
   b. Absent.

Time is quantified since the surgical procedure – implantation moment – until experimental animal euthanasia and histopathological examination of the tissue fragments.

**Results**

Morphologic lesions were studied and interpreted in relation with the implant localization, absence or presence of perichondrium and the period of time until harvesting and histopathological analysis.

The cartilage fragments suffered morphologic modifications in relation with presence or absence of perichondrium, place of implantation and time passed from the moment of the implantation.

For the subcutaneous implant, after one month (M1) the elastic cartilage presented quasnormal properties. For the fragments covered with perichondrium CB/CC were present in a proportion of 90 to 100%. The cellular density was 1–4 cells per cavity.

CP showed uniform characteristics, round, ovoidal shaped equal measurements both central and peripheral, without wall rapture. ECM had an eosinophilic color, as it is normal for the elastic cartilage. Fragments without perichondrium presented similar findings, with cells present in 80 to 100% of the cavities, a little lower cellular density – 1–2 per cavity and small basophilic outbreaks in ECM (Figure 1).

For the intramuscular implant after one month the cartilage was already modified. In the fragments covered in perichondrium CB/CC were present in 70 to 80% of the cavities and the cellular density was 1–2 per cavity. CP from the middle of the fragment were hypertrophied. ECM was eosinophilic. In the fragments not covered with perichondrium the cells were present in only 20 to 30% of the cavities, had bigger volume and a clear cytoplasm. The CPs were hypertrophic in the center of the fragment and mainly empty. ECM was eosinophilic with basophilic outbreaks around CPs.

The cartilage fragments implanted subperiosteal were considered to have normal findings. The team of researchers was confronted with technical difficulties when working with the bone and bone-cartilage junction.

CB/CC are present in approximately 50 to 70% of the cavities of the fragments implanted subcutaneously, after two months, no matter if the cartilage was covered in perichondrium or not. In the fragments not covered in perichondrium the cytoplasm is highly represented, eosinophilic. CPs dimensions are various, with hypertrophy of the central cavities, round or irregular shape. ECM is eosinophilic but basophilic or even calcification outburst occur.

For the intramuscular implantation, the number of cells drops dramatically to under 30% for the fragments covered in perichondrium and under 20–25% for the other ones. The nuclei are picnotic, situated peripheral.

The majority of the CPs are empty, with variable shape and size. In the center cavity walls with discontinuities can be seen, with eosinophilic liquid accumulation, finely granulated the newly formed spaces. ECM is eosinophilic, with an osteoid outburst in the periphery, with high eosinophilia in the fragments without perichondrium (Figures 2 and 3).
Subperiosteal implants, no matter if with or without perichondrium coverage show complete cell absence. CPs are empty, same shape and size, there is no wall discontinuity and hypertrophy. ECM is eosinophilic without calcification outbreak. A fibrous reaction with thickened collagen fibers, numerous connective tissue cells and vascular proliferation can be seen especially around the without perichondrium implanted cartilage.

After six months, for the subcutaneous implant all the cells are disjunct, no matter the presence or absence of perichondrium. ECM is still preserved as well as the phantom-like look of the cartilage. Most of the CPs are hypertrophied or have ruptured walls. The cartilage resorption and connective tissue organization begins.

Intramuscular the cartilage is fractured, almost resorbed in both variants. Fibrous connective tissue forming a surrounding barrier (pseudocapsule) can be seen marking the limit between the cartilage fragments and the striate muscular tissue around them.

Subperiosteal, the endochondral ossification begins starting with the third or the fourth month. The cartilage is completely replaced by osteoid tissue, with disappearance of the cartilaginous ECM and cells (CB/CC).

Figure 1 – (a) Elastic cartilage covered in perichondrium implanted subcutaneously – first month, HE stain, ×7.5; (b) Elastic cartilage not covered in perichondrium implanted subcutaneously – first month, HE stain, ×200.

Figure 2 – (a) Elastic cartilage covered in perichondrium implanted intramuscular – first month, HE stain, ×100; (b) Elastic cartilage not covered in perichondrium implanted intramuscular – first month, HE stain, ×100.

Figure 3 – (a) Elastic cartilage not covered in perichondrium, implanted intramuscular – third month, HE stain, ×200; (b) Elastic cartilage covered in perichondrium, implanted subperiosteal – fourth month, HE stain, ×200.
Discussion

The microscopic study of the cartilaginous fragments shows that no matter the implanting site (subperiosteal, intramuscular, subcutaneous) the original structure of the implanted tissue was changed. The occurring modifications were correlated in intensity and time with the implanting site and the presence or absence of a perichondrium cover. The results of our study show that the perichondrium can delay by one or two months the apparition of degenerative transformations, without the possibility to completely stop them. The presence of cells (CB, CC), the resembling aspects of the cavities (CP) and the eosinophilia of the ECM were kept longer by the fragments covered in perichondrium. There are at the same time radical and spectacular if we consider the complete resorption of the cartilaginous tissues when it reaches contact with muscular tissue [11]. The pieces implanted that reached the fat tissue developed the lesions more quickly.

The quickest and radical transformations were found for the intramuscular implants (striate muscular tissue – skeletal muscular fibers). These modifications appeared since the first month no matter the perichondrium. Six months since implantation the cartilage was found completely modified, fractured and with resorption or fibrous connective tissue organization phenomena.

Subperiosteal the cartilaginous cells (CB/CC) disappeared from the start – the first month, without influence by perichondrium, but ECM and cartilage architecture remained untouched.

Starting from the third month (fragments without perichondrium coverage) and fourth month (fragments with perichondrium coverage) enchondral ossification phenomena appeared. Six months since implantation, the cartilaginous fragments were completely merged in spongy bone tissue.

The eloquent modifications give us the possibility to state for the first time, based on the information gathered from our accessible sources [8–10], that the transformations of the cartilaginous implants are not influenced by the absence or presence of a surrounding perichondrium. At the same time, the quick transformation of the implanted tissue leads to sure and complete modifications of the original tissue, which was found in all 36 pieces with slightly and not so important differences. The modifications are at the same time radical and spectacular if we consider the complete resorption of the cartilaginous tissues when it reaches contact with muscular tissue [11]. The pieces implanted and harvested where big enough to be compared with the ones used for nasal plastic surgery [12, 13] and even auricular/typanic reconstructive procedures [14–16].

We wish to strongly state that under these circumstances the loss of cartilage elasticity is predictable. This leads to the idea that after such a transplant we should not count on nasal pyramid functional esthetics or normal gesture mobility, which may lead to post plastic surgery stiffening [17]. The general stiffening tendency together with possible enchondral ossification makes this method a perfect candidate for reconstruction and anatomic delimitation in the rhino-sinusal skeleton territory [18–22].

Conclusions

It is important to enhance the following: for the first time it has been done a follow-up for the autotransplanted auricle cartilage; we finally have the proof for the transformation that take place in same structural pieces transplanted inside three different histological tissues. The found and studied modifications were major and the autotransplantation determines complete cellular, tissue and mechanical reorganization. A special interest should be given to implantation site and especially its histological structure and the studied material suits best for reconstruction and strengthening of lamellar bony structures.

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