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Testing of an artificial modified bacterial cellulose auricle skeleton in an animal model

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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ABSTRACT:

The aim of this study was to assess whether modified bacterial cellulose can be used for an artificial auricle skeleton.

Introduction: The auricle is a part of the external ear. It consists of skin, cartilage, muscles and adipose tissue. The cartilage gives shape to the structure. There are several indications for reconstruction, such as congenital anomalies, mechanical injuries and burns, and a range of methods have been proposed for complete reconstruction of the auricle.

Material and methods: A bacterial cellulose membrane, at least 25 mm in thickness, was produced in vitro by Acetobacter xylinum culture. The entire artificial human auricle skeleton was made to scale to allow its implantation into an animal model - Wistar rats. Forty rats were divided into four groups of 10 animals. Each group was assigned a different resection time: 14 days, 30 days, 90 days or 720 days. After each resection, an examination of the artificial skeleton and the tissues surrounding it was conducted. The surgical procedure was based on the Nagata technique.

Results: Resection after 14, 30, 90 and 720 days shows progression of the healing process and integration of the artificial skeleton into the animal body. There are no signs of change in the shape or structure of the skeleton.

Discussion: Several surgical techniques and biotechnological methods have been developed over the past few years to improve the results of facial reconstruction. Other approaches can be used to create auricle cartilage, based on scaffolds and chondrocytes.

Conclusion: My findings indicate that modified bacterial cellulose can be used to form an effective artificial auricle which appears to maintain its shape and elasticity, with no signs of degradation.

KEYWORDS: Bacterial cellulose, modified bacterial cellulose, auricle, cartilage auricle skeleton, auricle reconstruction, Acetobacter xylinum

INTRODUCTION

The auricle is a part of the external ear. It consists of skin, cartilage, muscles and adipose tissue. The cartilage gives shape to the structure. Partial and total reconstruction of the auricle remains a challenge, mainly due to the complex three-dimensional structure of the external ear. There are several indications for reconstruction, such as congenital anomalies, mechanical injuries and burns, and a range of methods have been proposed for complete reconstruction of the auricle, of which the most commonly used are those developed by Avelar, Brent, Tanzer and Nagata [2, 3, 4, 18]; however, these are complex procedures that involve autologous cartilage graft from the abdomen - in most cases cartilage from 6 to 9 ribs [4, 5, 6] and can have two to four stages, depending on the decision of the surgical team and complexity of the reconstruction. Although few alternatives to patient's cartilage exist, complete reconstruction is possible using scaffolds with chondrocyte culture, or an artificial auricle made of silicon [13, 15, 19]. The present study describes the concept of an artificial cartilage skeleton made with modified bacterial cellulose, intended for creating an auricle implant, and determines whether it forms a stable and durable three-dimensional structure that will last in the rat body without any changes in the shape or physical proprieties.

MATERIALS AND METHODS

The project was approved by an Animal Bioethical Committee no. 40/ŁB 528/2010 and with an extended period no. 53/ŁB 528/2010

A bacterial cellulose membrane, at least 25 mm thick, was produced in vitro by Acetobacter xylinum culture. The membrane was then subjected to physical modification including mold compression and placed in a bath with a high concentration of sodium hydroxide. The resulting product consisted of modified cellulose with similar proprieties to cartilage [20]. The process of creation and modification of bacterial cellulose is the subject of patent application no. P.412169. The entire artificial human auricle skeleton was made to scale to allow its implantation into an animal model (Wistar rats with an approximate weight of 450 g).

Animas were kept separately in cages before and after surgical procedures.

Animas were sedated with Ketamine and Sedezine mixture administered according to their mass. After the procedure, no additional sedatives or pain killers were administered.

Final cellulose skeleton was sterilized in an autoclave in 120°C for 30 min. Afterwards, it was kept in sealed containers in neutral pH.

Forty rats were divided into four groups, 10 animals each. Each group was assigned a different resection time: 14 days, 30 days, 90 days or 720 days. After each resection, an examination of the artificial skeleton and the tissues surrounding it was conducted.

The surgical procedure schedule was based on the Nagata technique. A pocket in the back was made between the skin and muscles. The prepared and sterile cellulose skeleton was placed inside

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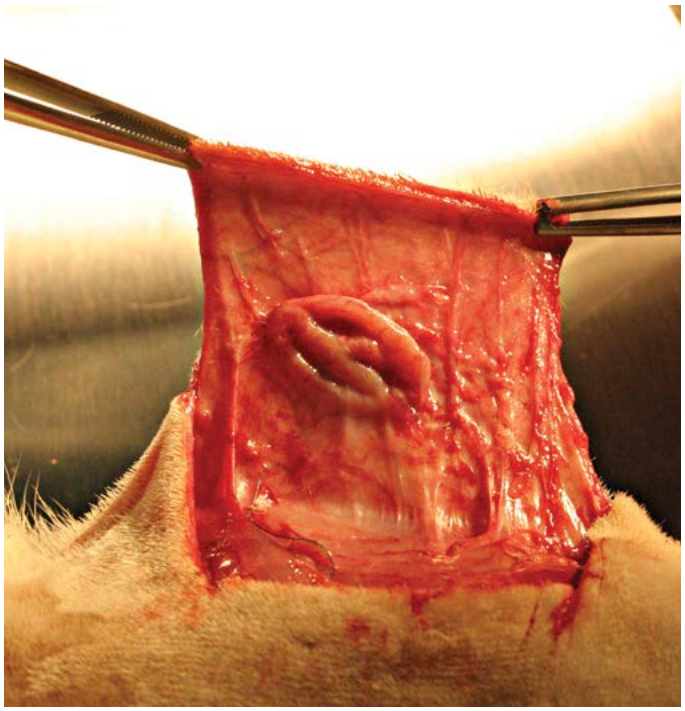

Fig. 1. Modified bacterial cellulose skeleton. Resection after 14 days.

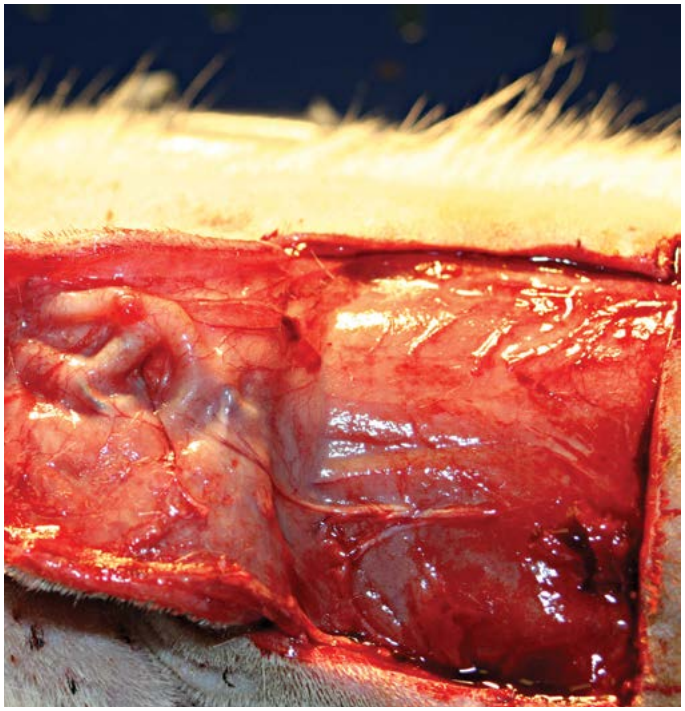

Fig. 3. Modified bacterial cellulose skeleton. Resection after 90 days.

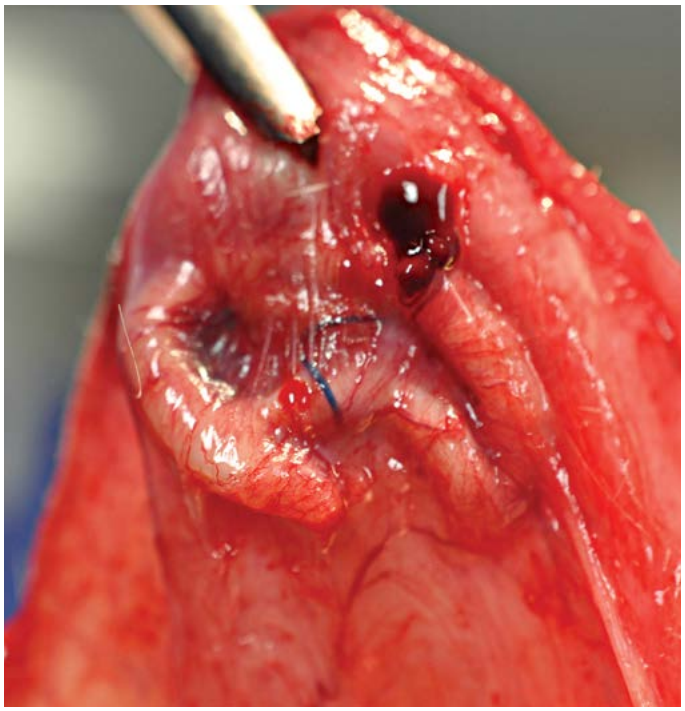

Fig. 2. Modified bacterial cellulose skeleton. Resection after 30 days.

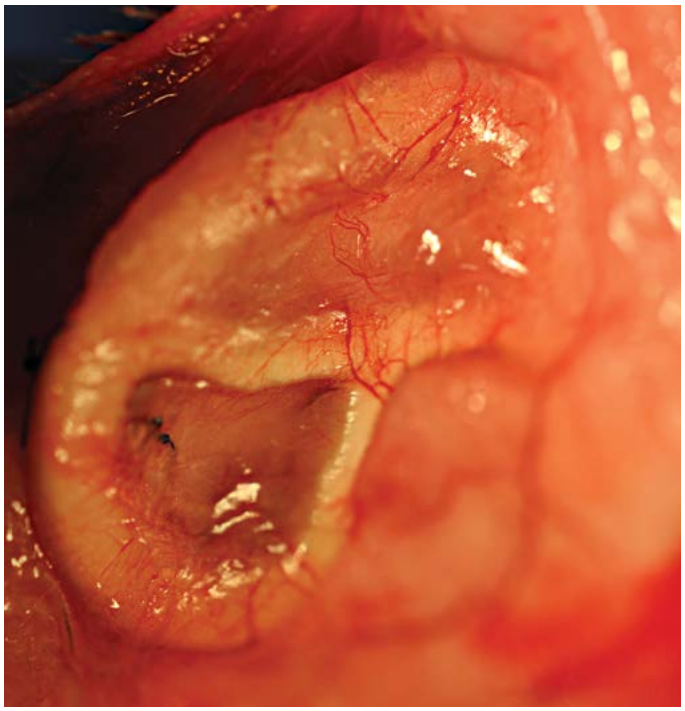

Fig. 4. Modified bacterial cellulose skeleton. Resection after 720 days.

the pocket before being lifted up and fixed to the skin using non-absorbable sutures (1/0). The cut was closed using absorbable sutures (2/0). The artificial cellulose skeleton was left in the body for a given period of time, depending on the group. During this period, all the animals were kept under careful supervision at all times. All were found to gain weight during the experiments. No deaths occurred that were associated with the presence of the implant in the body. Two animals died because they did not revive from anesthesia. After that time, the resection was performed and several factors were observed: the mobility of the implant, which was covered with connective tissue, limpidity of the connective tissue and any signs of swelling, the condition of the vascular system in the connective tissue covering the skeleton, the connection of the implant to the skin and connective tissue.

RESULTS

Resection after 14 days. A minor swelling is visible on the connective tissue covering the implant. A strong connection is observed with the skin and connective tissue. A simple vascular network is visible on the connective tissue. The connective tissue covered all

cavities of the implant, with no free spaces. The connective tissue with the implant inside displays low mobility. The implanted cellulose skeleton was not found to have changed its shape or elasticity compared to a non-implanted skeleton. Resection after 30 days. No swelling is visible on the connective tissue, nor on the tissue around the skeleton. The vascular network is larger than that seen in the resection after 14 days. The connective tissue is almost transparent and it covers all the cavities of the implant. The connective tissue surrounding the implant still demonstrates low mobility. After dissection of the artificial skeleton, it was found that cellulose retained the shape and elasticity of the non-implanted skeleton. Resection after 90 days. Similar to the earlier resections, with no visible swelling. The vascular network is extensive throughout the connective tissue covering the implant. The connective tissue itself is still transparent. The mobility of the implant remains within the elasticity limit of the connective tissue. There is strong connection to the skin but not to the perimysium. Dissection of the implant revealed no change in shape or elasticity as compared to the non-implanted skeleton.

Resection after 720 days. Still no changes can be seen regarding swelling. No other pathological changes either. The cellulose implant is strongly connected to the inner part of the skin, and the shape of the skeleton is still visible on the skin flap even after dissection. The vascular network is well spread and differentiated as regards the sizes of the branches. No physical or morphological changes can be seen in the modified cellulose. No signs of any degradation associated with the prolonged stay in the animal were visible.

DISCUSSION

External ear reconstruction is a highly challenging procedure and one of the most difficult reconstructions in facial plastic surgery. However, several surgical techniques and biotechnological methods have been developed over the past few years to improve the results of facial reconstruction. The first techniques were described by Converse [1], Tanzer [2] and Brent [3]. Later procedures were developed by Nagata [4–6] and Firmin [7], and reduced to two stages. The animal procedures, schedules, implantation place between the skin and muscles, horizontal placement followed by a vertical one, all used in the present study were based on those described by Nagata. The results indicate no problems associated with the implants in an animal model.

Storck et al. warned of the possibility of necrosis of the thin skin and insufficient vascularization [8]; however, this was not observed during or after the examination of the implants. The skin was found to be in physiologically good shape, with sufficient vascularization present under the skin. It should be mentioned that rat skin is extremely well vascularized and therefore it could heal and interact with the implant in a better way that in human body.

Cronin [9] experienced complications such as extrusion and infection when silastic material was used as a framework. However, no problems were experienced with infection of any kind during the present study, and no signs of extrusion of the modified bacterial cellulose skeleton were observed following implantation. In addition, studies by several medical centers where porous polyethylene was introduced [10, 11, 21] indicate that vascularization plays a key role in preventing extrusion. Other approaches can be used to create auricle cartilage, based on scaffolds and chondrocytes. [12–16]. Unfortunately, in contrast to the artificial cartilage described herein, these scaffolds are not able to maintain their form and deteriorate over time, which is the main obstacle in these techniques. No such problem was seen with modified bacterial cellulose: it maintains its shape, even after long periods of implantation, and does not demonstrate any signs of deterioration inside the animal body. Otto et al. also managed to create a chondrocyte scaffold which maintained its shape with no symptoms of degradation [17]. However, from a practical point of view, the technique described herein is simpler and based on a well-known biocompatible material; the sample was also implanted for a longer time in an animal body. Nevertheless, both methods appear very promising and could serve as the basis for future developments in auricle reconstruction.

CONCLUSION

My findings indicate that modified bacterial cellulose can be used to form an effective artificial auricle which appears to maintain its shape and elasticity, with no signs of degradation being observed. The skeleton becomes covered with physiological connective tissue with a delicate but well developed vascular system. Future studies are planned in which 3D scanning and CNC (Computerized Numerical Control) equipment will be used to create more precise molds for the skeletons. These techniques could personalize the process of fitting the skeleton to the patient.

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