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Repair of Articular Cartilage Full Thickness Defects with Cultured Chondrocytes Placed on Polysulphonic Membrane – Experimental Studies in Rabbits

MACIEJ PŁOŃCZAK*[,1], JAROSŁAW CZUBAK, GRAŻYNA HOSER[2], ANDRZEJ CHWOJNOWSKI[3], JERZY KAWIAK[2], KONRAD DUDZIŃSKI[3], KATARZYNA CZUMIŃSKA[1]

[1] *Postgraduate Medical Center, Otwock, Poland*
[2] *Postgraduate Medical Center, Institute of Cytophysiology, Warsaw, Poland*
[3] *Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland*

Autologous osteochondral transplantation is one of the methods that can be used to create hyaline or hyaline – like repair in the defect area. The purpose of the present study was to repair the full – thickness articular cartilage defects in nine rabbits' knee joints with autologous cultured chondrocytes. An articular cartilage defect was created on the patellar groove of the femur. The defect was filled with the chondrocytes cultured *in vitro* and placed into the knee on a polysulphonic membrane. 8 weeks after the operation the reparative tissue was analyzed macroscopically and histologically. The surfaces of the reparative tissue were smooth, and the defects were filled with mature hyaline cartilage in five cases. In two cases the reparative hyaline cartilage was immature and there was worse integration of the grafted tissue into the adjacent normal cartilage. The surface of the grafted area was irregular, the reparative tissue was desintegrated and incompletely differentiated. The results suggest that transplantation of the autologous chondrocytes cultured *in vitro* and placed into the knee on the polysulphonic membrane is effective in repairing of the articular cartilage defect.

K e y w o r d s : chondrocyte cultivation, polysulphonic membrane, repair cartilage defect

1. Introduction

Articular cartilage, which covers the distal parts of bones has a very limited capacity for regeneration. The untreated injuries of this tissue may lead to the osteoarthritis. Many trials to promote the repair of the articular cartilage defects, including trans-

* Correspondence to: Maciej Płończak, Postgraduate Medical Center, Hospital No. 2, Otwock, Poland, e-mail: maciej.plonczak@interia.pl

plantation of chondrocytes [1, 2, 5], perichondrium [3], periosteum and osteochondral fragments [4] have been studied in animals or clinical cases. Autologous osteochondral transplantation is a method that can be used to create hyaline or hyaline-like repair in the defect area. The purpose of the present study was to repair the full-thickness articular cartilage defects in nine rabbits' knee joints with the autologous cultured chondrocytes with the use of the bio-degradable, porous, polysulphonic membrane.

2. Materials and Methods

Chondrocyte Harvest and Culture Technique:
The articular cartilage slices were taken from the knee joints of 16-week-old White New Zealand rabbits. The denuded cartilage was cut into approximately 1-mm-thick slices. Cells were released from the matrix by enzymatic digestion, initiated not later than two hours after the surgery. The sliced cartilage was placed in a sterile glass tube containing 2 ml (0.25%) of trypsine and incubated by 1–2.5 hour. The isolated cells were washed in saline solution (0.9% NaCl) and were resuspended in 5 ml of culture medium (RPMI) containing 3.7% of autologous serum, 10% NCS and antibiotics (penicillinum and streptomycinum). After a few days parts of the polysulphonic membrane cut into approximately 5-mm slices were placed in the same culture flask. The cells were incubated in 5% CO_2 in air at 37ºC. Implantation was performed 14 to 30 days after the biopsy.

The scaffold used in our research was produced after the consultation between chemists and surgeons and its properties were established (Figs 1, 2).

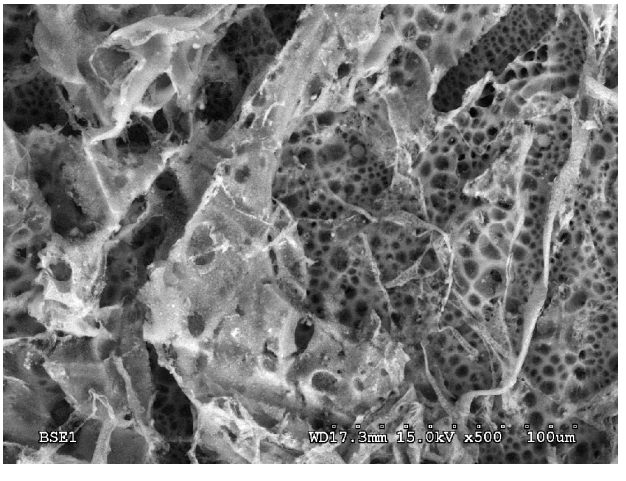


Fig. 1. Membrane of the fourth type – inside the membrane. The picture taken from biological, electron microscope. Enlargement: 500x

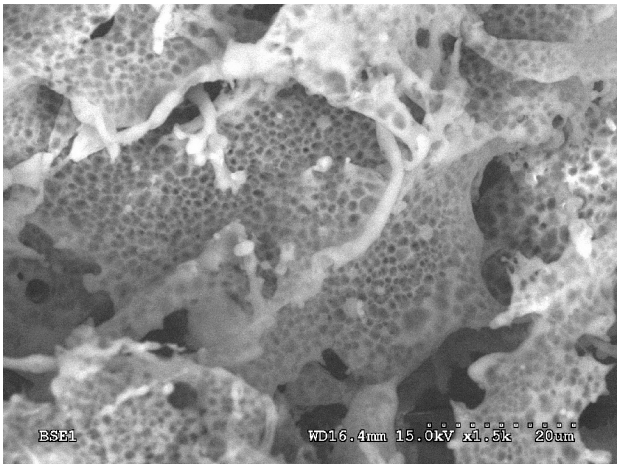


Fig. 2. Membrane of the fourth type – inside the membrane. The picture taken from biological, electron microscope. Enlargement: 1500x

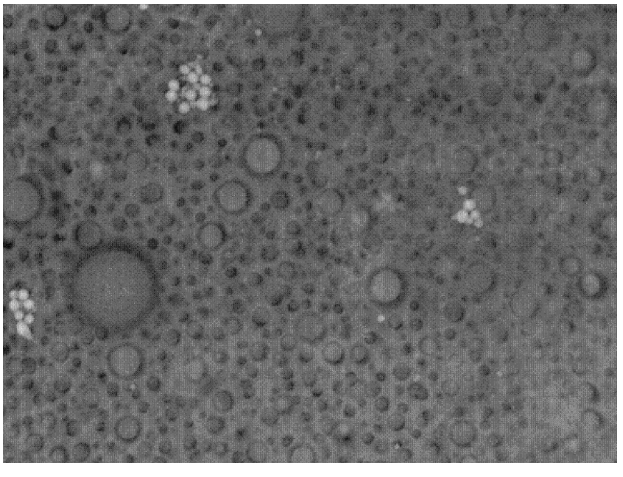


Fig. 3. Outer layer of the fourth type membrane with cells on the surface. Enlargement: 5000x. There are bacteria (white stains), which developed during the trip to Japan

- the scaffold has an appearance (shape) of a flat membrane with wide, open pores
- the diameter of a single slice is about 100 mm
- the scaffold is sterile and not cytotoxic
- it should have higher mechanical endurance in water environment than colagen
- it should not biodegrade too quickly
- it should be easy to attach with the use of surgical suture and tissue glue
- it should be easy to preserve without compromising its properties.

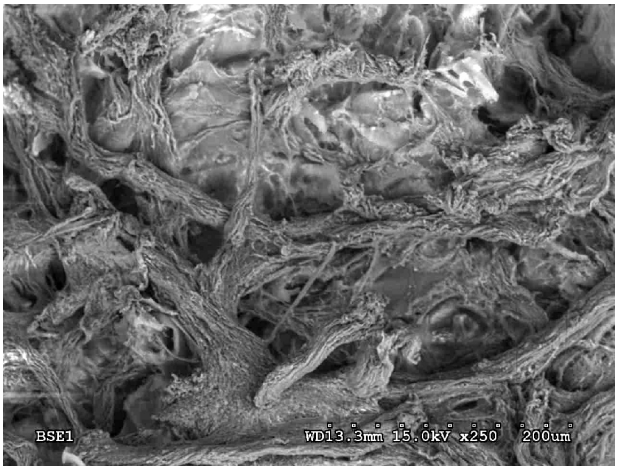


Fig. 4. Membrane of the fourth type – inside the membrane. Protein fibers composed of chondrocytes surrounded by matrix dried (liofilized) in vacuum. Enlargement: 250x

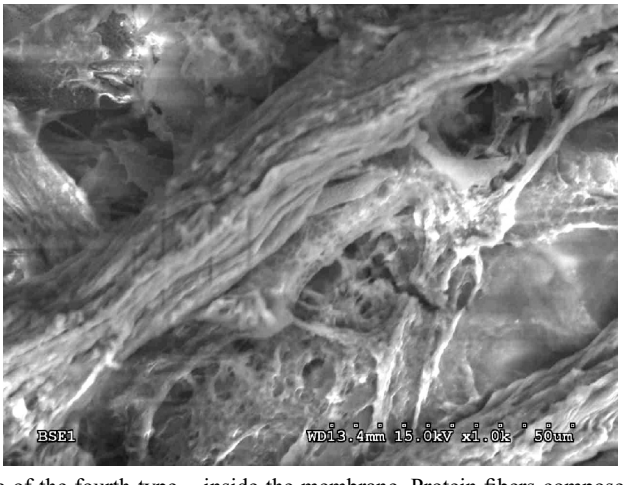


Fig. 5. Membrane of the fourth type – inside the membrane. Protein fibers composed of chondrocytes surrounded by matrix. Enlargement: 1000x

The wide-pore membranes with open pores were obtained in the process of saturation of chromatographic quality paper Whatman nr 3 or nr 2 by a membrane forming mixture composed of 18 g polysulfone Udel 1700 or polyethersulfone Ultrason E2020P and 8g of polyvinylpyrolidone with molecular weight 10 kD solved in 92 cm^3 of mixture 1:1 v/v N,N-dimethylformamide and N-methylpirrolidone. After saturation the paper was immersed in deionized water. When coagulation and washing of the pore precursor was complete, the cellulose was dissolved in ammonium copper II complexes. After solving of the pore precursor (cellulose) the completely prepared membrane was washed in 5% solution of nitric V acid and in sterilized,

demineralizated water till until cooper ions disappeared in both solutions. Finally the membrane was preserved in 70% etanol-water solution sealed in medical grade polyethylene bags.

Chondrocyte transplantation:

A group of 10 White New Zealand rabbits weighting 2–3.5 kg was used in the experiment. One rabbit was excluded from the analysis of the results because of a infection due to a surgical error. The surgeries on the rabbits were performed under combined general anesthesia using 30 mg/kg ketamine hydrochloride and 2 mg/kg xylazine administered intramuscularly. After a medial parapatellar incision, each patella was dislocated laterally and the articular cartilage defect, penetrating into the subchondral bone was created on the patellar groove of the femur. The size of the defect was 5 x 5 mm in width and 4 mm in depth. The defect was filled with the autologous chondrocytes cultured *in vitro* and placed on the polysulphonic, porous membrane (Fig. 3÷5). After the operation, all the animals were allowed to walk freely without any splinting in the cages. 8 weeks after operation, the reparative tissue was analyzed macroscopically and histologically.

3. Results

In the monolayer culture all of the cells presented a fibroblast-like morphology after 2–3 weeks of the culture. The cell number after 4 weeks increased approximately 4 to 5 times the initial number.

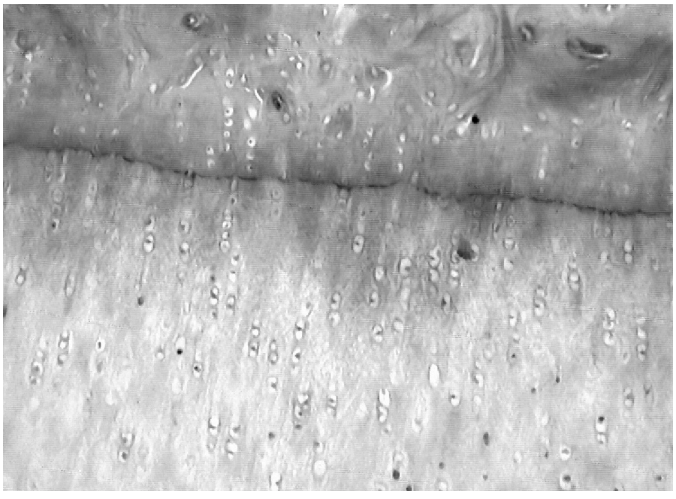
In macroscopic examination in six cases the total fulfilment of the defect with regenerated tissue was revealed. The tissue had smooth, white, glossy surface and was completely integrated with the surrounding cartilage. In three cases the surface of the newly formed tissue showed irregularities, the defect was partially filled and incompletely integrated with the residual cartilage.

In microscopic examination 8 weeks after operation, the surfaces of the reparative tissue were smooth, intact and the defects were completely filled with mature hyaline cartilage, showing good structural integrity in five cases (Fig. 6a and b). In two cases the reparative hyaline cartilage was immature and there was worse integration of the grafted tissue into the adjacent normal cartilage. In two cases the surface of the grafted area was irregular, the reparative tissue was desintegrated and incompletely differentiated.

4. Discussion

This is the first report describing the effectiveness of chondrocyte transplantation placed on the polysulphonic membrane. In the present study the osteochondral defects healed successfully in only five of nine cases due to a rather short period between the implantation and macroscopic and microscopic examinations.

a)



b)

Fig. 6. a) The linear order of chondrocytes in the tissue is well visible on both sides b) The border-line between the regenerated and the surrounding cartilage

Brittberg [1] reported the treatment of full-thickness defects of the cartilage in the knee with chondrocyte transplantation with monolayer culture. This method seems to be ideal in the treatment of the cartilage defects with the autologous chondrocytes, but there is a risk that transplanted cells in suspension may leak out of the defect as a result of joint motion.

Allogeneic chondrocytes might be rejected from the defect due to an immune response. The articular cartilage has been thought to have low immunogenicity because the cellular antigens of chondrocytes are covered with the extracellular matrices [5].

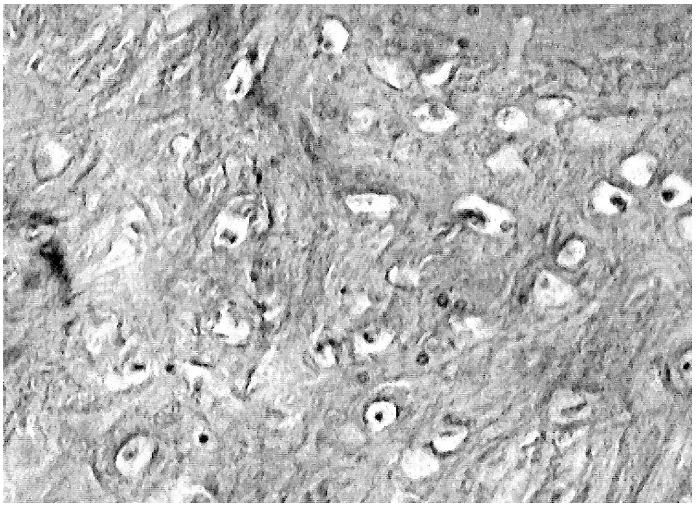


Fig. 7. The regenerated mature tissue. Chondrocytes in isogenic groups placed in cavities

However, once the chondrocytes are isolated from the extracellular matrices, they do show immunogenicity. The chondrocytes used in our experimental model were autologous and an immunogenic rejection could not be induced.

5. Conclusion

The results suggest that transplantation of autologous chondrocytes cultured in vitro and placed into the knee on a polysulphonic membrane is effective in repairing of the articular cartilage defect.

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