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Culture of Human Autologous Chondrocytes on Polysulphonic Membrane – Preliminary Studies

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This work investigated an effective method of isolation and culture of human autologous chondrocytes placed on a polysulphonic membrane. The cartilage was taken from the hip joint of 78 years old woman who underwent total hip arthroplasty due to idiopatic arthrosis and from the knee of 46 years old man with cartilage lesion from non-weight bear area. The cells were released from the matrix in the course of enzymatic digestion. The isolated cells were placed with parts of polysulphonic membrane in the same culture flask and incubated. Due to evaluation the weight of tissue grown on the polysulphonic membrane the elementary analysis was performed. The elementary analysis of the polysulphonic membrane slices after ten weeks of the culture revealed higher concentration of the tissue on one part of the membrane in case of the older woman – 0.726 mg of protein per 1 mg of the membrane then in case of man – 0.513 mg per 1 mg. The established method of isolation and culture of chondrocytes is effective enough to provide a sufficient number of cells that can be used as a transplant.

K e y w o r d s: autologous chondrocytes, polysulphonic membrane, burning analysis

1. Introduction

Articular cartilage, which covers the distal parts of bones, provides sufficient structural stability that enables transmission of large loads from one bone to the other. The cartilage is almost frictionless for bearing surfaces. It can be deformed and regain its orginal shape, it also protects the subchondral bone and has remarkable durability [1].

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The articular cartilage has a very limited capacity for regeneration due to the lack of blood vessels and low mitogenic potency of the chondrocytes. The untreated injuries of this tissue cause pain and loss of joint function in young people and may lead to osteoarthritis [2]. Available methods of treatment are not effective enough to restore quantity, quality and durability of the normal joint surface. Repair is provided by infiltration of stem cells from the bone marrow, through perforation of the subchondral bone. The reparative tissue consists of fibrocartilage which has worse biomechanical properties than hyaline cartilage [3–5].

Limited capability of the articular cartilage to regenerate has gained attention and clinical interest in recent years. Many attempts to promote repair of the articular cartilage defects, including transplantation of perichondrium, periosteum and osteochondral fragments have been studied in animals or clinical cases [6–8]. Autologous chondrocytes transplantation placed on a scaffold is one of the methods that can be used to create hyaline or hyaline – like repair in the defected area [9, 10]. The cells isolated from the pieces of cartilage, taken from a non-weightbearing area of the articular surface, cultured *in vitro* and transplanted into the lesion have a capacity to produce an extracellular matrix, chemically and biomechanically similar to the normal hyaline cartilage [11, 12]. Filling a cartilage defect with the scaffold containing chondrocytes may also facilitate the cartilage repair. Chondrocytes may invade, adhere to it and proliferate and synthesize a new matrix [13–16]. Experimental evidence suggests that implantation of the cells placed on biocompatible matrices based on natural or synthetic polymers may enhance quality of the repair tissue [17, 18]. Many kinds of materials, such as collagen or fibrin gel, carbon fibers, chitosan, hydrogel, or other synthetic gels, can be used as implants into the lesions in the joint surface [14–23].

Both natural and synthetic materials have been researched in this experimental study. The autologous cultured chondrocytes were placed in the articular lesion on a synthetic, bio-degradable, porous, polysulphonic membrane, as well as on a natural, absorbable collagen membrane. The purpose of the present study is to evaluate the effect of culture of chondrocytes placed on the polysulphonic membrane, especially to establish an effective method of isolation and culture of the articular cartilage chondrocytes.

2. Material and Methods

The cartilage was taken from the hip joint of 78 years old woman who underwent total hip arthroplasty due to idiopatic arthrosis and the knee of 46 years old man with a cartilage leasion from the non-weight bear area.

The articular cartilage slices taken from the articular surface of hip and knee joint of 78 years old woman and from 43 years old man were cut into approximately 1-mm-thick slices. In the next phase, the cells were released from the matrix in the course of 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%) collagenase and incubated for 1–2.5 hours. The isolated cells were washed in saline solution (0.9% NaCl) and were resuspended in 5 mL culture medium (RPMI) containing 3.7% autologous serum, 10% NCS, and antibiotics (penicillin and streptomycin). The isolated cells were placed with parts of a polysulphonic or collagen membrane cut into approximately 5-mm slices in the same culture flask and incubated in 5% CO_2 in air at 37ºC.

In order to estimate weight of the tissue grown on the polysulphonic membrane the elementary analysis was performed. Measuring of N-content provides information on the concentration of tissue on the 5 mm diameter slice of the polysulphonic membrane.

3. Results

The elementary analysis of the cartilage culture on the polysulphonic membrane after two weeks revealed concentration 0.094 mg of protein on the 1 mg of membrane in the case of the man and 0.128 in the case of the woman. The mass of proteins on the polisulphonic membrane increased more intensive in the case of the younger man until sixth week – 0.318 mg and 0.259 in the case of the woman. After ten weeks larger mass of protein in the older woman case was observed – 0.726 mg of protein per 1 mg of the membrane and 0.513 mg per 1 mg was observed in the man case.

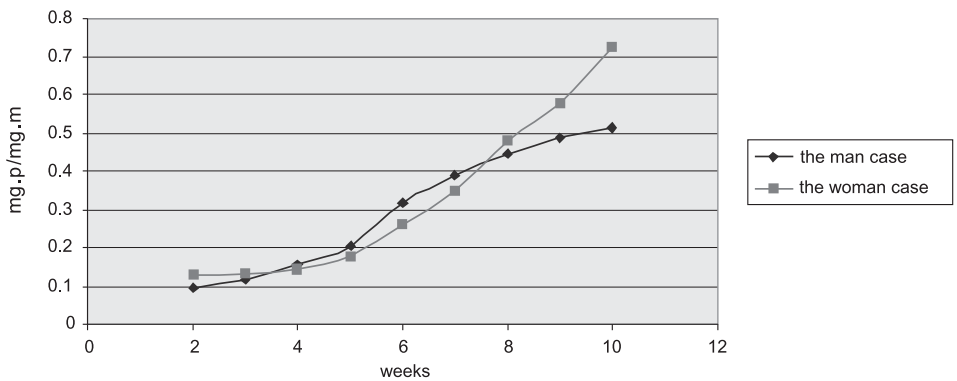

Fig. 1. The mass of protein on the 1 mg of membrane after the two to ten weeks culture of the chondrocytes

4. Discussion

The polysulphonic, semipermeable membrane consists of monomers of sulfon. It possesses a bilayer structure, with compact and porous sides. The compact layer has protective properties for the cells attached to the porous layer where the large

inner surface promotes the cell invasion and proliferation. Due to its wide-meshed interconnecting pores, intensive contact between the cells is possible. The porous structure of the spongiosa material with its interconnecting pores promotes development of collagen fibrils.

The polysulphonic membrane contains sulfate groups [SO_2] concerned with the main chain of polimer. These groups provide an environment similar to matrix of hyaline cartilage where anionically charged glycosaminoglycan chains contain similar groups [SO_2]. This semipermeable material can be held in position by sutures and/or tissue glue which prevent the membrane mobilization under mechanical loading.

The porous polisulphonic membrane is comparable with Chondro-Gide membrane where the compact membrane layer which has a smooth surface is cell occlusive, preventing the injected cultivated cells from diffusing into the synovial fluid and also protecting them from mechanical impact. The other layer of the membrane consists of collagen fibers in a loose, porous arrangement that favours the cell invasion and attachment. The collagen matrix stimulates the autologous cultured cells to differentiate into the chondrocyte phenotype and to produce collagen II and GAG.

The elementary analysis of the polysulphonic membrane slices performed every week during the ten weeks culture and pictures from electron microscopy revealed a high concentration of the tissue on each part of the membrane. Similar research concerned with culturing of any cells on polysulfonic scaffold is unavailable. Three dimensional arrangement of this material *in vitro* conditions enhances the cell proliferation.

5. Conclusion

The established method of isolation and culture of chondrocytes is effective enough to provide a sufficient number of the cells that can be used as a transplant. This method gives the opportunity to culture the chondrocytes in elderly people as well.

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