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Chondrocytes isolation from hyaline cartilage by continuous monitoring method

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Abstract

Background: Articular cartilage has poor regenerative capacities. Numerous cartilage repair techniques are known, including implantation of autologous chondrocytes.

Material and methods: From 18 rabbits pieces of cartilage were harvested from femoral condyle. Minced cartilage was treated with 0.25% trypsin-EDTA. In the 1st group (n=9) the cartilage was digested with 0.6% collagenase in 15 ml tubes by shaking in incubator at 37°C, 5%CO₂. In the 2nd group (n=9) digestion was performed in 25cm² cell culture flasks placed on the lateral side, monitoring the process under a microscope after 120 minutes. The isolated cells were cultured to a 80-90% confluence. The chondrocytes were identified using histochemical staining after culturing for 16 days in overconfluence.

Results: Chondrocytes isolation in the 1st group lasted a fixed 360 minutes, in the 2nd group – 140±10 minutes. In the 1st group were isolated $9.2 \times 10^4 \pm 3.1 \times 10^4$ chondrocytes with a viability of $85.36 \pm 16.41\%$, but in the 2nd group – $1.6 \times 10^5 \pm 3.4 \times 10^4$ chondrocytes with a viability of $98.09 \pm 3.85\%$. The mean period of cell culture in the 1st group was 15 ± 2 days, in the 2nd group – 11 ± 3 days. In first passage of the 1st group were obtained – $1.2 \times 10^6 \pm 4.3 \times 10^5$ chondrocytes and in the 2nd group – $2.92 \times 10^6 \pm 3.6 \times 10^5$ chondrocytes. The secreted extracellular matrix by chondrocytes was stained specifically for cartilaginous tissue.

Conclusions: The method used for chondrocytes isolation has a direct impact on the number of isolated cells, their viability, but also upon the culture period and the number of cells obtained during the first passage.

Key words: cartilage, chondrocytes isolation, continuous monitoring.

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