ISOLATION AND CULTURE OF PORCINE CORNEAL CELLS

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EXPERIMENTAL METHODS

• Primary cultures

Eyes from 6 month old swine (isolation of endothelial and stromal cells) or 80 days old porcine fetus (isolation of epithelial cells) were obtained from the slaughterhouse of Institute for animal science and animal husbandry (D-Braunschweig and D-Mariensee). The cornea was excised with an attached 1-2mm wide, scleral ring and was rinsed three times in Ca²⁺ and Mg²⁺ free PBS (ICN, D-Eschwege). To obtain endothelial cells the inner side of the cornea was treated with trypsin 0.5g/l - EDTA 0.2g/l -solution (Gibco, D-München) and incubated for 10 min at 37 °C. The cells were detached with a rubber spatula and cultivated in 25 cm² tissue culture flasks (Costar, D-Fernwald). To obtain corneal fibroblasts, both endothelial and epithelial sheet were dissected off, the stroma was cut into small pieces and 3 mm explants were attached to a 100 mm diameter plastic dish (Costar, D-Fernwald). Fibroblasts showed outgrowth after 6-7 days. The isolation of porcine epithelial cells was performed with fetal tissue. The cornea was treated as described above. The posterior half of the stroma and endothelium were removed and the anterior portion of the cornea was incubated in 0.8 units/ml Dispase II (Roche Diagnostics, D-Mannheim) at 4°C for 16 hours. Next the cornea was washed twice in PBS and an intact sheet of epithelium was lifted from the stroma. The epithelial sheets were treated with trypsin-EDTA-solution for 10 min at room temperature. Single cells and small clumps were seeded on a 35 mm diameter plastic dish (Costar, D-Fernwald) coated with rat tail acid-extracted collagen. Endothelial cells and fibroblasts were cultivated in DMEM (ICN, D-Eschwege) supplemented with 10% new born calf serum (Life Technologies, D-Egggenstein), 4mM L-glutamine and 1% antibiotic solution (Gibco, D-München). Epithelial cells were feeded with MCDB 153 Keratinozytenmedium (Biochrom KG seromem®, D-Berlin) supplemented as described above.

RESULTS AND DISCUSSION

Isolation and cultivation of stromal and endothelial cells were successful without any problems. Figure 2 and 3 show a primary culture of endothelial cells and fibroblasts with their typical hexagonal and spindle shapes respectively. Isolation of epithelial cells was more difficult. Success in cultivation depends on enzyme treatment, collagen coated substrate, medium MCDB 153 and fetal material. Figure 4 shows a culture of epithelial cells.

Cultivation of each cell type was possible until passage of 4 and more. Growth and morphology upon subculture were similar to that in primary culture and in vivo. Corneal cells were subcultivated in 75 cm² tissue culture flasks at 1:6 split ratios. Cells were successfully frozen in medium containing 10% dimethylsulfoxide as cryoprotectant at -196 °C.

CONCLUSIONS

Both outgrowth technique and selective enzyme treatment have been employed in this work to achieve separation and isolation of the three corneal cell types. We finally succeeded in establishing cultures of different porcine corneal cells. These will be used for the reconstruction of a porcine cornea tissue in vitro for permeation studies.

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REFERENCES