Isolation and Characterization of a Canine Corneal Epithelial Cell Population

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The Canine Cornea

• Functions:
  • Structural support
  • Protection
  • Surface immunity maintenance
  • Optical clarity

• No blood supply to corneal cells
  • Tear film is crucial

• Four primary layers present in canine corneas
Clinical Relevance

• Several types of corneal issues seen in clinics:
  • Keratoconjunctivitis Sicca ("Dry eye" syndrom)
  • Ulcers
  • Abrasions

• In need of mechanism for testing new medications

• Downfalls of in vivo studies:
  • Costly
  • Ethical considerations of animal usage
  • Extensive coordination required
Experimental Objectives

1. Establish population of canine corneal epithelial cells for future treatment studies

2. Determine ideal conditions for culture of epithelial cells from canine corneas

3. Characterize corneal epithelial cell line

4. Cryopreserve corneal cell populations for future studies
Methods

1. Corneal cells isolated from 4-6 corneas from recently euthanized dogs (<1 hour)

2. Cells cultured in T75 flask (+/- collagen coating) fed every 1-3 days

3. Passaged every 7-14 days or until grown to confluency

4. After 2-4 passages, cells either cryopreserved for future use or passaged into smaller plates for immunostaining
Results

• Mixed cell population

• Distinct morphological differences between keratocytes and epithelial cells

• 7-14 days to reach confluency

• Decreased proliferation after 5 passages
Results

Epithelial cells

Keratocytes
Results

• Collagen-coated flasks key to growth of epithelial cells

Non-collagen flask at P2
Results

- Collagen-coated flasks key to growth of epithelial cells
Results: Immunostaining

- Selected antibodies:
  - DAPI – cell nuclei
  - Vimentin – Keratocytes
  - Cytokeratin—epithelial cells

http://www.molvis.org/molvis/v17/a288/yl-fig1.html
Results: Immunostaining

- DAPI: failed at 1:50 dilution

- Cytokeratin: too light, non-specific staining at 1:100 and 1:400

- Vimentin: minimal staining (including controls) at 1:75 and 1:250
Goals for Future Studies

• Successful immunostaining of collagen-derived CEC lines

• Biochemical analysis of cells for vimentin and cytokeratin

• Experiments studying effects of plasma, serum, and other treatment modalities on growth and viability of CECs

• Development and testing of a novel electrolyte solution matching the content of canine tears
  • Compare results to those of plasma and serum
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