The Technique of Impression Cytology in Dry Eye Disease: A Review

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1 Introduction

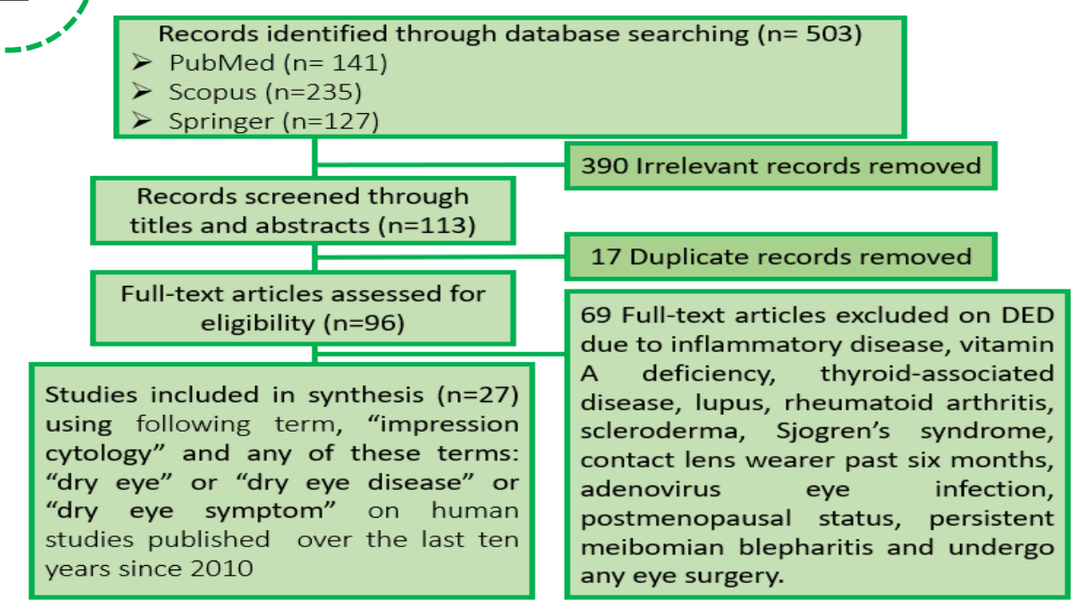
Background

- Impression cytology (IC) is a useful technique to assess ocular surface diseases. This technique can evaluate the epithelial cells morphology changes.
- This review examines numerous techniques of IC are employed in dry eye disease (DED) researches.

Objectives

- Aim of this review were to scrutinise each IC technique with specific modification and its advantages, and to identify the most used IC technique in DED research.

2 Methodology



3 Results

Collection of IC Specimen

- One and 24 studies performed impression at tarsal and bulbar conjunctiva respectively, and two studies did not clearly describe the location of impression.
- Superior bulbar conjunctiva is the most frequent location to collect the sample because the high cells density. Whereas interpalpebral conjunctiva exposes to environment[1] and more desiccated[2].
- Cellulose acetate was preferred filter paper used for IC because it enables to take off up three cell layers of epithelium, preserving the cell morphology[3] and still have possibility to observe goblet cells.
- The pore sizes of filter paper reported were 0.022, 0.025, 0.1, 0.22 and 0.45 µl. The most used filter pore size was 0.22 µm. It affects the consistency of cell collection and the preserves detail better[4-5].

Fixation of IC Specimen

Fixative	Advantages	Disadvantages	Application
Formaldehyde	✓ The penetration of formalin is high. ✓ Cell morphology well preserved in formalin. ✓ Cheap. ✓ Stable. ✓ Easy to make the solution. ✓ Effective fixation for routine laboratory staining of the tissue.	✓ Slow fixation ✓ Formalin reaction with the tissue is reversible, and it can be removed by washing. ✓ Fail to preserve acid mucopolysaccharide. ✓ Highly vascular tissue may have dark-brown granules (artefact). ✓ Exposure to the skin may cause dermatitis. ✓ Chronic inhalation may cause bronchitis.	Effective for routine laboratory staining.
Methyl and Ethyl alcohol	✓ Fast penetration	✓ Inflammable. ✓ Needs licence.	Good for cytology smear

- The filter paper was fixated with immersion fixation to preserve the tissue nearest to its living state and prevent any bacterial growth in the tissue[6].
- The fixation that frequently performed using a mixture of ethyl alcohol, formaldehyde, and glacial acid of 20:1:1 for 10 minutes. Coagulant fixatives such as ethanol or glacial acid act as a primary fixative that fast-acting combined with a crosslinking agent (formaldehyde) will stabilise the precipitated protein[7]. The mixture found to work well with cell morphology in eye samples[8].

Staining of IC Specimen

- The cell sample was commonly stained with periodic acid Schiff (PAS) and counterstained with haematoxylin and eosin.
- PAS identifies goblet cells by their mucin contains[6] which stained in dark pink (PAS-positive) or by their eccentrically placed nuclei, plump shape and large size[9].
- Haematoxylin stains the epithelial cells' nuclei with a blue-black hue, making it easier to observe the specimen's epithelial cells[10]. Eosin is used to stain the cytoplasm by stains rosy red to pink colour.

Staining	Tissue to Bind
Eosin	Cytoplasmic proteins and collagen
Giemsa	Both nucleus and cytoplasm
Haematoxylin	Nucleus and cytoplasm
PAS	Mucin
Papanicolaou (PAP) - Haematoxylin - Orange G - Eosin Azure	 Nuclear Cytoplasmic and keratin component The cell cytoplasm is stained as blue-green colour

Analysis of IC Specimen

- The specimen is evaluated under a light microscope:
  - ✓ the goblet cell density
  - ✓ the cytoplasm's diameter
  - ✓ the nucleus to cytoplasm ratio of non-secretory cells,
  - ✓ the presence of nuclear chromatin.
- Most of the grading scale that been used in this review was introduced in 80, such as Nelson (1983) later improved in 1988, Tseng (1985) and Natadisastra (1987) grading. The latest grading was by Oraza and Marube (2002), and Haller-Schober EM (2006).
- Nelson's was the frequently used grading scale. The cell size and nucleus/cell ratio of Nelson grading have high agreement with planimetry appraoch[11].

4 Conclusion

This review concluded that the cellulose acetate paper of a 0.22 µm pore size is sufficient for specimen collection observing cellular morphology changes. The fixation using ethyl alcohol, formaldehyde and glacial acid of 20:1:1 mixture for 10 minutes works well with conjunctival cell. The PAS staining, haematoxylin and eosin as counterstaining enable highlighting all the epithelial cell and goblet cell structures. Nelson's grading is suggested for grading purpose to assess the conjunctival epithelial morphology behaviour and response in DED studies.

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