The evolution of impression cytology of the ocular surface.

Konstantinos Tourmouzis[1], Adam Master[2], Robert Honkanen[3], Basil Rigas[2*]

[1]Royal United Hospital, Combe Park, Bath, United Kingdom
[2]Department of Preventive Medicine, Stony Brook University, Stony Brook, USA
[3]Department of Ophthalmology, Stony Brook University, Stony Brook, USA

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Abstract

Impression Cytology (IC) allows the collection of cells from the superficial layer of the ocular surface. It consists of three main steps: cell acquisition using membranes placed on the ocular surface, cell transfer from the membrane onto a glass slide or tube, and finally cellular analysis. Since the technique was first introduced in 1977, each of these steps has undergone significant improvement or modification, resulting in a method that is easy, simple, and non-invasive compared to other cell acquisition methods. This mini review examines the gradual improvements to the steps of IC to overcome its limitations, as well as to the analysis of the acquired cells. The remaining limitations of IC are also discussed. Currently, IC is used, among others, in understanding the pathophysiology of ocular surface diseases such as dry eye disease through identification of its inflammatory mediators. It is also the main method of staging squamous metaplasia in ocular cells and is used as a diagnostic tool for multiple ocular conditions.

Keywords: Impression, Cytology, Ocular surface.

Introduction

Impression Cytology (IC) is a method of collecting cells from the most superficial ocular surface layer to allow their evaluation. The initial application of IC was the identification and staging of squamous metaplasia in ocular cells through cytological evaluation. Current IC use includes the identification of inflammatory markers in dry eye disease, in efforts to better understand its pathophysiology. The expectation is that these markers will improve its diagnosis and generate novel measurable outcomes for therapeutic interventions [1]. Other applications include the diagnosis of chronic conjunctivitis, limbal deficiency, vitamin A deficiency, documentation of sequential changes on ocular surface cells as well as immunostaining and DNA analysis [2,3]. Of note, we have recently developed a novel simple process for the preclinical evaluation of candidate drugs using conjunctival cells harvested by IC and grown on mixed cellulose ester membrane filters [4].

IC has multiple advantages compared to other methods of ocular surface cell acquisition. It can provide intact epithelial cells from eyes that are normal as well as from those with ocular disease. IC is non-invasive, rapid, and inexpensive. Discomfort to the patient is minimal and no side-effects have been reported. Finally, it allows for easy repeated sampling to monitor cellular changes over time [3].

Modern IC can be conceptualized as having three distinct main steps, each of which has been improved over time. The first step is cell acquisition of upper layer conjunctival or corneal cells through application of a membrane on the ocular surface. Next, the collected cells are transferred onto a glass slide or into a tube containing buffers for protein or nucleic acid extraction. Finally, the cells can be evaluated with multiple methods, assessing their morphology or molecular features [5].

Since the inception of IC in 1977, there have been significant efforts to improve each of these 3 steps, which have resulted in improved efficiency of cellular transfer, increased chance to acquire an adequate sample suitable for detailed cellular analysis [5,6]. These advances ultimately lead to a process that is faster, simpler, and robust. Here, we highlight recent advances in IC and consider how it can be further improved.

Literature Review

The efficiency of epithelial cell acquisition, the first step of IC, is highly dependent on the type of membrane used. What differentiates the various membranes is the amount of cells they can retrieve, how well they can maintain the integrity of retrieved cells, and how successfully they can facilitate the transfer of cells to a glass slide or tube for evaluation [5].

Two pioneer groups in IC simultaneously introduced in 1977 methods to efficiently acquire cells from the conjunctiva. Thatcher et al. used a plastic applanation cytometer that was pressed on the conjunctiva to collect cells. This approach was an alternative to the scraping and cotton swab techniques that were used to harvest cells at the time [7]. Egbert et al. applied cellulose membranes to the conjunctiva. Their initial aim was to remove the secretions of goblet cells, but later realized that they were able to consistently remove epithelial cells as well, a technique on which modern IC is based [6]. IC allowed faster cell acquisition with a greater amount of cells extracted, while

being easier, simpler and less uncomfortable for the patient than comparable techniques [3].

Several limitations have been reported regarding the original method of manually applying the membrane on the conjunctiva. The amount of cells collected from each application of the membrane depends on the skill of the examiner in applying appropriate pressure to the membrane while collecting a sample [8]. To create a standardized method, an ophthalmodynamometer was first used, demonstrating increased yield when a force of 60g was used instead of 40g or 80g [9]. A second limitation of the original method is the application of a topical anaesthetic to minimise patient discomfort. Such an application, however, dilutes samples and reduces the sensitivity of subsequent PCR testing [10]. In an effort to overcome these limitations, a sampling device called Eyeprim®, was developed, which controlled the application surface and pressure while not requiring topical anaesthesia [11]. However, it seems that there is no significant difference in the amount of RNA collected between use of traditional IC and the Eyeprim® [8].

In the original IC method using cellulose membranes cells were analysed while still attached to the membranes [6]. In 1997, Krenzer and Fredo reported that this generated significant background staining when attempting to stain cells. They unsuccessfully tried to replace cellulose membranes with various membrane materials. This necessitated an entirely new protocol where a nitrocellulose membrane was used to gather cells followed by its dissolution with acetone and cellulase on a separate poly-L-lysine coated glass plate. The dissolution of the membrane significantly reduced background staining without downgrading specimen quality. However, the complete dissolution of the membrane required its sequential exposure to multiple solutions over 3 hours, followed by overnight incubation of each sample, rendering the process time-consuming and complicated [12].

To improve on the previous limitations of the cell transfer process from the membrane to the glass slide, a new glass slide coating was recently developed by Master et al [5]. The coating consists of a novel triblock copolymer of collagen with polyethylenimine and poly-L-lysine, which markedly improved the transfer of cells from the membrane to the glass slide (quantitative). A mixed cellulose ester membrane is initially used to collect conjunctival cells, which is then immersed into the triblock copolymer solution before being applied on a glass slide. The glass slide is then rapidly heated and cooled over 6 minutes, causing polymerization of the triblock components, leading to the binding of cells, copolymer and glass. This makes the cells immediately ready for staining. In contrast to previous methods, cells can be transferred and be ready for staining within 10-15 minutes, greatly reducing the overall time of IC. The efficiency and integrity of cell transfer with this method was significantly improved compared to traditional poly-L-lysine covered slides and albumin-paste covered slides. Overall, this intervention resulted in a quicker and simpler IC method while increasing its diagnostic yield.

The third and last stage of IC involves the processing and analysis of the cells collected. The first and most common mode of cellular analysis used is light microscopy. Earlier staining methods used Periodic Acid-Schiff (PAS) to stain goblet cells and hematoxylin as a counterstain for epithelial cells [6]. This was modified using Gills' modified Papanicolaou stain to replace the previous hematoxylin counterstain, which allowed improved interpretation of squamous metaplasia, and is still in use today [3,13].

The next modality of cellular analysis used in IC was electron microscopy [14]. This technique was first used to investigate patients with mucopolysaccharidoses, the diagnosis of which is best when cells are evaluated at a subcellular level. The study demonstrated a potential for analysis of subcellular, cellular and intercellular morphology thanks to the more powerful magnification of electron microscopy.

Immunocytochemistry, first used in IC in 1990, employed transparent Biopore membranes placed on gelatin-coated slides, without transfer of cells [15]. The difficulty in manipulating these thin membranes (they roll during their manipulation), prompted the use of cellulose acetate membranes, which were firmly applied on gelatin coated slides to permit cell transfer, thereby simplifying their subsequent examination [16]. Immunofluorescence, the primary method of understanding and investigating the immune processes underlying dry eye disease [3], has been attempted with multiple types of membranes and glass slide coatings to improve cellular yield and integrity.

Other methods of cellular analysis used in IC include PCR and flow cytometry. PCR was first used to investigate the presence of inflammatory cytokines in Sjogren's patients [17]. Flow cytometry in IC was initially attempted for HLA-DR and CD23 analysis in ocular surface diseases [18]. The advantages of flow cytometry are its increased standardization and objectivity compared to immune staining, but they are counterbalanced by the requirements for specialized equipment and experienced personnel to process the samples [19].

Discussion

Since its first application, IC has been consistently improving across all its steps. Cell acquisition is now using membranes that allow the non-invasive harvesting of cells with increasingly standardized methods, while also permitting efficient transfer of the cells to a glass slide; the latter has become quantitative with the method recently developed by our team [5]. The potential of cellular analysis has also significantly increased thanks to the ability to use glass slides and the introduction of multiple modes of analysis.

The main limitations of IC are the current need for topical anesthesia due to patient discomfort during sample collection, as well as the difficulty in consistently obtaining an adequate number of cells due to dependance on the examiner's skill [5]. The Eyeprim® device, expected to have solved these issues, may not yet have reached the level of being superior to existing methods [8].

When evaluating each step individually, the step of cell acquisition appears to be the one with the most room for improvement. A device or method that could standardize the pressure applied to the ocular surface, overcome the need for topical anesthesia, and ensure robust cell acquisition will greatly enhance the clinical applicability of IC. In this context, the quantitative cell transfer recently developed by us may

resolve one of the most significant limitations of IC, providing consistently high yields compared to previous methods in a fraction of the time [5].

Conclusion

IC has proved a very important diagnostic and research tool in ophthalmology. It has significant applications in research of ocular surface diseases and the evaluation of squamous metaplasia, in enhancing our understanding of many ocular conditions, and in the assessment of new therapeutic interventions. The continual improvement of IC since its inception has made it the simplest, fastest, and least invasive method for the acquisition and study of ocular surface cells.

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*Correspondence to:

Dr. Basil Rigas,

Department of Preventive Medicine,

Stony Brook University,

Stony Brook, USA

E-mail: basil.rigas@stonybrookmedicine.edu

Tel: + 631-444-9538