Commentary on recent advancements in impression cytology.

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Abstract

Impression Cytology (IC) has dramatically advanced since its introduction as a method for collecting cells from the eye's outer layer. This commentary discusses two significant recent methodological improvements of IC. The first, using a co-polymer, overcomes the quantitatively limited transfer of cells from the eye to microscope slides, making it quantitative. The second is a method for the reliable *exvivo* culture of ocular surface cells that allows the study of the pathophysiology of ocular and systemic diseases and the assessment of candidate therapeutics, accelerating drug discovery.

Keywords Impression Cytology; CPP copolymer; *Exvivo* cell culture; Ocular surface

Description

Impression Cytology (IC) is a pivotal development to the field of ophthalmology, introduced in 1977 as a method for obtaining cells from the superficial ocular surface [1]. Its role has evolved and expanded, with recent advances in the diagnostic landscape of both ocular and systemic diseases potentially reshaping its clinical applicability [2]. Initially developed for identifying squamous metaplasia in ocular cells, IC's versatility has been underscored by its role in diagnosing ocular pathologies such as dry eye disease and ocular surface diseases. The primary advantage of IC lies in its non-invasive nature, minimal discomfort to patients, and its capability to allow repetitive sampling. Recent scientific developments in high throughput bioinformatic analyses (transcriptomic and proteomics) highlight the potential for IC to have a larger and more impactful role in the diagnosis of systemic conditions and scientific discovery.

The IC process is compartmentalized into three distinct steps: cell acquisition, transfer to a medium for examination, and cell evaluation. The original IC method utilized cellulose membranes that required cells to remain attached to the membrane to be analyzed. Keeping cells on the membranes leads to significant background staining making the analysis of specimens with light microscopy difficult, and immunohistochemical methods nearly impossible. Nitrocellulose membranes were later used to avoid this concern as these membranes could be dissolved in a time-consuming process using acetone and cellulase. Although direct staining of cells transferred onto glass allowed for improved analysis, the time required to process the samples using these older methods prohibited rapid assessment of a large number of samples.

Addressing these challenges, Master et al. introduced a new transfer technique utilizing a novel triblock copolymer-glass slide coating composed of collagen, polyethylenimine, and poly-L-lysine [3]. With this method, cells are completely transferred from a mixed cellulose ester membrane to a glass slide that can be prepared for staining within 10 minutes. This novel "glue" method outperforms previously described methods using poly-L-lysine-covered slides and albumin-paste covered slides capturing approximately 60% and 250% more conjunctival cells than these other methods in a rabbit model, respectively. Removal, rather than dissolution of the membrane using a "membrane softening solution" leaves the cells adhered to the slide in the desired location without alterations in their morphology. Depending on what needs to be visualized, after transfer and removal of the mixed cellulose ester membrane, cells are ready for staining using any standard lab protocols including immunohistochemical methods. This improvement in IC technique not only streamlines and accelerates the IC process but also augments the diagnostic yield possible from each specimen.

Drug development is a resource-intensive process that relies heavily on cell-based assays to evaluate potential drug candidates in its early stages, and on costly human trials before final approval is obtained. There is a need for better methods to predict which candidate therapeutics will actually succeed. While immortalized cell lines are commonly used, they often lack predictive accuracy in part because diseases do not typically affect just a single cell, but an organ or system. *Exvivo* models offer a more accurate representation of a disease process but are difficult to establish.

In response, Master et al. developed a simple *exvivo* cell culture process, specifically for ocular surface cells [4]. Conjunctival

cells (both goblet and superficial epithelial cell layers) harvested using impression cytology can be maintained on mixed cellulose ester membrane filters for up to 72 hours with remarkable viability and stable gene expression. Analysis of the expression of 84 interferon and interferon-related genes from cultured cells showed that approximately 70% remain unchanged after 8 hours. Perhaps more importantly, the *exvivo* cultures remain physiologically responsive and informative making them useful for studies of disease pathways or novel drug discovery. The authors showed that cultured cells remained responsive to key inducers such as interferon-gamma, and that *exvivo* cultured cells can be efficiently transfected with plasmids, amplifying their research potential. Additionally a major benefit of *exvivo* cultures is that they can effectively be used to screen for effects of novel therapeutics. Harvested samples from animals or humans can be exposed *exvivo* to novel therapeutics and then assessed for effect using a variety of-omic methodologies, thus greatly reducing the time and cost of this endeavor. This has been done with the novel drug phosphosulindac, revealing its potential to suppress the expression of the cytokine CXCL10, a known participant in the pathophysiology of dry eye disease.

Additional benefits may be realized from these ever-expanding IC advances. A significant advantage, these two novel approaches allow repeated sampling of the same subjects over time preparing the way for longitudinal studies that monitor disease pathophysiology or drug effects. Application of these IC advances also allows its more realistic implementation to assess systemic disease. For example, recent reports have described the plausibility of ocular IC samples to assess for Zika virus in neonates [5]. However, systemic assessments utilizing IC and the recent advances reviewed here need not be limited solely to ocular conditions. The described advances might have utility testing susceptibility of systemic malignancies, biopsied by other means for diagnostic purposes, to chemotherapeutic agents in an *exvivo* setting possibly streamlining therapeutic planning.

The greatest impact of the two methodological advances we reviewed here may perhaps be in accelerating drug discovery. One can envision, for example, the rapid and efficient screening of large compound libraries at an early preclinical stage, saving significant effort and expense, and ultimately bringing new therapeutics to the patient in record times. IC continues to advance and improve as a diagnostic and investigative tool. The importance of IC cannot be overstated. One can only anticipate its ever-growing significance in the world of ophthalmology and medicine in general.

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