Impression cytology of the ocular surface—research tool or routine clinical investigation?

Impression cytology, with cellulose acetate filters, was introduced in 1977[1] as a minimally invasive conjunctival biopsy. It provides an alternative to conjunctival diagnostic excision biopsy or conjunctival smears made from scrapes taken with a blunt spatula. It was found that cellulose acetate filter paper pressed onto the ocular surface removed one to three cell layers of the surface epithelium, preserving its morphology and permitting the use of a limited range of histological techniques.[2 3] Impression cytology provides a flat mount of an area as large as the size of the applied filter paper with well preserved morphology. By comparison, conjunctival smears destroy much of the morphological information and conjunctival biopsies provide information on a relatively small sample of the surface epithelium, both because of the difficulty of preparing flat mounts and because of their necessarily small size. For these reasons impression cytology is ideal for sampling the corneal epithelium. Impression cytology is therefore the sampling technique of choice, providing the surface epithelium is the target tissue of interest rather than the basal epithelium or basement membrane.

Not surprisingly the technique has made an increasing contribution to our understanding of surface diseases of the eye. It has been modified by different authors. Initially it was used for qualitative and quantitative evaluation of the degree of squamous metaplasia and goblet cell numbers in ocular surface diseases such as dry eye, Stevens–Johnson syndrome, pemphigoid, and vitamin A deficiency, and the effect on these of various therapies.[2–5] It has also been used to demonstrate corneal epithelial abnormalities, including the presence of goblet cells indicating a stem cell deficiency disorder[6 7] and corneal squamous neoplasia.[8] Papanicolaou or haematoxylin and periodic acid Schiff's stains are commonly used on these specimens. Typically, impressions are taken by pressing a disc or strip of filter paper onto the conjunctival and/or corneal surface in four quadrants from each eye. These may be placed on clear double sided tape on a microscope slide and fixed with a spray fixative or fixed in 95% alcohol and stained free floating. It is probable that the latter technique provides better resolution of the cytology although specimen handling is more cumbersome. Filter pore size affects the consistency of epithelial cell collection and the resolution of cell detail. Larger pore sizes collect cells better but with less well preserved cell detail. Surfactant treatment of the filter paper reduces cell pick up. Most authors have compromised by using surfactant free filter papers of a pore size between 0.22 μm and 0.44 μm.[9]

This technique of cell collection has also been adapted for other ocular surface investigations. The filter paper can be fixed and prepared for transmission electron microscopy where it has been used to demonstrate the features of mucopolysaccharidosis[10] and, in AIDS patients, viral particles in the conjunctiva.[11] It has also been used to harvest cells from the cornea for investigation of DNA polymorphisms using the polymerase chain reaction.[12]

However, it is only recently that the technique has been adapted to allow immunohistochemistry and expand its role still further.[13] Cellulose acetate filters are opaque and may have to be chemically cleared for cytological examination with xylene, destroying cell surface antigens. This, together with background fluorescence and staining of the filter paper, has precluded immunohistochemistry. Recently these problems have been addressed by using either Biopore membranes manufactured by one of the makers of cellulose acetate filters (Millipore Corp, Bedford, MA, USA) or by transferring impressions from the cellulose acetate filter to a gelatin coated glass slide The latter is a more convenient technique than the use of unmounted Biopore membranes which have proved difficult to handle.[14] Using these techniques a variety of cell surface markers and cytokines have been detected in impression cytology specimens.[13 14]

All these methodologies have been invaluable as research tools but have not yet become routine diagnostic tools in most clinics because they are relatively cumbersome and time consuming for the clinician and pathologist alike. However, Thiel *et al*, in this issue of *BJO* (p 984), describe the use of mounted Biopore membranes that combine ease of use with good epithelial cell collection and permit application of all the potential investigational techniques described here. It is to be hoped that this technique will bring impression cytology from the clinical laboratory into routine clinical use.

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