Goblet Cells of the Normal Human Bulbar Conjunctiva and Their Assessment by Impression Cytology Sampling

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ABSTRACT **Goblet cells of the conjunctiva are the main source of mucus for the ocular surface. The objectives of this review are to consider the goblet cells as assessed by various histological, cytological and electron microscopy methods, and to assess the consistency of published reports (over more than 25 years) of goblet cell density (GCD) from impression cytology specimens from nominally healthy human subjects. Reported GCD values have been notably variable, with a range from 24 to 2226 cells/mm^2 for average values. Data analysis suggests that a high density of goblet cells should be expected for the healthy human conjunctiva, with a tendency toward higher values in samples taken from normally covered locations (inferior and superior bulbar conjunctiva) of the open eye (at 973 +/- 789 cells/ mm^2) than in samples taken from exposed (interpalpebral) locations (at 427 +/- 376 cells/mm^2). No obvious change in GCD was found with respect to age, perhaps because the variability of the data did not allow detection of any age-related decline in GCD. Analyses of published data from 33 other sources indicated a trend for GCD to be lower than normal across a spectrum of ocular surface diseases.**

KEY WORDS **conjunctiva, conjunctival impression cytology, goblet cell density, goblet cells, impression cytology, mucus, transmission electron microscopy**

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I. INTRODUCTION

Based on most models of the micro-"structure" of the tear film and its interface with the ocular surface cells, a mucus layer or gel should form part of the normal tear film structure.[1] This should be part of the double layer associated with the palpebral surface of tear films when a contact lens is worn.[2] A mucus covering provides support for the overlying tear film, helping to keep the ocular surface moist and lubricated. The epithelial cell layer that makes up a healthy conjunctival cell surface includes the mucus-secreting cells of the conjunctiva, namely the goblet cells.[3-6] Goblet cells contain a specific mucin type called *MUC5AC*. In rabbit eyes ex vivo, special electron microscopy preparation techniques can show that the corneal surface (and probably the bulbar conjunctiva) is covered with a thin layer of a porous gel-like mucus complex.[7] In contrast, a much thicker amorphous layer, with less obvious microscopic pores, is present across the palpebral conjunctiva.[8]

The main source of this mucus layer is the goblet cells, present across most parts of the ocular surface.[9] Goblet cells were first noted because of their unusual shape (*becher-zellen*) compared to surrounding epithelial cells rather than because they were recognized as a source of mucus.[10] However, the mucus content is the main feature of these cells and the feature by which they are likely to be identified in tissue specimens subjected to standard histological examination, even to the extent of staining for different types of mucus content.[9-14]

Goblet cells of the human conjunctiva can also be observed by conjunctival impression cytology (**CIC**), a technique usually credited to Egbert et al, who considered it to be a form of "conjunctival biopsy."[15] Such a descriptive term reflects the fact that a small sample of tissue is indeed removed from the conjunctiva, but the technique differs markedly from true biopsy sampling methods or whole tissue samples used to study the actual structure of the goblet cells. Furthermore, the sample of cells obtained by CIC does not need to be embedded and sectioned, as it is in standard histological examinations of conjunctival

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material. With impression cytology, cells are removed from the ocular surface and placed onto a filter, where staining reveals the cells, including the mucus (goblet) cells. The technique has become a popular research tool and is by far the most common method of assessing the conjunctiva, including the goblet cells.

Over the years, some 475 publications have reported use of CIC to assess the morphological characteristics of cells obtained from both bulbar and palpebral conjunctiva, as well as the cornea and limbus surfaces (PubMed January 1977 through December 2011). Various investigators have published reviews highlighting their own perspectives on the utility of the technique as a simple and noninvasive method to analyze the ocular surface cells.[16-24] While the main use of CIC has been to assess the structural (morphological) features of conjunctival epithelial cells (including goblet cells), the material collected on the filters can be subjected to a range of biochemical (molecular) marker labels (tags) to assess whether the cells are constitutively different in disease conditions, eg, in keratoconjunctivitis sicca (dry eye).[24]

This review concentrates on the application of CIC to the bulbar conjunctiva, especially its use in evaluating the goblet cells. While developing the CIC method that would allow them to visualize the goblet cell secretions, Egbert et al did not provide any data on the actual numbers of goblet cells observed (goblet cell density [**GCD**]), although they did indicate that the density of the goblet cells was dependent on the number of conjunctival cells that were sampled onto the filter. Earlier, when other investigators were trying this novel method to assess goblet cells of the ocular surface, two slightly different grading schemes were developed to facilitate subjective comparisons of the CIC samples. These schemes were proposed by Nelson and colleagues[25-27] and by Tseng and colleagues.[28,29] The Nelson scheme is outlined in Table 1. Since it has become the most widely used (based on citations in published articles), it forms a commonly used basis for deciding whether or not a CIC specimen is considered normal or abnormal. This proposed designation is partly based on making a subjective assessment of the numbers of goblet cells visible across the CIC sample.

The primary purpose of this systematic review of goblet cells, as assessed by CIC, is to allow for two broad issues to be considered as objectively as possible. First, is there now sufficient data available from CIC of the bulbar conjunctiva to allow investigators to objectively determine whether or not their samples show "normal" GCD values, as compared to abnormal values of GCD that might be observed in diseased eye states, such as dry eye? Second, using the data that has been published since the technique and the grading schemes were introduced, is it possible to actually derive a set of normative values for conjunctival GCD, including consideration of the potential effects of age and gender? In this review, some consideration will be given to other methods of assessing goblet cells and their density in an effort to present the CIC data with a balanced perspective.

II. APPEARANCE, FINE STRUCTURE AND ULTRASTRUCTURE OF THE CONJUNCTIVAL GOBLET CELLS

A. Light Microscopic Appearance and Assessment of Goblet Cell Density (GCD)

Goblet cells are best known from their appearance in conventional histology, although their shape can also be apparent in tissue sections examined by transmission electron microscopy (see section II.B). The conventional histological approach, perhaps using mucus-detecting stains, allows the investigator to see goblet cells from the side and to see some detail of the cells (Figure 1).[6] With suitable sections and higher magnification imaging, it is possible to see where the goblet cells are located within the conjunctival epithelium, ie, whether they are more superficial or deeper.

Figure 1 shows a number of features worthy of comment. In this specimen, taken from the upper palpebral conjunctiva of a rabbit, it can be seen that the conjunctiva is generally four cells thick. It is not distinctly stratified, and normally there is little morphological difference between the more superficial versus the deeper lying (basal) cells in close proximity to the underlying basement membrane and connective tissue. In the diseased conjunctiva (eg, moderate-to-severe dry eye), distinct stratification and even keratinization of the superficial conjunctival cells can develop, but these are not normal features.