

Comparative scanning electron microscopy of bullous diseases*

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Abstract: The purpose of this study is to compare scanning electron microscopy findings of the blister roof in three distinct bullous diseases: one intraepidermal acantholytic (pemphigus foliaceus); one due to hemidesmosomal dysfunction (bullous pemphigoid); and one secondary to anchoring fibril dysfunction – type VII collagen (dystrophic epidermolysis bullosa). In pemphigus foliaceus, acantholytic phenomena were readily demonstrated. In bullous pemphigoid, the epidermis had a solid aspect. In dystrophic epidermolysis bullosa a net was seen in the blister roof.

Keywords: Microscopy, electron, scanning; Pemphigus; Skin diseases, vesiculobullous

INTRODUCTION

Scanning electron microscopy (SEM) is a technique used for high-resolution three-dimensional analyses. It is very useful to examine the surface of solid specimens, e.g., in dentistry. In dermatology, one application includes the diagnosis of hair conditions.¹ However, few reports were found on the use of SEM for bullous diseases.^{2,4}

Bullous diseases have different pathogenic factors, and may be due to mutations of proteins involved in intercellular or dermal-epidermal adhesion or to autoimmune, acquired damage to these proteins.^{5,6,7} The cleavage level depends on the proteins involved and their resulting dysfunctions (Figure 1).

The purpose of this study was to compare SEM findings of the blister roof in three distinct bullous diseases: (1) one acantholytic intraepidermal (pemphigus foliaceus, PF); (2) one due to hemidesmosomal dysfunction (bullous pemphigoid, BP); and (3) one secondary to anchoring fibril dysfunction – type VII collagen (dystrophic epidermolysis bullosa, DEB).

MATERIALS AND METHODS

A blister roof from each condition was cut using iris scissors, fixed in 10% glutaraldehyde solution, routinely processed for SEM, with critical-point drying and metal-coating, and subsequently inverted to

expose the inner side of the blister to the scanning electron microscope.

The three diseases were diagnosed by the current gold standard: PF by direct immunofluorescence, which demonstrated intercellular IgG deposition; BP by direct immunofluorescence, which demonstrated linear fluorescence in the basement membrane zone; and DEB by both immunomapping, which showed cleavage below the basement membrane, which remained attached to the blister roof, and DNA sequencing for type VII collagen, which confirmed mutation in the *COL7A1* gene leading to a glycine substitution in protein synthesis.

RESULTS

Pemphigus Foliaceus – under low magnification, in the inverted roof of the PF blister, it was observed that the keratinocytes lost intercellular contact, becoming polygonal; demonstrating acantholytic phenomena, some keratinocytes became rounded, resembling the classic appearance of acantholytic cells under light microscopy (Figures 2 and 3).

Bullous Pemphigoid – under SEM, the blister roof showed a “solid” epidermis, with a smooth surface and no loss of adhesion among keratinocytes; the

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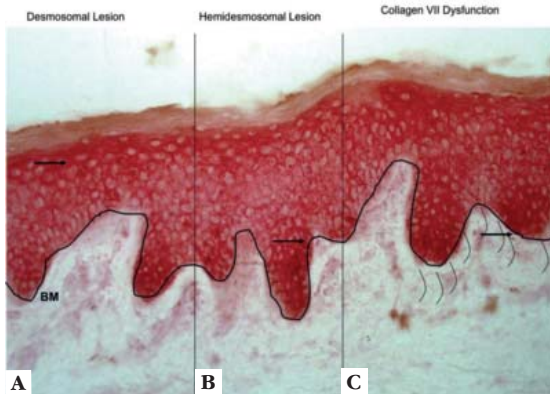


FIGURE 1: Schematic representation of cleavage levels (arrows) in a normal epidermis stained with the APAAP technique. The black line represents the basement membrane (BM). **a)** Intraepidermal lesion in desmosomal defects. **b)** Dermoepidermal cleavage in hemidesmosomal defects, with persistence of the BM in the blister floor. **c)** Dysfunction of type VII collagen (represented by thin black lines) with cleavage under the BM, which remains attached to the blister roof.



FIGURE 2: SEM of a pemphigus foliaceus blister roof, showing isolated acantholytic keratinocytes (original magnification x800)

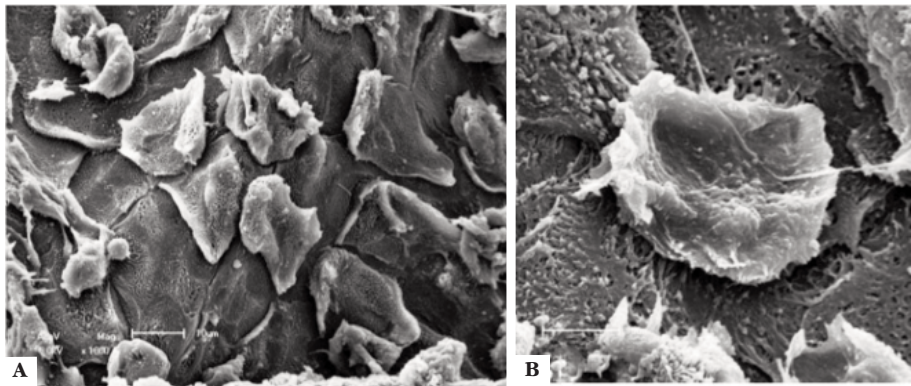


FIGURE 3: SEM of a pemphigus foliaceus blister roof. **a)** acantholytic keratinocytes (original magnification x1,000) with irregular contour. **b)** detail of an acantholytic cell (original magnification x3,000)

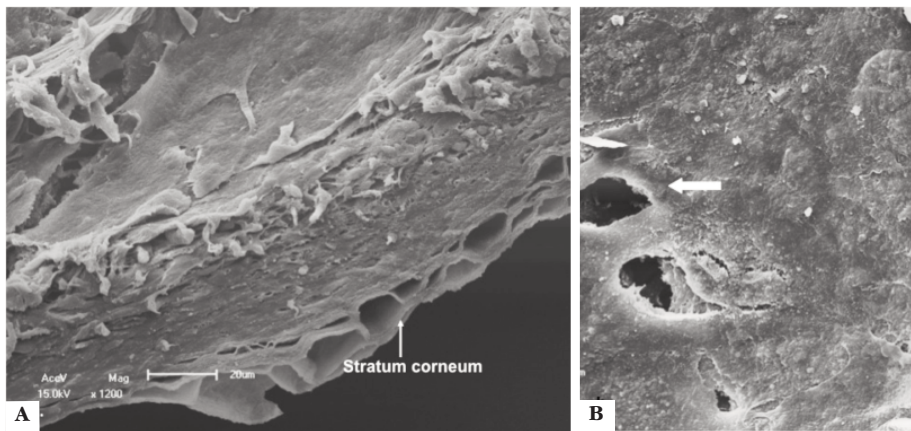


FIGURE 4: SEM of a bullous pemphigoid blister roof. **a)** epidermis without acantholytic changes (original magnification x1,200). **b)** detail of the cell membrane with a "solid" aspect and a visible intercellular space (arrow) (original magnification x3,000)

basal keratinocyte layer could be visualized, showing a regular, flat surface (Figure 4A). In some areas, the intercellular spaces were visible (Figure 4B).

Dystrophic Epidermolysis Bullosa – examination of the inverted blister roof in a DEB case allowed identification of a collagen net attached to the roof, corroborating immunomapping findings, which demonstrated the basal membrane – collagen IV on the blister roof (Figure 5). At higher magnification, an artifactitious detachment of this collagen net was observed in the border of the examined fragment, showing its interwoven and reticular characteristics (Figure 6A). In the area located behind the detachment, the basal cell membrane could be observed (similar to Figure 4B). In some areas, under very high magnification, the attachment of the collagen net to the cell membrane could be seen (Figure 6B).

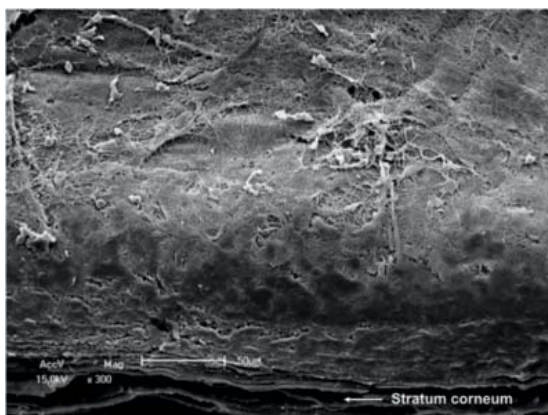


FIGURE 5: SEM of dystrophic epidermolysis bullosa, showing a collagen net attached to the blister roof (original magnification x300)

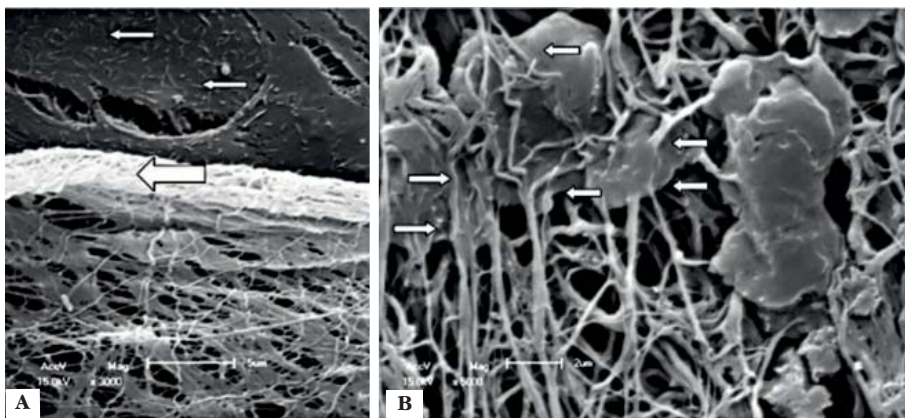


FIGURE 6: SEM of dystrophic epidermolysis bullosa. a) Border of the examined fragment, with artifactitious detachment of the collagen net (large arrow) showing the solid aspect of the cell membrane, with intercellular clefts similar to those shown in Figure 4b (original magnification x 3,000). b) adhesion of the collagen net to the cell membrane, showing normal hemidesmosomal function (arrows) (original magnification x5,000)

DISCUSSION

In biological research, SEM usually provides very illustrative three-dimensional images showing the varied aspects of the specimen.

We did not find any published studies comparing the ultrastructural aspects of blister roofs in the literature. We chose three diseases with different pathogenetic mechanisms leading to distinct tissue cleavage patterns: one intraepithelial acantholytic; one by hemidesmosomal dysfunction, with loss of the epidermis; and another due to type VII collagen damage, leading to loss of the epidermis along with the basal membrane.

In PF, we were able to document the acantholytic phenomena caused by the desmosomal lesion, showing three-dimensionally acantholytic keratinocytes. As expected, the findings were poorer for BP, without acantholysis, and demonstrating the aspect of the basal keratinocyte cell membrane.

In DEB, due to the pathological loss of the basal membrane, we were able to document the ultrastructure of type IV collagen, under pathological cicatricial conditions which is classified as net-forming collagen, in agreement with our findings.⁸ In addition, we were able to observe adhesion of the collagen fibers to the cell membrane in some areas, three-dimensionally demonstrating the hemidesmosomal function of attaching the basement membrane to the basal keratinocyte.

Although SEM is difficult to use in the diagnosis of bullous dermatoses, it proved to be valuable in demonstrating the patterns of tissue injury found in these diseases. □

REFERENCES

1. Suga H, Tsunemi Y, Sugaya M, Shinkuma S, Akiyama M, Shimizu H, et al. Hair shaft abnormalities in localized autosomal recessive hypotrichosis 2 and a review of other non-syndromic human alopecias. *Acta Derm Venereol.* 2011;91:486-8.
2. Almeida HL Jr, Monteiro LM, Goetze FM, Silva RM, Rocha NM. Clinical variability in dystrophic epidermolysis bullosa and findings with scanning electron microscopy. *An Bras Dermatol.* 2012;87:127-30.
3. Hietanen J. Acantholytic cells in pemphigus. A scanning and transmission electron microscopic study. *Acta Odontol Scand.* 1982;40:257-73.
4. Palleschi GM, Torchia D, Pacini P. Scanning electron microscopy in ultrastructural morphology of perilesional keratinocytes in pemphigus foliaceus. *Acta Cytol.* 2007;51:941-3.
5. Almeida Jr. HL . Molecular Genetics of Epidermolysis Bullosa. *An Bras Dermatol* 2002; 77:519-32.
6. Cunha PR, Barraviera SRCS . Autoimmune bullous dermatoses. *An Bras Dermatol* 2009;84:111-24.
7. Oliveira ZNP, Perigo AM, Fukumori LMI, Aoki V. Immunological mapping in hereditary epidermolysis bullosa *An Bras Dermatol.* 2010;85:856-61
8. Gordon MK, Hahn RA. Collagens *Cell Tissue Res.* 2010;339:247-57.

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