MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructure of Gingival Sulcus Mucosa after Application of Hybrid Heliocomposites

V. A. Shakhlamov, T. G. Solnyshkova, and N. V. Artamonova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 1, pp. 95-98, January, 2006
Original article submitted October 5, 2005

Hybrid heliocomposites were used for repair of the carious tooth crown. Ultrastructural study of mucosa biopsy specimens from sites adjacent to the filling 3 weeks after treatment showed that Charisma, Herculite, and Esterfil Ca filling materials led to degenerative changes in the mucosa, while Filtek Z-250 heliocomposite caused no pathological changes in the gingiva at the site of contact.

Key Words: *gingival sulcus; heliocomposite; ultrastructural changes*

Filling materials hardening under the effect of halogen light are now widely used in practical dentistry. These materials are characterized by sufficiently high mechanical strength, chemical stability, they are esthetic and easily polished [1,3]. Composite materials occupy the leading position among repair materials.

Traditional materials are Charisma (Heraeus Kulzer), Filtek Z-250 (3M), Herculite XRV (Kerr), and Esterfil Ca. Sometimes heliocomposite directly contacts with gingival sulcus mucosa during tooth filling, which makes preservation of the morphological structure of the sulcular mucosa after treatment an important problem.

The development of modern esthetic dentistry results in the creation of new filling materials, and improvement of the criteria for their choice becomes an important task [5].

MATERIALS AND METHODS

Fragments of the gingival mucosa were collected at sites of contact with the heliocomposite filling in healthy 25-30-year-old men 3 weeks after the filling was made. Specimens from similar sites of the sulcular mucosa, not contacting with the filling materials, served as the control. The severity of gingival tissue inflammation was evaluated using gingivitis index (PMA) and index of need in periodontitis treatment (CPITN) after AIMANO [2]. Gingival status was evaluated visually and by staining with Schiller—Pisarev solution. In the latter case PMA index was 0 (no inflammation). Probing showed CPITN index equal to 0, which corresponded to normal tissue. The treatment was prescribed after determination of the gingival indexes.

The treatment was carried out under infiltration anesthesia with Sol. Ultracaini D-S (1:200,000, 1 ml). Defects in the tooth crown were repaired by optically hardened filling materials (Filtek Z-250, Charisma, Herculite XRV, and Esterfil Ca). Three weeks after the treatment the gingival mucosa was light-pink in all patients, without signs of hemorrhages, with zero PMA and CPITN indexes.

Fragments (1 mm^2) of sulcular mucosa for electron microscopy were taken under local anesthesia in areas adjacent to the filling from Charisma, Filtek Z-250, Herculite XRV, and Esterfil Ca materials, fixed in 2.5% glutaraldehyde in cacodilate buffer (pH 7.3), postfixed in 1% osmium tetroxide, and embedded in epon and araldite mixture. Semithin sections for target slicing were stained (in succession) with

Laboratory of Experimental Cell Pathology, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow

methylene blue, Azur II, and fuchsin. Morphological structure of the sulcular epithelium and adhesion epithelium was studied. Morphometrical evaluation of the structural components was carried out using Scion Image software. The data were processed by methods of variation statistics. The significance of differences was evaluated using Student's *t* test.

RESULTS

Photooptic picture and ultrastructure of the sulcular mucosa at the site of contact with Filtek Z-250 heliocomposite corresponded to the morphology of normal sulcular mucosa without disorders in its histoarchitecture.

Staining of semithin sections of control samples and samples from patients with fillings showed even basal membrane of the adhesion epithelium, lining the bottom of the gingival sulcus. Basal membrane of the sulcular epithelium was sharply twisted, this appreciably enlarging the area of its contact with gingival liquid.

Basal membrane in patients with Charisma filling was flat, though with perinuclear edema in the basal cells. Perinuclear edema increased in the pricky cell layer (Fig. 1). Focal edema was observed in the connective tissue stroma of patients with fillings from this heliocomposite. Ultrastructural studies confirmed the presence of morphological changes indicating cell degeneration. Acantholysis phenomena, characterized by not only lysis of the cell-cell contacts between the pricky cells, but also by the presence of numerous vesicles in the cell-cell spaces, were detected in the pricky cell layer of the sulcular mucosa at the site of contact with Charisma filling material (Fig. 2, *a*); sharp dilatation of the cisterns of granular endoplasmatic reticulum was observed here (Fig. 2, *b*). These dilatations were situated near the cell plasma membrane and presumably disordered fibril fixation to the membrane, which was seen from irregular width of the cell-cell space (shrinkage to 0.57±0.21 μ at sites of the greatest damage; $p \le 0.05$). Vesicles were filled with homogenous gray matrix and were located between plasma membranes of neighboring pricky cells. Cell-cell contacts were absent in this region. Contacts between the pricky cells without degenerative changes were preserved; the width of cell-cell space was 1.40±0.06 μ ($p \le 0.05$), which virtually did not differ from control biopsy specimens (1.20±0.15 μ).

Surface layer cells were characterized by degenerative changes in the cytoplasm and disappearance of contacts between the cells. The cells acquired a flat shape; their main area presented as a nucleus with a large nucleolus. The degree of keratinization increased in some patients; the epithelium acquired a horny layer, which seemed to improve the mechanical resistance of the sulcular mucosa at the site of contact with the filling material.

The structure of the epithelium in patients with fillings from Herculite XRV heliocomposite was in some cases normal. Pricky cells in sites with normal structure had sharply pronounced cell-cell connections, axons from one cell wedging into spaces between axons of the neighboring cell like the elements of a "zip" fastener. However, at other areas of the epithelium (Fig. 3) focal acantholysis of cell-cell contacts was observed, with sharply extended (to 1.70±0.08 μ; $p \le 0.05$) intercellular space.

Ultrastructural study of the sulcular mucosal epithelium at the site of contact with the Russian heliocomposite Esterfil Ca showed intact contacts between

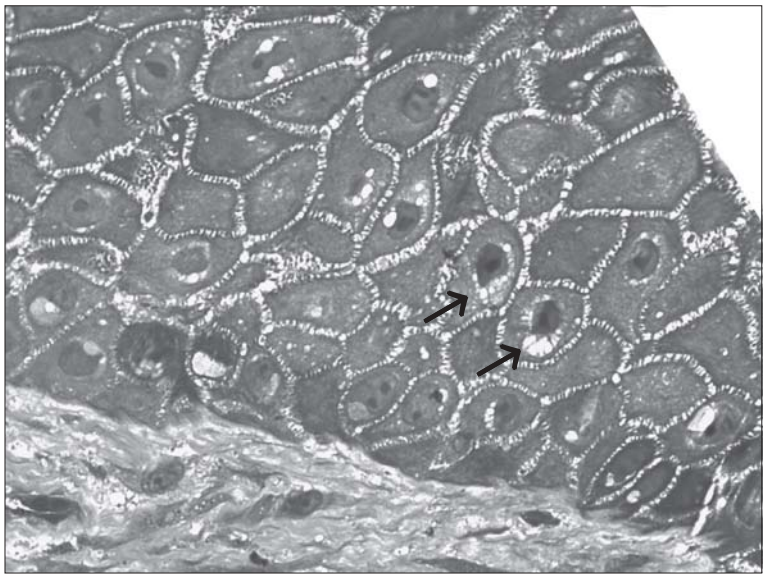

Fig. 1. Biopsy specimen of sulcular mucosa at the site of contact with Charisma heliocomposite. Pricky layer of adhesive epithelial cells, perinuclear edema of basal and pricky layer cells (arrows). Semithin section. Here and in Fig. 3: methylene blue, Azur II, and fuchsin staining; ×1000.

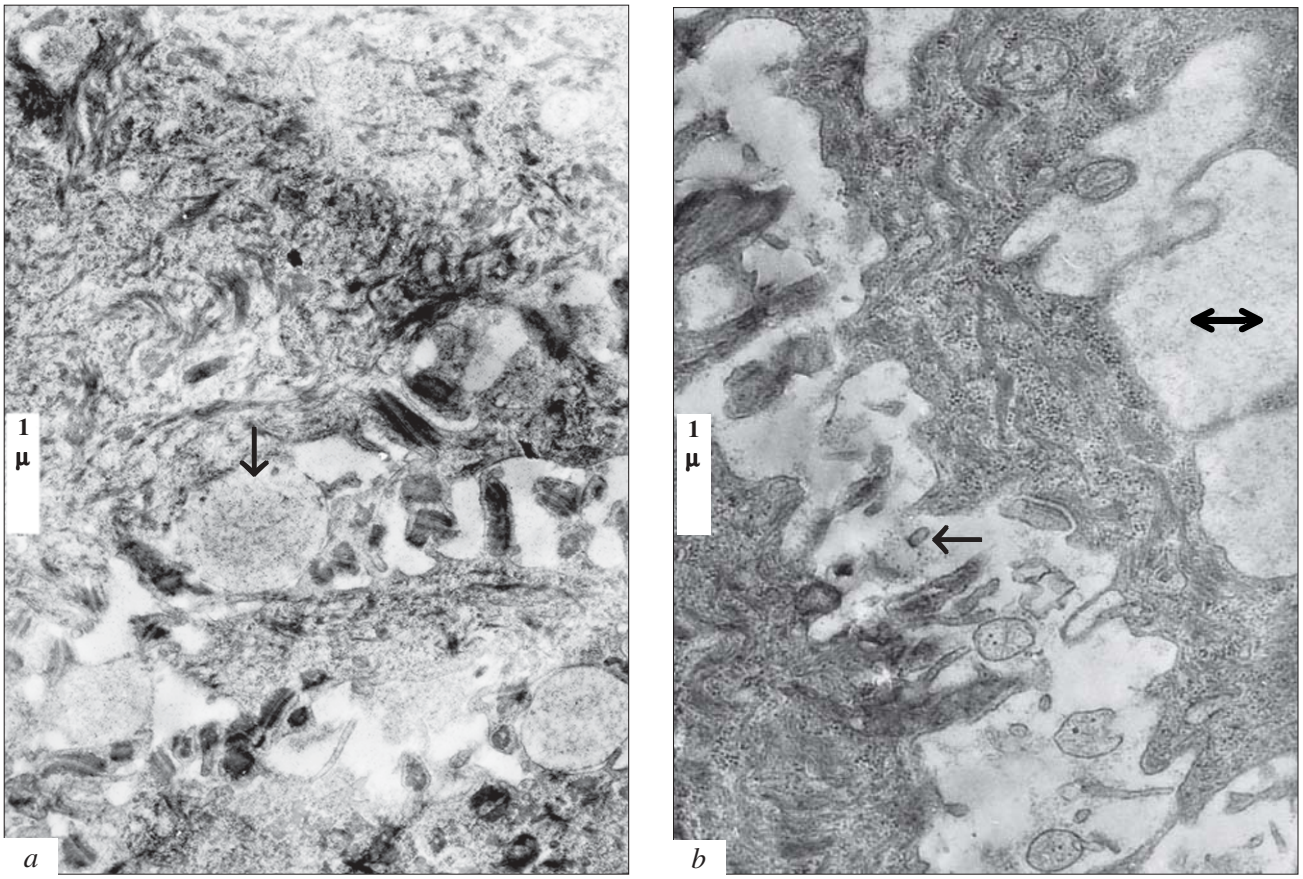

Fig. 2. Ultrastructure of sulcular mucosal pricky cells at the site of contact with Charisma heliocomposite. *a*) vesicles in the cell-cell space (arrow); *b*) sharp dilatation of granular cisterns of endoplasmatic reticulum near the plasma membrane (bold arrow). Destruction of cell-cell contacts (thin arrow).

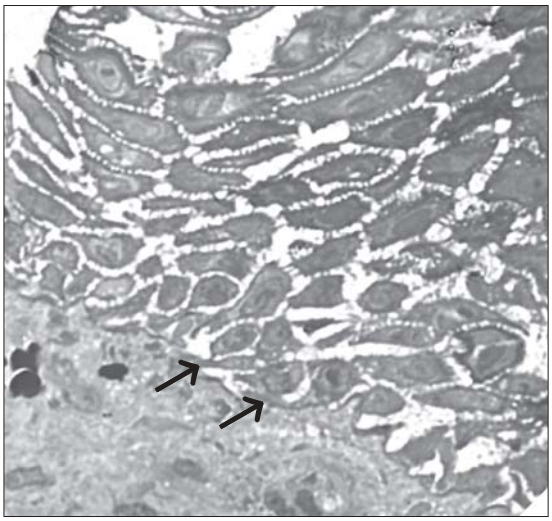

Fig. 3. Biopsy specimen of sulcular mucosa at the site of contact with Herculite heliocomposite. Basal cell layer (arrow); intercellular edema in the pricky layer, dilatation of cell-cell spaces and acanthosis. Semithin section.

pricky cells and shrinkage of cell-cell spaces to 0.55± 0.04 μ ($p \leq 0.05$). Numerous large (up to 0.37 μ^2) vesicles were seen between pricky cells. Cell-cell contacts were completely absent in these areas. Similar vesicles were present in the cytoplasm of pricky cells. Leukocytes penetrating into the oral cavity through the gingivodental fixation epithelium or sulcular epithelium play an important role in the defense function of the oral mucosa [4,6]. Ultrastructural findings indicate drastic shrinkage of cell-cell spaces in the latter case and hence, reduced leukocyte penetration into the oral cavity at the site of Esterfil Ca filling application.

Hence, tooth filling by Charisma, Herculite XRV, and Esterfil Ca materials leads to the development of degenerative changes in the sulcular epithelium at the site of contact with the filling material. Intact histoarchitecture of the sulcular mucosa in patients with fillings from Filtek Z-250 indicates that the use of this filling material is preferable for the treatment of dental crown caries.

REFERENCES

1. L. G. Borisenko and G. G. Chistyakova, *Sovr. Stomatol.*, No. 2, 39-41 (1999).
2. N. V. Kuryakina and N. A. Savel'eva, *Preventive Dentistry* [in Russian], Moscow (2003).

3. I. K. Lutskaya, O. G. Chukhrai, and E. I. Marchenko, *Sovr. Stomatol.*, No. 4, 3-9 (2001).
4. Yu. M. Maksimovskii, L. N. Maksimovskaya, and L. Yu. Orekhova, *Therapeutic Dentistry* [in Russian], Moscow (2002).
4. A. K. Nikolishin, *Modern Filling Materials* [in Russian], Poltava (1998), pp. 3-14.
5. M. D. Perova, *Periodontal Tissues: Health, Disease, Approaches to Restoration* [in Russian], Moscow (2005).