Corneal Biofilm Plaques: A Novel Clinical Presentation

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Purpose: To report a novel clinical presentation of corneal biofilms, consisting of formation of superficial and recurrent corneal plaques.

Methods: Interventional case report. A 9-year-old boy presented with subepithelial, whitish, avascular, and recurrent corneal plaques without any clinical manifestations of active corneal inflammation and/or infection. He had a history of minor ocular trauma; otherwise, his medical history was unremarkable.

Results: An excisional biopsy was performed under topical anesthesia. Histological analysis identified these plaques as clusters of gram-negative bacilli surrounded by an extracellular matrix. Samples were further evaluated with special stains (calcofluor white, Flamingo fluorescent dye, propidium iodide, and Gomori–Grocott) that demonstrated biofilm structures.

Conclusions: Corneal plaques are a very rare clinical presentation of corneal biofilms that allow prolonged survival of microorganisms even in the absence of prosthetic material and clinical signs or symptoms of corneal active inflammation and/or infection.

Key Words: biofilm, corneal plaque, corneal biofilm, ocular biofilm, crystalline-like keratopathy

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Biofilms are clusters of microorganisms that produce a polymeric extracellular substance, which form 3-dimensional structures that adhere to living or nonliving surfaces. Compared with microorganisms in their planktonic form, biofilm formation confers greater antibiotic resistance and greater resistance to the host immune system.[1,2] In humans, formation of biofilms has been detected in up to 65% of microbial infections, which causes concern because these pathogenic structures are challenging to treat.[2]

At the ocular level, previous studies have reported biofilm formation to be associated with biomaterials such as contact lenses, sutures, scleral buckles, valvular tubes, and keratoprostheses,[3–5] as well as with infectious keratitis in animal models.[5]

In this article, we describe for the first time—to our knowledge—a novel clinical presentation of biofilms in the human cornea, consisting of formation of corneal biofilm plaques in the absence of prosthetic material and in the absence of clinical changes suggestive of active corneal inflammation and/or infection.

CASE REPORT

A 9-year-old boy presented to our cornea department with a 2-year history of asymptomatic lesions in his left eye; his medical history was otherwise unremarkable. His mother mentioned that 6 months before the appearance of the first lesions, the patient had minor trauma to this eye with a pencil, without any visual changes, which resulted only in a transient red eye. Two years ago, when they first detected the lesions, they consulted a different ophthalmology clinic, and the lesions were removed during slit-lamp examination on 2 occasions, both times recurring 1 month after removal.

On examination, visual acuity without correction was 20/20 in both eyes; On biomicroscopy, the right eye was within normal limits, and the left eye presented 2 whitish, 2.8×1.6-mm and 1.2×0.6-mm avascular corneal plaques with well-defined borders, with negative fluorescein and rose bengal staining. Lesions were located between the 6 and 7 o'clock meridians at the midperipheral and peripheral cornea, without compromising the visual axis or the limbus (Figs. 1A, B). Anterior segment optical coherence tomography (Triton; Topcon Medical Systems, Inc, Oakland, NJ) showed hyperreflective subepithelial lesions (Fig. 1C).

Excisional biopsy was performed under topical anesthesia. Lesions were removed with 0.12-mm forceps, followed by scraping of the resection area with a crescent blade to guarantee complete removal of possible remnants. Subsequently, microsponge-assisted impregnation of the area with 30% alcohol for 30 seconds and then with 0.02% mitomycin C (MMC) for 30 seconds was performed, followed by extensive saline washing of the ocular surface and bandage contact lens placement, which was removed after 48 hours (Fig. 2). Alcohol and MMC were used because, although the etiology was uncertain at that time and their use is controversial, these substances have been used to avoid recurrences of some ocular surface pathologies. During the postoperative period, 0.4% sodium hyaluronate (Lagricel; Laboratorios Sophia, Guadalajara, Jalisco,

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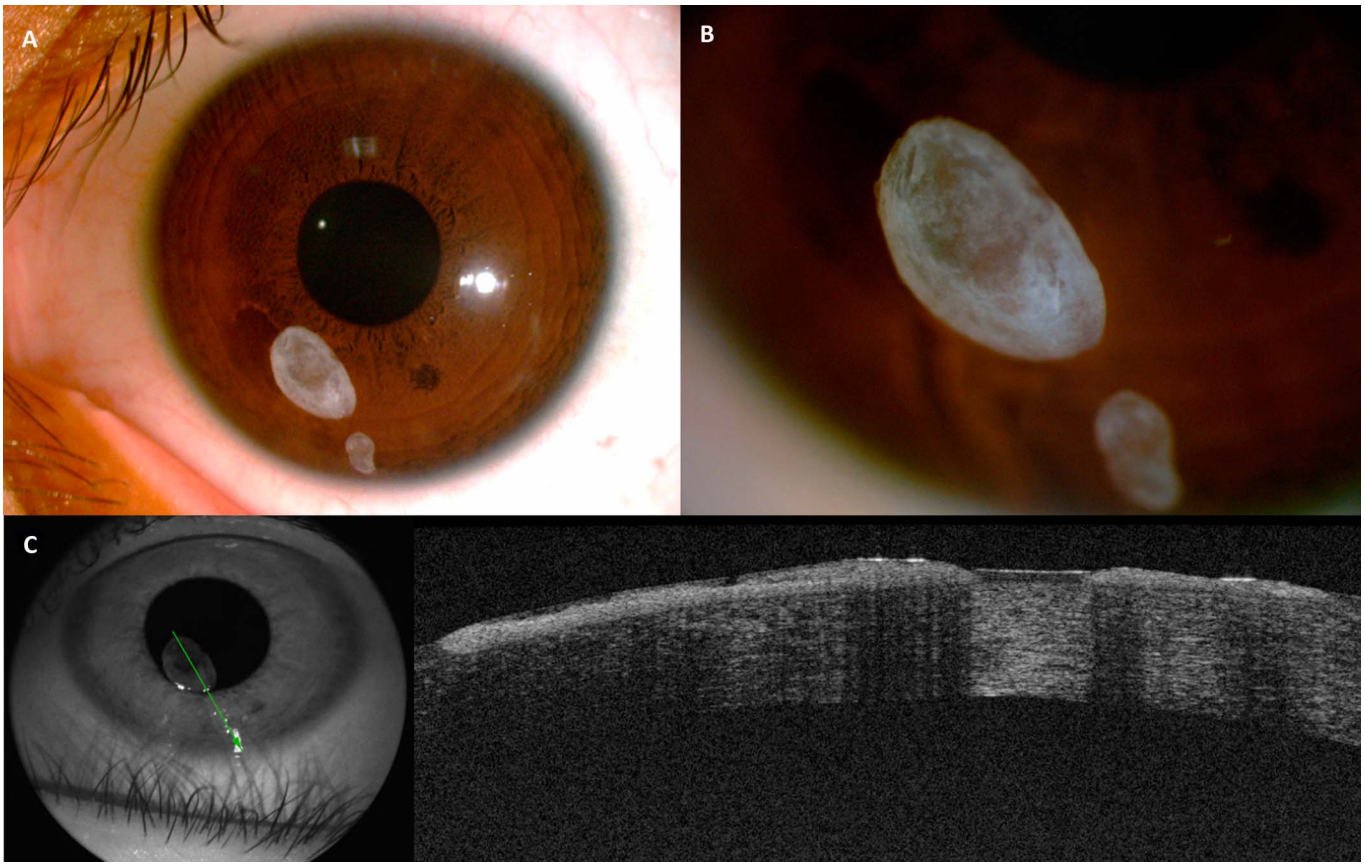

FIGURE 1. A, Slit-lamp photograph showing a left eye with 2 whitish, avascular corneal plaques with well-defined borders. No clinical evidence of inflammation or infection is observed. B, Slit-lamp photograph showing a close-up view of corneal plaques. C, Anterior segment optical coherence tomography evidences 2 subepithelial and hyperreflective corneal plaques on the evaluated axis denoted by a "green line."

Mexico) every 2 hours and moxifloxacin 0.5% (Vigamoxi; Alcon Laboratories, Inc, Fort Worth, TX) every 4 hours were used for 1 week. In addition, fluorometholone 0.1% (Flumetol; Laboratorios Sophia, Guadalajara, Jalisco, Mexico) was used every 4 hours and gradually tapered over 6 weeks.

The removed plaques were fixed in formaldehyde and sent to the pathology laboratory. Histopathological hematoxylin–eosin staining analysis showed that samples were subepithelial lesions, formed by multiple clusters of basophilic deposits surrounded by amorphous material, and scarce inflammatory cells toward the most superficial

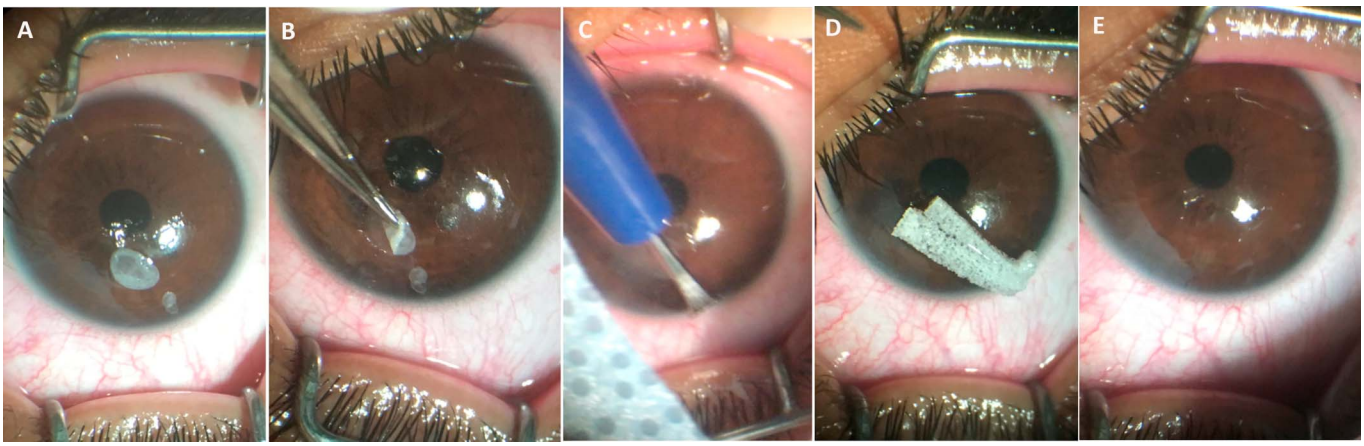

FIGURE 2. A, Corneal plaques before removal. B, Corneal plaque removal with 0.12-mm forceps. C, Scraping of the resection area with a crescent blade. D, Microsponge-assisted impregnation with 30% alcohol and 0.02% mitomycin C. E, Cornea immediately after removal of the plaques.

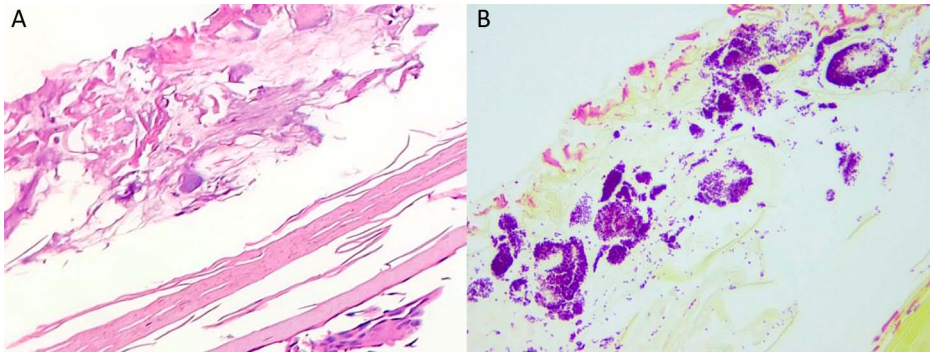

FIGURE 3. Histological characterization. A, Hematoxylin–eosin staining at ×400. Examination discloses an extraceulllar matrix with basophilic deposits overlying the Bowman layer. B, Gram staining at ×400. This stain highlights gram-negative bacilli organized in clusters overlying the Bowman layer.

portion of the tissue. In the deepest part of the tissue, the Bowman membrane accompanied by a few microns of the superficial stroma of the cornea without inflammatory findings was evident (Fig. 3A). Gram staining confirmed that the previously described basophilic material corresponded to gram-negative bacilli clusters (Fig. 3B).

In addition, some of the obtained tissue lamellae were deparaffinized, and special staining with calcofluor white, Flamingo fluorescent dye, and propidium iodide was performed. These stains indicated that the amorphous material surrounding the bacterial colonies corresponded to an extracellular matrix formed by polysaccharides, proteins, and nucleic acids, confirming biofilm plaques. Gomori–Grocott staining was also performed to identify fungal structures; however, we observed black staining of bacterial aggregates, probably because the carbohydrates form the extracellular matrix of the biofilm; fungal structures were not observed (Fig. 4). Cultures were not performed because the entire tissue sample was sent for pathology analysis because an infectious etiology was not initially considered. From the material recovered from the deparaffinized plates, microorganism genotyping was attempted using polymerase chain reaction, albeit unsuccessfully.

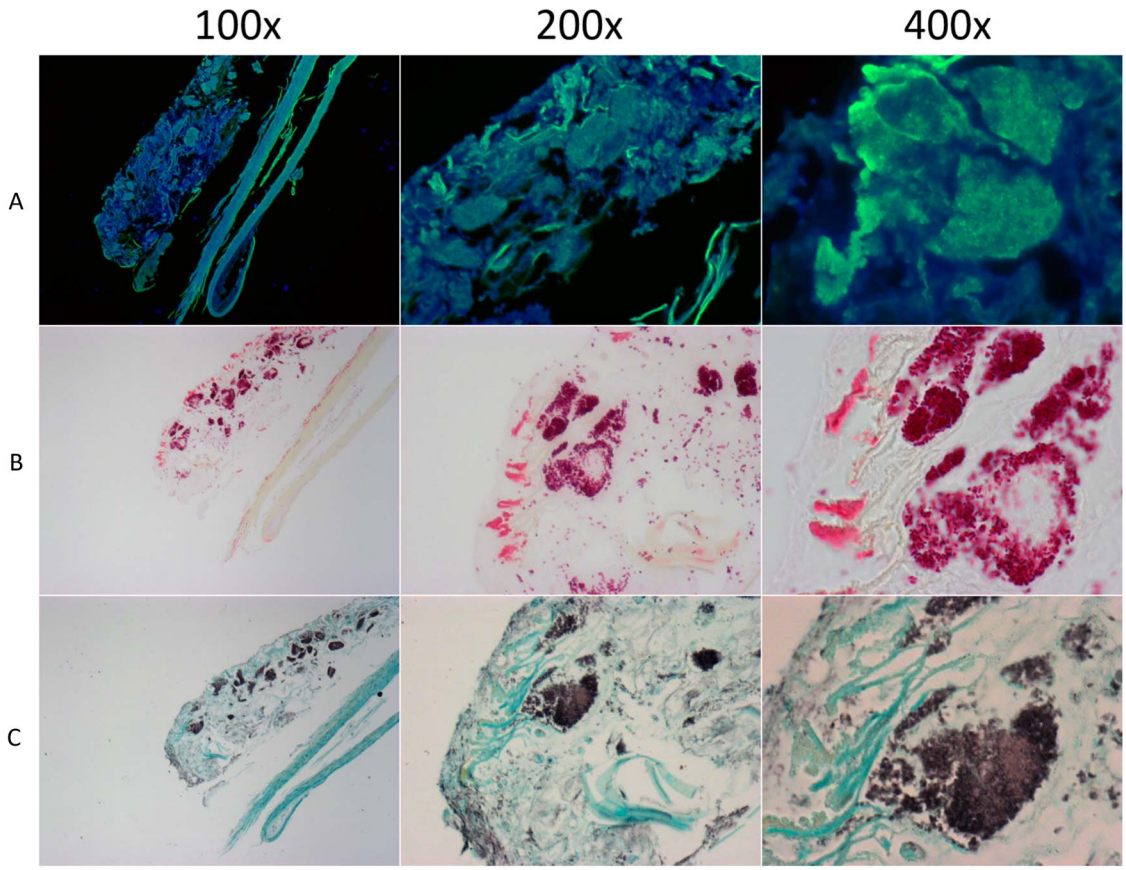

FIGURE 4. Special staining. Tissue was stained with (A) calcofluor white (carbohydrates), Flamingo fluorescent dye (proteins), and propidium iodide (DNA). Merged fluorophore staining is shown at ×100, ×200, and ×400 magnifications. (B) Gram staining at ×100, ×200, and ×400 magnifications. (C) Gomori–Grocott staining at ×100, ×200, and ×400 magnifications.

On the basis of these findings, our patient was diagnosed with recurrent biofilm plaques secondary to a possible bacterial inoculation after minor trauma. Since diagnosis, monthly clinical and photographic follow-ups have been performed over the past 5 months. Currently, the patient is in good health without any evidence of recurrence.

DISCUSSION

Formation of biofilm plaques on the corneal surface is a novel clinical presentation, which could be easily confused with noninfectious diseases because of their noninflammatory clinical appearance. In 2004, Mihara et al[6] described biofilm formation on the ocular surface (limbal conjunctiva) in the absence of biomaterials for the first time in the literature. However, to our knowledge, the case described herein is the first to report biofilm plaque formation on the corneal surface, and because the patient's only previous history was minor trauma with a pencil 6 months before the onset of lesions, we postulate that this event led to inoculation of the microorganisms growing in a biofilm.

It is remarkable that the patient never presented clinical findings suggestive of active corneal inflammation and/or infection, despite being colonized for such a long time. This absence of inflammation occurs by 2 mechanisms described in chronic infections associated with biofilm formation: the first is the action of the extracellular matrix as a physical barrier that prevents microorganism detection by the immune system and blocks phagocytosis, and the second is the genetic capacity of biofilm-producing microorganisms to release regulatory factors that affect the cellular immune response, leading to an imbalance between T helper 1 (Th1) and T helper 2 (Th2) lymphocyte populations, which does not allow microorganism clearance.[7]

Now that the etiology has been identified, it is not possible to determine whether the current lack of recurrence is due to the effect of alcohol or MMC on the causative organism, or due to complete removal and scraping of the lesions, and because lesions had been previously removed during slit-lamp examination on a pediatric patient, there is a high probability that the lesions, microorganisms, or biofilms had not been completely removed, which could also explain the previous recurrences.

For this type of corneal lesions, some differential diagnoses must be considered: infectious crystalline keratopathy, retained graphite, and medication deposits. Compared with infectious crystalline keratopathy, biofilm plaques were subepithelial, not intrastromal, and have no stellate appearance; however, physiopathologically, these conditions are similar because both correspond to indolent microorganism colonization due to biofilm formation. Graphite remains inert when retained in the corneal stroma, but a recent study demonstrates that graphene oxide could act as a biofilm formation enhancer[8]; however, we did not find clinical, histopathological, or microbiological data that suggest retained graphite remnants. And last but not least, multiple medications can cause similar deposits (plaques) on the ocular surface, but our patient had no history of chronic use of topical or systemic medications.

In conclusion, corneal plaques are an extremely rare clinical presentation of biofilms that allow prolonged survival of microorganisms with the absence of clinical manifestations of corneal active inflammation and/or infection.

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