

# Bacterial biofilm on the sinus mucosa of healthy subjects and patients with chronic rhinosinusitis (with or without nasal polyposis)

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## Abstract

**Objective:** To study the presence of bacterial biofilm on the sinus mucosa of healthy individuals (controls) and patients with chronic rhinosinusitis with or without nasal polyposis.

**Methods:** An analytical, prospective and observational study was conducted. Tissue samples were obtained from the sinus mucosa. The bacteria were isolated and typified, and the material was examined for biofilm formation using tissue culture plate, Congo red agar detection and tube methods.

**Results:** A total of 100 cases were analysed for the presence of biofilm. Bacterial biofilm was present in 26 of 50 disease group cases (52 per cent) and in 4 of 50 control group cases (8 per cent) ( $p < 0.01$ ).

**Conclusion:** The presence of biofilm on the mucosa of chronic rhinosinusitis patients offers a possible cause for the persistent inflammation, and for antibiotics resistance and antimicrobial therapy failure. These findings could change the approach to treatment.

**Key words:** Bacterial Biofilm; Chronic Rhinitis; Nasal Polyposis

## Introduction

The Sinus and Allergy Health Partnership Task Force has defined rhinosinusitis as ‘a group of disorders characterised by inflammation of the mucosa of the nose and the paranasal sinuses’.<sup>1</sup> The International Classification of Diseases divides rhinosinusitis into acute and chronic forms, according to the duration of the symptoms. Acute rhinosinusitis lasts up to 12 weeks with complete resolution of symptoms, whereas the chronic form persists beyond 12 weeks. Chronic rhinosinusitis is now considered a multifactorial disease, associated with extrinsic or non host related factors (e.g. allergy, asthma and aspirin intolerance; microbial factors; osteitis; bacterial biofilm; and super antigens), and intrinsic or host-related factors (e.g. anatomical variations, immunodeficiency, ciliary dysfunction or Kartagener’s syndrome, and other associated diseases such as cystic fibrosis).<sup>2</sup>

In the field of otolaryngology, biofilms have been documented in cases of otitis media with effusion, cholesteatoma, tonsillitis and rhinosinusitis, and on adenoids removed from children with chronic rhinosinusitis. They have also been isolated on some prosthetic devices, such as tracheotomy and tympanostomy tubes, frontal recess stents, and cochlear implants.<sup>3</sup>

Bacterial biofilm is a three-dimensional structure composed of aggregates of bacterial cells (micro-colonies) and the extracellular matrix released by them, which adheres to an organic or inorganic surface. Biofilms are defined as an organised community of bacteria, adherent to a surface, that are contained in an extracellular polymeric substance composed of exopolysaccharides, nucleic acid and protein.<sup>4–7</sup> It is estimated that 99 per cent of all bacteria exists in biofilm and only 1 per cent live in a free-floating state (planktonic).<sup>8</sup>

Biofilms are highly resistant to host defence mechanisms, both innate and specific immunity mechanisms. Because of their exopolysaccharide matrices and reduced metabolic rate, they are less susceptible to phagocytic macrophages and are resistant to antibiotics that attack only dividing cells.<sup>9–11</sup> The National Institutes of Health has estimated that at least 65 per cent of all bacterial infections in humans are related to biofilm.<sup>9</sup>

Biofilm development is a cyclical process (Figure 1), involving initial attachment and mature biofilm formation, followed by detachment and potential reseeded of other parts of an implanted medical device, or perhaps other organs and tissues in the human body.<sup>12</sup>

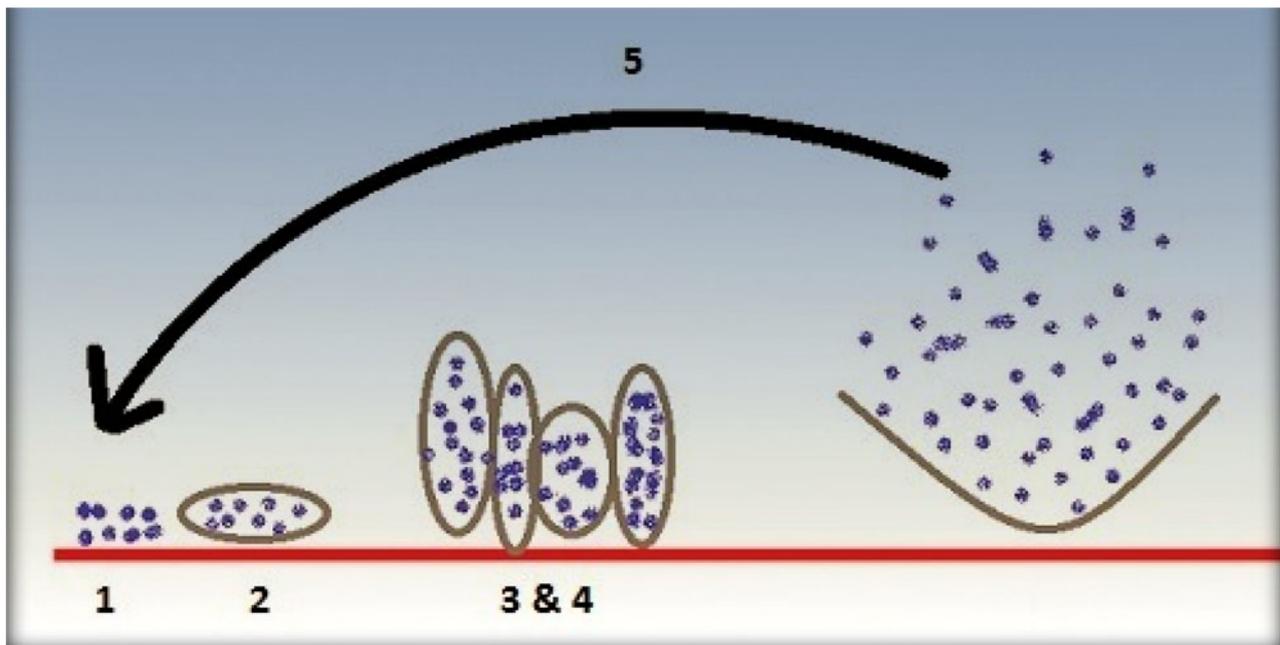


FIG. 1  
Various stages of biofilm formation.

Sanclement *et al.* demonstrated the presence of biofilm in 24 out of 30 affected individuals and in 0 out of 4 control subjects.<sup>13</sup> However, the authors did not identify the organisms present within these biofilms, nor did they establish whether the presence of biofilm plays a role in the pathophysiology of chronic rhinosinusitis. Mladina *et al.* stated that biofilm is nothing other than a normal, otherwise abundantly colonised, mucous blanket.<sup>14</sup> Their study aimed to establish whether bacterial biofilm exists exclusively at the diseased mucosal surfaces or at the healthy mucosa of paranasal sinuses too.

The current study was performed to determine the presence of bacterial biofilm on the sinus mucosa both of healthy subjects (control group) and of patients with chronic rhinosinusitis with or without nasal polypsis (disease group).

### Materials and methods

This prospective, randomised study was conducted on 100 patients (50 in the control group and 50 in the disease group), who attended the Sawai ManSingh Hospital in Jaipur (Rajasthan, India) between September 2011 and October 2012. Informed consent was obtained prior to enrolment for the study.

The data collected included demographic information, symptoms, clinical examination findings, pre-operative medical treatment, nasal endoscopy and pre-operative computed tomography (CT) scan findings, and any history of nasal surgery. The exclusion criteria were fungal sinusitis and rhinological granulomatous disease.

Specimens were obtained intra-operatively from normal sinus mucosa (control group) and from the

mucosa of diseased sinuses as evidenced by pre-operative CT scans and nasal endoscopy. The samples were immediately transported to the hospital microbiology laboratory.

The samples were ground and then inoculated on a fresh agar plate (Blood agar, MacConkey agar and thioglycollate broth), and incubated for 24 hours at 37°C. After incubation, the culture plates were examined for growth. Clinical specimens were identified after Gram staining, and standard biochemical tests were conducted on isolated colonies on nutrient agar. Those colonies with positive test results were further observed to assess their ability to form biofilm, using tissue culture plate, Congo red agar detection and tube methods.

The tissue culture plate assay described by Christensen *et al.* is the most widely used method and has been considered the standard test for the detection of biofilm formation.<sup>15</sup> Isolates from fresh agar plates were inoculated in brain-heart infusion broth with 2 per cent sucrose dispensed in 2 ml amounts in test tubes, and incubated for 18–24 hours at 37°C in a stationary condition. The broth with growth (visible turbidity) was diluted 1:100 with fresh medium. The individual wells of sterile, polystyrene, 96-well, flat-bottom tissue culture plates (HiMedia Laboratories, Mumbai, India) were filled with 0.2 ml aliquots of diluted cultures. Broth on its own served as a control to check sterility and non-specific binding of media.

The tissue culture plates were inoculated for 24 hours at 37°C. After incubation, the contents of each well were gently removed by tapping the plates. The wells were washed four times with 0.2 ml of phosphate buffer saline (pH 7.2) to remove free-floating

'planktonic' bacteria. Biofilm formed by adherent 'sessile' organisms in plates were fixed with sodium acetate (2 per cent) for 30 minutes and stained with crystal violet (0.1 per cent weight/volume) for 30 minutes. Excess stain was rinsed off by thoroughly washing with deionised water, and the plates were kept for drying.

Adherent bacterial cells usually formed biofilm on all side wells and were uniformly stained with crystal violet. The optical density values of stained adherent bacteria were determined with a micro enzyme-linked immunosorbent assay auto reader at a wavelength of 570 nm (optical density of 570 nm). These optical density values were considered as an index of bacteria adhering to the surface and forming biofilm.

## Results

In the present study, 100 cases (50 control group and 50 disease group cases) were analysed for the presence of biofilm. Bacterial biofilm was present in 26 out of 50 cases (52 per cent) in the disease group and in 4 out of 50 cases (8 per cent) in the control group ( $p < 0.01$ ) (Table I).

Among the disease group ( $n = 50$ ), 20 patients (40 per cent) had chronic rhinosinusitis, 18 (36 per cent) had ethmoidal polyps and 12 (24 per cent) had antrochoanal polyps. Bacterial biofilm was detected most commonly in those with ethmoidal polyps (12 out of 18 patients; 67 per cent).

Common isolated bacteria are coagulase-positive staphylococcus (with a previously reported rate of 24 per cent), coagulase-negative staphylococcus (20 per cent), pseudomonas (16 per cent), *Enterobacter aerogenes* (16 per cent) and *Enterobacter cloacae* (16 per cent).<sup>16</sup> In our study, coagulase-positive staphylococcus was the most common bacteria (Table II).

## Discussion

Chronic rhinosinusitis with or without nasal polyposis is a multifactorial disease, but the associated persistent inflammation is a major component of the disease. This study demonstrates the presence of another chronic factor, bacterial biofilm, which might contribute to nasal mucosa damage, increase numbers of inflammatory cells in the tissue, and lead to recurrent or chronic infections, including those which are not responsive to culture-appropriate antibiotic therapy.

Bacterial biofilm is a three-dimensional structure made of aggregates of bacterial cells (micro-colonies)

TABLE I  
PRESENCE OF BIOFILM IN CONTROL AND DISEASE GROUPS

Group	Positive biofilms ( $n$ (%))
Control*	4 (8)
Disease*	26 (52)

\*Total  $n=50$

TABLE II  
BIOFILM PRODUCTION OF ISOLATED BACTERIA

Organism	Isolates ( $n$ )*	Positive on biofilm ( $n$ (% <sup>†</sup> ))
Coagulase-negative staphylococcus	10	5 (10)
Coagulase-positive staphylococcus	12	7 (14)
Pseudomonas	8	5 (10)
<i>Enterobacter aerogenes</i>	8	4 (8)
<i>Enterobacter cloacae</i>	6	3 (6)
<i>Proteus mirabilis</i>	2	0 (0)
Hafnia	2	2 (4)
Acinetobacter	2	0 (0)

\*Total  $n=50$ . <sup>†</sup>Percentages based on total number of isolates

and the extracellular matrix released by them, which adheres to an organic or inorganic surface. It is estimated that 99 per cent of all bacteria exist in biofilm and only 1 per cent live in a free-floating state (planktonic).<sup>8</sup>

Biofilms are highly resistant to host defence mechanisms, both innate and specific immunity mechanisms. Because of their exopolysaccharides matrices and reduced metabolic rate, they are less susceptible to phagocytic macrophages and are resistant to antibiotics that attack only dividing cells.<sup>9–11</sup>

Sanclement *et al.* demonstrated the presence of biofilm in 24 out of 30 affected individuals and in 0 out of 4 control subjects.<sup>13</sup> Galli *et al.* demonstrated the presence of biofilm in 10 of 24 affected individuals (42 per cent); there was no evidence of biofilm in the control group ( $n = 20$ ).<sup>17</sup>

In the present study, 100 cases were analysed for the presence of biofilm. Bacterial biofilm was present in 26 out of 50 disease group cases (52 per cent) and in 4 out of 50 control group cases (8 per cent).

- This study aimed to determine the presence of bacterial biofilm on the sinus mucosa of healthy individuals and chronic rhinosinusitis patients
- Bacterial biofilm was present in 26 out of 50 disease group cases and in 4 out of 50 control group cases ( $p < 0.01$ )
- The most common organism found was coagulase-positive staphylococcus (14 per cent)

In our study, the common organisms (based on the total number of disease group isolates,  $n = 50$ ) were coagulase-positive staphylococcus (14 per cent) and enterobacteriaceae (14 per cent), followed by pseudomonas (10 per cent), coagulase-negative staphylococcus (10 per cent) and hafnia (4 per cent).

This study shows that biofilm is not only present in chronic rhinosinusitis patients but may also exist in patients who are free of sinus diseases. However, the

presence of biofilm on healthy control samples may simply reflect colonies of bacteria.

### Conclusion

The presence of biofilm on the mucosa of patients with chronic rhinosinusitis offers a possible cause for the persistent inflammation, and for antibiotics resistance and antimicrobial therapy failure. These findings could change the approach to treatment.

Our study demonstrated the presence of biofilm on the mucosa of healthy sinuses. However, the presence of biofilm on healthy control samples implies that the biofilm may simply be colonies of bacteria.

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