THE DARK SIDE OF BIOFILM

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PERSPECTIVES

Bacterial biofilms are structured multicellular microbial communities embedded in a matrix of self-produced extracellular polymeric substances (EPSs) [1,2]**.**

Anthony van Leeuwenhoek first defined biofilm as an aggregate of bacteria in dental plaque. About 100 years later, Louis Pasteur described aggregates of bacteria that he hypothesized to be the cause of the wine becoming acetic. Since then, for the next century, research has shown no interest in microbial biofilms, long unknown to medical microbiologists[3]. Only recently, the rates of antibiotic resistant Gram-negative bacteria associated with biofilm-forming activity have increased worrisomely, so that biofilms are nowadays considered a serious public-health problem worldwide, capturing the attention of research again[4]. As a matter of fact, although the observation of biofilms can be considered as old as microbiology itself, the mechanisms by which biofilms contribute to the pathogenesis and chronicization of several diseases are still unknown[5]. Moreover, the lack of therapeutic strategies that are truly effective in eradicating them imposes the identification of more effective antimicrobial treatment options[6]. This explains the growing emphasis on biofilms over the past decade, particularly regarding the complex mechanisms involved in biofilm life cycles and their strategies to resist infections[7]. Indeed, biofilms represent more tolerant forms of existence for bacteria than planktonic forms, being protected from several stressors. Their life cycle begins with planktonic microorganisms, which are free-living species that propel themselves to a surface or a tissue, and then produce the EPS matrix, which protects them from a wide variety of stressful conditions. Moreover, microorganisms produce small signaling molecules involved in quorum sensing (QS), which is an intercellular communication mechanism that ensures intercellular communication[8]. The maturation of biofilm ends with the development of a three-dimensional structure that guarantees the efficient transportation of nutrients and signaling molecules. When bacteria leave the biofilm in the planktonic form, they attach to new surfaces and start a new life cycle, and become susceptible to host defenses and antibiotics[9]. On the contrary, pathogenic biofilms are 10- to 1000-fold more drug-resistant than planktonic microorganisms and cause about 80% of all chronic infections worldwide[10,11]. Given the recognized involvement of biofilms in the pathogenesis of difficult to treat acute and chronic infections, due to the elusion of host defenses along with the inherent antibiotic-tolerance, there is a there is a critical need to accurately diagnose, prevent, and treat biofilms[12]. In this context, their early and correct detection, which can be performed by scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM) combined with live/dead staining or fluorescent in situ hybridization (FISH), plays a pivotal role in the diagnostic path of patients suffering from several infectious disease[13]. In the last decade, as regards the field of Rhinology, nasal cytology (NC) has been introduced as a crucial part of the rhino-allergologic

diagnostic path, since it is an easy-to-apply, reproducible and non-invasive diagnostic tool that allows to classify the different sino-nasal pathologies according to the cytotypes of the nasal inflammatory infiltrate and to assess the presence of biofilm identification with good accuracy and lower costs[13]. In particular, in NC samples, which are obtained by Nasal Scraping® from the middle part of the inferior turbinate, air-dried, stained with May-Grunwald-Giemsa (MGG) and then read with optical microscopy, biofilm appears as a cyan-stained "infectious spot" whose polysaccharide nature can be confirmed by periodic acid-Schiff staining[14].

Interestingly, just by observing some nasal cytological samples, we came across a very evocative image (***Fig. 1***) which, in our opinion, symbolizes the dark side of the biofilm. Indeed, it represents a bacterial biofilm present on the surface of the nasal mucosa which, on the cytological sample, recalls the profile of a man with an evil and frightening aspect. This man seems to have a threatening appearance, just like the biofilm, which poses a threat to human health, resisting the common therapies available today and contributing to the recalcitrance of infections. This dark side could also refer to everything that is not yet completely clear about biofilm and which, therefore, represents an important barrier to the identification of new therapeutic strategies. In this sense, just as we have to look for the good in every person, we would also like to find the good in the biofilm. In fact, biofilms are characterized by self-regeneration, sustainability, scalability, and tunability, which are favorable features that make them candidates for diverse applications, including catalysis, electric conduction, bioremediation, and medical therapy[15]. As regards the latter application, we would highlight that the human microbiome consists of different microorganisms and properly the overgrowth of "bad" bacteria, which displaces those microorganisms that constitute a healthy microbiome, leads to microbial dysbiosis, mucosal and chronic diseases[16,17]. In the future, the characteristics of some bacteria considered "good" could be exploited, including for example *Corynebacterium accolens,* which has antimicrobial activity against both planktonic and biofilm forms, paving the way for innovative probiotic therapies and also to the possible use of "good" biofilms[18].

Further studies are therefore required to understand the still unclear mechanisms of biofilm formation and eradication as well as its possible positive applications, to finally bring to light the dark side of biofilm.

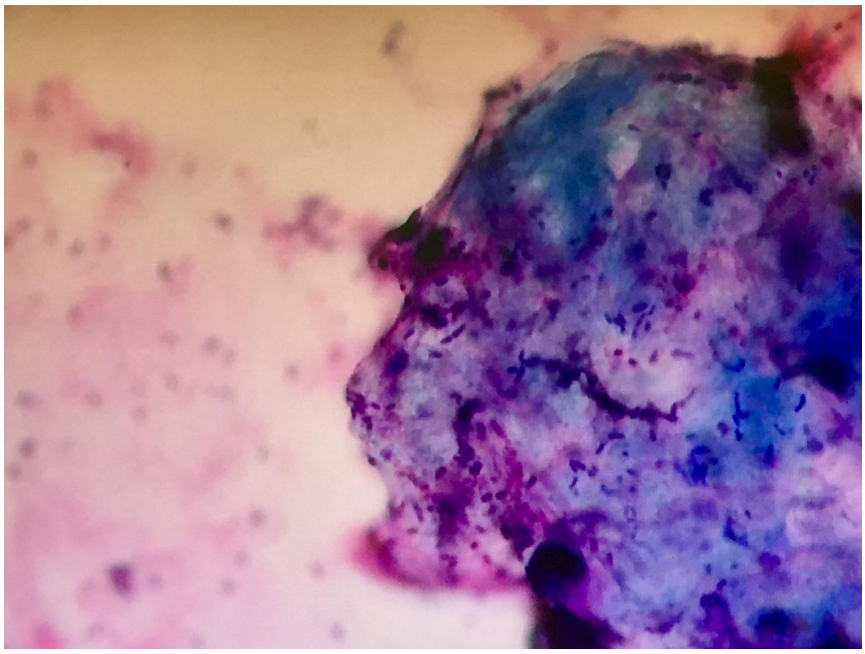

FIGURE LEGEND

Figure 1 - Nasal cytology in a patient with chronic rhinosinusitis. Numerous bacteria incorporated in an exopolysaccharide matrix (biofilm) MGG staining. Magnification 1000X

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