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The Most Influential Historical Aspects in Biofilm Evolution

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Abstract
About 3.25 billion years ago, in the early fossil record, the development of biofilms is widespread across several Archaean and Bacterial groups, including live fossils found in the deepest branching phylogenetic tree divisions. The formation of biofilms is undeniably a historic and essential part of the developmental cycle of prokaryotes, and plays a crucial role in survival under diverse environmental conditions. The concept of biofilm development was a very gradual process having many small perceptions that have now merged and synthesized and created a major "wave" that will bring microbiology into the new millennium. Such early findings that bacteria really grow in surface-attached matrix-enclosed biofilms fit into a growing set of observations made by many groups. The primary instances are the microscopic observations by Antony van Leeuwenhoek from Delft, The Netherlands (1632–1723), mentioned by Dobell in 1960, which were published in letters between September 1683 and June 1708, where Leeuwenhoek observed microorganism aggregation in the teeth scurf samples collected from his own mouth. Biofilms formed by microorganisms are an organized community of cells embedded in the polymer matrix secreted from the cells itself. Biofilms are capable of cohesion to host cells or infused into tissue or cell secretions, and sometimes may contain host components. The molecular mechanisms and ecological forces of bacterial socialization can be analyzed using the blend of booming field of microbial social evolution and molecular microbiology alongside evolutionary theory. Knowledge of the history, nature and evolution of biofilm aid in the design of better preventive strategies for infections linked to biofilm. This article highlights the major milestones in the evolutionary aspects of biofilm.

Key Words: *Biofilm, bacteria, slime, microbiology*

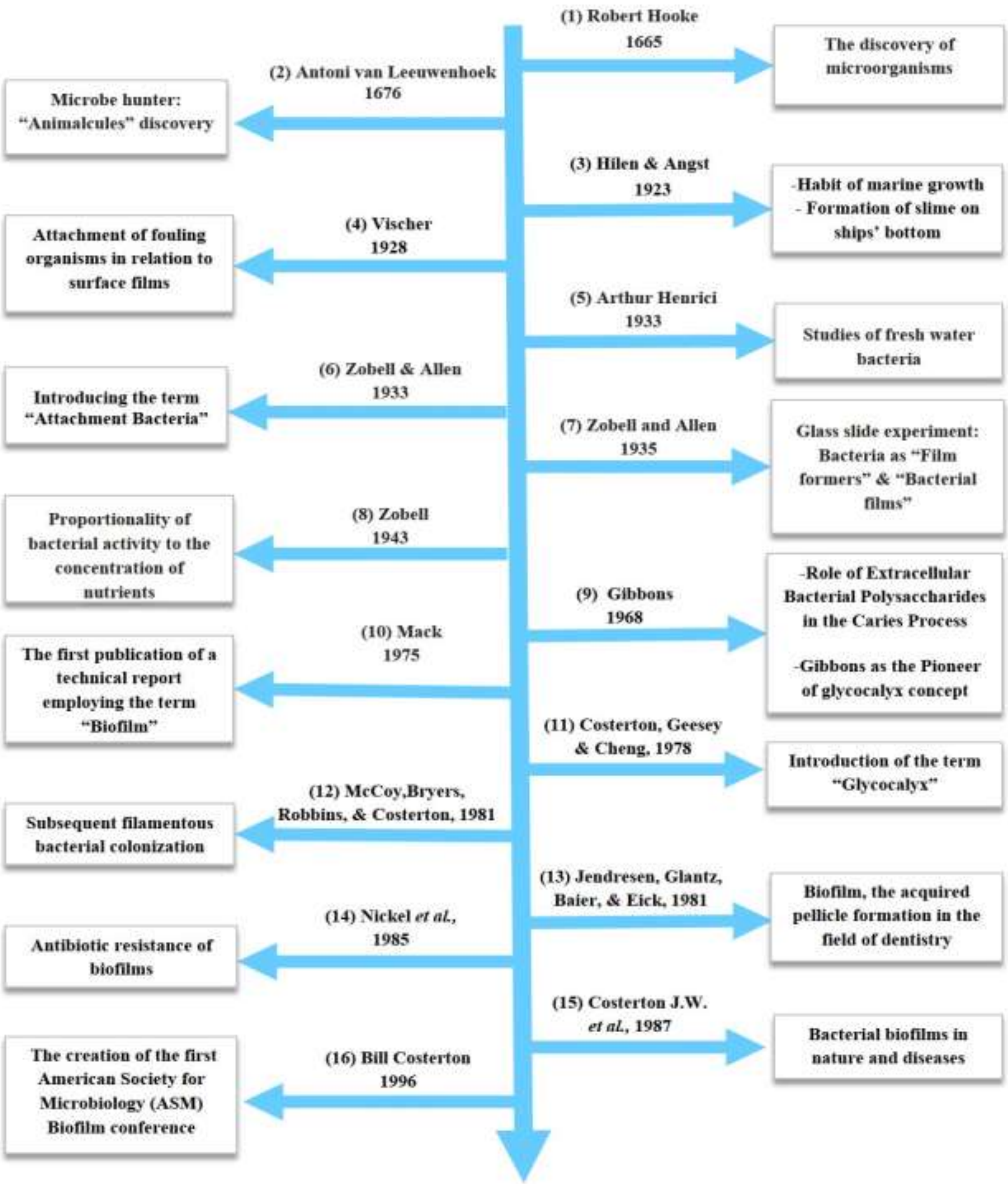
Introduction

The realm of microbiology has tremendously evolved over the years, but till date, one of the subtlest advances has been the realization of the extent to which bacteria can grow and develop on surfaces in unique and complex communities(1). During the years (1665-1678), Robert Hooke and Antoni Van Leeuwenhoek of the Royal Society revolutionized the microbial universe through the use of microscopes. As early as 1665, Robert Hooke (1635-1702) was the first to depict the microorganism known as the microfungus Mucor in Micrographia, but his publication was often overlooked by that of Antoni Van Leeuwenhoek who designed the single lens microscopes and contributed to extraordinary discoveries in microbiology. In 1676, Antoni Van Leeuwenhoek (1632-1723) was referred to as one of the first "microbe hunters "of his century by the historical accounts. With the help of his primitive, self-built microscope, Antoni was the first to unmistakably observe small organisms which he termed as "animalcules" (or little eels). These animalcules were found in some pepper water while investigating the taste of spices during his microscopic study of infusions and later a white matter was scrapped from the surface of his own teeth(2)**.**A few centuries later, while investigating the habit of marine growth, Miss Hillen at the University of Washington, observed the formation of slime on surfaces exposed to sea water and reported the presence of bacteria, yeast and molds. She rightly confirms the bacterial composition of the slime which, according to her, is of crucial importance for the settlement and development of the young barnacles (3). Supporting similar studies in the same year, Angst observed the formation of slime on some ship's bottom and concluded that "the slime bears a direct relation to the appearance of the barnacles"(4)**.** In 1928, still in the marine domain, Visscher provided a detailed report on the nature and extent of fouling of ship's bottom, and it greatly highlights the valuable study of the attachment of fouling organisms in relation to surface films (5)**.**

Figure 1 provides a timeline of the milestones in discoveries and studies related to biofilm. Although the term "biofilm" was coined in 1975, previously researchers have conducted studies and discovered the various properties exhibited by the biofilm under various conditions. The early instances of biofilms were shown during the 1930s, after which further studies were carried out which evidently supported the biological aspects of biofilms.

Figure 1: A chronological outline of significant events related to "biofilm"

In 1933, Arthur Henrici and Claude Zobell published two most influential papers in the Journal of



Bacteriology (JB) by introducing the concept of bacterial growth on glass slides, but this time, fresh water was used as a medium (6). By investigating a portion of the aquatic microflora, Henrici from the laboratories at the University of Minnesota, introduced microscope slides in an aquarium which previously developed algae on its walls. After a week's immersion, he observed that "in addition to the algae, a thin and uniform coating of bacteria of various forms, some of unusual morphology" were present. This experiment was repeated with other aquaria, with the lily pond in the greenhouse of University of Minnesota, and finally with the waters of Lake Alexander, Minnesota where Henrici was able to show that after a few days, the thickness of the deposit of bacteria onto the glass slide progressively increased. Distinguishing the cells became a tedious task and that was indicative of the presence of micro colonies of steadily increasing sizes. A sheath of gum surrounded the cells were found to be responsible for the firm adherence to the glass slide while resisting removal by running water. The study reported that "It is quite evident that for the most part the water bacteria are not free floating organisms, but grow upon submerged surfaces; they are of the benthos rather than the plancton" (7).

Zobell and Allen in the same year, managed to use a similar procedure to study marine bacteria on the coast of Southern California where standard slides were submerged off the end of the Institution pier and were carefully removed for examinations. Their results indicated the attachments of bacteria, and lower amounts of actinomycetes and diatoms preceding barnacles, hydroids, bryozoa and other fouling organisms. They categorized the firmly attached bacteria as "attachment bacteria" and concluded that "Some of them appeared to be definitely thigmotactic because they grew only in adherent films on surfaces" (8). A minimum of 2 to 4 hours is required for the firm attachment of the cells, making it resistant to washing off by running water and any staining procedures (9).

The accumulation of bacteria was observed to be varying with respect to time indicating either a multiplication process or a favoring influence of the film-formers upon subsequent attachment". By submerging both the sterile and film coated glass slides simultaneously in the same medium for the same amount of time, Zobell and Allen noticed that a greater number of attached organisms were present on the film coated slide. Therefore, they confirmed the role of bacteria as "primary film formers" along with the latter's influence for subsequent attachment of larger virulent fouling bacteria. They successfully introduced the term "bacterial film" along with the "organic detritus" that sticks to it while providing the fouling organisms with suitable nutrients (9).

In 1943, the work of Zobell and Grant explains the proportionality of bacterial activity with respect to nutrient concentration. The amount and quality of organic matter present in the water seem to affect bacterial populations and it was calculated that more bacteria were firmly attached to the walls of submerged glass bottles than the number found in water. The increasing size of the film, due to the secretion of a faintly staining material, revealed to be proportional to its age after different submerging durations. This mucilaginous slime appeared to be "part of or the product of the bacterial cells" themselves, and played a crucial role in the fouling of submerged surfaces. In addition, Zobell *et al.*, were also successful in showing that not only glass but other solid surfaces could potentially promote bacterial growth (10).

As the historical account goes on, the concept of "bacterial film" started to impact various fields and even reports relating to sewage treatment contained a few observations regarding the study of the basic slime layer developing in trickling filters. Cooke's studies involved 20 filters at the Dayton Ohio sewage treatment plant which was supporting separate communities of macro and microorganisms, which he described using the term as "biocoenose". Using an inoculum from another filter, the time taken for the growth of the microbial film to an optimum size was still uncertain. After 57 weeks of observation, a maximum thickness of about 2-3mm was reached and Cooke's concluded that "this film was teeming with life of many types" He added that this microscopic community requires careful continuous irrigation, without which drying followed by flaking off would inevitably occur. Furthermore, Cooke innovatively used three ecological concepts to describe the composition of this micro community, namely; Fidelity, Constancy and dominance, where the species association in a uniform manner was considered along with an estimation of the total count of colonies (11).

In 1975 the concepts of bacterial films were enlightened when N.W Mack at the Institute of Water Research and Department of Microbiology, from Michigan State University, used scanning electron microscopy to visualize the stages of bacterial film development in trickling wastewater filter (12)**.** A word search on biofilm in PubMed reveals that Mack's report was the first to employ the term "biofilm" to describe this technical issue (13). He successfully describes the microbial cells' attachment and development of the matrix which is the base onto which biofilms develop (12). Between 1960 and 1967, Gibbons was the pioneer in the development of the concept of "glycocalyx" which he understood to be a polysaccharide which is an extracellular polymer possessing insoluble and adhesive nature contributing to streptococcal plaque formation(14)(15)**.**
Defining and characterizing biofilms started in 1978 when Geesey and Costerton investigated "How Bacteria Stick". They introduced the term "Glycocalyx" which is generated and maintained by the cells to promote protection and adhesion to desirable surfaces. The glycocalyx is crucial for the survival of bacteria on surfaces and it acts as a food reservoir while conserving and concentrating digestive enzymes. This glycocalyx was believed to arrange the bacteria in an organized community which helps them to act together as a "consortium" (15)Costerton was credited with the title of "pathfinder of biofilms" when he explained that this niche provides necessary nutrients and promotes the perfect microbial lifestyle with unique responses to changes in environmental conditions. Costerton *et al.*, thus concluded that bacteria in the wrong niche would simply die, leaving space and nutrients for other bacteria more suited to the location (15)**.** Costerton's paper was a kick start to the biofilm era where he highlights their tenacious nature while sticking to a diverse number of surfaces "ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream" (15)**.**

The first biofilm related publication in PubMed was reported by Mack and Ackerson in 1975 defining the technical biofilm problem. In the year 1981, the two medical articles were published simultaneously reporting the term biofilm by dentists from the University of Lund, Sweden (Jendresen and Glantz; Jendresen *et al.,)* (16) (17).

In the same year McCoy *et al.*, reported the term biofilm in a technical microbiology report (non-medical article) and also investigated the effect of high fluid velocity on subsequent filamentous bacterial colonization (18)**.**
Jendresen *et al*., were unsatisfied about the previous studies relating to the formation of biofilms onto the human tooth surface, commonly called pellicle. He understood the implications of this clinically relevant nuisance in the field of dentistry and his innovative studies successfully shed light on the role of tooth surface roughness with respect to clinical adhesiveness (17)**.**
In 1985**,** biofilms took new dimensions in the field of medical microbiology. Nickel *et al.,* were among the first to assess the degree of resistance of biofilm bacteria to antibiotics in medically relevant domains (19). This discovery triggered many questions regarding the nature of this new, yet potentially dangerous mode of microbial lifestyle. In a survey in 1987, as a result of various epidemic outbreaks, Costerton started to examine pathogenic ecosystems and summarized the concept of Biofilm as an "Irreversible adhesion of bacteria to a surface supported by the exopolysaccharide glycocalyx polymers". Cell division produces sister cells which lead to the formation of micro colonies in a continuous biofilm structure with the new recruitment of bacteria from the surrounding planktonic state for further colonization". A contrast was also put forward between planktonic cells and biofilm bacterial cells depending on their structural and functional differences (20).
In 1996, Bill Costerton was organized the first American Society for Microbiology (ASM) biofilm conference in Snowbird, Utah, USA. As rightly depicted by Hoiby in the year 2014, the yearly number of biofilm reports seems to have increased with time (21). A current survey on PubMed biofilm papers has been done to evaluate the status of publications till date and is represented graphically below in Figure 2**.**

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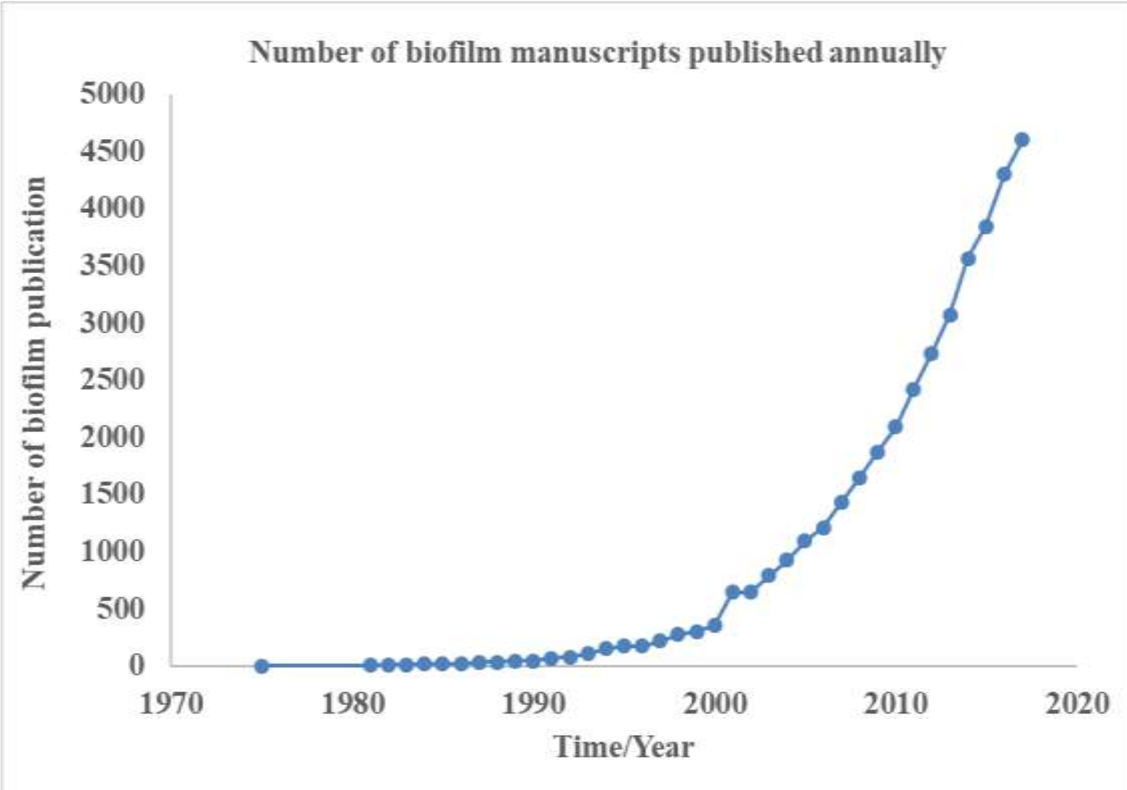

Figure 2: Number of Biofilm manuscripts published annually from 1975 to 2020 (45 years, PubMed search word: biofilm), Y-axis: Number of biofilm publications, X-axis: Time/year). [Total number of biofilm publication is 50616]

Figure 2 depicts that studies on biofilm came into focus since 1975. The period of 1970–80 was the initial phase of biofilm studies, while the research gathered more attention towards the end of 1980. Studies related to biofilm have witnessed an exponential increase after 2000, by the end of which, properties of biofilm such as antibiotic resistance and quorum sensing have gained a lot of attraction from the researchers. By the end of December 2019 about 50616 articles related to studies on biofilm have been published in PubMed. The ill-effects of biofilm in healthcare have attracted the researchers' attention to scrutinize the data on biofilms for better understanding and planning of future therapies.

Undeniably, Costerton tremendously contributed to the advances in medical microbiology and since the establishment of his hypothesis, biofilm related infections have become alarming issues (21). Early biofilm research aimed at detecting and quantifying bacteria in several ecosystems by employing basic morphological techniques to describe their formation within a unique safeguarded neighborhood. The field of medical microbiology was revolutionized with the advent of device related chronic infections and the refractory nature of these infections(22). As rightly stated in a review in 2004 by Hall Stoodley *et al.*, this protected mode of growth of bacterial cells, has made biofilms one of the most demanding problems in the industrial and medical field (1). Device related infections have made prosthetic heart valves, catheters, cardiac pacemakers and joint prostheses highly life threatening and as a result, biofilm was defined as a new source of infection isolated from medical implants or tissues (1). According to Costerton, the contemporary era of biofilm research began with the introduction of the confocal scanning laser microscope by Caldwell and his group of dedicated researchers (22). In Osaka, at the fifth International Society for Microbial Ecology meeting, they introduced the world to the discovery of the ability to investigate the hydrated biotic specimens existing on opaque surfaces (23). As the years passed by, essential contributions aiming at understanding and exploiting biofilms have been successful and we globally prioritize controlling and eradicating the scourges related to this safe haven for bacterial species.

CONCLUSION

In conclusion, biofilm infection and its impacts in various disciplines have gained attention in less than 50 years, even though it is an old concept. Biofilm related infections are causing nuisance in all the fields of microbiology. Historical aspects of biofilm help to understand the evolutionary nature and characteristics of biofilm that in turn helps in designing novel strategies to combat biofilms.

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