# Biofilms

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May 22, 2000
Period 4

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Biofilms are communities of bacteria or other single-celled organisms organized in "slime." You can find them in plaque, in many persistent infections, on slippery rocks in streams, and in many other everyday places. Perhaps you might wonder why infections or plaque never go away (or always come back). Those infections are caused by bacterial communities of bacteria within a strong biofilm.

Microbiology is not a very old subject of study. However, Anton van Leeuwenhoek was curious about things he could see with his primitive microscope, so he scraped the plaque from his teeth and looked at it. It was then that he saw the "animalculi" (bacteria) that composed this type of biofilm. Even though Leeuwenhoek studied this biofilm, no one really paid attention to them. Then, in the 1970s, scientists began to realize the importance of bacterial biofilms. They discovered that 99% of bacteria live in sessile bacterial biofilm communities, but it was not until the 1980s and 1990s that we began to appreciate the complexity of them. We are still studying these things with more advanced technology to find out more about them.

Not all bacteria are capable of forming biofilms. In a study of one species, the Pseudomonas aeruginosa, it was shown that the bacteria with *sad* (surface attachment defective) genes were the only bacteria of the species that formed biofilms. Others were not able to adhere to solid surfaces and/or form microcolonies. This is because they did not twitch. The bacteria with *sad* genes twitch slightly, allowing them to form bonds with surfaces and other bacteria. These *sad* planktonic (independent) bacteria form very strong and stable biofilms as they undergo the process of biofilm formation.[1] ([1] means *see Appendix 1*) First, the *sad* bacteria must adhere to a solid surface (be it bone, plastic, or tooth). Then, when they get settled, they must spread and reproduce to form a microlayer across the surface. When this is completed, a microcolony can be formed. After the colony is established, the mature biofilm (bacteria in a polymeric matrix that they secrete) is finally formed.

Biofilms are complex bacterial “neighborhoods” that have a definite structure. For one, the bacteria nearer the interior of the biofilm have a slower metabolism. When bacteria are far away from the wall of the biofilm, the channels within the matrix cannot deliver much oxygen to them, hence their metabolic state. However, the cells nearer the exterior of the biofilm have a more ample supply of necessary nutrients, so they grow more rapidly.

Do not think that biofilms are limited to just one species of bacteria (or other organisms). Many biofilms have a community of different bacterial species, such as plaque.[2] After the biofilm is formed, other types of bacteria can attach to form a more complex and diverse biofilm. Their self-defense mechanisms are the main reason that biofilms cause such persistent infections. Ordinarily, if bacteria invaded a host, antibiotics, antibodies, and phagocytes (bacteria attacking cells, such as white blood cells) would destroy the bacteria before they could cause harm. However, biofilms are very resistant to these kinds of attacks.[3] For one thing, their transfer properties help them. The antibodies and antibiotics cannot penetrate the polymeric matrix that they create, so they are useless against them. Chemicals that would destroy planktonic cells (such as chlorine, hydrogen peroxide, etc.) would only kill surface cells, leaving the cells in the interior unharmed. Not only that, but increased use of such materials would cause the bacteria to grow resistant to them, making them useless in the future. As a result of their decreased metabolism, antimicrobial agents that rely on the intake of it by the bacteria do not work either.

Even though it may seem as though these bacterial biofilms are completely resistant to antimicrobial agents, they are not. When phagocytes attack biofilms, they excrete phagocytic enzymes. These enzymes destroy everything around it. Because of that, the enzymes penetrate the biofilms to destroy the outer layer of bacteria. Though this injures the biofilm, it does not severely harm it, because the cells in the inside are still healthy. Then the cells in the interior can reproduce to replace the cells that were killed. If the phagocytes keep on excreting these enzymes, the biofilm will eventually be killed. However, the phagocytes will eventually run out of the enzyme and allow the biofilm to regrow. Even so, the release of these phagocytic enzymes destroys human cells, in addition to biofilm cells. Therefore, this method of destroying biofilms is not very effective.

Biofilms also release planktonic cells periodically.[4] This serves two main purposes. One is that it allows free planktonic cells to colonize elsewhere, and the other is that it gives away "bait" for antimicrobial agents. The cells released occupy the antibiotics while the biofilms remains unharmed (at least for a while).

Different bacteria can handle different kinds of attacks. In the study of the Pseudomonas aeruginosa, it was found that bacteria of the species with two different types of sad genes created different biofilms. One produced a biofilm that was very resistant to antimicrobial agents, and another did not. If a small difference in genetics such as this created such a different biofilm, imagine what would happen if you had two different species. This is a reason why bacterially diverse biofilms are somewhat more stable than those with only one type of bacteria. (This is similar to an ecosystem; with more organisms there is more stability.)

By now you might think that biofilms are all bad, but they have their up and down sides. Biofilms actually have some good uses, such as in sewage treatment plants. They are used in sewage treatment plants to remove excessive nutrients from the water. They are a great alternative to costly chemical cleaning. However, they have some drawbacks. They corrode the pipes. They attach themselves to pipes, and bond metal ions within the polysaccharide matrix. This causes a pH difference between cells in the biofilm of up to 1.5 units, causing the metal to move away from the pipe surface to other areas of the biofilm where there is a higher pH, and thus causing the corrosion of the pipe. This can be very expensive to fix. The biofilms must be physically detached from the pipes. This usually results in the scraping the pipes, which is time consuming as well as costly.

Biofilms are also unwanted for water treatment because they harbor bacteria that are released into drinking water. This can be hazardous. A very common biofilm bacteria is the Pseudomonas aeruginosa, which can be dangerous to the health of people with weakened immune systems.

Perhaps I forgot to mention the necessity of water for a biofilm to form. If there is not enough water, the biofilm will not form properly. This is why they form so well in aquatic environments. They live dwelling on the water's surface in streams, rivers, and other bodies of water. Many of them also live on rocks, forming a slimy layer on the rock's surface. This trait of needing water also applies to the human body. This is one reason why bacteria can form such strong biofilms in the human body.

There are many infections that involve biofilms, such as dental caries, musculoskeletal infections, contact lens infections, etc. A major concern about biofilms comes about when artificial biomaterials (such as artificial skin, knee replacements, artificial hearts, etc.) are surgically inserted into the body. It is in the interest of the patient and the doctors that human cells will colonize the new biomaterials first, so that they can become integrated with the body. However, biofilms may colonize it first. If this happens, the body will reject the new implants. Because it is very, very difficult (or impossible) for the body to destroy the biofilm, the implants would then have to be removed. This rejection puts the patient at risk, as well as increasing the need of repeated surgery. This is why biofilms may be very harmful and annoying to patients with biomaterial implants.

Not all biofilms have humans as their haven. Some biofilm-forming bacteria are methanotrophs (methane-utilizing bacteria). These bacteria produce an unusual biofilm. They spread out to form a thin layer.[5] They form their biofilms using energy from methane that comes from the wastes of bacteria that decompose cow droppings, landfill garbage, as well as other things. The methane the decomposing bacteria release goes into the ground, where it is used by the methanotroph biofilms.

Biofilms are very interesting. They have unusual properties, such as their unusual strength and their ability to withstand conditions in which planktonic cells would die. Many infections that seem impossible to remove are caused by biofilms, as well as the reduction of methane in the atmosphere. These unique characteristics make biofilms have many applications, like water treatment. Perhaps in the future we may harbor bacterial biofilms to be more efficient in humans' benefit. Who knows? We may even find a way to kill biofilms within a human without harming the host. Imagine what results that would bring! At this point in time, research is conducted by microbiologists to answer these questions and bring forth results. In the three-hundred years of study of biofilms, the most progress has been made in the last thirty years of it. Perhaps with increased technology we might accelerate our rate of discovery to tame these vicious biofilms. That is our goal. We must find out if we will succeed.

# Works Cited

(References are listed in order of amount of usage.)


1. Costerton, J. W., Stewart, Philip S., and Greenberg, E.P. “Bacterial Biofilms: A Common Cause of Persistent Infections” Science Vol. 284 21 May 1999: 1318-1322

2. “Biofilms” <http://www.bact.wisc.edu/ScienceEd/Biofilms.html>

3. “The Microbial World: Biofilms” <http://helios.bto.ed.ac.uk/bto/microbes/biofilm.htm>

4. “An Introduction to Biofilms” <http://www.edstrom.com/Lab/WaterQualityBulletins/biofilm/biofilm01.htm>

5. Beuling, Evelien. “Mass Transfer Properties of Biofilms” <http://www.ct.utwente.nl/~ospt/minipost96/uva/bpk1.html>

6. “Oral Biofilms” <http://deep13.ra.utk.edu/ceb/proj10.htm>

7. “Introduction to Biofilms” <http://utweb.utampa.edu/faculty/kbeach/Mar327Lect/mchapla/BIOFILM1.htm>


# Photo & Illustration Credits


1. Picture 1, Appendix 1: <http://www.personal.psu.edu/faculty/j/e/jel5/biofilms>

2. Picture 2, Appendix 1: Science Vol. 284 21 May 1999: 1321

3. Appendix 2: <http://deep13.ra.utk.edu/ceb/proj10.htm>

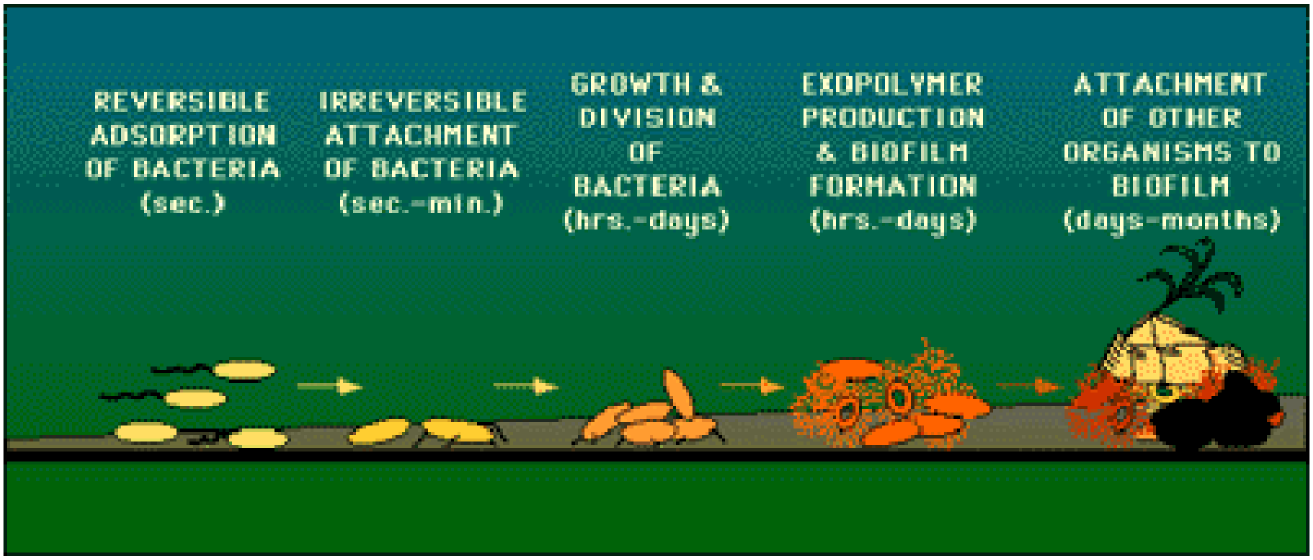
4. Appendix 3: Science Vol. 284 21 May 1999: 1320

5. Appendix 4: Science Vol. 284 21 May 1999: 1321

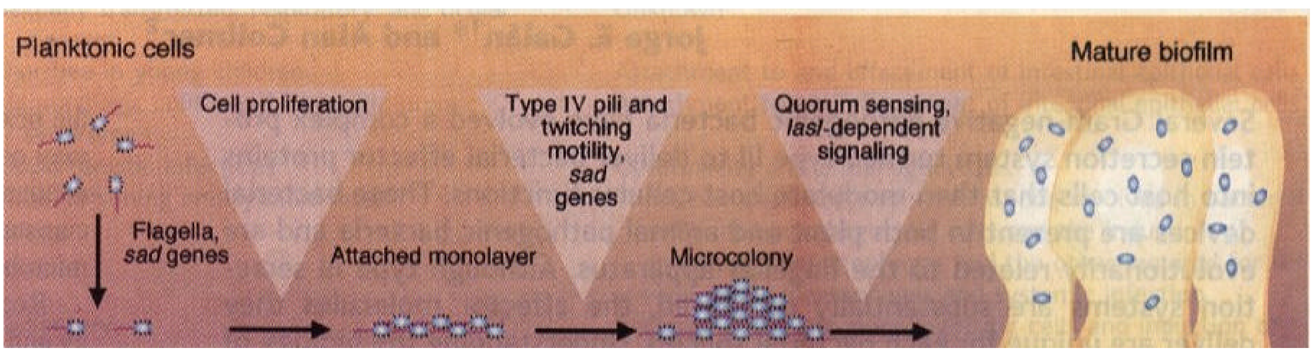
6. Appendix 5: <http://helios.bto.ed.ac.uk/bto/microbes/biofilm.htm>

# Appendix

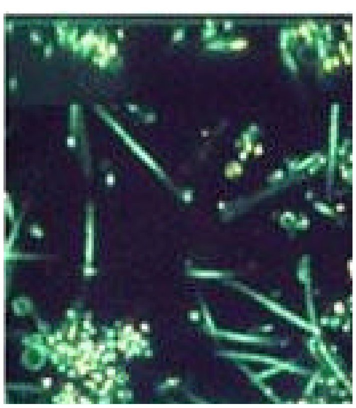
Appendix 1: Biofilm Formation




These pictures show how biofilms form and the duration of each step.



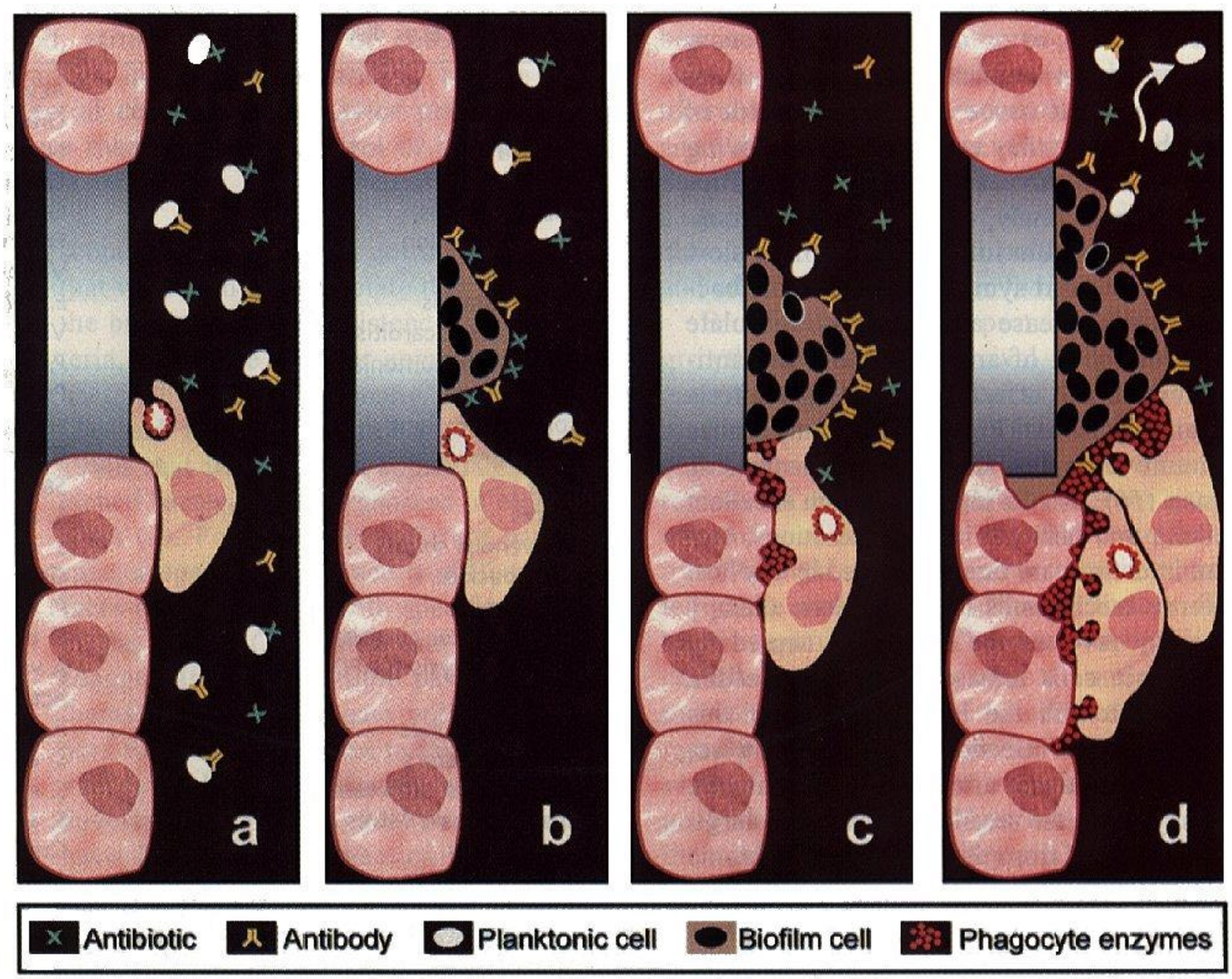

Appendix 2: Plaque



This picture depicts oral plaque biofilm as seen under a microscope.

The bacterial diversity is clearly shown.

Appendix 3: Attack on Biofilm




Shown here is how a biofilm withstands attacks by the human immune system.

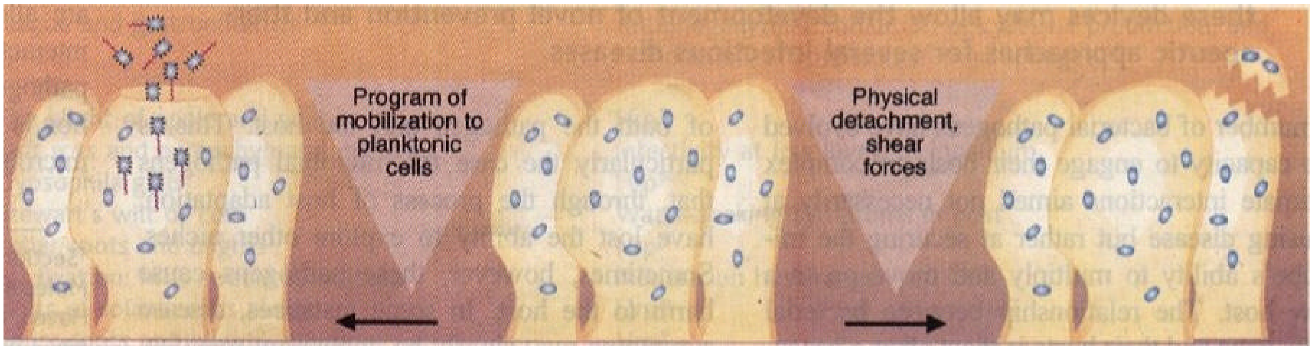
a) Initial Colonization. Planktonic bacteria are susceptible to antibiotics and phagocytes.

b) Biofilm First Forms. Antibodies and antibiotics are attracted but cannot penetrate.

c) Planktonic Cell Release. Individual bacteria are released, and phagocytes try to kill it.

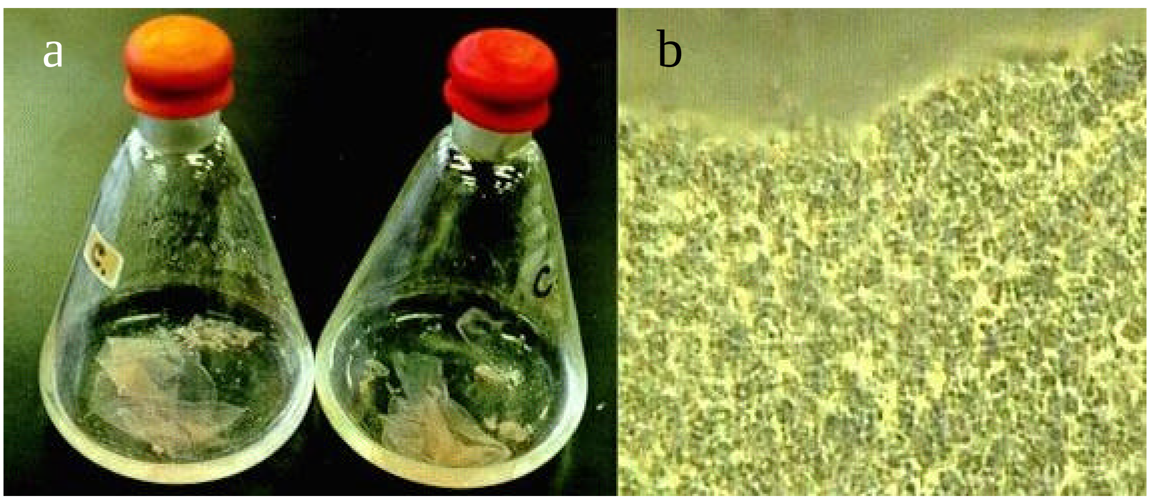
d) Phagocytes Release Enzymes. Phagocytes are frustrated, but enzymes are released and kill the outer layer of bacteria. The bacteria continue to be released, causing infection in other areas.

Appendix 4: Release of Planktonic Cells




This diagram shows two different ways the planktonic cells can be released from a biofilm.

Appendix 5: Methanotrophs




The two pictures show the methane-utilizing bacteria.

a) Two flasks containing an atmosphere of methane, carbon dioxide, and oxygen. The films have been disturbed, causing them to fold.

b) Bacterial biofilm as seen under a microscope. The block-like patterns show the planes of division.