

Biofilms, GALT and Dysbiosis

A new understanding of microbial biofilms and drug resistance

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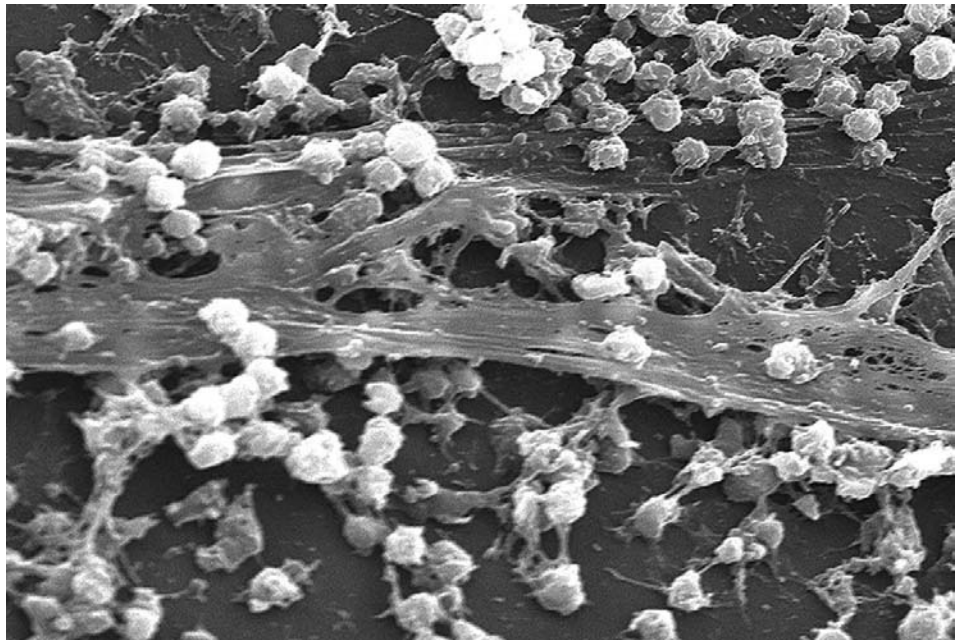
Biofilm (scientific definition): A multilayered bacterial population embedded in a polysaccharide matrix and attached to some surface.

Biofilm (literal translation): Bugs in bomb shelters

The Centers for Disease Control and Prevention (CDC) estimates that up to 70% of the human bacterial infections in the Western world are caused by biofilms, yet not everyone agrees that biofilms are the predominant form of bacteria.

An understanding of communication among bacteria, including those within bacterial communities, is shaping medical treatments of the future. This previously unrecognized but prominent form of the bacterial community is the biofilm. Current microbiology is experiencing a shift in how bacteria are conceptualized. In naturopathic microbiology a medical student witnesses firsthand the conventional way to grow bacteria, inoculating a flask that contains a broth of nutrients. By stirring the broth constantly, the cells have plenty of oxygen and a homogeneous distribution of food. Under these optimal growth conditions, a nice batch of planktonic bacteria will appear, floating in the solution. Because the vast majority of laboratory methods used today examine cultured microorganisms in their planktonic mode, we miss the reality of biofilms. It is important to note that 99% of all microbial species from most environments are unculturable (Lewis, 2008). Once a group of bacteria sets up a "bomb shelter" or biofilm inside the human body, it may be 10 to 1,000 times less susceptible to an antimicrobial substance than the same organism in suspension in a planktonic medium.

It wasn't until the past decade that the term "biofilm" was used to describe bacterial growth on a plant's surface. Most people are familiar with the slippery substance covering rocks in a river or stream. This slime is an aquatic biofilm made of colonies of bacteria, fungi and algae. They form streamers – gooey assemblages of microbes tethered to a surface. Our understanding of the pathogenicity of a biofilm is critical, since it enhances a microorganism's resistance to antimicrobial substances such as pharmaceutical drugs.



Staphylococcus aureus bacteria found on the luminal surface of an indwelling catheter. An erythrocyte is present with its biconcave cytomorphology. The biofilm woven between the round cocci bacteria has been found to protect the bacteria that secrete the substance from attacks by antimicrobial agents such as antibiotics (magnified 2,363x). Image: Janice Carr, CDC

An appreciation of the significance of biofilms is a relatively recent phenomenon. Only within the past 15-20 years have biologists begun to examine the physiology of these microbial communities. This is quite significant considering that Dutch microscopist Antonie van Leeuwenhoek first described biofilms in the late 1600s. Biofilms may represent one of the greatest breakthroughs since Pasteur to the medical community. We predict that when fully understood, this new biofilm perspective of how microorganisms live and respire will have a fundamental impact on medicine (ecology, industry and agriculture). In the advent of antibiotic-resistant bacteria, this information has particular relevance. The biofilm plays a central role in helping microbes survive and spread within the host because the slimy matrix of a biofilm acts as a shield, protecting pathogenic bacteria from antibodies and white blood cells.

Hundreds of research articles exist in hydrology and industrial journals on biofilms

and how they impact these areas. Bacterial biofilms contribute to the corrosion in metal piping and contamination of medical devices such as urinary catheters, hemodialysis equipment, and medical and dental implants; and biofilm colonies are involved in decreasing the drag on a ship, compromising its speed. Biofilms are responsible for more than two-thirds of all infections that physicians encounter, including *Pseudomonas* infections suffered by cystic fibrosis patients, tuberculosis, Legionnaire's disease, periodontal disease and some infections of the middle ear (CDC, n.d.). Biofilms have been a prime topic at recent dental conferences since it is the Strep mutans (or dental plaque) in the biofilms that can compromise teeth, causing accelerated decay (Chen et al., 2007). Minimal research is available on how biofilms impact medicine (Broun et al., 2000).

Perhaps one of the most extraordinary environments where one can find a good example of a biofilm is in the belly of a dairy

cow. Presence of these microbial communities in ruminants provides a rich example of the interactions within a biofilm. In fact, the rumen, which is the largest compartment of the bovine stomach, is filled with many biofilms that microbiologists refer to as "mobile fermenters." Cells in this biofilm thrive in the mucous layer of the stomach and grow on the food the animal ingests. The cellulose, a simple carbohydrate in grass that we cannot break down with our digestive enzymes, is a perfect fuel for the bacteria in the biofilm; the bacteria in the biofilm in turn convert the grass into a microbial biomass that supplies the proteins, lipids and carbohydrates the cow needs (Lappin-Scott and Costerton, 2003).

Evasive Mechanisms Used by Biofilms

Life inside the "gated community" of a biofilm confers many advantages to the individual

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ual cell, including protection from a number of environmental stresses: UV radiation, desiccation, rainfall, temperature variations, wind and humidity (Parsek and Singh, 2003).

Other mechanisms bacteria use to evade antibiotics arise from the remarkable heterogeneity inside the biofilm. Microbes closest to the fluid that surrounds the biofilm have greater access to nutrients and oxygen compared with those in the center of the matrix near the substratum. As a result, the bacteria in the outer layers of the community grow

cells in the center of the biofilm community are further protected since the entry of positively charged substances such as metal ions and certain antibiotics is restricted.

One of the most intriguing defense mechanisms enabled by the formation of a biofilm is called quorum sensing. Quorum sensing may be involved in the defense against antibiotic drugs. The enzymes superoxide dismutase and catalase are both regulated by quorum sensing in *Pseudomonas aeruginosa* and the quorum sensing mechanism employed by

the biofilm survive assaults not only from disinfectants but also from the cells of a host's immune system that typically kill bacteria by unleashing antimicrobial agents, including the reactive oxygen species (ROS). In addition, quorum sensing enables the multidrug efflux pumps, which reduce the accumulation of antibiotics within the bacterium.

Most major medical models in the world agree that disease begins in the gut. In medical school the GI immune system took a back seat to the humoral immune system. It was significant when in gastroenterology class Lise Alschuler, ND, FABNO spoke of the digestive tract as a lymphoid organ unveiling the Gut Associated Lymphoid System (GALT), the collective immune system of the digestive tract, on an overhead projector complete with Peyer's patches.

Recent evidence substantiates that 70%-80% of the immune system is located in the gut. Microbes flourish there. They replicate quickly, exchanging genetic material with each other and with other organisms. Where there are microbes, there is immune activity; the gut is rife with immune activity. Because the lumen of the gastrointestinal tract is exposed to the external environment, much of it is populated with potentially pathogenic microorganisms. Lymphoid tissue in the gut is comprised of the tonsils, adenoids, Peyer's patches, lymphoid aggregates in the appendix and large intestine, lymphoid tissue accumulating with age in the stomach, small lymphoid aggregates in the esophagus and diffusely distributed lymphoid cells and plasma cells in the lamina propria of the gut. Microscopically, Peyer's patches appear as oval or round lymphoid follicles (similar to lymph nodes) located in the lamina

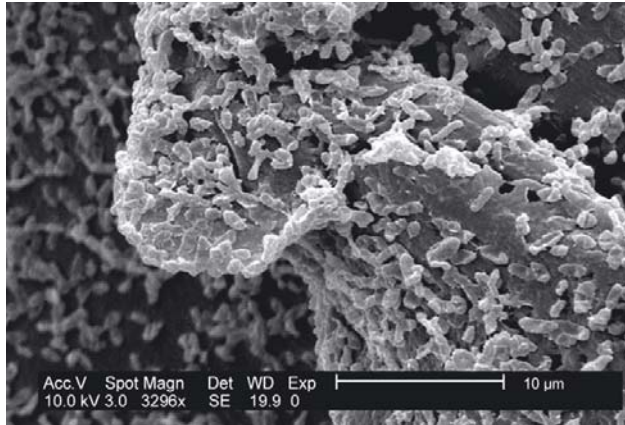
propria layer of the mucosa and extending into the submucosa of the ileum. In adults, B lymphocytes are seen to predominate in the follicles' germinal centers, and T lymphocytes are found in the zones between follicles. Their function is to establish immune surveillance of the intestinal lumen and facilitate the generation of the immune response within the mucosa. In order to deal with the harshest of alien invaders, the mechanism of a secretory IgA "gatekeeper" also evolved.

Host vs. Pathogenic Bacteria: Biofilms and Secretory IgA (sIgA)

Biofilms appear to have an amphoteric role in the gastrointestinal tract. Some evidence exists to demonstrate that sIgA supports normal gut flora through biofilm formation, which is a beneficial factor. In normal or host flora, biofilms enhance adhesion, increasing the stability of the colonies. In pathogenic bacterium such as *E. coli*, the sIgA also enhances biofilm formation. This function inhibits translocation and enhances agglutination of the bacteria to protect the host. The nature of the mechanism of the two sIgA mediated responses – beneficial flora vs. pathogenic biofilms – may be different in execution, but the results are both beneficial to the host (Bollinger et al., 2003).

Possible Role of Biofilms in Chronic Inflammatory States in the Gut

Are biofilms involved in longstanding, unresolving gut inflammation, such as IBS or Crohn's disease? Does environmental



a *P. mirabilis* biofilm growing on polycarbonate coupons using a cdc biofilm reactor. Image: Janice carr, cdc

more quickly than those on the inside. Many antibiotics are effective only against fast-growing cells, so the slow growers within the biofilm tend to be spared. Also, the biofilm matrix is negatively charged, so the

biofilm bacteria, the first enzyme promotes destruction of the harmful superoxide radical and the second enzyme converts the equally toxic hydrogen peroxide molecule into water and molecular oxygen, thereby helping

stress produce mutants adapted for biofilm growth? Can certain nutrients (or lack thereof) impact this? Biofilm tolerance is largely due to the presence of a small fraction of persister cells essentially invulnerable to antibiotics (Brooun et al., 2000). A dormancy program may be turned on in these cells, and this program may be responsible for the tolerance of *M. tuberculosis* to antibiotics, leading to a latent form of the disease that can then relapse into a life-threatening infection. We do not have definitive answers on this yet, but this same concept may someday be applied to understanding why unresolved gut inflammation occurs, possibly being fueled by similar persister cells in the biofilms causing resistance to antibiotics. Biofilm bacteria have an unusual ability to resist chemically unrelated antimicrobials, including ones they never encountered in nature. This ability is largely due to the presence of Multidrug Resistance Pumps (MDRs), which are membrane translocases that pump out antibiotics from the cell (Lomovskaya and Lewis, 1992). Medicinal plants make different types of antimicrobials, but these are weak (individually) when tested in vitro. This is due to their efflux by MDRs (Tegos et al., 2002). Plants are smart and have developed MDR inhibitors that act synergistically with antibiotics. Barberry plants make the fairly ineffective antibiotic berberine. Barberry also produces 5'-methoxyhydrnocarpin-D (5'-MHC) that is a potent inhibitor of MDRs (Stermitz et al., 2002). It is interesting to note that 5'-MHC has minimal antimicrobial activity on its own, but when combined with berberine, 5'-MHC is a potent inhibitor of MDRs. This finding provides an important precedent for the idea that synergistic interaction among different compounds (antimicrobial or not) explains the frequent failures to isolate single active substances from medicinal plants. Plants have faced the problem of microbial multidrug resistance far longer than we have, and their solution is apparently to use a combination of an antibiotic with an MDR inhibitor.

Research at the University of California – Davis has shown that the HIV virus has a gut mucosal connection. HIV enteropathy is well documented in chronic HIV-1 infection. There is evidence of viral replication in GALT resident CD4 T-cells; CD4 T-cell depletion is correlated with decreased expression levels of genes regulating epithelial barrier maintenance and digestive/metabolic functions. One study showed HIV-induced pathogenesis in GALT emerges at both the molecular and cellular levels prior to seroconversion in primary HIV infection, potentially setting the stage for disease progression by impairing the ability to control viral replication and repair/regenerate intestinal mucosal tissues. This is a major element in the limitation of HIV treatment, as resistant gut colonies provide a reservoir of re-infection. This is completely consistent with the biofilm phenomena (Verhoeven, 2008; Sankaran et al., 2008).

Flavonoids: Natural Inhibitors of Microbial Resistance

Key concept: Dr. Bill Mitchell, who specialized in biochemistry in his practice, frequently emphasized in his books and manuals that flavonoids had an affinity for the microcirculation and stabilized CT. From a biochemical point of view, the hydroxyl (OH) groups on all flavone structures and their larger complexes, the tannins, precipitate proteins through the formation

of ionic, hydrophilic and hydrogen bonds with the groups on the proteins. This may explain why any epithelial or endothelial formations with biofilm lesions could be structurally reinforced.

The search for compounds active against antibiotic-resistant strains of bacteria shows promising activity specifically with flavonoids, a majority of which are non-toxic.

For example, Xu and Lee (2001) tested 38 flavonoids (flavones, flavonols and flavonones) for activity against strains of methicillin-resistant *Staphylococcus aureus* (MRSA). The growth of MRSA was inhibited by the aglycones of the flavonols and flavones tested. Their order of activity was as follows: flavones > kaempferones > datiscetin > quercetin > luteolin > myricetin. The flavones acacetin, chrysin and rhoifolin and flavanones pinocembrin, hesperidin, naringenin and eridictyol were

inactive. In the testing of the flavonoids, the only antibiotic active against MRSA was vancomycin (MIC=1.56µg/ml). When 38 flavonoids were tested against resistant gram-negative bacilli, Xu and Le found that myricetin was active against *K. pneumoniae* and *P. aeruginosa*. Xu and Lee noted that the wide range of myricetin activity both against gram-positive and gram-negative bacteria was related to its inhibition of protein synthesis. They also reported that only the polyhydroxylated derivatives of flavonoids (except flavones that contain no hydroxyl groups) were active against MRSA. Flavonoids – flavanones (hesperidin) in particular – were found to inhibit the growth of *H. pylori*.

When approaching the complexities of the intestinal milieu, a comprehensive treatment plan with multiple interventions may offer the best outcome for patients. ■



Nita Bishop, ND is a recognized authority on the flavonoids in plants and berries. She co-developed the first BS degree in herbal medicine at Bastyr University and continues her research on flavonoids as adjunct research professor at SCNM. During the last 10 years, she has studied medicinal plants on a global level, formulating new medicines, including utilizing the highest-testing flavonoids. Her articles have been published in *NutriNEWS/Douglas Labs*, *Naturopathic Digest*, plus peer-reviewed herbal magazines. Dr. Bishop maintains practices at two clinics in Washington, NW Naturopathic Skin & Detox Specialists and Granite Falls Naturopathic.

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