Biofilm Growth and Infiltration

Biofilms present a challenge to any system which processes and distributes fluids across uncontrolled environments. This is particularly the case in the deployment and operations of public drinking water systems. The biofilm is the result of the colonization and extension of micro-organisms which adhere to the surfaces and in turn create a colony of more micro-organisms and the agglomeration of microbiological products in an extracellular matrix producing and ever increasing colony of microorganisms which result in impairments to the flow of the water and putatively could result in significant contamination of the water as well. In this paper we examine the issue of the creation and sustaining of biofilms as a function of surface roughness. It has been demonstrated that the application of nano Se or the application of lipase to create and equivalent nano roughness can act as a bacteriostatic environment and deter the growth of bacteria. The explanation of this phenomena has been presented in a multiplicity of ways all having substantial difficulties. At one extreme one uses the concept of surface energy in terms of Gibbs free energy. This assume a large scale phenomenon and fails to adequately account for the detailed physics of adhesion. At the other extreme we have the DLVO model which includes a combination of van der Waals forces along with dual barrier forces creating double energy catching levels, one for reversible and one irreversible. However, it appears that all such models are deficient. We examine these models, and make some observations that may assist in a process leading to some clarity. We also present this in the context of biofilm control for potable water systems, a significant problem.

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Contents

1 THE PROBLEM

Clean drinking water is an essential in any modern society. As has been well known, even the well managed drinking water may be contaminated as a result of the presence of biofilms[1]. External contaminants are carefully restricted but the growth of biofilms is almost inevitable. The growth is often fostered by the pipes themselves with iron based pipes providing a fertile ground for the biofilm[2].

Biofilms are the development of an integrated bio mass resulting from the growth of bacteria and other microorganisms. The biofilm aggregates and builds into a large mass which can degrade pipes, inhibit flow, and initiate bio hazards if the fluid such as water is to be used in a potable manner. Biofilms are a significant cost in the operations and maintenance of various water flow mechanisms in residential and commercial facilities.

There are many nano bacteriostatic mechanisms for surface treatment have been demonstrated to inhibit bacteria and the resultant biofilm growth. The application of additive nano-Se appliqués or other extractive nano-surfacing have been shown to inhibit biofilm growth via the bacteriostatic actions. The nano technology can be applied to the repair and maintenance of existing distribution systems.

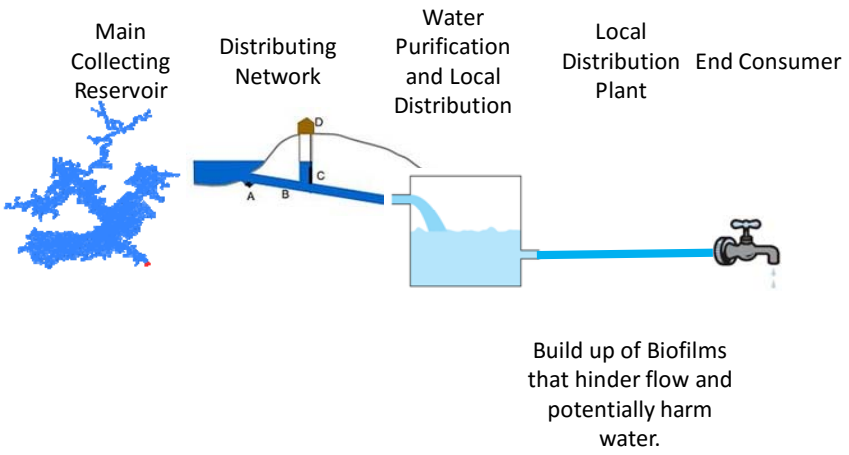
This proposal focuses specifically on the use of the nano technology to public water supply systems. Specifically, it addresses the issue of system remediation and the need for a low cost and fast method to purge the biofilm contaminants as well as shielding other contaminants such as lead from public water supply systems. The proposal focuses on the use of PVC treated with the nano surfacing technology as well and the development of the insertion and installation methodologies to achieve a very low cost remediation system.

1.1 A SIMPLE EXAMPLE

The problem being proposed for study here is the mitigation and inhibition of biofilms in the transport and distribution of water in public water distribution systems. We demonstrate the typical system below.

[1] See EPA Report 1992.

[2] See NRC 1998 report in detail.



As the above demonstrates water is generally collected from aquifers, or other storage areas, and at times directly from flowing bodies. It is then treated and purified and perhaps stored in local facilities. Then as demand occurs it is distributed across a local network. It is that local network which is extensive and in many cases aged that biofilms occur. It in this network that seeks to examine the efficacy and cost efficiency of deploying PVC nano-treated insertions.

The proposed installation is demonstrated below:

Old Pipe with biofilm: The pipe must first be purged and cleaned of biofilm and reported.

The old pipe is then covered internally with a nano surfaced PVC sleeve.

The process is simple:

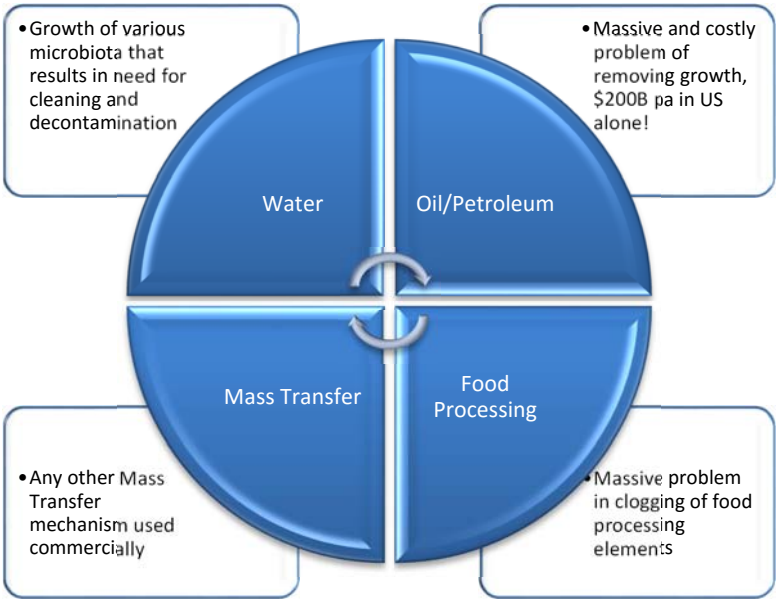
1. Purge old pipe and clean with a standard "pigging" device and then repurge for all removed biofilm.

2. Insert nano treated PVC sleeve for a new pipe.

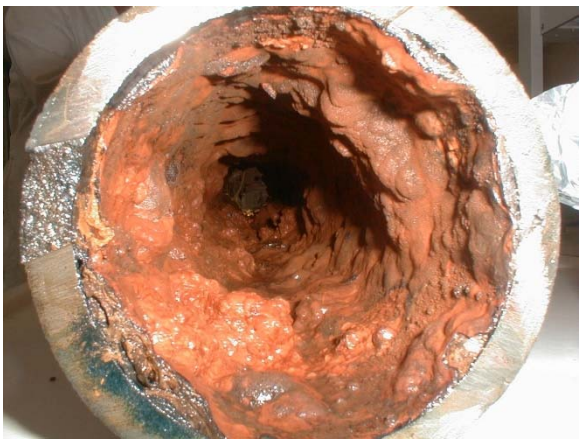
Preliminary analysis indicates that this nano treatment will inhibit regrowth of biofilms for extended periods and further the PVC sleeve will inhibit outflow of such elements as lead from the old pipe. The primary purpose of this study therefore is to validate this approach using the existing nano technology based upon data and sample obtained from actual systems in situ.

1.2 THE PROBLEM

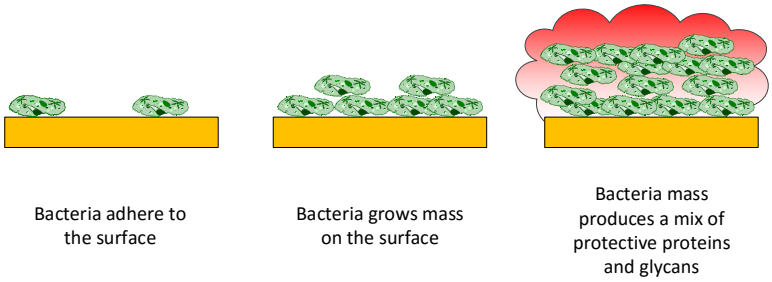
Biofilms are created by the adhesion of bacterial aggregates on the surfaces of various fluid processing, transport and containment mechanisms. The basic physics surrounding this phenomenon was presented by van Loosdrecht et al and it is based upon the construct of surface energy. The small biological particles can adhere to surfaces and one attaching can create via extracellular membrane extension the foundation for a developing biofilm. The problems with this biofilm are significant in a variety of areas such as oil pipelines as discussed by AlAbbas et al and in desalination as discussed by Elimechem et al in desalination plants. Srey et al provide an excellent survey of the impact of biofilms in the food industry.



A typical biofilm encrustation is shown below:

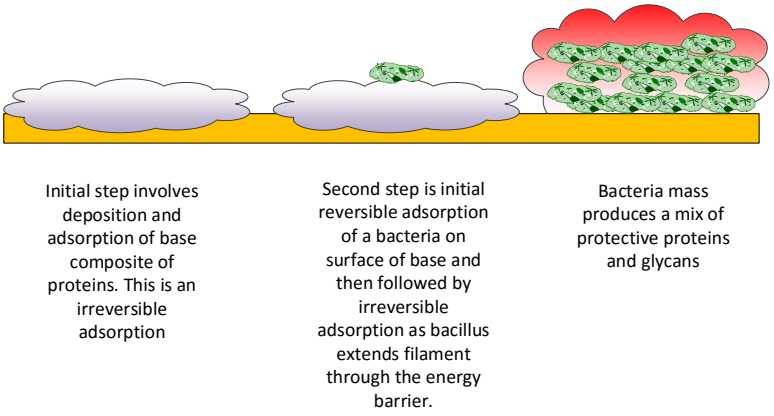


Note the significant growth of biofilm. In the reference by Pervical et al they have extensive discussions regarding the development process in potable water. The effect of chlorine in the water does diminish the growth slightly but it is a common factor not only in loss of flow but in contamination. Fundamentally the process is some three steps as shown below:



The details on the above are also in Kanematsu and Barry. The first step is the development of a protein film on the surface. Then the bacteria reversibly attach and finally the bacteria irreversibly attach and additional biofilm mass grows to cover the bacteria.

Another more detailed view will incorporate a combine reversible and then irreversible process initiated by the coating of the surface by a protein like substance shown below.



Note in the above we must have the layer irreversibly adsorbed to allow an initial reversible bacterium to attach. We will explain this later when examining the issues regarding forces.

From Fleming and Ridgeway, we have:

The term "biofouling" is referred to as the undesired development of microbial layers on surfaces. This operationally defined term has been adapted from heat exchanger technology where "fouling" is defined generally as the undesired deposition of material on surfaces, including:

– Scaling, mineral fouling: deposition of inorganic material precipitating on a surface
– Organic fouling: deposition of organic substances (e.g. oil, proteins, humic substances)
– Particle fouling: deposition of, e.g., silica, clay, humic substances and other particles

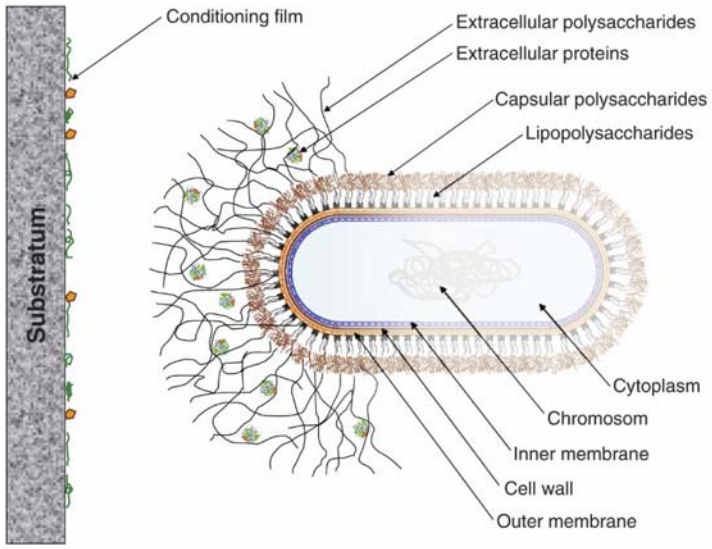
– Biofouling: adhesion of microorganisms to surfaces and biofilm development

In the first three kinds of fouling, the increase of a fouling layer arises from the transport and abiotic accumulation on the surface of material from the water phase.

What is deposited on the surface originates quantitatively from the water. In these cases, fouling can be controlled by eliminating the foulants from the liquid phase. However, this is different in the case of biofouling: microorganisms are "pseudo-particles", which can multiply. Even if 99.99% of all bacteria are eliminated by pre-treatment (e.g. microfiltration or biocide application), a few surviving cells will enter the system, adhere to surfaces, and multiply at the expense of biodegradable substances dissolved in the bulk aqueous phase.

Thus, microorganisms convert dissolved organic material into biomass locally, through metabolic transformations. These metabolic processes, i.e. biodegradation and surface growth, form the basis of biofilm reactors (e.g. membrane bioreactors) that have been introduced in the past decade. Biofouling can be considered as a "biofilm reactor in the wrong place and time".

The Figure below is from the above reference and depicts this effect with bacterial growth as we have also described it above.



The conditioning film shown above is essential. It is a laying down of proteins and water which adhere to the surface via an adsorption process. We generally suspect that such an adsorption is due to the van der Walls forces from the surface to the structure of the specific proteins. We will expand this discussion latter. Upon completion of the surfacing then the proteins extending from the bacillus manage to penetrate this barrier and also become attached via van der Waals forces. Darouiche discusses the impact of biofilms on medical implants as well. He notes:

The essential factor in the evolution and persistence of infection is the formation of biofilm around implanted devices. Soon after insertion, a conditioning layer composed of host-derived adhesins (including fibrinogen, fibronectin, and collagen) forms on the surface of the implant

and invites the adherence of free-floating (planktonic) organisms. Bacterial cell division, recruitment of additional planktonic organisms, and secretion of bacterial products (such as the glycocalyx) follow.

As Batte et al note:

Most of the pipes used in drinking water distribution systems are made of plastic (PVC, PE, etc.) or metal (copper, cast iron) which can become highly corroded (Figure 11). A recent survey of public distribution system pipes in France showed that a large proportion of them are PVC (40%), while the rest are grey iron (22%) or ductile iron (20%) (Cador 2002).

Below are samples of Iron and PVC pipe as used by the authors.

They continue:

The effects of the organic nutrients released by plastic pipes on bacterial growth in drinking water have long been questioned. Organic additives which leach out of plastic have a measurable impact on biofilm accumulation, and are known to promote the multiplication of opportunistic, pathogenic bacteria in laboratory tests. However, no field studies have looked at these events…

The lack of information on biofilm dynamics is a limiting factor in managing the quality of water in distribution system and conducting drinking water surveys. In spite of the difficulty of gaining access to the inner surfaces of distribution pipes, biofilm measurement on pipe walls is indispensable if more information on the water contamination risks is to be obtained. New methods need to be developed, adapted, evaluated and optimized. Such methods will create important advantages: continuous, non-destructive, simple, in situ, online information on biofilm location and development.

However, biofilm monitoring requires laboratory-scale tools adapted to biofilm sampling, so that the hydraulics of real distribution systems can be reproduced as closely as possible.

From Preedy et al we have:

Biofilms are defined as a layer or layers of cells adhered to a substratum which are generally embedded in an organic biological matrix, i.e., extracellular polymeric substances (EPS). It is

due to biofilm formation that many bacteria survive in highly diverse and adverse environments as a result of the polymicrobial ecosystem.

Not surprisingly, biofilms have formed on a variety of surfaces and are not only restricted to attachment at a solid—liquid interface but have been observed at solid—air and liquid—liquid interfaces, with some having beneficial results as well as detrimental; for example, in industry biofilms are used successfully to separate coal particles from mineral matter. On the other hand, biofilms have been known to cause biofouling reducing mass and heat transfer and effectively increasing corrosion; also from a medical point of view, biofilm colonized implanted medical devices often lead to implant failure.

Furthermore, the food industry has had a major interest in biofilms as a result of their resistance to cleaning and disinfection because spoilage and pathogenic bacteria pose a risk to public health and product quality.

1.3 CURRENT TECHNOLOGY

Current technological areas focus on several areas. The areas are:

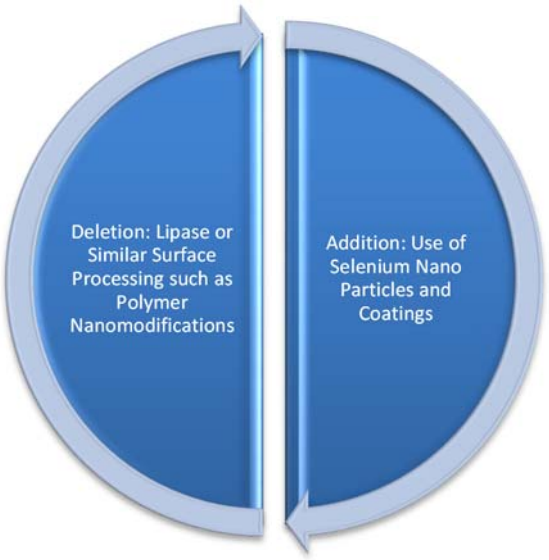
Nano Surface Enhancements: These are nanotechnology enhanced titanium surfaces which demonstrate reduction in bacterial infection potential and also demonstrate enhanced tissue and bone growth ensuring improved human acceptance.

Surface Bactericidals for Intracorporeal Applications: These are nanotechnologies for surface coatings of various catheters and the like that result in dramatically reduced risks of infection by inhibiting bacterial growth.

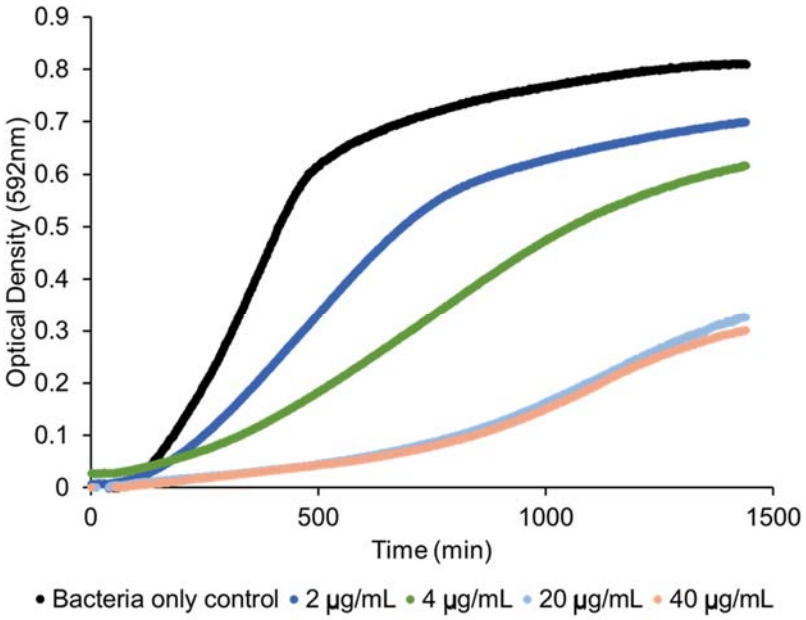
Selenium Enhanced Bactericidals: This is a selenium based product which enables the control of bacterial growth. It appears to function as a bacteriostatic agent. Combined with a bactericidal agent the combination may affect dramatic control for long periods of bacteria on surfaces. This area of product development appears to have several areas of application: (1) Those applications which can be seen to be applied directly to the skin (cosmetics, wound dressing, etc.), and (2) Those applications which can be used in clinical and consumer applications to treat surfaces for anti-bacterial purposes, (3) The control of growth on various surfaces of harmful flora or fauna.

1.4 THE APPROACH

Treatments have developed an approach to mitigate the growth of biofilm. This is via the treatment of the surface by nano processing. The treatments may be either by addition of materials such as nano Se or by the selective deletion of surfaces to create a similar nano surfacing effect by the use of lipase and other similar surface treatments.

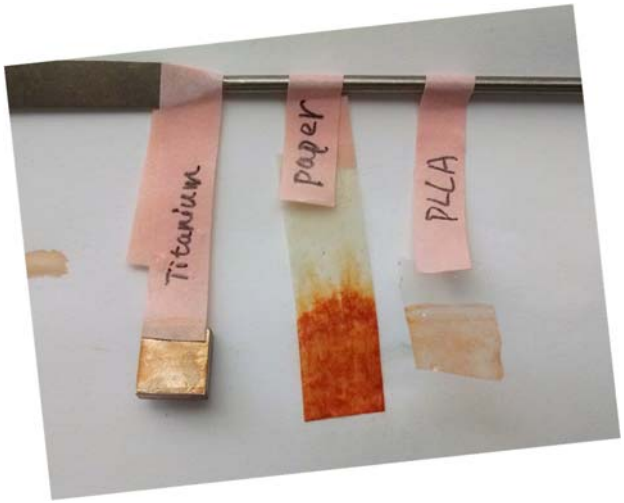


For example, nano Se has been demonstrated to slow down S. aureus proliferation at a dose-dependent rate. Increased lag time (in 40 and 20 µg/mL doses) would allow for the body's immune system to attack bacteria before exponential growth. We demonstrate some of these results below:



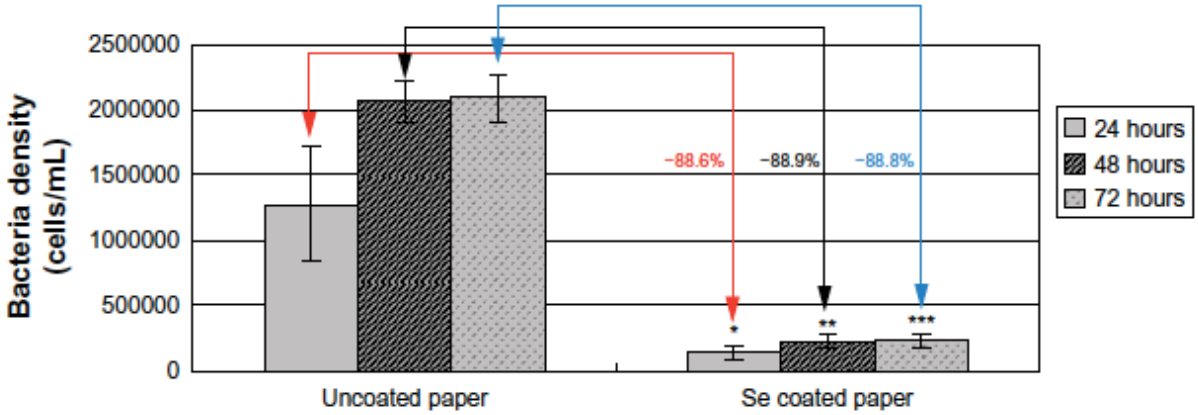
In the above we note that the regrowth of bacteria is dramatically reduced by the application of a Se surface at concentrations of 20-40 micrograms/milliliter. It has also been observed that surface coatings of a density of 100 ng/sq cm of 20-70 nm diameter nano Se on the surface are also adequate. The question we pose herein is; what is the physical process that causes this to occur?

All types of surfaces and materials can be coated with SeNP, including typical biomaterials (titanium, polymers, etc) and household items (paper towels, tablet screens, keyboards).



Bacterial efficacy has been demonstrated as shown below:

- Gram positive Staphylococcus epidermidis was decreased by several logs on SeNP-coated paper towels
- SeNP coatings have also reduced gram negative Pseudomonas aeruginosa, E. coli, MRSA, and ampicillin resistant E. coli



2 BASIC PRINCIPLES

We now examine the physical processes which may account for this twofold process. Namely:

1. Nano Se coatings and lipase nano processing of surfaces tend to create a bacteriostatic environment.

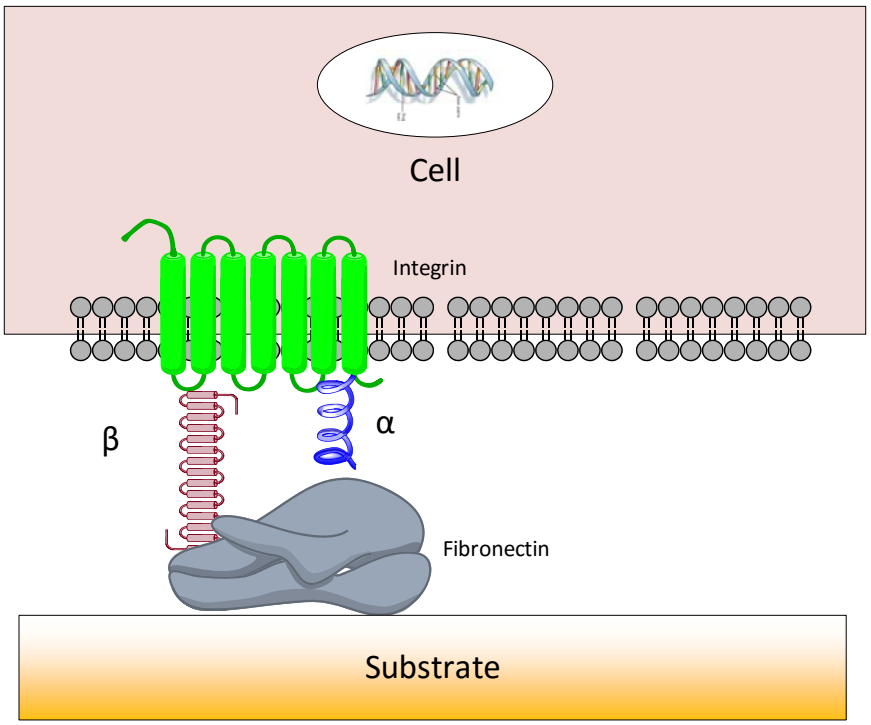
2. Nano Se coatings and nano surface processing tend to create an environment that enhances tissue adhesion.

These appear to be contradictory results. It would appear that both processes are controlled by the same physical mechanism. Yet the outcomes are dramatically different. We attempt to explain some of these effects. However, it should be noted that in our analysis the explanation is yet far from clear.

The following is a brief discussion of some of the basic principles and specific technologies. Details are contained in the papers by Webster and his team at Northeastern and Brown University. We have also examined the literature in general and provides a summary regarding that as well.

2.1 SURFACE ENERGY

The principal basis for the technology is understanding surface energy as relates to bacterial adhesion and subsequent biofilm growth. We demonstrate the basic principle below for a eukaryotic cell using the approach by Webster. On a smooth surface we have with cells a fibronectin, a glycoprotein, which binds integrins. This allows the pathogen cell to attach to the surface and commence biofilm growth.



The issue then is to create a surface which is not conducive to the binding. This can be accomplished by manipulating the surface energy by mechanical means. We can show that the protein absorption is proportional to the surface energy. This is shown below[3]:

$P_{i,absorption} \approx a_i S$

P = Protein absorption

S=Surface Energy

Now the surface energy can be written as follows:

$$E_s(r_{eff}) = E_{0,s} + \rho r_{eff}$$

This depicts the surface energy as a combination of the chemical or ground surface energy for a perfectly flat surface plus a contribution due to roughness. We will try to rough the surface to increase the surface energy to increase the protein absorption. Now it can be shown that the effective roughness is given by:

[3] See Butt and Kappl pp 220-223

$$r_{eff} = \frac{S_{unit}}{S_{measured}} \sqrt{\frac{\sum_{i=1}^{N} \left(z_{i,filtered} - z_{avg,filtered} \right)^2}{N}}$$

Thus we then have:

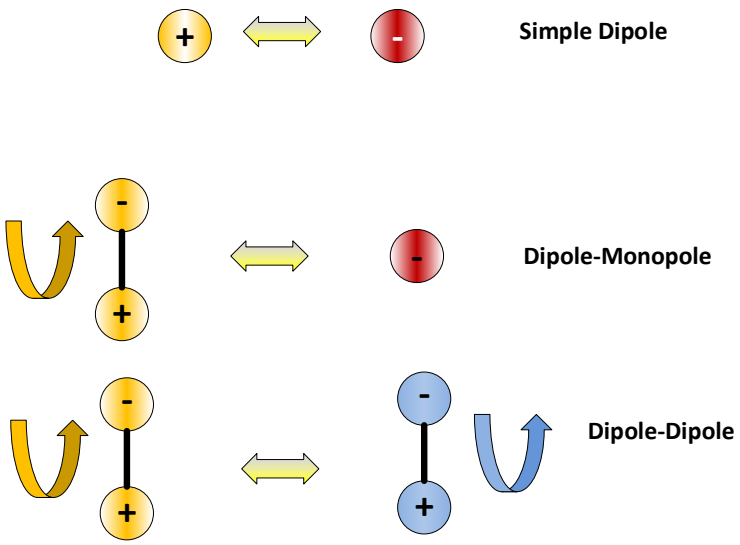
$$F_{adsorption}(r_{eff}, E_{0,s}) = Ar_{eff} + \beta E_{0,s}$$

Which is the contribution due to roughness first and then the contribution due to surface energy. This equation is for fibronectin, which we have seen can decrease bacteria and macrophage functions. Similar equations and relationships can be developed for other proteins. Thus we can increase nanoscale roughness and not change chemistry to increase protein adsorption.

In effect the technology allows for the fine tuning of the surface characteristic to inhibit bacterial and biofilm growth.

2.2 VAN DER WAALS FORCES

We can briefly examine van der Waals forces as discussed by Butt and Kappl. Let us first consider simple Coulomb forces. We can consider three types of surface to external adhesion for vdW. They are shown below:



For each we can determine the attractive force. They are:

$$F_{Monopole} = \frac{K}{d^2}$$

$$F_{Monopole-Dipole} = \frac{K}{d^4}$$

$$F_{Dipole-Dipole} = \frac{K}{d^6}$$

Note the dramatic differences. Later we shall see that many argue for the simple connection of inverse square where one surface is positive and the other is negative. This is a simple vdW approach. However, the other two can be equally valid depending on the nature of the molecules connecting. Namely in the case of proteins the protein structure can be quite complex depending on the specific amino acid construction. Note that for proteins the bonds generally are inverse fourth power strength due to the dipole-monopole configuration. There may even be cases in certain protein structures where the bonds are inverse sixth power[4].

2.3 BACTERIAL ADHESION

The adhesion of bacteria to surfaces is a complicated and yet to be satisfactorily answered phenomenon. There are several theories and we will examine one herein. We use the DLVO approach which is a force or energy approach. Alternative approaches using thermodynamically defined terms and Gibbs Free Energy, G, have also been proposed but they do not seem to provide adequate answers. Let us first review some general principles.

The DLVO (Derjaguin, Landau, Vervey, Overbeek) approach uses the two forces; van der Waals and Ionic. The paper by Trefaly and Borkovec is an excellent summary of this and we shall follow its approach. The total free energy is defined as:

$$W(h) = W_{vdW}(h) + W_{DL}(h)$$

Where DL refers to the double layer force/energy between the surface and the medium and h is the distance from the surface to the contact point on the bacterium. The above can be shown to be:

$$W(h) = -\frac{C}{h^2} + B\exp(-kh)$$

The first term is van der Waals and the second the double layer[5]. Note negative energy means attraction and positive is repulsion. We can determine the points where W(h) is zero, namely the points where we go from attraction to repulsion of the reverse. Namely we equate W(h) to zero.

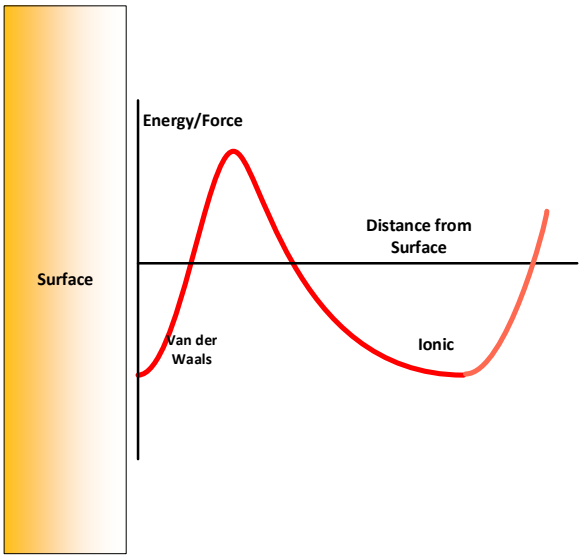
[4] See Petsko and Ringe, Protein Structure and Function, Sinauer (Sunderland, MA) 2004. Pp 8-11.

[5] Note as we have mentioned above the inverse square may be inadequate for proteins.

$$\frac{(C/B)}{h^2} = \exp(-kh)$$

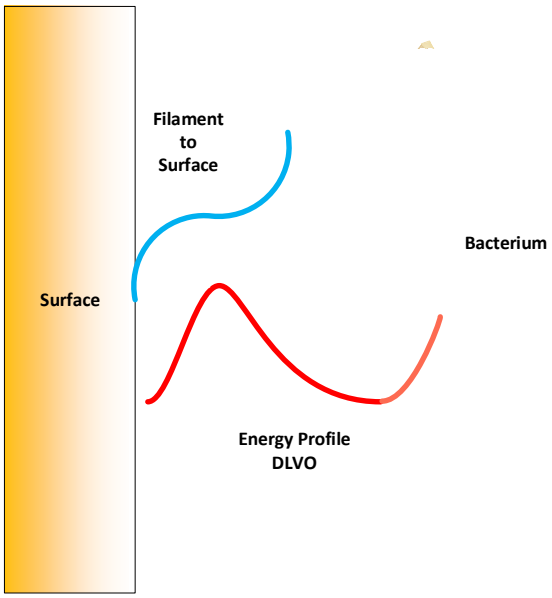
That is, we can plot the left and right equations and determine the intercepts and this shows when we go from negative to positive forces. An example is sown below:

Now the surface may be seen as below with these two forces:

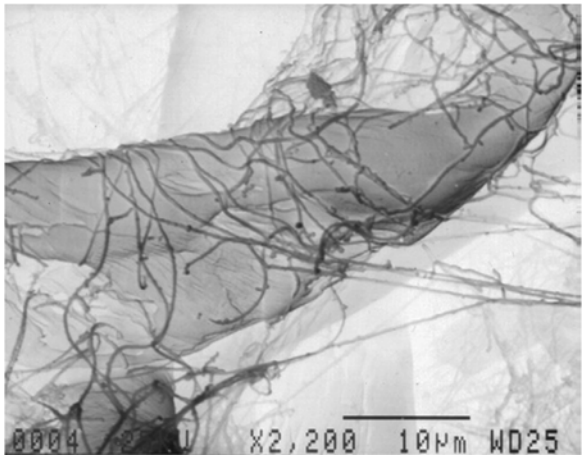


Note that in close we have an attraction due to van der Waals and then at a distance we have the double layer effect. The scales are not precise but just descriptive. The vdW force is much stronger but there is a positive "barrier" between it and the outer layer. Brownian motion can get a bacterium close to the surface and catch it reversibly in the ionic or DL area. However, to have an irreversible bond something must get to the vdW section, a much stronger section.

Now the bacterium sends out a filament to try to bond to the surface via the vdW forces. It must penetrate the barrier and then bond as shown below.



In the Boland et al paper we have an example of such bonding showing the extending filaments:



They discuss what they term cellulose binding domains, CBD, areas of the protein which do the binding in this case to cellulose. They state:

These CBDs have been classified into 10 families (I-X) on the basis of amino acid sequence homology. The amino acid sequences of CBDs in C. cellulovorans and C. josui show high homology with those from other cellulolytic genera such as Bacillus. CBDs in this family contain several highly conserved amino acid sequences:

1. Tryptophane-asparate-phenylalanine-asparagine-asparate-glycine-threonine
2. Isoleucine-alanine-alanine-isoleucine-proline-glutamine
3. Isoleucine-leucine-phenylalanine-valine-glycine

We can then ask; what if we roughen the surface, what will that do? The specific answer is not known and even less understood conceptually. A logical conclusion is that by roughing the surface we increase the positive side by moving the inner vdW in and out and thus make it more difficult to adhere. The Thermodynamic argument is a hand waving discussion of surface energy. But we tried that argument above without success on adhesion of human tissue cells.

As Bok noted in his Thesis:

The forces that govern microbial deposition, adhesion and detachment are still not fully understood, and difficult to relate with each other. In a previous study we successfully investigated the characteristic shear force to prevent adhesion of microbial strains. In the current research we used a more systematic approach by including not only the shear forces to prevent adhesion, but also those that stimulate detachment of adhering bacteria, as well as theoretical adhesion forces calculated using the extended DLVO theory.

In addition, the effect of a passing air-liquid interface, which invokes a high, perpendicularly oriented detachment force on adhering bacteria, was determined. Furthermore, all experiments were carried out with six different bacterial strains in order to allow general conclusions to be drawn. As a first step in the experimental analysis, the gravitational force and its impact on bacterial deposition and adhesion was eliminated by averaging the deposition rates on bottom- and top plate. At low shear rates, deposition efficiencies (α) exceed unity especially for the S. epidermidis strains, indicating that deposition is more favorable than theoretically predicted. Often such deviations are ascribed to the presence of surface structures.

With respect to possible relations between the different forces distinguished, we test the following hypotheses:

1) ***A strong hydrodynamic shear force to prevent adhesion relates to a strong hydrodynamic shear force to detach an adhering organism.***

This hypothesis implies a positive correlation between attachment and detachment. Comparison between Fprev and Fdet show that regardless of the substratum involved, Fdet is always larger than Fprev. In the experimental set-up used, bacteria are adhering to the substratum surface for at least half an hour before being subject to high shear. Therewith, over time the bond between a bacterium and the substratum surface may become stronger. Supporting evidence for this is provided by others who have used AFM and found that the adhesion force increases with prolonged contact time.

Thus, even though initial adhesion forces are rather weak, they may be indicative for forces after a prolonged time, i.e. a relatively strong Fprev might be expected to correspond with a relatively strong Fdet. However, … it is clear that no correlation exists between Fprev and Fdet. It implies that attachment and detachment should be regarded as independent processes and the hypothesis of an unambiguous relation between attachment and detachment forces should be rejected.

2) ***A weak hydrodynamic shear force to detach adhering bacteria implies that more bacteria will be stimulated to detach by a passing air-liquid interface through the flow chamber****.*

… clearly indicates that Fdet for hydrophobic DDS-coated glass is larger than for hydrophilic glass, indicating stronger interaction forces on the hydrophobic substratum. Table 3 summarizes parameters involved in air-bubble-induced detachment. An air-liquid interface exerts forces 104 times larger than Fdet, yet it does not result in complete detachment. Combining the data … reveals the absence of a clear relation between shear-induced detachment and detachment by passing an airbubble. Thus a weaker Fdet does not result in higher air-bubble-stimulated detachment and this hypothesis has to be rejected too.

In this respect it must be realized that different mechanisms of detachment are involved in both processes. Hydrodynamic detachment forces are measured while the system is completely submerged in liquid whereas an extra phase is introduced in air-bubble-induced detachment. Furthermore, Fdet is a force acting parallel to the substratum surface, whereas the air-liquid interface acts perpendicularly to the substratum surface.

3) ***DLVO interactions determine the characteristic hydrodynamic shear forces to prevent adhesion and to detach adhering micro-organisms as well as the detachment induced by a passing air-liquid interface.***

Further analysis revealed the absence of quantitative relations between FDLVO and Fprev, as well as between FDLVO and Fdet. DLVO-predictions have often been demonstrated to deviate from experimental observations of bacterial interaction phenomena, which is usually ascribed to the presence of surface appendages or chemical surface heterogeneities. However, the direction of action of the DLVO-forces should be taken into account as well.

DLVO-forces act perpendicularly to the substratum surface, whereas both Fprev and Fdet are directed parallel to the substratum surface. When the fluid flow is increased to high enough values, the bacterium most likely detaches in a rolling fashion. It can be argued that in this mode of detachment, forces normal to the surface (i.e. DLVO- and lift forces) are related to forces directed parallel to the surface.

However, in similar detachment studies it was found that lift forces are negligible and surface roughness may play a decisive role in determining the hydrodynamic force to remove adhering particles from the surface.

This feature is not accounted for in the DLVO-theory. Table 2 shows only slight differences between the theoretical FDLVO-values for the various microbial strains, but substantial differences between the experimentally obtained forces Fprev and Fdet. If a correlation between DLVO forces and shear forces would exist, an increase of these parallel directed forces implies an increase in normally directed forces.

However, this is not observed in FDLVO. Hence, the parallel detachment forces do not correlate to the perpendicularly directed DLVO-forces. The DLVO theory predicts a secondary minimum of interaction at a distance of about 30 to 40 nm away from the surface … On hydrophilic glass, closer approach is impossible due to strong repulsion and adhesion can only occur in the secondary minimum. On DDS-coated glass, also primary minimum interactions are predicted.

However, due to the prohibitive high barrier of the free energy (ranging from 229 kT to 1030 kT depending on the strain used), it is very unlikely that adhesion in the primary minimum can occur. Therefore, also on the hydrophobic DDS-coated glass, only adhesion in the secondary minimum is expected to occur. …Fdet values are much higher than FDLVO.

Often, a transition of adhesion from the secondary interaction minimum towards the primary minimum is used as explanation. However, in this study this is considered to be impossible as on glass a primary minimum is absent and on DDS-coated glass it is considered to be unreachable due to the high energy barrier.

It is therefore more likely that the higher Fdet values are the result of attachment of surface appendages, or "extracellular polymeric substances" produced, capable to reach the surface. These structures are known to extend as much as hundreds of nm away from the bacterial cell wall, which is more than enough to bridge the distance between secondary minimum and the substratum surface. Unfortunately, although it is known for instance that some streptococci may possess surface fibrils, structural information about the cell surface of far most all strains studied in the literature are lacking, let alone detailed knowledge about the length, diameter and micro(nano-)scopic physico-chemical properties of these structures. The use of the DLVO-theory as currently done in the literature as well as in this chapter, can therefore only pertain to long-distance approach, where fine surface structures do not play a role.

Up to what distance of approach and up to what extent this statement is valid, is hard to say. However, while the DLVO-theory predicts interactions for the entire micro-organism, it is likely that the experimentally obtained detachment forces are related to a number of distinct (hydrogen) bonds. When these linkages break, due to parallel directed forces, the bacterium can be transported away from the surface due to lift forces which are induced by the tangential flow. In this respect, parallel directed hydrodynamic forces (i.e. Fprev and Fdet) can serve as useful parameters to indicate adhesion strength…….

There is no unambiguous relation between the hydrodynamic forces (Fprev and Fdet) directed parallel to the substratum surface and perpendicularly oriented parameters (FDLVO, air-liquid interface detachment), because these forces act in different directions.

DLVO forces maybe wrongfully estimated because of local charge heterogeneities and bridging between cell appendages and/or exudates on the one hand and substrate surface on the other. Furthermore, air-liquid interface induced detachment relies on a three-phase system, whereas the other forces are obtained for a two-phase environment, complicating establishment of a possible correlation.

Thus from the above experimental analyses the DLVO has some merit but it clearly does not describe the entire process. There are significant issues still outstanding to be explained theoretically. Bacterial adhesion and the formation of biofilms is still in the process of being fully understood. Kanematsu and Barry provide an exceptionally strong discussion here but we must resort to experimental data for phenomenological insight. Boland et al also provide a substantial discussion on this but fail to provide a strong analytical basis. Their analysis is useful to better understand some of the phenomenology.

2.4 THE THERMODYNAMIC PARADIGM

The thermodynamic paradigm is based upon certain principles that aggregate large collections of common particles like gas, steam, or a fluid. Thermodynamic principles work in the large like those used in reactors or distillation columns or heat exchangers. We shall review some of these principles and then demonstrate their lack of efficacy in this model.

For example, when considering the process of wetting, one can generally use thermodynamic and surface tension methods. There is a homogeneity on the surface and on the wetting materials. Tran and Webster (2013) have provided an interesting analysis for nano scale wetting. They explain it via the Wenzel and also the Cassie-Baxter models. They all involve surface tension as is done in the core Young's analysis[6].

van Loosdrecht et al were one of the first to explain the adhesion via thermodynamic principles. They argue that one should use the Gibbs Free Energy, G as follows:

$$\Delta_{adhesion} G = G_{Bacterium\_Surface} - G_{Bacterium\_Liquid} - G_{Solid\_Liquid\_Interface}$$

Then they state:

The Concept of Short-Range Interactions

If adhesion is performed at constant pressure and temperature, and if the molecular composition of the surface does not change, all G's can be replaced by the corresponding interfacial tensions. This concept is restricted to those cases where bacteria and the solid surface are in direct contact and the original phase boundaries are replaced by a new one, namely, the bacterium solid interface. When this new interface is formed, interfacial tensions may be used for a direct estimation of the adhesion Gibbs energy. Many authors have found a good correlation between contact angle measurements (which have been used to estimate the solid/vapor and solid/liquid interfacial tensions) and bacterial adhesion and have therefore applied this concept to discuss bacterial adhesion.

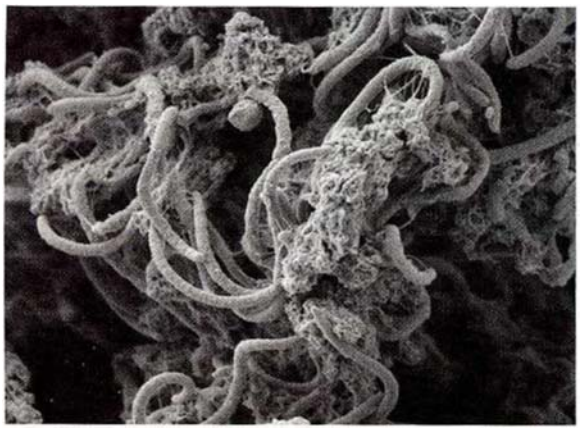
The Concept of Long-Range Interactions

The DLVO theory for colloidal stability can be used to calculate the interaction Gibbs energy between a particle and a surface as a function of the separation distance (H). The balance of interracial Gibbs energies ... is the basic premise of this theory. The net interaction Gibbs energy is interpreted in terms of Van der Waals interactions (which are usually attractive) and an electric interaction due to the overlap of the electrical double layers at the charged surfaces. The most important parameters determining the van der Waals interaction are the Hamaker constant, which is a material property, the distance (H) between bacterium and substratum, and the geometry of the system. For simple systems there is an approximate relation between the Hamaker constant and the interfacial tension. Because at short range other interactions (e.g.,

[6] See Butt et al, pp161-180.

steric repulsion and hydrogen bonding) also play a role, the DLVO theory can only be used if the separation distance (H) between the surfaces is greater than approximately 1 nm.

alAbas et al demonstrate oil pipeline biofilm as below:



Now in contrast alAbas et al note:

Thermodynamic approach

The thermodynamic approach assumes the system is in equilibrium and the bacterial attachment is a reversible process. The interfacial free energies between the interacting surfaces are compared and calculated, …. This comparison is expressed in the so-called free energy of adhesion.

Based on that, the work of adhesion (Wadh) and free energy of adhesion (AGadh) is obtained. The work of adhesion can be calculated by the Dupre Equation as follows:

$$W_{adhesion} = \left(-\gamma_{solid\_microorganism} + \gamma_{microorganism\_liquid} + \gamma_{solid\_liquid}\right) dA$$

…The free energy of adhesion … is calculated by the following:

$$\Delta G_{adhesion} = -W_{adhesion} = -\left(-\gamma_{solid\_microorganism} + \gamma_{microorganism\_liquid} + \gamma_{solid\_liquid}\right) dA$$

The microbial adhesion will be favorable when the change in G, is negative (< 0) and will not be energetically favorable if … positive.

They then continue:

DLVO Approach: The drawback of the thermodynamics approach is that it ignores the electrical double-layer interaction with the bacteria… This assumption is invalid as the bacterial cells have a surface-negative or-positive charge. In contrast, the DLVO approach displays a balance between attractive Lifshitz- van der Waals… and repulsive or attractive electrostatic forces …

These two forces are function of the distance (d) between the bacteria and surface. In order to calculate the adhesion free energy … the electrostatic interactions between surfaces should be included. The inclusion of electrostatic interactions requires that the zeta potentials of the interacting surfaces be measured, in addition to measuring contact angles. So the total free energy expression is:

$$\Delta G_{adhesion} = \Delta G_{vanderWaals}(d) + \Delta G_{electrostatic}(d)$$

The attractive Lifshitz-van der Waals AGLW is calculated by:

$$\Delta G_{vanderWaals}(d) = -\frac{AR}{6d}$$
$$A = 24\pi d_0^2 \gamma_i^{vanderWaals}$$

The repulsive or attractive electrostatic forces is shown .. below:

$$\Delta G_{electrostatic}(d) = \pi\varepsilon_r\varepsilon_o\pi a\left[2\phi_a\phi_s \ln\left[\frac{1+\exp(-kd)}{1-\exp(-kd)}\right](\phi_b+\phi_s)\ln\left[1+\exp(-kd)\right]\right]$$

The term A is the Hamakar constant, ϕ are the zeta potentials of the bacteria and the flat surface, R is the sphere radius assuming the bacteria is sphere shapes and ε are the electrical permittivity of the vacuum and medium respectively, K is Debye- Huckel parameter, and d is the distance in nm. It has been found that the medium ionic strength has no influence on the Lifshitz-van der Waals attraction, whereas both the range and the magnitude of the electrostatic interactions decrease with increasing ionic strength due to shielding of surface charges. In case of high ionic strengths, electrostatic interactions have lost their influence. Extended DLVO approach The extended DLVO theory relates the origin of hydrophobic interactions in microbial adhesion and includes four fundamental interaction energies: Lifshitz-van der Waals, electrostatic, Lewis acid-base, and Brownian motion forces …

The above approach makes semi-macro thermodynamic assumptions. Specifically, a large mass of surface, liquid and biofilm concentrate. In fact, the dynamics of the process are totally overlooked. This is the general failing of thermodynamic approach; they assume some form of steady state along with homogeneity. In reality we have a dynamic process in a highly heterogeneous environment.

3 THE TECHNOLOGY

We briefly discuss the technology to be employed. The details are contained in the references by Webster discussed herein.

3.1 NANO TECHNOLOGY

Nano surface treatments can be accomplished by treating the surface itself or adding nano materials to the surface. The result is a stable nano surface that inhibits bacterial growth and ensuing biofilm development. The Gecko has nano fibrils on its feet that allow it to climb any surface by means of van der Waals attraction as we see in nano material surfaces. The production of nano Se is performed via a proprietary process but fundamentally is the following:

Glutathione +NaOH + Se --→ Nano Se

The image below is an example of the (red) Selenium produced in this manner.

As Mendonca et al note, using reference to Webster's work, the details of surface energy effects and adhesion or lack thereof:

The changes in initial protein–surface interaction are believed to control osteoblast adhesion. This is a critical aspect of the osseointegration process. When implants come into contact with a biological environment, protein adsorption (e.g. plasma fibronectin) that occurs immediately will mediate subsequent cell attachment and proliferation. Cell binding to protein domains of adhesive extracellular matrix proteins involves receptors termed integrin receptors that transmit signals through a collection of proteins on the cytoplasmic face of the contact, termed focal contacts.

Surface effects are often mediated through integrins that bind the RGD motif in cell attachment proteins. The RGD motif of cell adhesive proteins such as fibronectin or vitronectin is important in mediating cell adhesion of osteoblasts and other cells to synthetic material surfaces.

Nanofeatures could alter the conformation of these RGD containing proteins, a phenomenon known to affect cell adhesion and behavior. Changing the ***surface energy*** *or wettability of a biomaterial represents a classical approach to altering cell interactions with the surface.*

Extracellular matrix protein adsorption onto surfaces (to subsequently modulate cell adhesion) is dramatically affected by surface energy. Interestingly, many studies of self-assembled monolayers (SAMs) have demonstrated that hydrophobic groups are more likely to adsorb albumin and that albumin is not replaced by ECM proteins, blocking cell adhesion. Hydrophobic surfaces adsorbed fibrinogen, while hydrophilic surfaces allowed an interchange of adsorbed albumin by ECM proteins. Nanoscale topography is a powerful way of altering protein interactions with a surface.

Webster and colleagues observed an increased vitronectin adsorption on nanostructured surfaces when compared to conventional surfaces. They also found an increased osteoblast adhesion when compared to other cell types, such as fibroblasts, on the nanosurfaces.

Another study suggested higher adsorption of fibronectin on hydrophilic SAMs surfaces with greater focal adhesion formation (integrin binding) evident in the osteoblast cells adhered to the hydrophilic SAM treated surfaces.

Lim and colleagues more directly related protein adsorption, cell adhesion and the active process of attachment by measurement of increased focal adhesion kinase (FAK) activity.

In a study using SAMs biofunctionalized with RGD, Cavalcanti- Adam and colleagues also found that the spacing among the nanofeatures modulates focal adhesion (FA) formation; cells cultured on a 58 nm nanopattern formed normal FA, whereas those plated on a 108 nm nanopattern failed to develop FA.

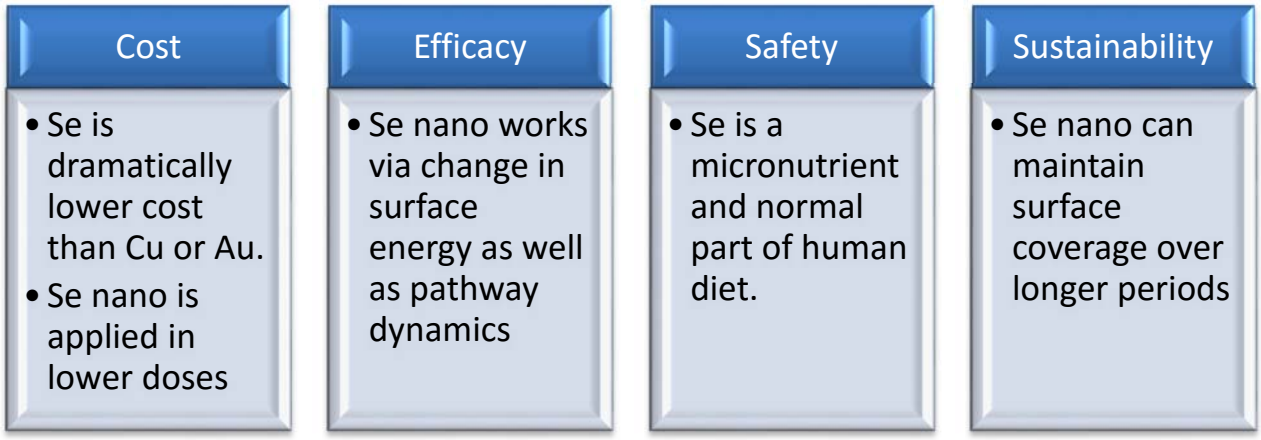
Surface roughness at the nanoscale is an important determinant of protein interactions that ultimately direct cell activity in control of tissue formation at implant surfaces.

3.2 MATERIAL PROCESSING

To obtain a proper nano surface there are two methods. The additive method uses nano Se which can be made at specific nano size and in a very well controlled and defined distribution so as to assure the proper surface energy. The second approach is the deletive approach whereby a nano surfacing has a process that removes materials in such a controlled manner so as to achieve the same desired surface energy.

3.2.1 Additive

Nano Selenium has been demonstrated as highly effective. In addition, as we demonstrate below it is also safe and sustains the effect on the surface for an extended period of time.



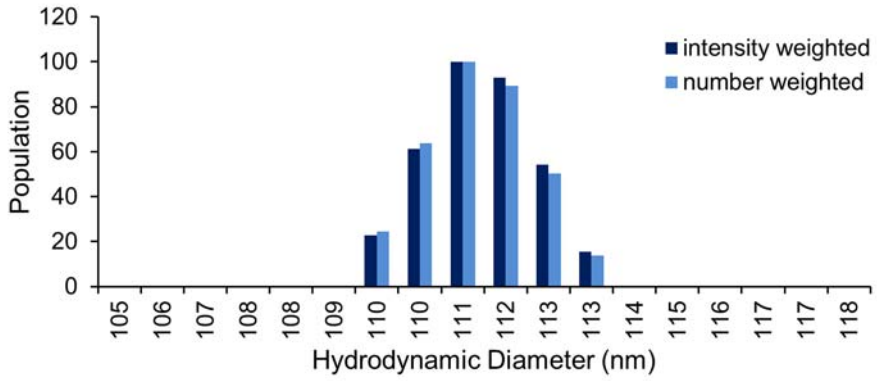
Why SE? The reasons are as follows:

- Essential micronutrient metalloid
- Component of several key antioxidants, detoxifying and metabolic enzymes, in form of selenocysteine, selenomethionine
- Two allotropes: red (bioactive) and grey (crystalline)
- Strong associations with reduction of Reactive Oxygen Species1,2,3 (ROS)
- Cofactor of glutathione peroxidase
- Antibacterial activity to a broad range of pathogenic strains3
- First proposed to have a role in cancer in I9692
- Cancer mortality rates were inversely associated with geographic distribution of selenium
- Since then, many studies have reviewed Se's connection to cancer prevention in humans
- Selective cytotoxicity to cancer cell lines in nanoparticle form4

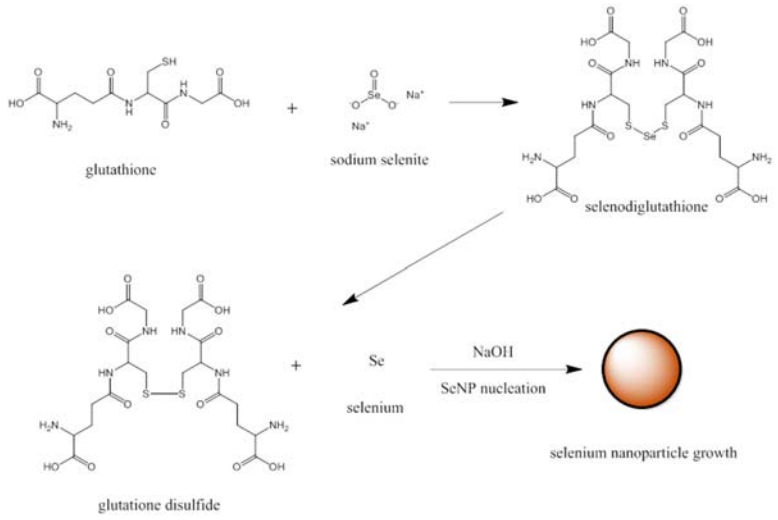
Nano Se can be produced at specific nano diameters with minimal dispersion.

- Spherical in shape
- Monodisperse—size distribution fits within one bell curve
- Negatively charged (uncoated)

We demonstrate some results below where we show how tightly we can obtain specific nano sized coatings.



The overall process for the production of nano Se is graphically demonstrated below.

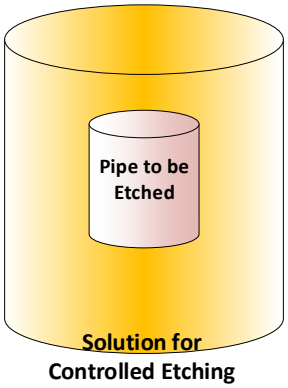


Thus using the proprietary method developed by Prof Webster and his students we have an extremely effective and cost efficient result.

3.2.2 Deletive

The deletive approach used extraction mechanisms which produce similar effects to the additive mechanism of nano Se. The advantage of such an approach is that it does not add anything to the material. The disadvantage in certain active biological surfaces such as human skin is that it causes immunological effects. However, its used in stable media such as PEEK, Titanium, steel, and other materials used for water flow and containment is that it can be readily effected and at low cost.

The processing of a deletive method is depicted below:



There are numerous processes to implement nanoscale surface features on metallic or polymeric surfaces. We then utilize one of our processes to create such nanoscale features:

1. Anodization
2. Chemical etching

The deletive approach provides comparable results to that for Se coatings.

3.3 BIOFILM INHIBITION RESULTS

There have been a variety of biofilm inhibition methods. As Garrett et al note:

Bacterial adhesion has become a significant problem in industry and in the domicile, and much research has been done for deeper understanding of the processes involved. A generic biological model of bacterial adhesion and population growth called the bacterial biofilm growth cycle, has been described and modified many times.

The biofilm growth cycle encompasses bacterial adhesion at all levels, starting with the initial physical attraction of bacteria to a substrate, and ending with the eventual liberation of cell clusters from the biofilm matrix. When describing bacterial adhesion one is simply describing one or more stages of biofilm development, neglecting the fact that the population may not reach maturity. This article provides an overview of bacterial adhesion, cites examples of how bacterial adhesion affects industry and summarizes methods and instrumentation used to improve our understanding of the adhesive properties of bacteria.

The NRC report states[7]:

The pipe surface itself can influence the composition and activity of biofilm populations. Studies have shown that biofilms developed more quickly on iron pipe surfaces than on plastic PVC pipes, despite the fact that adequate corrosion control was applied, the water was biologically treated to reduce AOC levels, and chlorine residuals were consistently maintained…In addition to influencing the development of biofilms, the pipe surface has also been shown to affect the

[7] See p 230-234.

composition of the microbial communities presents in the biofilm. Iron pipes supported a more diverse microbial population than did PVC pipes. The purpose of these studies is not to indicate that certain pipe materials are preferred over another but to demonstrate the importance of considering the type of materials that come into contact with potable water.

4 UTILIZATION OF BIOFILM CONTROL

One of the motivations for the reductions in biofilms is the remediation of the existing systems of pipes in water systems containing lead, a well know toxic element. At the time of the disclosure of the lead problem in Flint, MI, one reporter asked why they did not replace all the water pipes in Flint. Since the reporter had no real understanding of the issue or the costs and apparently the local Government leader was unprepared to answer the result has been uncertainty and confusion.

4.1 BIOFILM ISSUES IN POTABLE WATER

As we have indicated biofilms are a major source of contamination and inhibitors of performance of potable water distribution systems. As discussed in the National Research Council report in 1998:

Growth of coliform bacteria in distribution system biofilms could be considered a nuisance if they had no public health significance. Coliform bacteria have traditionally been used to indicate the adequacy of drinking water treatment. A new interpretation of this indicator concept implies that drinking water is not adequately treated if coliform bacteria can proliferate in distribution system biofilms.

One concern is whether opportunistic pathogens such as Legionella pneunophila, Mycobacterium avium complex (MAC), or other microbes can also proliferate. Members of the M. avium complex (i.e., M. avium and Mycobacterium intracellulare) have been shown to occur in drinking water distribution systems with levels ranging between 0.08 and 4S,000 CFUs/ml.

The greatest increase in M. avium complex infections has been with acquired immunodeficiency syndrome (AIDS) patients, approximately 25 to 50 percent of whom suffer debilitating and life-threatening MAC infections.

The organism infects the gastrointestinal or pulmonary tract, suggesting that food or water may be important routes of transmission for AIDS patients. In an ongoing research study, examination of eight, well-characterized, drinking water systems showed that slow-growing mycobacteria were frequently detected in raw water and in distribution system samples using a chloramine residual. Either free-chlorine or ozone treatment appeared to be sufficient to eliminate mycobacteria to below detectable levels in plant effluent levels…

The conclusion that chloramines provide a selective advantage for mycobacteria may be premature because of the higher rates of mycobacteria detection in raw water and possible elimination of bacteria that overgrow the selective medium. The reason for the frequent detection of slow-growing mycobacteria in the free-chlorinated groundwater site is unclear but may be due to the low chlorine residuals (average of 0.15 mg/L) observed at this location. The levels of slow-growing mycobacteria detected in raw water samples and in distribution system...

The results show that high levels were detected in a small number of samples. These levels could occur following flushing or other activities that could dislodge biofilm samples or resuspend

distribution system sediments. Biofilm Mycobacterium levels ranged from nondetectable to >1,500 CFUs/cm2.

4.2 BASIC GOALS

Some thoughts may be worthwhile.

1. Most old cities have no idea where all these pipes are. Records are not that great nor are they accurate and things move. So trying to find them is extremely problematic.

2. The cost to even dig and install new pipes is fantastic. Estimates are about $5 million per mile of pipe, possibly even more.

3. However, the old pipes can be used, somewhat. Namely they provide rights of access if they can be cleaned and resurfaced.

4. In the oil industry they face the same problems. Oil pipeline get what is called a biofilm growth. Like arteries in old folks. What they do is use a device called a "pig" that has razor sharp blades that rotate and cut off all the old biofilm. Let's assume you can do that in the old lead pipes. The tools exist and they work. Much less that the replacement costs. And the pigs collect the biofilm masses and expels them as it moves forward.

5. But one still has lead problem in the old pipes. What one could do is install a PVC or equivalent pipe sheath inside the lead after the pipes have been cleared. This keeps the lead out.

6. You must either coat the PVC with nano Selenium or use a deletive process with a selective lipase to inhibit future biofilm growth. This can be an extremely low cost alternative. The installation is a section by section insertion, not requiring massive redigging.

7. This replaces 95% of the lead pipe and provides a stable bacteriostatic flow path. Unfortunately, any lead pipes on residential sites would not be included by lead leeching from them would most likely be within acceptable limits.

8. It may leave some residual lead local pipes in homes but they were there anyway. You will reduce the lead burden by well over 99.9%, well within EPA limits.

9. When using the pigs, you also use modified GPS to fully digitized map the system for future reference.

10. Estimates of this process are about $250,000 per mile of pipe.

The overall goals of a study should be as follows:

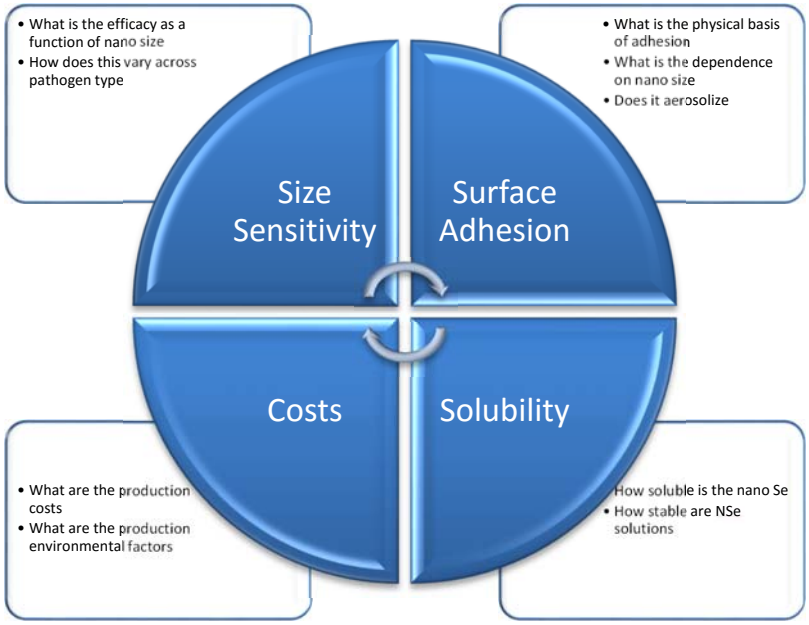
1. To examine the ability of nano surface modifications to minimize and possibly eliminate biofilm growth by changes in surface energy of pipes.

2. Determine the efficacy of the elimination of biofilms based upon a wide variety of pathogens.

3. Examine and determine the efficacy of additive and deletive approaches.

4. Determine the means and methods to treat surfaces based upon costs models.

5. Ascertain possible long terms efficacy rates.



4.3 PHENOMENOLOGY AND UNDERSTANDING

As we have discussed there does not appear to be a well-defined theoretical understanding as to the mechanisms that a bacterium uses to bind to a surface. Our conjecture uses the DLVO as a framework, albeit as we have discussed it has limits, and sees that in a DLVO world the bacterium needs to develop a proximity via some filament. Yet as we know the filaments are most likely proteinaceous in nature and thus there are bonds that may act positively and negatively as the filament tries to establish an adherence.

5 A PROPOSED RESEARCH EFFORT

The overall goal of the Research conducted herein is to determine what the optimal efficacy-cost treatments should be to minimize the development of biofilms on the distribution networks for potable water. The specifics of the research are to ascertain the change in biofilm buildup on various surfaces and with various treatment methodologies. The pathogens to be studied in this Research cover the span of those common in biofilm production as well as those seen in health care venues.

From the 1992 EPA Report on Biofilms it states[8]:

Biofilms provide protection for microorganisms, including disinfectant-resistant microorganisms and opportunistic pathogens. These microorganisms may be present in water obtained at the tap. Therefore, it is possible to drink disinfected water and still become ill.

Thus the goal is to mitigate biofilms and especially create a bacteriostatic environment that inhibits the growth of the protective cover that biofilms provide for continued bacterial growth.

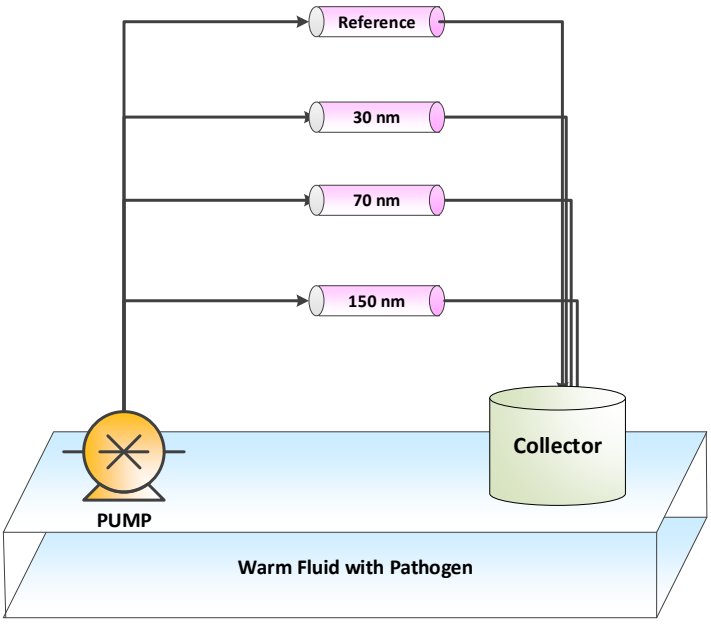
5.1 BIOFILM ANALYSIS

The first portion of the Research will include the development of biofilms from sets of pathogens and on collections of common surfaces found in potable water distribution systems. This will entail the in situ collection of biofilms and the identification of constituents as described in the next section.

5.2 TRANSPORT MEDIUM COATINGS

The test will include the determination efficacy of various surface energy targets against a reference of no coatings and detailed by pathogens separately and in combination. An experimental set up is shown below.

[8] See EPA 1992 p 8.



In the above configuration the following will be performed:

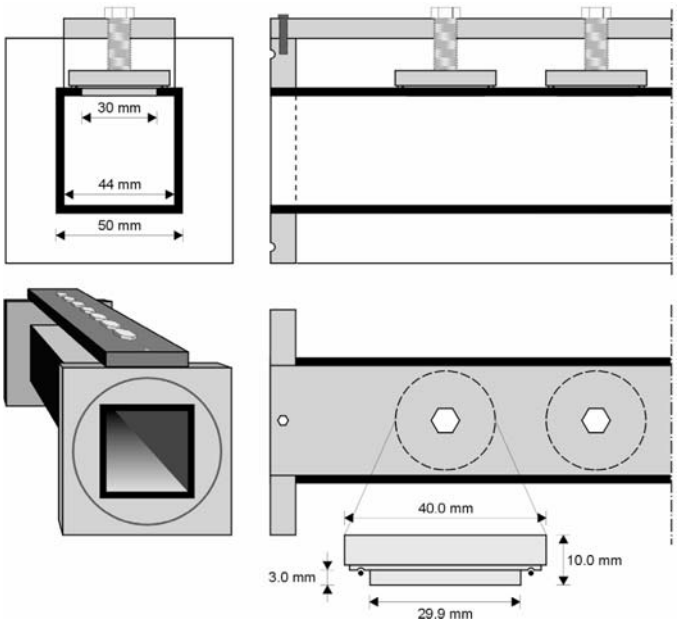
1. In situ collections of biofilms will be made at six to twelve locations from existing water supply systems in the New England area. These samples will then be tested using protocols generally accepted for the identification of biofilm constituents.

2. Selected biofilm agents will be selected. It is anticipated that somewhere between six and twelve such agents or combinations will be selected based upon the results obtained from field in situ tests.

3. Warn water baths will be contaminated by selected amounts of biofilm agents and water so infected will be pumped through treated and untreated pipes for a period of time, most likely six months. The system will also allow for the real time visual inspection of biofilm buildup. The pipes will be treated and untreated PVC. There will be a spectrum of pipes ranging from 10 nm to 15o nm as well as a reference pipe which shall be untreated.

4. At the completion of the tests the pipes will be disassembled, the biofilms removed and the constituents analyzed and the results compared.

Specifically, the Research will test various surface treatments a various surface energy levels on various surface types and across various pathogen baths. Note that this scheme is a modification of the method used by Boe-Hansen et al. as shown below:



As Boe-Hansen et al note:

The model distribution system was continuously fed with drinking water from a municipal distribution network. The drinking water was produced from groundwater without disinfectants with an inlet AOC content of 6.1 ± 3.3 µg ac-C/L (n = 18).

The hydraulic retention time and the flow velocity were fixed at 2 hours and 0.07 m/s respectively. Biofilm samples were collected by removing test-plugs and subsequently swabbing the entire surface of the plugs with a sterile cotton bud to collect the attached bacteria.

The bud was then transferred to cell-free water, and vortexed vigorously for 1 minute to release the bacteria. The model system was operated at constant conditions for more than one year to allow a quasi-stationary biofilm to form, and during this period the biomass on the surfaces was frequently quantified using total microscopic counts (AODC), heterotrophic plate counts (HPC, R2A, 15°C, 7 days) and ATP (adenosine triphosphate) measurement. Later, the mature biofilm (>1 year of age) was examined by a suite of techniques to evaluate the most suitable one for biomass quantification (HPC, AODC and ATP), activity measurement (leucine incorporation, AOC removal, CTC and live/dead stains), and microbial diversity profiling (clone libraries, DGGE).

In addition, some structural studies were performed directly on the test-plugs using a DAPI stain visualized by confocal laser scanning microscopy (CLSM). Additional information regarding the specific procedure for each technique is given in Boe-Hansen et al. (2001, 2002a, 2002b…

It is estimated that the total duration of this efforts will be one year.

5.3 PATHOGEN TESTING

The research will involve the testing of the following pathogens. We believe that it is essential to isolate the pathogens ab initio in order to ascertain the most effective surface energy depletion levels. We have selected the first group based on their prevalence in biofilm generations in pipes.

Organism	***Persistence***
Coliforms	max. 3 weeks
E. coli	*1-3 days*
Yersinia	*up to 2 weeks*
Campylobacter	*up to 4 weeks*
Helicobacter pylori	*2-3 weeks*
Aeromonads	*Could not be established*
Atypical mycobacteria	*persistent*
Pseudomonas aeruginosa	*> 4 weeks*
Giardia cysts	*1-3 days*
Cryptosporidium oocysts	*1-3 days*
Entero viruses	*7 days*
Legionella	*persistent*

From the perspective of human contamination, we would also consider the following.

Organism	*Persistence*
Bordetella pertussis	
Burkholderia cepacia	
Campylobacter jejuni	
Escherichia coli (E. coli)	
Escherichia coli O157:H7	
Klebsiella pneumoniae	
Pseudomonas aeruginosa	
Salmonella enterica	
Staphylococcus aureus	
Acinetobacter baumannii	
Enterobacter cloacae NDM-1 positive	
ESBL Producing Escherichia coli (E. coli)	
Klebsiella pneumoniae KPC-2 positive, ST258 (multi-drug and carbapenem resistant)	
Methicillin Resistant Staphylococcus aureus (MRSA)	
Vancomycin Resistant Enterococcus faecalis (VRE)	

Testing of the growth of these pathogens and the development of biofilms on water pipes will be performed according to the following protocols:

1. One proposal is to employ the RNA testing as discussed by Barghouti. As Barghouti states:

The Universal Method (UM) described here will allow the detection of any bacterial rDNA leading to the identification of that bacterium. The method should allow prompt and accurate identification of bacteria. The principle of the method is simple; when a pure PCR product of the 16S gene is obtained, sequenced, and aligned against bacterial DNA data base, then the bacterium can be identified. Confirmation of identity may follow. In this work, several general 16S primers were designed, mixed and applied successfully against 101 different bacterial isolates. One mixture, the Golden mixture7 (G7) detected all tested isolates (67/67). Other golden mixtures; G11, G10, G12, and G5 were useful as well.

The overall sensitivity of the UM was 100% since all 101 isolates were detected yielding intended PCR amplicons. A selected PCR band from each of 40 isolates was sequenced and the bacterium identified to species or genus level using BLAST. The results of the UM were consistent with bacterial identities as validated with other identification methods; cultural, API 20E, API 20NE, or genera and species specific PCR primers. Bacteria identified in the study, covered 34 species distributed among 24 genera. The UM should allow the identification of species, genus, novel species or genera, variations within species, and detection of bacterial DNA in otherwise sterile samples such as blood, cerebrospinal fluid, manufactured products, medical supplies, cosmetics, and other samples.

2. A second methods as discussed by Barron is more classical and is generally been employed at the Laboratory at Northeastern.

3. Other more advanced techniques may be employed as described by Emerson et al:

The advent of new molecular technologies in genomics and proteomics is shifting traditional techniques for bacterial classification, identification, and characterization in the 21st century toward methods based on the elucidation of specific gene sequences or molecular components of a cell. We discuss current genotypic and proteomics technologies for bacterial identification and characterization, and present an overview of how these new technologies complement conventional approaches.

The new methods can be rapid, offer high throughput, and produce unprecedented levels of discrimination among strains of bacteria and archaea. Remaining challenges include developing appropriate standards and methods for these techniques' routine application and establishing integrated databases that can handle the large amounts of data that they generate. We conclude by discussing the impacts of rapid bacterial identification on the environment and public health, as well as directions for future development in this field.

However, these newer techniques will require use of an Illumina system and we propose using the Illumina MiniSeq system because of costs and capability as well as throughput efficiency[9].

[9] http://www.illumina.com/content/illumina-marketing/amr/en_US/systems/miniseq/applications.html

4. There is also a US Government standard used by FWS which states[10]:

Unless otherwise specified, all of the materials and techniques described in 3.8 Bacterial Identification Techniques are described in detail in MacFaddin's[11] (2000) and or the 11th Edition Difco Manual (1998). Each of the tests listed are provided with a set of control bacterial species available from ATCC, which will provide quality control for each biochemical test. It is not necessary, however, to set up control isolates for every test run in these protocols. It is strongly suggested that newly prepared batches of media and reagents be tested using the control bacterial isolates listed for each.

[10] https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=0ahUKEwj4-9HFrqfMAhUCMj4KHejVD3UQFghPMAQ&url=http%3A%2F%2Fwww.fws.gov%2Fpacific%2Ffisheries%2Ffishhealth%2Fdocuments%2Fbluebook%2Fcdr_pdfs%2Findexed%2Fb3_08.pdf&usg=AFQjCNGbL5FE3e0woiPb-DfOk-xEKRBKqA&sig2=aszx3gob3awPK32KVVj35A&cad=rjt

[11] MacFaddin, Biochemical Tests for Identification of Medical Bacteria 3rd Edition, Lippincott (New York) 2000.

6 CONCLUSIONS

In this paper we examined several issues. Specifically:

1. What is a Biofilm? This we have answered by reference to various studies.

2. How do biofilms form? The answer to this may often depend but it is clearly a dynamic process.

3. What is the physical phenomenon that allows biofilms to adhere and have strong adsorption? This is a work in progress. We believe the thermodynamic approach is problematic at best. It is necessary to consider more detailed dynamic physical phenomenon. We make some suggestions here.

4. What is the effect of nano-surfacing on biofilms? This appears to be uncertain at best. There are contrasting phenomenological results.

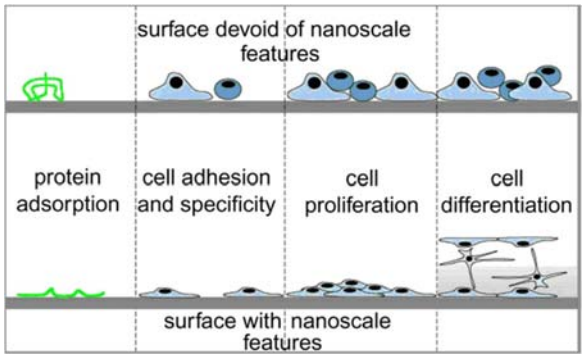
5. Why does nano-surfacing enhance adsorption of certain eukaryotic cells such as bone and ligaments while inhibiting the adsorption of prokaryotic cells such as bacteria? This appears not to have been examined.

6. How can nano-surfacing be optimized to minimize biofilms? Argument from surface energy have been proposed but are problematic.

These questions can and have been answered in part but there remains a set of uncertainties that challenge the effective utilization of nano technologies.

6.1 NANO SURFACING FOR ENHANCED GROWTH

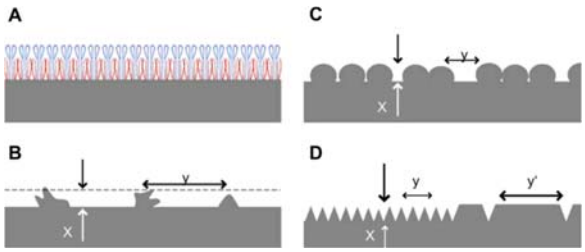
Our focus has been on nano surfacing to inhibit bacterial growth. At the same time, it has been argued elsewhere based on experimental demonstrations that nano surfacing enhances cellular growth. From Mendonca et al we have the following process proposed for the enhancement of nano growth. They show the following graphic and make the subsequent following statement:



Depiction of broad range of nanoscale topography effects observed in cellular protein adsorption is altered by nanoscale modification of bulk material. Both cell specificity and extent

of cell adhesion are altered. Depending on the nano-architecture cell spreading may be increased or decreased. By presently undefined mechanisms, cell proliferation appears to be enhanced by nanoscale topography. For osteoblast, several investigators have shown nanoscale topography enhances osteoblast differentiation…. Across a micron rough titanium surface, 50–100 nm surface accretions of titanium oxide are observed by scanning electron microscope (SEM) analysis Greater osteoblastic gene expression (Runx2, Osterix, Alkaline Phosphatase and Bone Sialoprotein) was measured in cells adherent to the nanoscale HF treated surface compared to the micron-scale surface. This nano topography is associated with the elevated levels of gene expression that indicate rapid osteoblastic differentiation. Most recent investigations show that this nanoscale surface modification promotes high levels of IGF-2 and BMP2 and BMP6 expression by adherent human mesenchymal stem cells for prolonged periods of time in culture.

Thus the creation of nano roughness enhances adhesion as well as bone reformation. They describe the nano surfaces as shown below:



Nanoscale surface modification. (A) Self-assembled monolayers (SAMs) can change the topography and chemistry of a surface to impart novel physical and/or biochemical properties. (B) Deposition or chemical modification techniques can apply nanoscale features (x < 100 nm) in a manner that are distributed in micron-scale (y > 100 nm). (C) Other deposition or compaction methods can place nanoscale features in nanoscale distribution. The cell response to surfaces represented by (B) or (C) may be different. (D) Isotropic surfaces can be created in the nanoscale (x < 100 nm) by subtractive or additive methods. The distribution can be in either the nano- (y) or micron-scale (y). It is thought that some nanosurfaces mimic natural cell environments.

Thus there is the question; why does nano surfacing enhance one set of growth while inhibiting another. We could argue that it is the dynamics of the process.

6.2 POSSIBLE EXPLANATION

We can possibly argue the following explanation from what we have developed herein.

1. The first coating of a surface is by the protein layer. Generally, this is done by some local van der Waals forces since the proteins are close to the surface and are well known to exhibit such forces. Also the protein layer seems to be a prerequisite for adhesion. However, the type of protein layer may very well depend on the surface structure. They structure of proteins vary widely and perhaps if we adjust the nanostructure we selectively change the type of protein adhering to the surface.

2. We know that bacteria seem phenomenologically to require proteins to adhere for them in turn to reversibly adhere to the proteins. This the proteins must be electrostatically and vdW wise strongly attracted to the surface and the cell.

3. After a reversible adhesion then we seem to have the appearance of protein filaments extruding from the bacteria and down through the protein layer, most likely using the protein to overcome the barrier wall normally between van der Waals and electronic forces. Once the filament hits the surface then it adheres irreversibly and the biofilm commences growth.

4. The supposition is that by changing the roughness of the surface we change the types of proteins or the nature of their adhesions on the surface. There does not appear to be any research determining this one way or the other at this time.

7 REFERENCES

1. alAbbas, F., et al, Bacterial attachment to metal substrate and its effects on microbiologically-influenced corrosion in transporting hydrocarbon pipelines, Paper Best Practices in Pipeline Operations Conf, Bahrain, 2012.
2. Barghouti, S., A Universal Method for the Identification of Bacteria Based on General PCR Primers, Indian J Microbiol (Oct–Dec 2011) 51(4):430–444
3. Barron, E., Rapid Identification of Bacteria and Yeast: Summary of a National Committee for Clinical Laboratory Standards Proposed Guideline, MEDICAL MICROBIOLOGY • CID 2001:33 (15 July) 221
4. Batte, M., et al, Biofilms in drinking water distribution systems, Reviews in Environmental Science and Bio/Technology · January 2003
5. Boe-Hansen, R., Albrechtsen, H.-J., Arvin, E. and Jørgensen, C. (2002a). Dynamics of biofilm formation in a model drinking water distribution system. J. Water Supply Res. Technol–Aqua., 51, 399–406.
6. Boe-Hansen, R., Albrechtsen, H.-J., Arvin, E. and Jørgensen, C. (2002b). Bulk water phase and biofilm growth in drinking water at low nutrient conditions. Wat. Res., 36, 4477–4486.
7. Boe-Hansen, R., Albrechtsen, H.-J., Arvin, E. and Jørgensen, C. (2002c). Substrate turnover at low carbon concentrations in a model drinking water distribution system. Wat. Sci. Tech.: Water Supply, 2(4), 89–96.
8. Boe-Hansen, R., et al, Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions, Water Sci & Tech, Vol 47 No 5, 2003.
9. Boks, N., Bacterial interaction forces in adhesion dynamics, PhD Thesis, Groningen, 2009.
10. Boland, T., et al, Molecular Basis of Bacterial Adhesion, Handbook of Bacterial Adhesion: Principles, Methods, and Applications, Edited by: Y. H. An and R. J. Friedman, Humana Press Inc., Totowa, NJ
11. Butt H et al, Physics and Chemistry of Interfaces, Wiley (New York) 2013.
12. Butt, H., M. Kappl, Surface and Interfacial Forces, Wiley (New York) 2010.
13. Combs Jr, Gerald F., and William P. Gray. "Chemopreventive agents: selenium." Pharmacology & therapeutics 79.3 (1998).
14. Costerton, J., et al, BACTERIAL BIOFILMS IN NATURE AND DISEASE, Ann. Rev. Microbiol. 1987. 41:435
15. Darouiche, R., Treatment of Infections Associated with Surgical Implants, NEJM 2004, 350;14
16. Davis, M., Water and Wastewater Engineering, McGraw Hill (New York) 2010.
17. Elimelech, M., W. Phillip, The Future of Seawater Desalination: Energy, Technology, and the Environment, Science, Vol 333, Aug 2011.
18. Emerson, D., et al, Identifying and Characterizing Bacteria in an Era of Genomics and Proteomics, Bioscience, November 2008 / Vol. 58 No. 10

19. EPA, Control of Biofilm Growth in Drinking Water Distribution Systems, EPA/625/R-92/001, Environmental Protection Development June 1992
20. Fleming, H., H. Ridgeway, Biofilm Control: Conventional and Alternative Approaches, Biofilms (Springer) 2008
21. Garrett, T., et al, Bacterial adhesion and biofilms on surfaces, Progress in Natural Science 18 (2008) 1049–1056
22. Gitai, Z., The New Bacterial Cell Biology: Moving Parts and Subcellular Architecture, Cell, Vol. 120, 577–586, March 11, 2005
23. Gorth, D., S. Puckett, B. Ercan*, T.J. Webster, "Decreased bacteria activity on Si3N4 surfaces compared with PEEK or titanium," International Journal of Nanomedicine, 7: 4829-4840 (2012).
24. Gupta, R., Hydrology and Hydraulic Systems, Waveland (Long Grove IL) 2008
25. Huang, T., et al, Composite Surface for Blocking Bacterial Adsorption on Protein Biochips, BIOTECHNOLOGY AND BIOENGINEERING, VOL. 81, NO. 5, MARCH 5, 2003
26. Irvine, D., et al, Simulations of Cell-Surface Integrin Binding to Nanoscale-Clustered Adhesion Ligands, Biophysical Journal Volume 82 January 2002 120–132
27. Kanematsu, H., D. Barry, Biofil and Materials Science, Springer (New York) 2015
28. Katsikogianni, M., Y. Missirilis, Concise Review of Mechanisms of Bacterial Adhesion to Biomaterials and of Techniques Used in Estimating Bacterial Material Interactions, Euro Cells and Materials, Vol 8 2004
29. Khang, D., S.Y. Kim, P. Liu-Synder, G.T.R. Palmore, S.M. Durbin, T.J. Webster, "Enhanced fibronectin adsorption on carbon nanotubes/poly(carbonate) urethane: independent role of surface nano roughness and associated surface energy," Biomaterials, 28(32):4745-4768 (2007).
30. Krasowska, A., K. Sigler, How microorganisms use hydrophobicity and what does this mean for human needs? Frontiersin Cellular and Infection Microbiology, August 2014, Volume4, Article112
31. Kulkarni, S., Nanotechnology: Principles and Practices, Springer (New York) 2011
32. Kumar et al, "Selenium nanoparticles involve HSP-70 and SIRT1 in preventing the progression of type 1 diabetic nephropathy", Chemico-Biological Interactions 223 (2014).
33. Machado, M., et al, Decreased Staphylococcus aureus biofilm formation on nanomodified endotracheal tubes: a dynamic airway model, International Journal of Nanomedicine 2012:7 3741–3750
34. McFaddin, J., Biochemical Tests for the Identification of Medical Bacteria, William and Wilkens, (Baltimore) 1980.
35. Mendonca, G., et al, Advancing dental implant surface technology – From micron to nano topography, Biomaterials 29 (2008) 3822–3835
36. National Research Council, Identifying Future Drinking Water Contaminants, 1998, http://www.nap.edu/catalog/9595.html

37. Percival, S., et al, Microbiological Aspects of Biofilms and Drinking Water, CRC (New York) 2000.

38. Perla, V., T. Webster, Better osteoblast adhesion on nanoparticulate selenium— A promising orthopedic implant material, Published online 29 July 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30423

39. Preedy, E., et al, Surface Roughness Mediated Adhesion Forces between Borosilicate Glass and Gram-Positive Bacteria, www.pubs.acs.org/Langmuir Langmuir 2014, 30, 9466—9476

40. Ramos, J., T.J. Webster, "Cytotoxicity of selenium nanoparticles in rat dermal fibroblasts," International Journal of Nanomedicine, 7: 3907-3914 (2012).

41. Schkolnik, S., et al, In Situ Analysis of a Silver Nanoparticle-Precipitating Shewanella Biofilm by Surface Enhanced Confocal Raman Microscopy, PLOS One December 28, 2015

42. Srey, S., et al, Biofilm formation in food industries: A food safety concern, Food Control 31 (2013) 572e585

43. Srivastava et al, In vivo synthesis of selenium nanoparticles by Halococcus salifodinae BK18 and their anti-proliferative properties against HeLa cell line. Biotechnology Progress (2014)

44. Steel, E., Water Supply and Sewerage, McGraw Hill (New York) 1953.

45. Tran, P, T.J. Webster, Antimicrobial selenium nanoparticle coatings on polymeric medical devices, Nanotechnology, 24 (15): 155101 (2013).

46. Tran, P. and T.J. Webster, Selenium nanoparticles inhibit Staphylococcus aureus growth, International Journal of Nanomedicine, 6:2001-2011 (2011).

47. Tran, P., and T.J. Webster, "Enhanced osteoblast adhesion on nanostructured selenium compacts for anti-cancer orthopedic applications," International Journal of Nanomedicine, 3(3): 238-247 (2008).

48. Tran, P., L. Sarin, R. Hurt, and T.J. Webster, Titanium surfaces with adherent selenium nanoclusters as a novel anti-cancer orthopedic material, Journal of Biomedical Materials Research Part A, 93A (4): 1417-1428 (2010).

49. Tran, P., T.J. Webster, Understanding the wetting properties of nanostructured selenium coatings: the role of nanostructured surface roughness and air-pocket formation, International Journal of Nanomedicine, 8: 2001-2009 (2013).

50. Trefalt, G., M. Borkovec, Overview of DLVO Theory, www.colloid.ch/dlvo (2014)

51. U.S. Fish and Wildlife Service, 3.8 Bacterial Identification Techniques, https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=0ahUKEwj4-9HFrqfMAhUCMj4KHejVD3UQFghPMAQ&url=http%3A%2F%2Fwww.fws.gov%2Fpacific%2Ffisheries%2Ffishhealth%2Fdocuments%2Fbluebook%2Fcdr_pdfs%2Findexed%2Fb3_08.pdf&usg=AFQjCNGbL5FE3e0woiPb-DfOk-xEKRBKqA&sig2=aszx3gob3awPK32KVVj35A&cad=rjt

52. Van der Kooij, Potential for biofilm development in drinking water distribution systems, Journal of Applied Microbiology Symposium Supplement 1999,85, 39-4s

53. van Loosdrecht, M, et al, Bacterial Adhesion: A Physiochemical Approach, Microb Ecol 1989 V 17

54. Voutchkov, N., Desalination Engineering, McGraw Hill (New York) 2013.

55. Wang, Q., T. Webster, Nanostructured selenium for preventing biofilm formation on polycarbonate medical devices, Published online 15 June 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34262

56. Wang, Q., T.J. Webster, Short communication: Inhibiting biofilm formation on paper towels through the use of selenium nanoparticles coatings, International Journal of Nanomedicine, 8: 407-411 (2013).

57. Webster, T, A.A. Patel, M.N. Rahaman, Anti-infective and osteointegration properties of silicon nitride, poly-ether-ether-ketone, and titanium implants (vol 8, pg 4447, 2012)," Acta Biomaterialia, 10 (3): 1485-1486 (2014).

58. Werber, J., et al, Materials for next-generation desalination and water purification membranes, NATURE REVIEWS MATERIALS, 2016

59. Xu, X., D., Mosher, Fibronectin and Other Adhesive Glycoproteins, R.P. Mecham (ed.), The Extracellular Matrix: an Overview, Biology of Extracellular Matrix, DOI 10.1007/978-3-642-16555-9_2, # Springer-Verlag Berlin Heidelberg 2011

60. Zhang and Gladyshev, "General Trends in Trace Element Utilization Revealed by Comparative Genomic Analyses of Co, Cu, Mo, Ni and Se", J. of Biol. Chem (2010).