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Biofilm formation, control and novel strategies for eradication

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Biofilms are communities of microorganism that are form on solid or fluid interfaces and are designed to protect the individual cells, such as bacteria, from the environment. The mass is formed by microorganisms attached to a surface, such as a surface of a medical device, and the associated extracellular substances produced by one or more of the attached microorganisms. The adhesion of bacteria to a surface depends on a number of microbiological, physical, chemical, and material-related parameters. Microbial adhesion and biofilm formation are major concerns in the control. Virulence and pathogenicity of microorganisms is often enhanced when growing as a biofilm, and new strategies are therefore required to control biofilm formation and development. Many pathogenic microorganisms reside within biofilms, which biofilms cause additional problems when designing new anti-microbial agents. The main strategies used today are based on nutrient control, pH control of biofilm, antimicrobial agents, chemical agents, furthermore we highlight the use surfactants and modify surface through a review of more recentness patented technologies.

Keywords: biofilms; antimicrobial, anti-adhesive surfaces, microbial adhesion, innovation.

1. Biofilm formation

A biofilm is an accumulation of microorganisms embedded in a matrix of polysaccharide. Biofilms may be form on solid biological or non-biological surfaces and are medically important, accounting for over 80 percent of microbial infections in the body. A biofilms composed by a mass of microorganisms attached to a surface, such as a surface of a medical device, and the associated extracellular substances produced by one or more of the attached microorganisms. These bacteria biofilms are prevalent on most wet surfaces in nature. Many biofilms are sufficiently thick to be visible to the naked eye. However, it was until the 1970s that the existence of bacteria was better comprehend into the biofilm. Sessile bacteria comprise a major component of the bacteria biomass in many environments and was possible verified that attached bacteria were organized in a sophisticated structure [1- 3].

Bacterial biofilms are integrated, multi-species communities of cells that adhere to almost any surface and are fundamental to the ecology and biology of bacteria. Biofilms constitute a protected mode of growth that allows survival in a hostile environment. The structures that form in biofilm contain channels in which nutrients can circulate [4], and cells in different regions of the biofilm exhibit different patterns of gene expression [5]. The complexity of the biofilm structure and metabolism has led to the analogy of biofilm to tissue of higher organism[6]. These sessile biofilm communities can give rise to non-sessile individuals, planktonic bacteria that can rapidly multiply and disperse. There are impressive numbers of chronic bacterial infections that involve bacteria biofilms, which are not easy eradicated by conventional antibiotic therapy.

The biofilm consist of microcolonies on a surface, and that within these microcolonies the bacteria have developed into organized communities with functional heterogeneity[3].The growth of the biofilm is slow, in one or more localization and biofilm infection are often slow to produce overt symptoms. Sessile bacteria cells release antigens and stimulate the production of antibodies, but the antibodies are not effective in killing bacteria within biofilms and may cause immune complex damage to surrounding tissue[7]. Even in health individuals with excellent cellular and humoral immune reactions. In this regard, bacteria and fungi growing as a biofilm rather than in free-floating (ie. planktonic) forms tend to be particularly resistant to anti-microbial agents and to be particularly difficult for the host immune system to render an appropriate response[8].

The bacteria in the biofilm are embedded in a matrix of extracellular polymeric substances (EPS) produced by the individual cells[9, 10]. This biofilm matrix is also commonly referred as glycocalyx; the extracellular substances are typically polymeric substances and commonly comprise a matrix of complex polysaccharides, proteinaceous substances and glycopeptides, lipids, lipopolysaccharides and other materials that serve as a scaffold holding the biofilm together[9, 11]. In most recent studies researchers discovered that as bacterial cell density within a biofilm increases, the bacteria may communicate with each other, in a cell-to-cell signals[12]. This can lead to the secretion of low molecular weight molecules that signal when the population has reached a critical threshold. This process, called quorum sensing, is responsible for the expression of virulence factors[13]. For example, *Pseudomonas aeruginosa* produces destructive proteases when the number of these bacteria reaches a high enough density in the biofilm infection is rarely resolved by the host defense mechanism[14]. Nevertheless under hostile environmental conditions spore forming bacteria are able to

form endospores. Endospores are extremely resistant survival forms that are intrinsically inert in the face of many environmental influences, and can also survive temperatures $> 100\text{ }^{\circ}\text{C}$ undamaged[15].

Biofilms can not only occur on insertable medical devices ("foreign bodies"), but also appear as a consequence of a bacterial infection on structures of the human or animal body itself. Biofilms growing in natural and industrial environments are resistant to bacteriophage, to amoebae, and to the chemically diverse biocides used to combat biofouling in industrial processes[3]. Of importance with respect to medicine, sessile bacterial cells can withstand host immune responses, and they are much less susceptible to antibiotics than their nonattached individual planktonic counterpart. It is likely that biofilms evade antimicrobial challenges by multiple mechanisms.

One mechanism of biofilm resistance to antimicrobial agents is the failure of an agent to penetrate the full depth of the biofilm[16]. Polymeric substances like those that make up the matrix of a biofilm are known to retard the diffusion of antibiotics, and solutes in general diffuse at slower rate within biofilms than they do in water. Antibiotics have been shown to penetrate biofilms readily in some cases and poorly in others, depending on the particular agent and biofilm[3]. Examples of biofilm-associated microbial infections include infections of: oral soft tissues, teeth and dental implants; middle ear; gastrointestinal tract; urogenital tract; airway/lung tissue; eye; urinary tract prostheses; peritoneal membrane and peritoneal dialysis catheters, indwelling catheters for hemodialysis and for chronic administration of chemotherapeutic agents (Hickman catheters); cardiac implants such as pacemakers, prosthetic heart valves, ventricular assist devices, and synthetic vascular grafts and stents; prostheses, internal fixation devices, and percutaneous sutures; and tracheal and ventilator tubing[17]. Both indwelling and subcutaneous biomedical implants and devices are potential sites for microbial infections and represent important targets for the control of infection, inflammation, and the immune response. Biomedical systems such as blood oxygenators, tracheal lavage, dental water units, and dialyzers are also susceptible to bacterial contamination and biofilm formation[18].

2. Microbial adhesion

Microbial adhesion and biofilm formation are major concerns in the control of biofilm associated infections and not biological surface colonization. Bacteria rapidly adapt to their extracellular conditions to survive in diverse environmental conditions forming communities including biofilms. Adhered microorganisms, microorganisms embedded in biofilms or microorganisms hiding in cracks or crevices may escape of cleaning and disinfecting procedures and be a source of recontamination of food products during processing; because of this, a major part of the pre-requisite programmed (Good Hygienic Practices Program) of a food manufacturing plant is therefore to ensure that microbial biofilms do not form or are efficiently removed[19].

In the native physiological state *in vivo*, microorganism demonstrated that the process of contamination of surface following a successive chain, including an initial microbial adhesion, strength of the binding of the attached microorganisms through exopolymer production, growth of attached microorganisms and continued secretion of exopolymers and localized detachment of biofilm organisms caused by occasionally high fluid shear or other detachment forces operative, allowing the colonization of closer surfaces[20]. Adhered and biofilm-forming microorganisms may also have other adverse effects in the colonized surface such as decreasing heat transfer[21, 22] or causing corrosion[23].

The mechanism of attaching to surfaces follows an organized sequence starting with the deposition of specific adhesive protein which binds to the surface reversibly. A successive deposition of cells creates a strong binding by cell to cell cohesion and cell-binding-proteins. Cell adhesion molecules involved in the process are first hydrolyzed by extracellular enzymes. Bacterial adhesion is directly related to protein adsorption[24].

2.1 Bacterial adhesion to surfaces: the influence of surface roughness.

Since the report in 1940 for Heukelekian H. et al, has been known that the surface characteristics are an important factor for the bacterial adhesion and development[25] and until nowadays this is central research area for the control of bacterial biofilm related disease. The adhesion of bacteria to a surface depends on a number of microbiological, physical, chemical, and material-related parameters; especially the surface topography has been widely discussed as a parameter influencing bacterial adhesion [26]. Bacteria embedded within biofilms are resistant to both immunological and non-specific defense mechanisms of the body. Contact with a solid surface induces the expression of a bacterial enzyme, which catalyzes the formation of exo-polysaccharides that promote colonization and protection.

Thus, the modification of surfaces can to reduce attachment surfaces to limit the adhesion of microorganism e.g. electropolishing of stainless-steel. Several parameters or measures have been used to characterize the material surface based on two-dimensional characteristics such as the Ra (roughness average), Rt (is the maximum peak to valley height in the sample length), and Rz values (the average maximum profiler height)[27].

Amongst the most widely used is the surface roughness Ra value (which is the arithmetical mean deviation of the profile) and an Ra value of $0.8\text{ }\mu\text{m}$ or less has been recommended for dairies and, in general, for food contact surfaces[28]. Although widely used, the Ra value will typically not characterize features of the surface such as soft or sharp topography or the presence of scratches or porosities. During recent years, scanning electron microscopy (SEM)

and atomic force microscopy (AFM) have been used to give a three-dimensional visualization of the surface topography including AFM determination of three-dimensional topographical parameters in the nanometer range[27, 29].

Through the recommendation of a minimum Ra value of 0.8 μm , a number of studies have evaluated if further reductions in numbers of adhering bacteria can be obtained by using even smoother surfaces with lower Ra values. However, experiments in milk showed no significant difference between adhesion on surfaces with Ra 0.4 and 0.035 μm [30]. Similar tendencies were found in a study of elements and base metal, where no significant differences were found in bacterial adhesion[31]. Conversely, it may be asked if rougher surfaces result in higher numbers of adhering bacteria, but *Flint et al* found similar levels on surfaces with Ra values in the range 0.5–3.3 μm [32].

2.2 Immune Response to Biofilm

Biofilms are difficult to treat with anti-microbials and bacterial resistance to antibiotics is enhanced up to 1000-fold over the level observed when grown under planktonic conditions. Immune responses are only directed towards those antigens on the outer surface of the biofilm. Antibodies and other serum or salivary proteins may fail to penetrate into the biofilm. Cells within the biofilm remain hidden from antibody and complement factor recognition, and thus from subsequent white blood cell phagocytosis[33]. The presence of biofilms can modulate cytokine synthesis, and can interrupt production of antibodies via synthesis of superantigens. Phagocytes are unable to effectively engulf a bacterium growing within a complex polysaccharide matrix attached to a solid surface. This may result in phagocytes releasing large amounts of pro-inflammatory enzymes and cytokines, leading to inflammation and destruction of nearby tissues[18].

In addition, biofilms increase the opportunity for gene transfer between bacteria and may be significant for the transfer of resistance genes to associated susceptible bacteria. Gene transfer can convert a previously avirulent strain into a virulent pathogen[34]. New and more virulent microbial phenotypes may be expressed when growing within a biofilm. Biofilms promote genetic diversity and maintain the high cell density needed for efficient genetic exchange. Thus, the community provides microbes protection from many forms of environmental insults, such as predatory attacks and chemical stress, for example from host immune system or antibiotics or disinfectants. Several bacteria have plasmids, giving the most diverse characteristics. These can be transferred horizontally by conjugation to different species in a biofilm[35]. Studies were performed with artificial dental plates, initially formed by bacteria called *Streptococcus*. A strain of *Bacillus*, containing conjugative transposon harboring a tetracycline resistance gene was inserted into this transposon system and transferred to cells of *Streptococcus*[36].

The transduction can theoretically be responsible for horizontal gene transfer in biofilms. This hypothesis is based on the fact that marine systems and fresh water containing an abundance of bacteriophages (about 108/ml), responsible for the lysis of a large number of bacteria. Daily, 10 to 20% of the bacterial population is lysed by phages, which have an important impact on microbial food chain since they may increase rates of mortality and / or reduce growth rates at all trophic levels. Recent studies show that phage can structure or restructure the microbial communities. In an analysis, where a population was nearly wiped out by cyanobacteria phages, was observed the presence of new species capable of degraded organic compounds that have emerged [37].

The most knowledge and evidences of the spreading phenotype comes mainly from in vitro studies in high numbers as pure cultures in the lab, although it could be a useful tool for studying biofilm dispersal in these and other nonflagellated bacteria and may have physiological relevance to biofilm dispersal in other environment.

3. New strategies to control biofilm formation and development

Virulence and pathogenicity of microorganisms is often enhanced when growing as a biofilm, and new strategies are therefore required to control biofilm formation and development. Many pathogenic microorganisms reside within biofilms, which biofilms cause additional problems when designing new anti-microbial agents[18]. Accordingly, alternative curative and prophylactic approaches for tackling microbial infections within a biofilm are required. Novel strategies are necessary because of the limitations to these current treatments such as inadequate control supply, potential for disease transfer and compliance issue. The capability and high resistance of sessile microorganisms to inhibitors, eradication of biofilm often requires high concentration of disinfectants or antibiotics, causing severe environmental damages, multiresistance emergence and nosocomial infections. Public health concerns, as well the economic loss associated to biofilm formation raise an urgent need for developing biofilm resistant systems.

The strategy that combines a broad spectrum microbial repellent agent with a surface coating that impairs bacterial growth has been investigated. This chief could be obtained through the modification of the surface by antibacterial compound which reduce or prevent the biofilm formation by either inhibiting bacterial adhesion and/killing bacterial cells which have adhered. Various antimicrobial substances such antibiotics, antiseptics, and/or metals and enzymes were grafted on various materials and these surface were shown display antimicrobial activities.

3.1 Host:guest strategies to increase the efficacy of antimicrobials

In order to reduce the toxicity caused by high concentrations of antiseptics inclusion compounds have been developed for use of more effective antiseptic agents. These compounds offer advantages in effectiveness, long-term activity, and low effective antimicrobial concentrations. The key component of the inclusion compounds is the cyclodextrin, which creates a hydrophobic cavity that surrounds the drug.

Cyclodextrins (CDs) are cyclic non-reducing, non-hygroscopic, water-soluble oligosaccharides, most commonly formed by six, seven and eight glucopyranose units, denominated α -CD, β -CD and γ -CD respectively[38, 39]. The structure of this class of compounds is often described as a truncated cone, which provides to CDs a hydrophilic exterior and a hydrophobic cavity that allows the formation of supramolecular complexes stabilized by noncovalent interactions such as van der Waals forces, hydrogen bonds and hydrophobic interactions[38]. The hydrophobic cavity of cyclodextrins can accommodate another molecule (a drug, for example) forming inclusion compounds through host-guest interactions. Formation of the inclusion complex can increase the guest's stability against hydrolyses, oxidation, photodecomposition, and dehydration. Upon inclusion the water solubility of the guest can increase as well as its bio-availability[39]. CDs can improve the therapeutic efficacy of poorly water-soluble drugs, enhance the physical and chemical stability, and also protect the guest molecules from degradation in the gastrointestinal tract [39-46].

Understanding the structure and properties of microbial surfaces at the nanometer level is of great importance for the development of novel antimicrobial compounds. Using polymers containing a chlorhexidine in cyclodextrin inclusion compounds, the researchers shown *in vitro* study higher antimicrobial activity and long-term of this devices against oral microorganism pathogens[47-51].

3.2 Physical and chemical surface modification.

Pioneers studies of Zobell and Henrici described for the first time in the literature that bacteria could attach to and thrive on surface[52]. A qualitative and quantitative measure for biofilm bacteria recovery in aquatic system was conducted by Geesey and colleague[53]. The existence of a surface is perhaps the most important prerequisite for biofilm formation involves the bacterial detection of a surface. The physicochemical factors govern the initial attachment and adhesion of bacteria to surface. The general rule-of-thumb is that bacteria will preferentially colonize surface that are hydrophobic, have surface roughness on the nano and micro scale, and are exposed to a conditioning layer in contrast to smooth, hydrophilic surfaces. The authors affirm that the key challenge in this area is the prevention of the formation of a conditioning layer that passives the exposed surface chemistry and provides a site of attachment for bacteria[54].

All materials are subjected to bacterial contaminations since exposed to air, humidity or diverse environmental conditions. To overcome these problems, several strategies have been used to create coatings that are either antimicrobial or nonbiofouling. Cheng et al., report a coating that combines both properties, switching from antimicrobials to nonfouling upon hydrolysis. Specifically, the application of polymethacrylate derivate with cationic side chain that becomes zwitterionic upon conversion of a terminal ester to carboxylate[55]. In this technology the bacteria attached after 1 hour of exposure to the initially prepared coating, 99.9% were dead. Over the course of the next 2 to 8 days, the coating slowly hydrolyzed, releasing 98% of the microbials cells. The nonfouling nature of the hydrolyzed coating prevents further attachments of microbial cells and formation of a biofilm. By tuning the hydrolysis rate of the coating, it should be possible to adapt it to range of applications in implantable medical devices[55].

A study on the effect of titanium surfaces on the attachment of bacteria demonstrated that roughness on the nanometer scale-and not micrometer scale- increases the attachment of bacteria. The authors compared all of the physical and chemical variables of their measurements (e.g., cell surface charge, surface energy and surface zeta potential) and concluded that topography is the most influential factor on bacterial adhesion, and other interfacial parameters had little or no influence in their study[56].

Earlier, copper-nickel (Cu/Ni) alloys were extensively used in marine environments due to their high corrosion and biofouling resistance. However, the choice of condenser material for the new fast breeder reactor in Kalpakkam is Ti to avoid steam side corrosion problems, which may pose a threat to steam generator parts having sodium as the secondary coolant. The surface modification of Ti using nano films of copper (Cu) and nickel (Ni) to utilize the antibacterial property of copper ions in reducing micro fouling was conducted by Vishwakarma et al [57]. The surface modification of Ti was carried out by the deposition of a Cu/Ni bilayer and (Cu/Ni) multilayer films using a pulsed laser deposition technique. Various surface characterization studies revealed that the deposited Cu/Ni films were thin and nanocrystalline in nature. The antibacterial properties were evaluated using total viable count and epifluorescence microscopic techniques. The results showed an apparent decrease in bacterial attachment on multilayered and bilayered Cu/Ni thin films on Ti surfaces. Comparative studies between the two types of films showed a bigger reduction in numbers of microorganisms on the multilayers[57].

The manipulation of individual environmental factors to prevent biofilm formation has been met with limited success. Control over surface chemistry has been used to reduce cell attachment, including the development of dynamic surface that degrade or reorganize in response to temperature and other environmental conditions and shed adsorbed bacteria into bulk fluid[58]. Specific surfaces must have antimicrobial properties and modify polymer coating also

reduce cell adsorption. Use of silver containing dressings has become prevalent in clinical practice to manage chronic wounds at risk for infections. The efficacy of using silver dressings in the chronic wound management and demonstrated that the studies about the emerging evidence base for this use of silver dressings in clinical practice on chronic wounds does not provide absolute evidence and more rigorously controlled long-term, randomized studies of human subjects with chronic wounds are needed[59].

Novel strategies are conducted in vitro to produce antimicrobials from bacteria mutant. An example of the modification of the surface was explored by Kaplan. In this work was development a mutant of *A. actinomycetemcomitans* that forms biofilm colonies which are tightly adhered to surfaces but which are unable to release cells into the medium or spread over the surface. The mutant strain (designated JK1023) was isolated. JK1023 colonies had a hard texture and were extremely difficult to remove from the agar surface. When cultured in broth, strain JK1023 produced biofilm colonies which were similar in size and shape to those of the wild-type strain, but which failed to produce satellite colonies on the surface of the culture vessel. In this study, biofilm colonies of strain JK1023 produced significantly less growth on the bottom of the well than the wild-type strain. These data indicate that mutant strain JK1023 exhibited a wild-type surface attachment phenotype but a decreased biofilm cell detachment phenotype when compared to the wild-type strain[60, 61].

3.3 Bacteriophage for the treatment of bacterial biofilms

Bacteriophage is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage. Typically, bacteriophages consist of an outer protein capsid enclosing genetic material. The genetic material can be ssRNA, dsRNA, ssDNA, or dsDNA ('ss-' or 'ds-' prefix denotes single-strand or double-strand) along with either circular or linear arrangement. To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means that a bacteriophage can infect only certain bacteria bearing receptors to which they can bind which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade bacteria. As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.)[62]. Each phage particle (virion) contains a nucleic acid genome that is enclosed in a protein or lipoprotein coat or capsid. Phages are obligate parasites. The phage nucleic acid encodes all the information necessary to direct its reproduction within the host bacterium. Virulent phage multiply by means of a lytic cycle in which the phage particle adsorbs to the host bacterial cell surface, injects its genomic material and takes over the host metabolic machinery, resulting in intracellular phage multiplication. Cell lysis and liberation of progeny phage complete the phage lytic cycle[63]. The first step in the lytic cycle, termed adsorption, is facilitated by tail fibers that bind to specific molecules, termed phage receptors, on the bacterial cell surface. The specificity of receptors for a single phage strain will determine its host range; some phage have specificity at the strain level whereas some are more broad-spectrum and could infect many bacterial strains within a single species or even multiple related-species. In the phage lytic cycle, infection of a bacterial cell with a single phage virion will result in production of multiple progeny phage, depending upon the burst size (number of progeny phage virions per infected bacterial host cell that are released upon cell lysis) of the particular phage strain. The lytic cycle of different phages can be characterized by the eclipse period, the period of time between initial phage infection and first appearance of infective phage particles, and latent period, the period of time between initial phage infection and release of infective phage particles by cell lysis[63].

Phages were discovered to be anti-bacterial agents, but the medical trials performed in western countries were sub-standard to the point of not being scientifically viable; this was because the early tests were conducted poorly and without an idea of what a phage was. Phage therapy was shortly thereafter ruled out as untrustworthy much because many of the trials were conducted on totally unrelated diseases such as allergies and viral infections. Antibiotics were discovered some years later and marketed widely. They quickly grew in popularity because of their broad spectrum and the ease with which they could be manufactured in bulk, stored, and prescribed. Hence development of phage therapy was largely abandoned in the West, but continued throughout 1940s in the Soviet Union for treating bacterial infections, with widespread use including the soldiers in the Red Army—much of the literature was published in Russian or Georgian, and unavailable for many years in the West. One of the first studies to examine the interaction of bacteriophage with biofilms was reported by Doolittle et al[64].

Researchers are working about to produce a bacteriophage that is capable of infecting a bacterium within of biofilm, and a first polysaccharide lyase enzyme that is capable of degrading a polysaccharide within of biofilm. The composition comprises a pharmaceutically-acceptable antimicrobial agent, and may also include a DNase. Also provided modified bacteriophages, methods of creating modified bacteriophages, compositions and bacteriophage for treating biofilms, and methods for treating biofilms using the bacteriophages and compositions. It was described a composition for treating a bacterial biofilm of a patient with bacteriophage that is capable of infecting a bacterium within biofilm. The first bacteriophage is capable of infecting *Pseudomonas sp.* by using a polysaccharide lyase enzyme that is capable of degrading a polysaccharide within biofilm[18].

Biofilms of *Escherichia coli* strains 3000 XIII developed on the surfaces of polyvinylchloride coupons in a modified Robbins device were infected and lysed using bacteriophage T4D. Similar studies with phage E79 infecting *Pseudomonas aeruginosa* indicated phage were infecting the surface organisms but access to the cells deep in the

biofilm was restricted. Temperature and nutrient concentration did not appear to affect susceptibility, but low temperature and low nutrients did prolong the time for lyses to occur and slowed the spread of infection within the biofilm[65].

Sutherland et al reported that, during the early stages of biofilm development, bacteriophage has an effect on biofilm development[66]. Surface decontamination of stainless steel and polypropylene contaminated with *Listeria monocytogenes* was evaluated using Listeriophage. Phage suspensions at concentrations up to 3.5.times.10.sup.8 pfu per ml were found to have an effect in reducing the bacterial count, which was comparable with 20 ppm solution of an industrial sterilizing agent, which is a quaternary ammonium compound[67].

The work present by Donlan, details the state of the art for efforts focused on new phages technologies. The nature of device-associated biofilms mandates that the most effective treatments will prevent rather than control existing biofilms, will target the extracellular matrix component of the biofilm and will avoid the overuse of antimicrobial agents. Phages can potentially meet these criteria, and it seems possible to develop treatments that are tolerated by the patient, are not inactivated by the patient's immune system and will not introduce bacterial virulence or toxin genes. Phage cocktails can also be formulated to avoid or minimize phage resistance. New phage strains can be readily isolated from the environment and phage could be genetically engineered for greater efficacy against biofilms. The persistence of healthcare-associated infections, many of them device-associated, and the rise in antimicrobial resistance continue to drive the quest for new treatment strategies that target biofilm-associated organisms. Such new anti-biofilm agents and treatment strategies could include the lytic bacteriophages[68].

3.4 Biofilm and antimetabolite

The studies of bacterial genetics of the biofilms identified numerous genes involved in biofilm formation, and sequencing of microbial genomes revealed the conservation of these biofilm-related genes across many organisms. In this way was described that bacterial are able to switch between a single cell (planktonic) lifestyle and biofilm. In pathogenic bacteria, growth as biofilm protects bacterial cells against the host immune system and increases tolerance to antibiotics treatment, thus resulting in chronic infections[69].The bacterial second messenger cyclic-di-GMP plays a pivotal role in biofilm formation, by promoting production of adhesion factors by promoting production[70].

The work presented by Hermans details on new antibodies technologies and, in effect, this patent reflects the advances with antibodies on treat specific bacterial infection. The antibodies can be used to coat medical devices which are inserted or implanted into the human body. Further these antibodies or vaccines comprising proteins that can be used to vaccinate subjects to prevent or treat natural occurring biofilm formation during infection. Also covered are medical devices coated with the antibody[71].Other technology encompasses the direction which biofilms researchers have been move going forward. A system in which an anti-microbial agent as an antibiotic and a biofilm-degrading enzyme (eg. alginate lyase) are each coupled to anchoring molecules, which help localize and maintain agent and enzyme at the site of the biofilm where they can exert their effect[72].

An isolated nucleic acid sequences and amino acid sequences for soluble, beta.-N-acetylglucosaminidase or active fragments or variants thereof which promote detachment of bacterial cells from a biofilm. In this technology an isolated mutant bacteria which forms biofilm colonies which tightly adhere to surface but which are unable to release cells into the medium or spread over the surface is also described. In additions, methods were described for modulating detachment of bacterial cells from biofilm by mutating soluble, beta.-N-acetylglucosaminidase or altering its expression or activity. Biofilm colonies of *Aggregatibacter actinomycetemcomitans* have been shown to release cells into liquid medium which then attach to the surface of the culture vessel and form new colonies, enabling the biofilm to spread[73].

Exciting discoveries about compositions, methods and devices for preventing, inhibiting or treating bacterial infections associated to biofilm have been proposed and summarized on Table 1.

Table 1. Strategies for biofilm control

Target	Technology	Level of Efficacy	of	Disadvantages	References
Bacteria	Antimicrobial or modified antimicrobial	Good	In vitro and in vivo studies	Resistance, efficacy limited,	[47 - 51]
			Strong of efficacy	Toxicity , high-dose	
	Antimetabolites, Antibodies	Little	In vitro	Low efficacy, High cost	[70-72]
				Difficult to control	
	Phages	Little	In vitro	Low efficacy, High cost	[63]
	Peptides	Little	In vitro	Good efficacy, High cost	[74]
	Natural Product	Little	In vitro	Good efficacy, low cost	[66]
Immune Response	Antibodies	Low	In vitro	Little evidences in vivo	[71]
Surfaces	Metal coatings	Good	In vitro and in vivo	Reduced mean- life	[57, 59]
	Polymers	Good	In vitro	Little evidences in vivo	[47-51]

In conclusion the main tool used today for the microbial control is the antibiotics. The role of this commonly substances is questioned because the resistance of the slime-like communities known biofilms. Inside this biofilms microorganism could produce bat substances to kill others. There is an imperative need in the near future to develop alternative antimicrobials to replace antibiotics for treating a whole spectrum of bacterial diseases. Action is needed due to an alarming increase of antibiotic resistance that poses a very real threat to modern medicine. Intermittent treatment and the re-emergence of infection tends to create selective pressure towards producing resistant microbial strains, and the nature of hospital environments and the sheer number of routine operations make the spread of infection more hazardous. Additionally, the ease and frequency of international travel assists in the spread of resistant bacteria throughout the world.

In addition to consideration of antimicrobial efficacy, concern of microbial contamination plays a crucial role when choosing a suitable and effective control agent. Thus a critical look and identification of actual or potential sources of contamination can provide a first indication of necessary disinfection measures. Although, many times control the contamination could be impossible to avoid.

Non-traditional ways to control the biofilm was related herein including modifications of surfaces, or altering the ways of depositions and durability of the biomass, use of antimicrobials and chemist agents, or by using biological modified molecules from the guest immune response. However, the benefits of increasing diversity for fostering innovation and economic success be needed to found the better source of biofilms control. Innovation needs novel thinking and demands that novel ideas be pursued, some long-held traditions and modes of operation need to be reexamined to ensure that the system is optimized as an engine for advancing society's goals. In addition, innovation often comes from non-traditional thinking, and many new ideas will come from new participants in science and engineering who often are less tied to traditional ways[75].

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