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Review Article

Biofilm Consortia on Biomedical and Biological Surfaces: Delivery and Targeting Strategies

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Microbial biofilms have been observed as congregates and attached communities on a diverse range of microecosystems of medicinal and industrial importance. Until recently, most investigations have been performed on planktonic (floating or fluid phase) microorganisms. After realization of the biofilm existence and their recalcitrance toward conventionally adopted preventive strategies and antimicrobial agents, research has been shifted toward novel therapeutics based drug delivery and targeting approaches. With the emergence of various biofilm models and methods to assess biofilm formation and physiology, it is pivotal to discuss various novel strategies that may become the therapeutic tools and clinically adaptable strategies of the future. This review explores various novel research strategies studied to date for their potential in effective biofilm eradication.

KEY WORDS: biofilm; antimicrobial agent; resistance; drug delivery; drug targeting; ultrasound; liposomes; microspheres.

INTRODUCTION

Research issues have been redefined and revolutionized by the fact that most bacteria in the bio-environment aggregate as biofilms. This is a growth domain in which bacteria behave very differently compared to free-floating (fluid phase, planktonic) bacteria growing in laboratory cultures (1). Biofilms can be considered as microbial ecosystems representing different microbial strains and species in aggregation, which efficiently co-ordinate and co-operate to protect themselves against environmental stresses and facilitate the nutrient uptake for survival (2). They are layer-like aggregates and stable synergistic consortia of microorganisms attached to the surface of biomaterials and biological sites. The interaction that occurs between biofilms and their physical and chemical micro- and macro-environment, largely determines the extent and manner through which these bacterial communities, cycle nutrients, degrade toxicants, survive in hostile environments and resist conventionally administered antimicrobial agents.

BIOFILM ARCHITECTURE

Bacteria in a biofilm grow in matrix-enclosed microcolonies interspersed with variably dense regions of the matrix that include water and nutrient channels (3,4). The bacteria (microorganisms) adhere and remain immobilized in matrix of polymeric compounds, which are generally referred to as extracellular polymer substances (EPS). Typical constituents of EPS are polysaccharides (major) and proteins (minor) often accompanied by nucleic acid, lipids or humic substances. The bacteria in biofilms generally bind together in a sticky web of tangled polysaccharide fibres (known as slime substances of EPS) which connect cells and anchor them to a surface and to each other (Fig. 1). Within this microcosm, anaerobic and aerobic bacteria can thrive alongside each other, sharing water passageways and a complex structure. The polysaccharide coating is like a shielding coat and one or different types of bacteria collaborate to make an eventual bacterial biofilm (5). Biofilm bacteria have been shown to be morphologically and metabolically distinct from those growing in liquid cultures, which are also capable of forming biofilms, once they find a locus point to stick. The later is mostly provided by the bio-surfaces in different bioengineering, biotechnology and biomedical settings (5,6). The sticking to a bio-surface sets off a genetic cascade that turns on specific genes to express polysaccharides and/or to express surface receptors needed to establish the biofilm colonization.

BIOFILM RESISTANCE TO ANTIMICROBIAL AGENTS

The possible mechanism(s) of biofilm formation in an aqueous environment are exhaustively reviewed (7–10). Various microscopic and physical methods have been proposed and documented for use to assess biofilm formation and to study biofilm physiology and the possible role of various genetic and environmental factors in biofilm formation (7). The formation of an infectious biofilm on biomaterials appears to involve several mechanistic and sequential steps. The mechanism(s) are based on the initial microbial adhesion or attachment to a biological (or biomedical) surface followed by a cascade of events leading to the development of different

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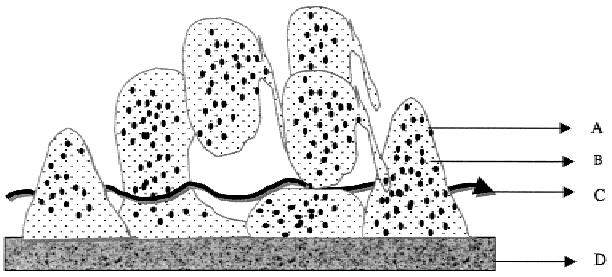

Fig. 1. Bacteria associated or attached as a biofilm with bio-surface (hypothetical)* A = Dense polysaccharide and epoxy-polysaccharide matrix, B = Microcosm and discrete micro-colonies of bacteria, C = Open water and nutrient channels, and D = Bio-surface to which the bacterial consortium is attached or adhered.

layers of biofilm micro-consortia. Figure 2 schematically presents the suggested mechanism, which is being extensively documented elsewhere (7–11).

However, the resistance of these biofilms to antimicrobial agents and/or biocides has been the major focus of research for last few years. The relative resistance of microbial biofilms to antimicrobial agents has been accounted due to transport-based and physiology-based mechanisms or a combination (11) (Table I). Transport-based mechanisms indicate that the biofilms act as barrier to antibiotic/antimicrobial diffusion (12). The main attributes of this mechanism rely on the features that govern transport rates and generate structural heterogeneity. External mass transfer resistance, which refers to the transport of a solute as it moves from the bulk fluid to the biofilm surface, further retards penetration. These solutes/materials can be soluble (microbial nutrients and organic solutes) or particulate (viable microorganisms, inorganic particles and antimicrobial agents). Structural heterogeneity is the most common feature of microbial biofilms (1–3). The biofilms are composed of different micro-colonies of bacterial cells in the bulk fluid, bulk fluid-biofilm interface and into the extreme interiors with different levels of dense exo-polymer matrix material and less dense water channels (5). These water channels transport oxygen (dissolved) to the biofilm, but limited diffusion and non-uniform oxygen use produce very low oxygen levels at the centers of cellular micro-colonies (13). This may explain the existence and even the physiological activity of fastidious anaerobes within mixed biofilms in an aerobic environment and further complicates the resistance to antimicrobial agents that are delivered and designed for single species based biofilms (3). The second explanation fo-

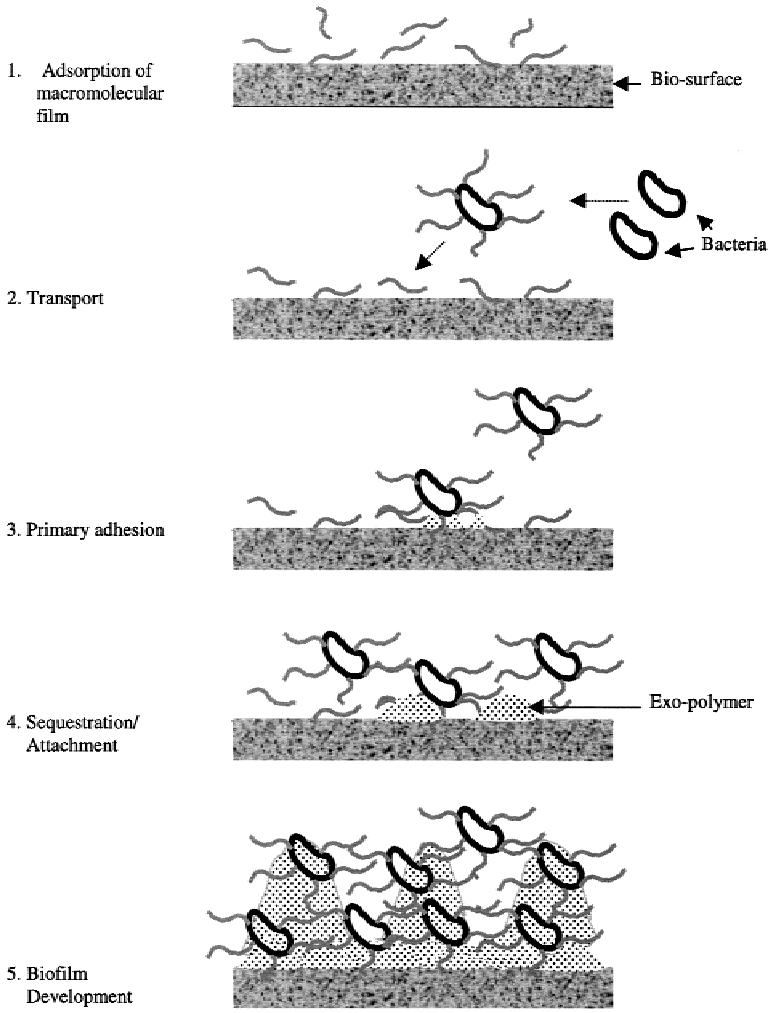

Fig. 2. Schematic diagram of biofilm formation on microbiota or any bio-surface with sequential steps (Modified from ref. 10).

Table I. Various Mechanisms Responsible for Biofilm Tolerance Toward Antimicrobial Agents[a]

Attribute(s)	Mechanisms
Transport-based mechanisms	
Extracellular polymeric substances (EPS)	Binding to charged components; irregular diffusion parameters
Surface hydrophobicity	Restricted or irregular transport of polar biocides
Structural heterogeneity of biofilm	Anaerobic micro-niche in a generally aerobic biofilm; reduced efficacy of selected antimicrobial agents
Physiology-based mechanisms	
Metabolic changes	Reduced efficiency of agents which obstruct active transport across membranes; Reduced efficiency of agents which interfere with enzymes involved in repair or regeneration
Reduced growth rate	Reduced efficiency of agents which interfere with enzymes involved in replication; Reduced permeability and altered structure/ organization of cell envelope
Miscellaneous	
By-stander effect	Neighboring cells protected by others producing neutralizing enzyme in mixed biofilms
Induction or repression of gene expression	Phenotype(s) with certain genes "switched off" exhibit an altered surface and hence a modified target

[a] Compiled from (3,12–15).

cuses on physiological, metabolic or genetic characteristics, which microorganisms acquire by growing within a biofilm. Thus bacteria in a biofilm are capable of making appropriate transformations due to environmental changes encountered in a typical biofilm. This subsequently imparts biofilm microorganisms a reduced susceptibility as compared to their freely floating counterpart (14).

Apart from the transport- and physiology-based mechanisms, it has also been suggested that induction or repression of gene expression in organisms constituting a biofilm could result in phenotype(s), which may exhibit a reduced susceptibility to an antimicrobial agent (15). The ability to disable or "turn off" and express or "turn on" certain genes might in turn disable the biofilm and thereby halt potentially lethal infections. Microarray techniques, also known as gene-chip technology, can be used to measure gene expression in the bacteria. This may prove to be useful in revealing the effect of some chemical signaling and structural molecules on gene expression and biofilm structure in same and different bacterial species.

BIOFILM CENTERED INFECTIONS

Biofilms are a major concern in the field of medical, pharmaceutical and biosciences especially in the case of a variety of biomaterial-centered infections in human (16). Fungal, protozoal and bacterial biofilms have been found on a variety of indwelling devices removed from patients with associated biomaterial-centered infections (17–19). The resistance of these biofilms to antibiotics and antimicrobial agents depends upon various factors. Not only do biofilms resist them, but also they are large enough to defeat the immune system. Biofilm bacteria have been protected from complement-mediated opsonic factors and phagocytic cells. The components of extra-cellular polymeric substances (EPS) of biofilm can also modulate the cellular immune responses (20). Consequently, infection of biomaterial implant may entail reoperation, osteomyelitis, amputation or may even lead to the death.

The use of antimicrobial agents and antiseptics is clinically feasible for the prevention and treatment of plaque-related oral diseases. Many workers have reported the results of studies in which the minimum inhibitory concentrations of agents for cariogenic and periodontal-pathogenic bacteria have been determined. However, such data are relevant only to situations where the organisms of interest are in aqueous suspensions (fluid phase or planktonic), whereas in caries and the inflammatory periodontal diseases the target organisms are in the form of biofilms, a form in which they behave very differently (21). Recently, Stark and coworkers (22) revealed that *Helicobacter pylori,* a causative organism of gastric ulcer and associated gastrocarcinoma accumulates at the air/liquid interface as water insoluble biofilms. The production of water insoluble biofilm by *H. pylori* may become an important parameter in elucidating resistance to host-defense factors and antibiotics. On the basis of micro-environmental pH homeostasis that regulates the growth and survival of *H. pylori in vivo,* a useful therapeutic and clinical strategy could be devised. The clinical efforts are waged with the development of preventive and therapeutic regimens to check the biofilm eliminating concentrations or biofilm killing concentrations of the antimicrobial agents in various conventional and local drug delivery devices (reviewed in 3). The following are the areas of biomedical and clinical sciences where the accumulation of biofilm needs special attention:

- Biomedical implants used for diagnostic and/or therapeutic procedures including cerebrospinal fluid shunts, orthopedic devices, artificial joints, wound drainage tubes, artificial hearts, prosthetic heart valves and cardiac pacemakers;
- Intravenous catheters especially continuous ambulatory peritoneal dialysis catheters;
- Contact lenses;
- Dental plaque mediated ailments (Caries and Periodontal pocket diseases); and
- Infected tissues of gastrointestinal tract, urinary tract, lungs, trachea and other organs.

Efforts and attempt are continuing to control and eradicate biofilms, using novel antibodies and the use of controlled and novel drug carriers. With the failure of conventional means to achieve therapeutic levels at the infectious sites of biofilm localization, either due to the ecological niche of the sites or the bacterial resistance toward the already existing therapeutic strategies, controlled and novel drug delivery strategies are appreciated.

BIOFILMS AND BIO-PHYSICALLY MODULATED DRUG DELIVERY

Biophysical means have been developed and adopted to modulate the antimicrobial therapy over last few years. Electric fields and ultrasound applications have been used to enhance the efficacy of antibiotics/antimicrobial agents via twofold action, i.e., biofilm penetration enhancement and killing bacteria through abrasive sterilization processes.

Ultrasound waves were investigated for biophysical modulation of drug release from delivery device into the bacterial biofilms. Ultrasonic irradiation *per se* enhances the killing of *Pseudomonas aeruginosa* biofilms when combined with gentamycin by nearly two orders of magnitude (23). The effects of ultrasound frequency and duration on biofilm killing using antibiotics were optimized (24). The studies inferred that lower frequency of amplification produces higher levels of killing of bacterial biofilms of *Escherichia coli,* where the study on duration indicated that complete sterilization of a 14-h biofilm could be achieved after 6 h of exposure. In another study, *Escherichia coli* biofilms on polyethylene disks were implanted subcutaneously into rabbits receiving gentamycin as a model antibiotic agent (25). Ultrasound was applied for 24 h and viable counts of the bacteria in the biofilm were made. Pulsed ultrasound significantly reduced bacterial viability to the excessively low level as compared against untreated biofilms.

Blenkinsopp *et al*. (26) proposed the term "bioelectric effect" to describe that small "dc currents" could be used to enhance the efficacy of biocides against *Pseudomonas aeruginosa* biofilms. Enhanced rate of biofilm elimination with antibiotic therapy was reported when a "dc current" was applied as part of the biofilm treatment (27). Photomechanical waves (PW) generated by ablation with high- pulsed lasers has been used as an extension to the electric field to synergies biofilm killing. While the heat and cavitations cause the ultrasound-assisted biofilm killing (28), the photomechanical wave-assisted effects were mainly attributed to the mechanical force (29).

Most of the studies have been focused on holding the electrical parameter (e.g., the dc current) constant and studying the result of varying biological variables (e.g., the level of biocides). However, McLeod and co-workers (30) kept the biology (level of biocides) constant and varied the applied electromagnetic field. These workers demonstrated a dose response curve for the current needed to produce increasing levels of killing of the bacteria in the *Pseudomonas aeruginosa* biofilm using tobramycin as a model antibiotic. Possible mechanisms for the bioelectric effect-mediated antibiotic control of bacterial biofilms have been proposed (30). The enhanced activity was proposed to be due to an increased delivery of oxygen to the biofilms as oxygen is generated by *in situ* hydrolysis. It is possible that when oxygen levels reach toxic levels in the biofilm, it weakens the bacterial cells and subsequently renders them more susceptible to the antibiotics. In contrast, another possibility is that increased oxygen supply could enhance the growth within the depth of the biofilm, which would negate the reduced susceptibility of the bacteria in the biofilm due to their slow growth (31). Moreover, the active oxygen intermediates such as peroxides that are generated during the process may also cause a bioelectric effect (30).

In recent studies, Soukos and co-workers (32,33) reported the use of photomechanical waves for effective drug delivery to bacterial biofilms with their possible clinical adaptability. In their study, photomechanical waves were generated by ablation of a target with a Q-switched ruby laser and subsequently exposed to *Actinomyces viscous* biofilms in the presence of methylene blue. These workers tested the hypothesis that photomechanical waves disintegrate and disorganize the structure of a microbial biofilm and thus increase the penetrability and permeability of simultaneously applied biocides. Simultaneously administered methylene blue penetrated the biofilm population as recorded using confocal scanning laser microscopy. These studies revealed that a single photomechanical wave was sufficient to produce a 75% increase in the penetration depth of methylene blue into the biofilm. The enhanced permeability of biofilm population by photomechanical waves was considered as a therapeutic tool with potentials for photodynamic therapy using photoactive compounds. These workers subsequently assessed the photodestruction of *Actinomyces viscous* biofilms after their sensitization with methylene blue followed by irradiation with photomechanical waves and red light at 660 nm.

The approaches of using either ultrasound or electric waves including photomechanical waves may prove useful in delivering biocides into biofilms of different species (caries, periodontitis, denture stomatitis, *Helicobacter pylori* infections of stomach, candidiasis) as well as in the treatment of prosthetic medical device and contact lens-associated infections.

BIOFILMS AND LIPOSOMAL DRUG DELIVERY

Drug delivery and targeting to the bacterial biofilms has received current interests. However, the potential of drug delivery in the localization and/or targeting of biofilms still remain to be proved and adopted in the field of pharmaceutical research. The targeting constructs could be realized using carriers, site-specific ligands and delivery of release modifiers. Targeting could be extended implicating intrinsic and inherent distribution profile of carrier (passive targeting). It can also be achieved using site-specific drug-carrier composite appended with suitable ligands to alter its distribution or uptake in the biological milieu and to release the drug in the proximity of bacterial biofilms (active targeting).

Among the various delivery systems directed against bacterial biofilm (mainly plaque or periodontal pocket flora, which represent the model biofilm in different studies) vesicular systems are found to be versatile in their disposition of the contained drug. These systems mimic the bio-membrane in terms of structure and bio-behavior, and hence are investigated intensively for targeting bacterial biofilms.

Vesicular systems in general and liposomes in particular are the highly investigated delivery and targeting devices designed and developed for bacterial biofilm targeting (34–44). Jones and Kaszuba (36) reported polyhydroxy-mediated interactions between liposomes constructed of phosphatidylinositol (PI) and bacterial biofilms. The targeting of liposomes to adsorbed films of bacteria was thought to be due to the interaction of the surface associated polymers of the bacterial "glyco-calyx" with polyhydroxy head groups of liposomal lipids. The theoretical basis of biofilm interactions of liposomes investigated by these workers was based on a three-

dimensional lattice model for the bacterial glyco-calyx and two-dimensional lattice for the liposome surface. The models were parameterized for the potential energy of interaction between surface of biofilm and liposome as a function of their separation. It was elucidated that a relatively small energy of interaction between the polyhydroxy-head groups (phosphatidylinositol) of the liposomal lipid and bacterium surface polymer residues (polyol phosphate polymers, teichoic acid) exists. This gives rise to a potential energy of interaction, which was found in excess than the classical double layer repulsive force and attractive dispersion force interactions. This potential energy of interaction exhibits quantitatively an energy minimum, which was found to be a function of the polyhydroxy-lipid concentration on the liposome surface. This model thus predicts an optimal liposomal composition for optimal adsorption of liposomes to bacterial biofilms. In accordance with the lattice model, these workers further demonstrated the adsorption of dipalmitoyl-phosphatidycholine (DPPC)-PI liposomes to a range of biofilms of oral and skin-associated bacteria on solid-support, where optimum levels of PI for biofilm-adsorption were determined and reported.

These DPPC-PI based liposomes were further studied for biofilm targeting with encapsulated enzymes, glucose oxidase (GO) and horseradish peroxidase (HRP) (41). The systems were termed as "reactive liposomes". These reactive liposomes exhibited significant localization to biofilms (due mainly to PI component) and in the process released encapsulated enzymes in the close proximity of the biofilm. This subsequently led to inhibition of further bacterial growth as released enzymes in presence of their substrates, i.e., glucose and iodide, release species like hydrogen peroxide and oxy-acids, which are antibacterial in nature.

In different studies, however, various liposomal versions like cationic liposomes (39,40), lectinized or proteoliposomes (44,46), immunoliposomes (38,43) and liposomal hydrogels (47) were investigated for their targeting potential using various models of attached or aggregated bacteria (Table II). Sanderson and co-workers (39,40,48,49) reported adsorption of cationic liposomes over biofilms of skin-associated bacteria. Cationic liposomes (dipalmitoyl-phosphatidycholine, cholesterol and stearylamine) were exposed to adsorbed biofilms of *Staphylococcus epidermidis* using a microtitre plate model (39). The interaction (as assessed by the apparent monolayer coverage of the biofilms by the liposomes) was described using Langmuir adsorption isotherm, which enabled the determination of maximum theoretical coverage of the bacterial surface and association/dissociation constants. Adsorption of SA-containing liposomes to biofilms is governed by several factors including hydrophobicity of bacterial strains, lipid compositions of liposomes, temperature and ionic strength of dispersion.

The results indicated that electrostatic effects mediate the attractive interaction between the cationic liposomes and negatively charged sites on the bacterial surface or the extracellular slime (e.g., teichoic acid). This was evident by two observations. Firstly, the increased ionic screening at higher ionic strength weakens the attractions between bacterium and vesicle (a decrease in dissociation constant) and secondly the compression of the diffused double layer surrounding these oppositely-charged surfaces leads to a decreased biofilm-vesicle dissociation constant. This subsequently results in to a maximum theoretical coverage and hence enhanced population of liposomes gets attached to bacterial biofilm. Subsequent to surface attachment the release of encapsulated biocides occurs within the vicinity of surrounding biofilms and thus a practical site-specific delivery could be negotiated.

To exploit the biofilm associated surface determinants (antigens) for target selectivity, Robinson and co-workers (43) reported the specificity and affinity of immunoliposomes toward *Streptococcus oralis* biofilms using two different surface-bound monoclonal antibodies (anti-oralis antibodies 4718 and 4715) raised against antigenic determinants of the same bacterium. The anti-oralis immunoliposomes showed the greatest affinity and percent monolayer coverage when targeted to a range of different oral bacterial biofilms, i.e., *S. oralis, S. sanguis, S. gordonii, S. salivarius,* and *S. mutans*. The targeting affinity of immunoliposomes for *S. oralis* however was largely unaffected by the number of antibodies conjugated to the liposomal surface or by the net charge on the lipid bilayer. Moreover, anti-oralis immunoliposomes were relatively less specific for *S. oralis* than the free anti-oralis antibodies because of the non-specific interaction of the liposomes with other bacteria of typical multi-species biofilm.

Similar attempts were made to exploit the surface glyco-conjugates/polysaccharide slime substances of the bacterial origin to target bacterial biofilm using lectin conjugated or

Table II. Various Liposomal Systems Studied for Delivery of Biocides to Bacterial Biofilms

Type of system	Composition	Bacteria (biofilm)	Ref.
PI-Liposomes	DPPC:PI	*Streptococcus mutans, S. epidermidis*	36
Cationic liposomes	DPPC: Chol: SA	*Staphylococcus epidermidis*	40
Reactive (enzyme bearing) liposomes	DPPC:PI:GO:HRP	*Staphylococcus gordonii*	41
Cationic liposomes	DPPC: Chol: DDAB	*Staphylococcus epidermidis*	48
Immunoliposomes	DPPC:PI:DPPE:MBS	*Streptococcus oralis*	43
Proteoliposomes (sCon A)	DPPG:PI:DPPE:MBS	*Staphyloccus epidermidis; S. sanguis; Proteus vulgaris; S. mutans*	46
Proteoliposomes (WGA)	DPPG:PI:DPPE:MBS	*Straphylococcus epidermidis; Proteus vulgaris*	46
Liposomal hydrogel	DPPC:PEG-DSPE	*Pseudomonas aeruginosa*	47

Abbreviations: DPPC = dipalmitoyl-phosphatidycholine, DPPG = dipalmitoyl-phosphatidylglycerol, PI = phosphatidylinositol, Chol = cholesterol, SA = stearylamine, GO = glucose oxidase, HRP = horse-radish peroxidase, DDAB = dimethyl-dioctadecyl-ammonium bromide, DPPE = dipalmitoyl-phosphatidylethanolamine, MBS = *m*-maleimidobenzoyl-*N*-hydroxysuccinimide, PEG-DSPE = PEG-diastearoyl-phosphatidylethanolamine, sConA = succinylated Concanavalin A, WGA = wheat germ agglutinin.

anchored liposomes. In most of these studies, *N*-succinimidyl-*S*-acetylthioacetate (SATA) derivatives of the lectins were conjugated through the reactive *m*-maleimidobenzoyl-*N*-hydroxysuccinimide (MBS) derivative of dipalmitoyl phosphatidylethanolamine (DPPE). This lipid derivative was then incorporated in to liposomes of DPPC (or DPPG) and PI by vesicle extrusion technique. Succinylated Con A (sCon-A)-bearing liposomes (proteoliposomes) have been found to be effective for the delivery of Triclosan to biofilms of skin associated bacteria, *Staphylococcus epidermidis* and *Proteus vulgaris,* and the oral bacterium *Streptococcus sanguis* (46). Even on exposure to a very short time the succinylated Con A bearing liposomes were retained by the bacteria biofilm and eventually delivered Triclosan in the cellular interiors of biofilms. The targeting was assessed by an apparent monolayer coverage (%amc) of the biofilms by liposomes and the optimum levels of phosphatidylinositol and Concanavalin-A were established using a biofilm model grown on microtitre plates. In contrast, inhibition or cell death rates for free Triclosan under the same experimental conditions following the exposure to the periodontal pocket bacteria was significantly less. The same group of workers (50) compared the role of surface bound lectins (succinylated Con-A and Wheat germ agglutinin, WGA) for their sensitivities toward various oral and skin-associated bacteria. The oral bacteria *Streptococcus mutans* and *S. gordonii* and the skin associated bacterium *Corynebacterium hofmanni* were successfully targeted using succinylated Con-A bearing proteoliposomes while the skin associated bacterium *Staphylococcus epidermidis* was targeted with WGA bearing proteoliposomes. In these experiments, both cationic and anionic as well as proteoliposomes were compared for their relative efficiency in delivering the bactericide Triclosan to biofilms. The concept of lectin-carbohydrate interaction accentuates the potential of lectin bearing liposomes as targeted delivery device for the control of dental plaque and gingivitis as established by Jones and co-workers (35,37,45). In different studies, a "lectin-target enhancement" factor (LTE, lectin liposomes binding per mole of lipid/ naked liposome binding per mole of lipid) was established and used as an indicator of targeting efficiency of different liposomal systems. The LTE was measured in terms of binding of liposomes to the target site either radiochemically using an appropriately labeled phospholipid or by inhibition of an appropriate ELISA using an antibody, which is specific for the target surface.

To further optimize the targeting as a function of mole percent of cationic and anionic lipids, Jones and associates (42) prepared liposomes of dipalmitoyl-phosphatidylcholine (DPPC) incorporating the cationic lipids stearylamine (SA), dimethyl-dioctadecyl-ammonium bromide (DDAB) and dimethylaminoethane carbamoyl cholesterol (DCChol) and the anionic lipids dipalmitoylphosphatidylglycerol (DPPG) and phosphatidylinositol (PI). The delivery of oil-soluble bactericide Triclosan and the water soluble bactericide chlorhexidine was studied for a number of liposomal compositions. Targeting was recorded to be most effective for DPPC-Chol-SA (for both bactericides), DPPC-DPPG and DPPC-PI based liposomes (for Triclosan). These systems were studied on *S. epidermidis* and *S. sanguis* biofilms. Double labeling experiments using ^{14}C-chlorhexidine and ^{3}H-DPPC suggested that there was an exchange between adsorbed liposomes, which as a result delivered bactericide to the biofilm and those in the bulk solution implying a diffusion mechanism for bactericidal delivery.

Recently the potential of ligand-mediated biofilm targeting has been explored using liposomes anchored with suitable site-directing ligands. Vyas and co-workers (51,52) proposed lectin-carbohydrate interaction as principle mechanism for the delivery of metronidazole against the bacterial flora of the periodontal pocket. Various engineered liposomes, i.e., mannan (polysaccharide) coated, sialo-mannan coated and lectinized (Con-A), were studied for their interaction with surface epitopes expressed on bacterial cell surface as glycocalyx. The targeting potential of these systems was expressed as % biofilm growth inhibition using a microtitre plate model for bacterial infection. Surface engineered liposomes provided excellent biofilm growth inhibition as compared against their plain counterparts however the mechanisms of their actions are yet to be fully elucidated.

Even though these developed liposomes are functional *in vitro* against bacterial biofilms, there are some problems associated with binding liposomes to the surface of medical devices. If drug loaded liposomes occupy all surface area, insufficient drug would be released to prevent bacterial adhesion for a significant period. In addition, the shear forces generated during the handling and insertion process would displace liposomes from the surface. DiTizio and co-workers (47) reported an approach to control bacterial biofilm formation on urinary catheters by sequestering the drug-loaded liposomes within a biocompatible matrix located on the surface of the catheter. The system consisted of a poly (ethylene glycol)-gelatin hydrogel in which liposomes consisted of DPPC and PEG-diastearoyl-phosphatidylethanolamine (PEG-DSPE) were sequestered. The three-dimensional gel matrix is capable of accommodating large quantities of drug-loaded liposomes, while simultaneously protecting the liposomes from membrane disrupting shear forces encountered during handling and insertion of the device. The prolonged release pattern of ciprofloxacin from these liposomal hydrogel preparations was approximated by zero-order release kinetics. Liposomal hydrogel coated catheters were also tested against *Pseudomonas aeruginosa* biofilms in terms of percent inhibition of bacterial growth and percent reduction in bacterial adhesion to treated catheter surfaces. It could be inferred that the zones of inhibition created by the ciprofloxacin loaded liposomal hydrogel preparations were approximately five-fold larger than the inhibition zones of control catheter sections treated with drug only. Similarly, hydrogel coating was found effective in preventing biofilm cells from adhering to the catheter surface as no bacterial cell viability was detected on these surfaces during a seven day treatment.

BIOFILMS AND MICRO-PARTICULATE DRUG DELIVERY

Biodegradable polymers for localized delivery of antibiotics have emerged as an important approach for treating biofilm infections associated with medically implanted devices. Several studies reported the microspheres prepared from biodegradable polymers and loaded with suitable antibiotics against *in vitro* developed biofilms. The relative effectiveness of poly (L-lactic acid) microspheres loaded with ciprofloxacin hydrochloride was investigated against peritoneal implanted biofilms of *Pseudomonas aeruginosa* in a rab-

bit model (53). The viable counts of *Pseudomonas aeruginosa* were markedly reduced or eliminated from the catheter, the device and the peritoneal wall in microsphere-treated rabbits as compared against rabbits treated with free drug. The viable counts were made from histological and scanning microscopic observations. A sustained release profile of antibiotics was recorded from a matrix type biodegradable device. The same group of workers investigated the need to maintain a sustained drug concentration above the biofilm eradication concentration to obliterate aged biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (54). In the specially developed modified open *in vitro* chemostat system, the drug was continually diluted at the site of administration (peritoneal cavity). The kinetics of release of ciprofloxacin as a function of drug loading and the dose of microspheres were correlated with the rate and extent of killing and eradication of the planktonic cells and aged biofilm cells cultured on pieces of silicone tubing in the chemostat. A correlation was established between sustained ciprofloxacin concentrations and the eradication of biofilms from both *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Habib and coworkers (55) used the microspheres for biofilm eradication to treat bone-associated bacterial biofilm infections, osteomyelitis. The microspheres of poly (glycolic acid-co-DL-lactic acid) loaded with ofloxacin provided a biphasic release profile with an initial fast release followed by a slow release phase, quite typical of a sustained release formulation. This release pattern was found to be pivotal for biofilm eradication in various studies carried out by these workers (53,54).

FUTURE PERSPECTIVES

The invention of biofilms as the target for biocides has revolutionized the approach of research in medical, pharmaceutical, and biosciences. A recent innovation in this regard is the search of biomaterials that resist bacterial congregation. Many new devices have been introduced, for example, with hydrophilic outer layers, antimicrobial coated surface, low surface energy and carbon-rich materials, highly biocompatible substances, biodegradable materials, and cell-or protein grafted surfaces (56,57). The use of phosphorylcholine (PC)-surfaced alloplastic materials improves the biocompatibility of medical devices in contact with blood or tears as it produces a non-thrombogenic surface (58). Recent experimental studies support the effectiveness of PC-grafted or surfaced medical devices to retard bacterial and crystal adhesion (biofilm formation) from the biological fluids (59). Not only the advances in polymer-grafted biomaterials but also the biofilm surface determinants (60,61) and genetic factors responsible for biofilm formation (62) are going to dominate the biofilm research of future.

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