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REVIEW ARTICLE **OPEN ACCESS**

Common and Innovative Strategy to Control of Microbial Biofilm Formation Through Modern Technology: Critical Review

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ABSTRACT
In recent years, the most of researchers and interesting ones in the field of microbial biofilm documented that nanoparticles like silver, metal ions play a significant role Nanoparticles are normally considered to be of a size no greater than 100 nm, and the exploitation of their unique attributes to combat infection has increased markedly over the past decade. The potential role of nanoparticles is to control the formation of biofilms within the oral cavity, as a function of their biocidal, anti-adhesive, and in biofilm approach. Here in the paper concentrate a light on the modern technique in biofilm and the role of nanotechnology in combating medical resistant microbial infection. The field that applies this technology and particles to understand and transform biosystems can be defined as nanobiotechnology. Further studies and investigation are still needed but the ability of nanoparticles to penetrate the biofilm, enter the cells and affect their biochemical functions makes them potential tools in biofilm control. In this paper we provide some Common and Innovative Strategy how to control of microbial biofilm formation through modern technology.
KEYWORDS: *Biofilm, Nanotechnology, Microbiology, Microorganism, Nanoparticals.*

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INTRODUCTION

Biofilms can form on solid or liquid abiotic surfaces as well on soft tissue in living organisms and are typically resistant to conventional methods of disinfection. These biofilms are predominantly composed of bacteria cells enclosed within an extracellular polymeric (EPS) matrix which they produce. The EPS matrix is the responsible for the cohesion (keeping the cells attached to one another) and the adhesion (to surfaces) of the biofilm and is composed essentially of polysaccharides and proteins. Biofilms are ubiquitous in nature and its formation is a strategy that bacteria use in order to survive in hostile environments.

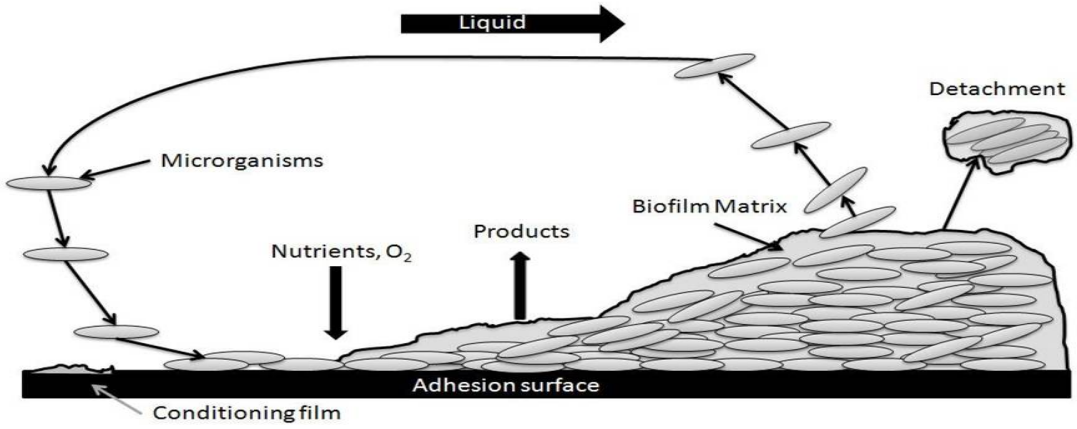

Figure 1: Schematic representation of biofilm formation

Biofilms are organized colonies of bacteria, fungi, or yeasts that form heterogeneous entities on biotic or abiotic surfaces by secreting extracellular polymeric substances (EPS). The genotypic and phenotypic characteristics of cells in biofilms differ from those of their free-floating counterparts, and these differences make them strongly resistant to antibiotics [1-5].

BACTERIAL BIOFILMS: FORMATION TO DISSEMINATION

It is now realized that most bacterially derived sessile communities are capable of forming irreversible biofilms on surfaces and interfaces by embedding themselves deep in a self-generated polymeric matrix. Furthermore, most of the fungal species that form biofilms do so in a similar manner; Candida and Aspergillus are fungal species of particular interest. The mechanism of biofilm formation depends on environmental stimuli and a series of genetic and phenotypic changes in planktonic cells. To date, five different stages have been suggested during biofilm development (Figure 2), namely, reversible-irreversible adherence, micro colony formation, 3D biofilm formation, maturation and dissemination [6-8].

In the earliest stage, biofilm development involves surface preconditioning and the adsorption of macromolecules, followed within seconds of surface exposure, by the formation of a conditioning layer. During the second stage, microorganism adhesion and coadhesion are strengthened by strong chemical attachments to the matrix polymer, and the unfolding of cell surface structures results in the exudation of a polysaccharide slime that attracts cells and debris. During the third stage, the nutrient rich biofilm environment promotes rapid microorganism growth that ultimately results in biofilm development in a 3D manner that substantially increases biofilm thickness. As film thickness increases, the forth maturation stage is reached, which is associated with antibiotic resistance. In the final stage, due to dynamic flux of the biofilm matrix, microorganisms detach, either actively or passively, and enter the surrounding environment as planktonic cells on a regular basis. Detached cells can also disseminate to fresh surfaces in the forms of detached biofilm clumps or fluid-driven cell clusters. Furthermore, bacteria originating from biofilm communities colonize new areas to produce new sessile populations[10-11].

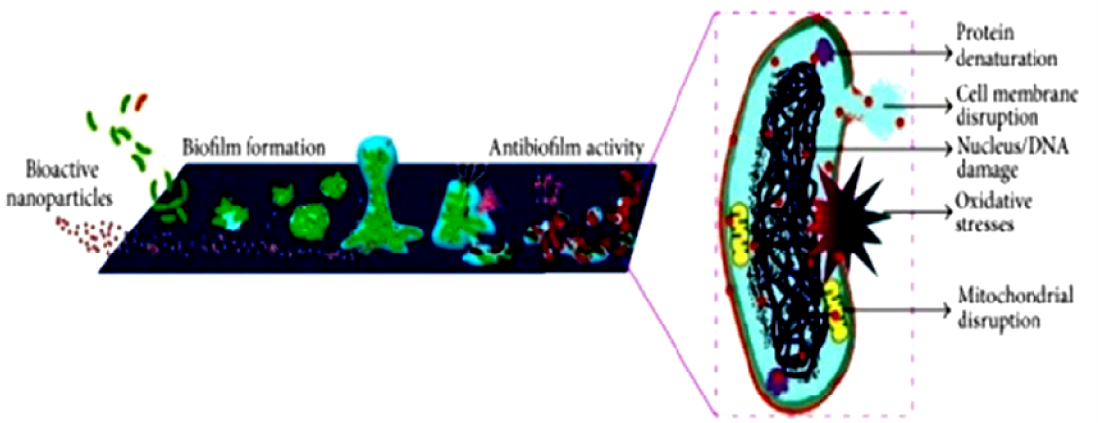

Figure 2: Schematic illustration of biofilm development and mechanisms responsible for the antimicrobial and antibiofilm effects of nanoparticles [9].

Biofilm Formation and Biofouling:

Biofouling is defined as the accumulation of unwanted proteins and other analytes or microorganisms on the surfaces of host materials. Microbial contamination and associated infections can have serious consequences in a number of environments, including hospitals and the food industry and in community-related settings. Fouling caused by marine organisms is also an issue of concern for industry and boating. After attaching to a surface, biofouling organisms can form a conditioning layer that provides an active platform for diatoms and algae, which results in increased operational and maintenance costs and the accelerated degradation of abiotic materials. Likewise, membrane fouling hampers pressure-driven membrane processes, such as reverse osmosis, microfiltration, ultrafiltration, and nanofiltration, used for water treatment and desalinization[12-14]. Membrane biofouling is caused by Aeromonas, Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, and Pseudomonas sp. and to a lesser extent by other microorganisms, like, fungi. In vivo, nonspecific protein adsorption facilitates bacterial attachment to surfaces and leads to colonization, subsequent biofilm formation, and infectious disease. Protein fouling followed by microbial attachment with biofilm development is a dormant factor of the failure of

biomedical devices and implants. Furthermore, microbial attachment reduces the sensitivities and an efficacy of devices, including those of in vitro diagnostic equipment, such as those required for immunological assays, and thus has therapeutic impacts [15-16].

APPROACHES TO BIOFILM CONTROL: Biological response to a biomedical device depends on the structure and surface functionality of the material used, and most device-associated infections are likely to originate from material surface contamination at time of implantation. Thus, the compositions or surface fictionalization of biomaterials are tailored to achieve desired results[17-18]. Surface engineering of materials can enhance device biocompatibility and functionality and material properties and surfaces can be modified to reduce microbial contamination and prevent biofilm infections. The different methodologies used include.

I. antifouling coatings,
II. Antiadhesive surface modifications,
III. Addition of antimicrobials to the surfaces of medical devices,
IV. coating devices with polymer products,
V. Surface engineering with chemical moieties,
VI. Coating, lamination, adsorption, or immobilization of bimolecules.

Strategies for biofilm control:

In industry, the operations of cleaning and disinfection are essential parts of the production process and the efficiency with which these operations are performed greatly affects the final product quality. Most cleaning regimes include removal of loose soil with cold or warm water followed by the application of chemical agents, rinsing, and disinfection. High temperatures can reduce the need for physical force. Chemical agents, usually surface active agents or alkali compounds, used as detergents, suspend and dissolve contaminant residues by decreasing surface tension, emulsifying fats, and denaturing proteins. These chemical agents are currently used in combination. Many situations require the occasional use of acid cleaners to clean surfaces soiled with precipitated minerals or having high mineral content. Mechanical action (water turbulence and scrubbing) are recognized as being highly effective in eliminating biofilms. An effective cleaning procedure must break up or dissolve the extracellular polymeric matrix associated with the biofilm so that disinfectants can gain access to the viable cells [19-21]. The cleaning process can remove 90 % or more of microorganisms associated with the surface, but cannot be relied upon to kill them. Bacteria can redeposit at other locations and, given time, water and nutrients can form a biofilm. Therefore, disinfection must be implemented. Disinfection is the use of antimicrobials chemicals to destroy microorganisms. This is required, since wet surfaces provide favorable conditions for the growth of microorganisms. The aim of disinfection is to reduce the surface population of viable cells after cleaning and prevent microbial growth on surfaces before restart of production. Disinfectants do not penetrate the biofilm matrix left on a surface after an in effective cleaning procedure, and thus do not destroy all the living cells in biofilms. Disinfectants are more effective in the absence of organic material (fat, carbohydrates, and protein based materials). Interfering organic substances, pH, temperature, water hardness, chemical inhibitors, concentration and contact time generally control the efficacy of disinfectants. The disinfectants must be effective, safe and easy to use, and easily rinsed off from surfaces, leaving no toxic residues that affect the sensory values of the product [22-24]. Table 1 resumes the properties of disinfectants commonly used in industrial systems. The disinfectants to be used should be chosen based on the following statements,

- Is the disinfectant effective in the pH range used?
- The disinfectant stable when diluted?
- Is the disinfectant toxic, safe or irritating?
- What is the microbial spectrum of the disinfectant?
- How does the temperature affect the activity of the disinfectant?
- Disinfectant corrosive at the surface?
- Is the disinfectant surface active?
- The disinfectant stable when reacting with organic material?

Table 1: Chemical disinfectants commonly used in industry

Disinfectant type	Applications
Chlorine	Neutral/alkaline conditions; stainless steel; food contact surfaces; floors/walls/air; clean-in-place (CIP), spray, soak, fog
Chlorine dioxide	Water treatment/slime/odour control; rinse for fruit/vegetables; acid form on food contact surfaces; stainless steel; CIP, spray, soak
Iodine	Acid conditions, < pH 3; stainless steel, plastics; food contact surfaces; floors/walls; CIP, spray, soak, manual; hand disinfectant; carbon dioxide atmosphere; helps dissolve mineral deposits
Anionic surfactants at acid conditions	Acid conditions, < pH 3; carbon dioxide atmosphere; stainless steel, plastics; foam on external surfaces; CIP, spray, soak, manual; carbon dioxide atmosphere; overnight disinfection; bilkstone/beer stone removal
Peracetic acid	Acid conditions; carbon dioxide atmosphere; stainless and mild steel, soft metals, plastic, rubber; food contact surfaces; CIP, spray, soak
Quaternary ammonium compounds (cationic surfactants)	Neutral/alkaline conditions; applicable to all materials; food contact surfaces; environmental areas/residue can extend activity; mildew and odour control; water treatment; spray, soak, manual, circulation
Amphoteric surfactants	Neutral/alkaline conditions; applicable to all materials; food contact surfaces; environmental areas; spray, manual soak; fog air; foam is suitable for external surface disinfection
Polymeric biguanides	Acid/alkaline conditions; applicable to all materials; food contact surfaces; environmental areas; can/bottle warmers, water treatment; spray, soak, manual, circulation; fog air
Glutaraldehyde	Neutral/alkaline conditions; non-corrosive to all materials; water treatment/slime control in can/bottle warmers, tunnel pasteurizers; glycol and Sweetwater systems in dairies; conveyor lines
Isothiazolinones	Acid, alkaline, neutral conditions; applicable to all materials; cooling water/towers, can/bottle warmers; long-term, continuous activity; conveyor lubricants
Phenolics	Lubricants for conveyor lines; water treatment
Hydrogen peroxide	Applicable to all materials; sporicide at high concentration at high temperature; aseptic packing of beverages

INNOVATIVE STRATEGIES FOR BIOFILM CONTROL:

Biofilms are a frequent source of infections and industrial process problems. Many studies have been performed in order to control biofilms in food industry. Some strategies are already common practice in industry.

Micro and Nanotechnology in biofilm control: The conventional methods hitherto applied for water disinfection and decontamination have been effective in the control of microbial pathogens. However, new problems are being associated to them. Besides requiring a considerable economic effort and expensive infrastructures, the chemical disinfectants are responsible for the production of harmful disinfection by-products (DBP). Chemicals such as free chorine, chloramines and ozone can react with diverse natural water constituents thus forming DBPs, many of which are toxic and/or carcinogenic. For these reasons, and in order to successfully control waterborne pathogens in water, it is imperative the development of new biofilm control strategies. Advances in the micro nanotechnology field promoted significant interest in its environmental and biological applications [25].

Layer-by-layer technique: The layer-by-layer (LBL) self-assembly of oppositely charged polyelectrolytes onto colloidal particles has been used to create novel nano- and microparticles with well controlled size and shape, finely tuned wall thickness and variable wall compositions. The original method was introduced in 1991 by Decher and co-workers for the construction of pure polymer multilayer films on planar supports. This technique uses electrostatic attraction and complex formation between polyanions and polycations to form supramolecular multilayer assemblies of polyelectrolytes. The first stage of shell fabrication involves step-wise deposition of polyelectrolytes from aqueous solutions. The polyelectrolyte multilayer film is formed by the alternate adsorption of oppositely charged

layers on to the particle. After each adsorption step, the non-adsorbed polyelectrolyte in solution is removed by repeated centrifugation or filtration and washing (Figure 3).

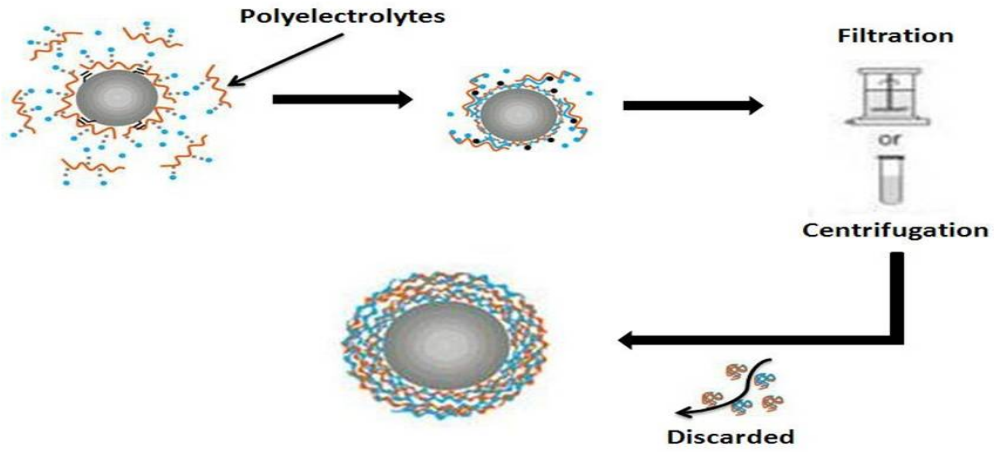

Figure 3: Schematic representation of LbL technique [25].

At present, there are two general approaches to encapsulate macromolecules into polyelectrolyte capsules using the LbL technique. The first method consists of formation of particles out of molecules subjected to encapsulation. Dyes and drug nanocrystals were used to template LbL assembly leading to encapsulation. The second approach for encapsulation of macromolecules exploits preformed hollow capsules and incorporates the macromolecules from the surrounding medium by switching the permeability of the hollow capsule shell [26-28].

Concerning biomedical biofilms, nanotechnology is emerging as one of the most auspicious methodologies for its prevention and control. The main nano approaches that have demonstrated the most promising results include: silver nanoparticles, drug delivery nanocarriers or phage therapy.

Silver nanoparticles: Antimicrobial activity of silver, copper and other metal ions is well known and, of all the elements, silver has been described as the one with the highest levels of toxicity for microorganisms and the lowest toxicity for animal cells. In fact, this metal has a broad antimicrobial activity spectrum against both Gram-positive, and Gram-negative bacteria, as well as yeasts. On the case of bacteria it is known that inhibits replication by binding to the microbial DNA and it also switches off important enzymes, leading to microbial death. The nanoscale materials have recently appeared as new antimicrobial agents due to their high surface area to volume ratio and unique chemical and physical properties. Silver nanoparticles (NPs), which are clusters of silver atoms, exhibit very strong bactericidal activity against both Gram-positive (GP) and Gram-negative bacteria, including multi resistant strains Thus, the advantage of impregnation of medical devices with silver NPs is that it protects both outer and inner surfaces of devices and there is continuous release of silver ions providing antimicrobial activity. Nanosilver, as particles, coating, or even impregnated on the medical device are thus emerging as a next-generation of antimicrobial agents. Although, some studies have raised some concerns regarding silver NPs biosafety, there are studies demonstrating the efficacy of silver NPs in reducing or preventing biofilm formation on catheter-materials both in vitro and in animal models [29-31].

Potent Antibacterial Nanoparticles against Biofilm Control: In this study, a simple and facile method was developed to generate stable GPA nanoparticles. These GPA NPs could effectively eradicate preformed biofilm and inhibit the biofilm formation, regardless of Gram-positive and Gram-negative pathogenic bacteria. In addition, GPA NPs had good biocompatibility and elicited a superior intracellular killing capability against multiple pathogenic bacteria in infected macrophages. The strategy may be useful to develop new therapeutics for treating chronic and stubborn infections related with biofilm and intracellular bacteria [32].

FUTURE WORK

Biofilms can be a problem in industries where water is involved in the manufacturing process. The chemical control of biofilms is an important issue because of the severe operation, management and public health impact. Biofilm control is proven to be even more challenging than the approaches taken against planktonic bacteria, however, the tests for chemicals efficacy are, most of the times, performed on planktonic bacteria. The selection of a suitable antimicrobial agent is of utmost importance for the development of a disinfection strategy. The antimicrobial efficacy should be tested against particular

contaminations, because the loss of efficacy depends on many factors. Thus, it should be tested in conditions as close to practice as possible, as in many cases, the influence of interfering agents could severely hinder the antimicrobial efficacy. Therefore, it is proposed to widen the list of disinfecting interfering substances, through the investigation of the mechanisms of action of the antimicrobial agents and their interactions with different cellular targets and soiling agents. Industrial processes have a need for biocides able to retain their activity in soiled conditions, work in low volumes, have low costs, and reduced corrosion [34]. Particularly in food industry, consumers and governmental agencies demand chemical agents that are less toxic, less susceptible to microbial resistance, and stable so that disinfection by-products do not enter natural systems. Therefore, anti-biofilm specific compounds should be sought as alternative drugs with the function to selectively blocking virulence, quorum sensing, and biofilm formation. Consequently, natural products such as phytochemicals, have already been introduced into the market, however, in general, their effects are limited compared to conventional disinfectants. In this work, the combination of enzymes showed biofilm control potential, the addition/ combination of phenolic or other new chemicals to potentiate the action of the conventional antimicrobials is suggested as follow up research. Both strategies (combinations with enzymes and new products) need optimization for complete control. When the biofilms were scaled up to the flow cell system, it was stressed that the way how biofilms develop is strongly connected with its degree of resistance [35-37]. The study of the process of biofilm formation is required from the early stages to maturation, by a combined perspective of their physical, chemical and biological phenomena. When the correlation of the processing characteristics is made with contamination occurrences, some solutions can be found:

- The study of the effects that the environmental parameters have on biofilm should be deepened and related with the food processing line characteristics. Plant performance should be optimized to find equilibrium when production is maximized and the microbial contaminations are minimized.
- Design materials with ability to inhibit soil accumulation must be sought, and
- The development of improved cleaning regimes, incorporated with conventional/new but efficient chemical agents is in demand.

A cleaning and disinfection plan should be developed complying with certain principals: the nature of the equipment (material and design), nature of the soiling agent, selection of a suitable antimicrobial agent, and optimum operational conditions at which the agent has maximum efficacy (temperature, concentration, hydrodynamics and exposure time). These suggestions should be performed not only for the model bacteria used for this thesis, but others such as Escherichia coli, Salmonella spp. or Listeria monocytogenes, commonly found in food industry, and their combinations. Understanding the different constituents that could emerge in food industry, will lead to a faster and more efficient response, by tailoring treatments to specific situations. New strategies are currently being researched and many more will appear as a response to new resistance mechanisms or technological advances[38].

CONCLUSION

Despite the advances made in the development of novel antibiofilm agents, devised biofilm treatment strategies are limited by their high costs and complexities, which means urgent development is required to identify cost-efficient alternatives. As is made clear by this thesis, recent developments in nanotechnology-based approaches aimed at preventing, controlling, and treating bacterial biofilm infections, especially of biomedical devices, are worthy of serious consideration. Different nanoparticle types and composites with demonstrated potential bactericidal and fungicidal properties have been shown to be efficient alternatives to antibiotics in terms of wound care and related biomedical issues. Nanomaterials are used as constituents of coatings, biomedical agents, and drug-delivery vehicles and of implant materials and research remains active in these areas. However, key issues like NP resistance and surface interactions between NPs, biofilms, and hosts need to be resolved to ensure successful clinical applications.

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CONFLICT OF INTEREST & FINANCIAL DISCLOSURE

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