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Interactions at Interface between Nanomaterial's and Biofilm: A General Survey

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Abstract

A biofilm is a consortium that exhibits three dimensions, syntrophic, and physiologically active-matrix. It displays a successful critical interdependency amongst producers and consumers. This association promotes microbial adherence, growth, antimicrobial resistance, and a high degree of persistence. The microbial cells are residing in the slimy extracellular medium consisting of polymeric polysaccharides, proteins, and lipids. The resident microbes adhere, float, or swim in the biofilm. This matrix can develop on non-living and living, including natural, industrial, or biomedical devices. The parameters such as cellular recognition, suitable attachment sites, nutritional signals, nature of earlier colonizers, etc., play a significant role in the formation of biofilm. This matrix is the most appropriate location to sustain a colony of microbes. Changes occurring in the ambient environment of biofilm enhance its development. The chemotherapeutic drugs or any other agents, like nanomaterials, have to overcome all these structural and functional aspects of biofilm to secure or cause the damage, as per the target. Commonly, a biofilm develops in an oral cavity, pulmonary system, in the form of cystic fibrosis, etc. Biofilms are also effective in causing pathogenesis in economically important plants and play an important significant role in geochemical cycles. The vast impacts of biofilms on plants are known phenomena but the concerning mechanism is still obscure. This review is an effort to understand the informative lacunae existing between interactions of nanomaterials and the biofilm.

Keywords: Biofilm; Biophysical Features of Biofilm; Growth, Inhibition of Biofilms; Microbial Colony; Nanomaterials; Syntrophic Matrix

Introduction

The formation of biofilm is among the primitive but secured modes of defense mechanism for the prokaryotes during the prehistoric era and even current times. During the antediluvian era, the conditions for life were harsh and survival was difficult. The microorganisms settle, aggregate, and adhere to suitable biotic and abiotic surfaces. These organisms produce extracellular polymeric substances which act as protection against the adverse ambient environment. The fossil studies support the antediluvian existence of biofilms [1]. Extracellular polymeric substances along with other components like polysaccharides, proteins, nucleic acids, and lipids, provide a specific macroscopic appearance to a biofilm or to slime. The extracellular polymeric substances of microalgae, specifically diatoms stabilize sediment, marine fouling, and bring about temporal synchronization between sand particles when the appropriate mechanical coupling is absent between them. There is an appropriate interaction involving the interfaces among extracellular substance and a substrate; substrate may be abiotic, biotic, organic, or inorganic. The concerned interaction involves varied types of bindings or bridging between the two. In nature, the soil is a common substrate for

biofilm formation. Its components influence the interaction between soil and biofilm. Humus or humic matter is one of the major components of soil (upper crest of the earth) and it is a product of the restricted breakdown of organic matter present in the soil. The humic substance is present as the main three fractions namely, humic acids, fulvic acid, and humin. These ingredients affect the structural and functional aspects of soil. The humic aspect contains larger and non-polar substances like paraffin, olefins, aliphatic substances, and terpenes while the fulvic component includes simple molecules like sugars, amino acid-related matter. These fractions bring about fluctuations in pH, and ionic strength of soil, further, these ingredients influence soil quantitatively and qualitatively. As a result, there are changes in the binding capacity and the nature of the soil; these bindings include hydrogen-bridges, metal-bridges, hydrophobic interactions, and aggregative behavior of a given sample of soil [2]. The insoluble ingredient of soil organic matter after the soluble part is retained in the aqueous base is humin. The humin primarily includes aliphatic hydrocarbon functionalities specifically those present in lipids, waxes, cuticular substances, cutin and cutan, suberin, and suberan; these are the minor plant components. There is a possibility of the presence of cellulose, peptide, and peptidoglycan but the presence of lignin derivatives is doubtful. The ingredients of humin are very much different from the base-soluble components constituting soil organic matter [3].

The extracellular polymeric substances produced by yeast and fungi, specifically, *Candida species*, cause flocculation, adhesion, formation, and development of slime. In the case of biofilm of *Sulfofolobus sulfatases,*-an archaeon, the extracellular polymeric substance responses to adhesion [4]. Biofilm does not possess fractional dimensions but exhibits self-similarity with size restrictions [5]. Biofilms are of common occurrence among biosystem and water associated surfaces in the environment. Microbes like bacteria, algae, viruses, and protozoa are common participants with biofilm communities [6]. Biofilms exhibit resistance, this feature restricts the entry of unwanted organisms. The biochemical nature of biofilm also attributes to prevent the entry of chemicals, and cellular invasion [7]. Biofilms show variations depending on their occurrences and the organisms present as colonies within. Wastewater treatment site (within trickling filters), solid substrata that provide support, nourishment, oxygen, water, and sufficient humidity, are the common location of biofilms. One can find such spots and extended zones on river banks, stones, and rocks under stagnant and dynamic water. Phototrophic prokaryotes, cynophytes form microbial mats at the interface in and around fresh, riverine, and marine aquatic habitats. The marine biofilms are also dynamic entities like freshwater biofilm and their microbes vary depending on the range of tidal zone [8].

The biofilms show variations depending on their occurrence, ambient environment, availability of nourishment, and oxygen [9]. Sites, like wastewater treatment, underwater mechanical structures, bio-fouling of ships, are suitable for the formation of biofilms [10]. Hydrodynamic status, the concentration of nutrients, primary components like exopolysaccharides, proteins, degree of microbial mobility, and intercellular signaling are the main parameters that control the architect of a given biofilm [4]. The resistant nature of biofilms is due to their extracellular matrix; it is an effective physical barrier for chemicals and cellular influx. This microbial alcove is a structurally and mechanically stable unit and retains a suitable degree of hydration for the inhabitants [7].

The biofilms are small patches of habitat and have microfluidic chambers. The inhabiting bacteria or microbes are physically restricted within and able to get nutrients. Since the bacteria are present in the form of non-adhering flocks within the matrix of biofilm; it is convenient to investigate their behavior, cellular density, and quantification of nutrients during the formation of biofilms. In the case of *Escherichia coli*, elevated cell density plays a significant role during the formation of biofilm in comparison to the availability of nutrition [5].

The biofilm and planktonic cells show different behavior. Biofilm cells associate with surface and get enveloped in the matrix made of extracellular polymeric substances, these occur in natural and engineering setups. The planktonic cells adhere to the surface and the matrix formed is of polysaccharides, proteins, DNA, and lipids. There can be a change in the spatial distribution of the components of extracellular polymeric substance during the maturation phase in biofilms. Biofilms exhibit viscoelastic behavior and detachment [11]. The planktonic cells are inhomogeneous environments while biofilm cells have varied microenvironments exhibiting electron donor and acceptor gradients. Biofilm cells have more of an extracellular polysaccharide substance than the planktonic cells. Some of the microbes recognize the surface contact and change the respective transcriptional mode involving their appendages like flagella. This behavior helps them to counteract oxidative stress, membrane transport, and ion uptake [11-13].

Biofilms exist in biosystems as cystic fibrosis of lungs, chronic wounds, dental plaque, vegetation infection on the heart valve, and as a crested form on the urinary and other catheters used during medical treatment. Such biofilms are small aggregates lodging small organisms adhering to the surface but enveloped by a thin tissue layer derived for the host. Sometimes, the biofilms are enlarged aggregates commingled with platelets, fibrinogen, and traces of minerals [14]. These slimy colonies are omnipresent and

able to sequestrate, accumulate, and transform the trophic environmental contaminators. These slimy aggregates act as a sorptive sponge and are able to abduct or snatch various chemicals and biological components. Extracellular polymeric substance (EPS) is one of the common components of microalgal, fungi like yeast, mould, and, archeal biofilm. In the case of microalgal biofilm, the EPS helps to stabilize and synchronize sediment and sand; it also causes marine fouling [15-17]. The EPS, in the case of heterotrophic bacteria, promotes growth, and itself acts as a substrate [18,19].

The biofilm acts as a physical barrier against undesired chemicals and organisms [10]. Biofilms are recalcitrant and antibiotic-resistant in nature. These alcoves have a high level of cohesive and adhesive strengths, these two together, easily cover the sheer stress they face during their removal or extraction [20]. Bacterial plant pathogens infest various parts of a plant and cause infection. These pathogens release degrading enzymes that digest or hydrolyze cell wall and cell organelles of the plant cells thereby ensuring infection. Commonly, the formation of biofilm is one of the virulent mechanisms resulting in initial bacterial colonization in a plant. This initiates the process of serious plant infection that causes great loss to the plants and crops [21]. The process of quorum sensing brings about cell to cell transmission and it is specifically important for biofilm sustenance. This function is density-based and small auto-inducers play functionally significant roles. These auto-inducers vary in different biofilms in a variety of microbes. N-octanoyl HSL (C8-HSL), N-3-hydroxydecanoyl HSL (3OHC10-HSL), and N-3-hydroxy-octanoyl HSL (20HC8-HSL) are the auto-inducers present in the biofilm of *Burkholderia* species [22]. Plant pathogens release extracellular enzymes that cause degradation of molecular aspects of plant tissues and enter invading various tissues like xylem. These enzymatic interactions are under the influence of quorum sensing [21].

Mostly, biofilms are considered to be as infelicitous biological entities but there are some promising features like the transformation of nutrition among rhizosphere plants, increased degree of biodegradation of organic carbon, and different pollutants during the wastewater treatment. Biofilms play an effective role during the soil remediation process. Their role in selected economic and inexpensive catalysis is evident. The energy conservation operation is effective in the production of biofuel and microbial dependent batteries [23].

Structural and Functional Aspects of Biofilm

The generalized structure of a biofilm is a syntrophic accretion of microbes. These microbes are Gram-positive and Gram-negative and may have one species or multispecies. The matrix of biofilm is slimy in nature; it adheres to the suitable substratum at an interface of consortium. The basic component of the biofilm is self-produced extracellular polymeric substance along with proteins, nucleic acids, and polysaccharides. The matrix consists of many porous layers having channels through which the microbes present in the interior of biofilm get nourishment and expel their waste. The early microbe settlers produce protein, this metabolite acts as a signal for the ambient microbes and helps to recruit fresh cells to join the existing accumulated microbes in biofilm. Thus, the proteins produced; act as the cell to cell chemical signals that induce the formation and development of biofilm. These proteins also send signals to produce and develop the polysaccharides. As a result, the biofilm becomes a multilayered, porous matrix [1]. There is enough water, and active and dead microbes within this porous matrix. These pores interconnect via channels and maintain the free flow of fluid. The concept of the Stokes and Continuity regulates this free flow of fluid while the concept of the Brinkman and continuity brings about the water flux within the porous matrix. The concept of the Brinkman equation regulates the flow of fluid and the flow relates to the kinetic potential. This kinetic potential depends on the velocity and pressure of the fluid, and gravitational potential. This concept plays a role during the transition between the slow flowing of the fluid under the influence of Darcy's law while the concept of the Navier Stokes concept regulates the fast flow of fluid in biofilm. The concept of continuity equation relates to the conservation of mass in such conditions. The process of convection and diffusion facilitates the distribution of nutrients [24].

The matrix of the biofilm consists of highly hydrated extracellular polymeric substances. The matrix exhibits cellular flocks of floating cells. Some biofilms are free-floating and in the form of sledges, and these do not anchor to any substratum. Most of the multispecies biofilms have a stable microbial congregation, physicochemical gradients, intense intercellular communication, and horizontal or lateral gene transfer. In this form of the gene transfer process, DNA transmission from parent to offspring does not occur among unicellular and multicellular organisms. This aspect of gene transfer plays an essential role during the evolution of an organism [4]. The structural aspect of biofilm provides a safe ambient environment against biocides and antibiotics; it is also responsible for the appropriate viscoelasticity and resistance towards dynamic shear stress. The feature of multiple species of biofilm results in a wide range of substrates, flexibility, and these are suitable for white biotechnology [23].

The marine biofilms show changes in their communities and structural aspects. These changes are the result of fluctuations in their ambient environment, diurnal, tidal, the prime composition of marine water, type of the substrate,

and episodic behavior (dysfunctional behavior); along with these, parameters like the hypersalinity, carbonate contents, and the geological profile of marine coast, act determinant factors during the formation of marine biofilms. The biofilms in the hypersaline lagoon and the Sabkhas (coastal, supra-tidal mudflat) have laminated sediment. The carbonate-rich marine water exhibits specific trapping, binding, and lithographic patterns establishing the concept of stromatolite. This concept explains the formation of layered mounds, columns, and sheets resembling sedimentary rocks. The layers are the result of the growth of cyanobacteria in a layered pattern. This concept also reflects on the prehistoric era of the earth [3]. Thus, biofilms are of ecological, environmental, and geological significance.

Functionalities of the Components of Biofilm

Polysaccharides, proteins, DNA, and amphiphilic molecules play an active role during the adhesive behavior of biofilm. These constituents induce and ensure a successful interface between abiotic and biotic interacting components, or cellular planktons. This coordinated interaction results in a firm attachment of biofilm for a prolonged duration. These ingredients of biofilm recognize, identify, and abridge the aggregated cells, thereby ensuring a successful cellular gathering. Amyloid and lectin proteins, DNA, neutral and charged polysaccharide bring about the cohesion between the ingredients and inhabitants of biofilm. These biomolecules form a network consisting of hydrated polymers and also conjugate with multivalent cations resulting in the mechanical stability of the biofilm. This specific network either encapsulates or unsheathes the extracellular matrix or slime, it also adds to the specific architecture of biofilm that helps the intercellular signaling. Hydrated polysaccharides and probably proteins restore an appropriate degree of hydration and tolerate the higher rate of desiccation in the water-deficient biofilm. These integrants are crucial in specific and non-specific host resistance relationship during the formation of biofilm and the biotic components. These components also provide a fairly good degree of tolerance towards antibiotics, disinfectants, and most of the antimicrobial agents. The components also protect biofilm from the destructive impacts of cyanobacterial nitrogenase enzyme, oxygen, and free moving protozoa. Proteins along with charged and hydrophobic polysaccharides facilitate the sorption of organic materials into biofilm; this process enhances the collection of nutrients and sorption of xenobiotics form ambient environs. Overall, this action adds to the detoxification in the given habitat. The same integrants of biofilm involve phosphate and sulfate ions of the slime to ensure the sorption of inorganic ions, promote processes such as gel formation of polysaccharides (polysaccharide gelation), accretion of minerals, toxic metallic ions, thereby contribute to the process of detoxification in the ambient environment. Proteins present in biofilm help in the hydrolysis of exogenous molecules and the accreted nutrients. These proteins also degrade the extracellular matrix of biofilm, thereby helping in the release of the microbes present therein. All the ingredients of the biofilm maintain a suitable supply of carbon, nitrogen, phosphorous, sulfur, etc., for the microbial community of biofilm. DNA present in the slime undergoes horizontal-gene-transfer among the inhibiting microbes and as a result appropriate genetic exchange takes place. Specifically, those proteins which constitute nanowires and pili, and humic matter function as electron donor or acceptors during redox interaction within the matrix of biofilm. The membrane vesicles that enclose nucleic acid, enzymes, lipopolysaccharides, and phosphates help the transportation of cellular metabolites resulting in a suitable metabolic turnover. Polysaccharides facilitate maintaining the specific C: N ratio and these components hold excess energy with them and act as a means to store energy. The polysaccharides accelerate, retain and stabilize the enzymes present in biofilm because of their specific enzyme binding ability [25-29].

Metals also have a role in biofilm formation. Metals like zinc, manganese, calcium, potassium, and copper facilitate the formation of hardy and tough biofilms *in vitro* [30,31]. Some of the microbial pathogen species, like *Xylella species,* make use and store the ionomes of the affected plants during the formation of their biofilms. Calcium ions nurture the biofilm formation in the case of *Xylella* species. The decline in the extra and intercellular calcium contents affects the formation of biofilm but this does not apply to other species or mutants [32]. Biofilms amass metal nanoparticles like gold nanorods (65 nmX15 nm), TiO_2 (20 nm) from their riverine and marine mesocosms, and are able to partition nanoparticle in the environment [33,34]. The biofilms act as effective chelating agents; these entities physically confine, trap metals, and colloidal forms of natural and unnatural organic matter present in aquatic media like wastewater, fresh and marine water, and potable water [33,35].

Biochemical and Molecular Aspects of Biofilm

The biofilms exhibit variations in the biochemical components of their matrix. These variations depend on the type, location, and types of organisms involved. Bacterial biofilm, algal biofilm, viral biofilm, and biofilm formed on the abiotic wet surfaces show differences in their biochemical structures. Even, *in vivo* and *in vitro* biofilms in biosystems have divergent biochemical composition; such features need specific attention while interacting with them [36]. Biofilms show a higher degree of resistance towards the undesired chemicals and organisms. This resistance depends on the specific biochemical nature of the matrix [7]. Biochemical aspects in the extracellular

matrix of biofilm include carbohydrates, proteins, and fatty acids. The common carbohydrates present are mono and polysaccharides, exopolysaccharides (EPSs), stachyose (a tetrasaccharide consisting of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose), mannose, maltose, etc. The protein components include oxoreductases (these metabolize glucocorticoids into their inactive keto-metabolites), chaperon proteins, some membrane proteins associated with virulence. The saturated and unsaturated fatty acids components are in higher percentage; the major ones are palmitic, stearic, and oleic acids [7]. Proteins and exopolysaccharides are some of the prime macromolecules that engage in the architecture of biofilm and also act as functional units.

For understanding purposes, let us consider the biofilm of *E. coli* as a typical structural model. Its matrix consists of extracellular polymeric substances, colonic acids, cellulose, and polyglucosamine. These structural components maintain specific proximity among the microbes and appropriate intercellular interaction. The extracellular DNA (eDNA), plays a dual role i.e., as a nutritional and brings about interconnection within the biofilm. The lattice of the biofilm becomes stronger with the help of extracellular organelles like flagella and pili; these structures make the lattice strong and ensure aggregation of microbes within a biofilm. The enzymes secreted, change the extracellular polymeric substance of biofilm as the environmental conditions change [37]. The primary functions of the biofilm are recognition of concerned organisms, prevention of unwanted organisms, cell to cell communication, and cell to matrix communication in the compact mass of biofilm [6].

Growth Rate and Inhibition of Biofilm

To find the growth rate and the inhibition of development and formation of biofilms helps to find a suitable approach for their treatment. With the help of combined r-RNA- targeted fluorescent hybridization probe and digital microscopy, it is convenient to quantify the amount of ribosome and compare the growth rate of a single cell in multispecies anaerobic biofilm. This technique provides a better understanding of the study of the growth rate of biofilms. The growth rate doubles in the established biofilms in comparison to the young biofilms. The estimation of the contents of RNA, DNA, and proteins in a young and established biofilm also depicts the related growth rates [38]. The relative standard curve between RNA/DNA and biomass of one species is a general indicator of the growth rate. The fluorescent oligonucleotide probe along with the DAPI staining technique helps to estimate the contents of RNA and DNA simultaneously [39]. The need for a standard curve for each organism might be alleviated in part by using the change in the relative contents of RNA and DNA (RNA/DNA ratio) to estimate the growth rate. The RNA/DNA ratio is a general indicator of growth rate and biomass in the environment. This ratio is also useful for estimating the relative growth rate of a specific organism. The fluorescent oligonucleotide probe in combination with the DAPI staining technique is useful to estimate the RNA and the DNA contents simultaneously. However, additional studies should be done to establish the character of this relationship for a variety of environmentally relevant organisms [39]. The type-1 fimbria expression plays a regulatory role during the formation of IscR regulated iron-dependent biofilm [40].

The inhibition of biofilm formation is under the control of either repression or expression of a protein associated with the extracellular matrix or release of repression of the promoters that help in the formation of the biofilm. The functioning of SinR, a transcriptional repressor, is turned off during the formation of biofilm in the case of *Bacillus subtilis.* There are three antagonists that form the complexes with SinR and *these complexes regulate the function of SinR transcriptional repressor. The helix-turn-helix motif of SinR gets associated deeply in the major groove of DNA and it identifies five specific bases of the 7-bp consensus motif. The promoters regulated by SinR play a significant role to form an intensely lopped DNA structure [41].

Biophysical Aspects and the Behavior of Biofilms

The biophysical aspects of biofilms help to understand and deal with their growth, impacts of physical parameters of the environment on the attachment between cells and biofilm, study of probable investigatory probes, imaging, developmental modes of biofilms, the formation of a colony of organisms, and to develop a suitable model for the future research. The biophysical principles regulate the collective behavior of the microbes and constitutional biomolecules of biofilms. The cells present in the matrix of biofilm perform movements; these cells either push or pull each other within the elastic matrix of biofilms. The movements of cells, nutrients, and other metabolites follow the basic concepts of physics and microfluidics. As a result, there develops a physicochemical biointerface between the motile cells and the molecular components of biofilms. Swarming and collective swimming of microbial cells within an active suspension plays a significant role during the formation of biofilms. There is a general acceptance that the concept of long-range fluid dynamics regulates the cell-cell and cell-surface scattering and these basic functions cause swarming and collective swimming of microbial cells during the formation of biofilm, at least in the initial stages. The internal and external noises continuously challenge the microbial functionalities like gene expression, varied motilities, and formation of biofilm [42]. The fluid dynamics and rotational diffusion are the two main parameters that influence the swarming and collective swimming of the microbes. The

thermal and intrinsic stocasticity among cells are under the control of long-range fluid dynamics; this indicates the role of physical interaction between the bacteria, the steric collision along with near-field lubrication forces. The collective microbial movements in suspension changes into a self-organized granular system, assorted biofilaments, and the flocks of animals, and these are correlated with the dominant short-range forces. The long-range fluid dynamic interactions are very weak prior to collision of microbes with surfaces but once these microbes move along the surface there is an aligning collision, thereafter, hydrodynamics impacts play their active role for a longer duration [42]. The complex fractal morphology of biofilm has a swarming pattern based on the coarse-grained-lattice model permitting restricted expansion. This restriction is because of cell division and the long-distance repulsion among the cellular flocks depending on the nutrients. Further, this small confinement is not due to any microbial motility and the concentration of any surfactant [5].

The state of turbulence is of common occurrence in varying degrees in any fluid system as seen in oceanic current and even biosystem and quantum system in microscale; it results in fluid mixing and transport of metabolites specifically in biosystems. Turbulence is also common in biofilms in the form of a self-sustained process. The characterization of turbulence in an active non-equilibrated fluid system is difficult. The statistical study on a microbial flow reflects on the existence of self-propulsion minimal model involving rod; the study also suggests that the concept of short-range interactions is prominent whenever there is a higher concentration of collective microbial movements [43]. Although most of the living fluids exhibit a self-sustained turbulence feature, still there is no established universally accepted quantitative theory regarding the same. The observations based on microbial velocimetry, their ambient fluid involving imaging and tracking of colloidal tracers, indicate a correlation between the velocity statistics and the kinetic energy of second-order of magnitude; further, there is a decline in the fluid memory when swimming action increases and also decline in linear scaling among kinetic energy and entropy of the system under consideration [44].

The phenomenon of biofilms possesses extreme biophysical forces and is overdesigned with a very high factor of safety (FOS). FOS represents a ratio of forces between structures, buildings, substrates, etc., and the forces applied. This factor of safety ensures its survival during extreme environmental, climatic and physiological conditions in most of the alcoves within and outside a biosystem, and also on the engineered structures. Biofilms withstand extreme physical and chemical forces that try to weaken those including natural and anthropogenic activities like brushing, scraping, flushing, and chlorination. Biofilms have extensive biomechanical cohesive strength that plays a crucial role and contributes to its high degree of recalcitrant ability. There are forces due to the morphological structural aspects of biofilm which reflects its roughness; the maintenance of the functional dynamic also results in the forces during the development of biofilms. The sum of these forces exceeds the sheer stress a biofilm experiences [20].

A micro-cantilever technique is suitable to investigate the mechanical testing of a biofilm. The cohesive strength represents and elaborates the effectivity of the linkage between biofilm to biofilm while adhesive strength is the degree of adhesion between biofilm and its substrate. The biofilm experiences shear stress (in Pascal units), and this stress is a common factor affecting it in an aquatic environment, and during water supply system involving engineered set-ups, like drinking water treatment plants, distribution system, and wastewater treatment systems. In case of biofilm formed by *P. aeruginosa, S. epidermidis,* and Mississippi river water biofilm are around 1,760 ± 400 Pa (n = 19), 1,470 ± 210 Pa (n = 47), and 27,510 ± 7,620 Pa (n = 13) respectively while these biofilms had shear stress 0.07 Pa, 0.18 Pa, and 1.9 Pa, respectively. It is very distinct that cohesive strength is very high than the respective shear stress during the growth periods of these biofilms [20].

Biofilms exhibit a structure-property relationship, and the optical coherence tomographic technique in combination with the atomic force microscopy illustrates this relationship *in vitro* effectively. The presence of sucrose and the age factor affect the chemistry, mesoscale morphology, mechanical properties, and microbiology of a biofilm. The elevated concentration of sucrose enhances the deposition in biofilm, degree of adhesion, and a sharp decline in the values of Young's modulus. The age factor affects the degree of adhesion, i.e., an increase in age results in a decline in adhesion. The ratio between the extracellular polysaccharides substance and the microbes also increases with the rise in the concentration of sucrose but this ratio decreases as the biofilm ages [45].

Biofilms are porous fluidic alcoves. Each biofilm differentiates into the annular base granular biofilm and the zone having streamers protruding in the space of pores. The microbial multispecies use the architectural plasticity of the biofilm for their sustenance. The flow of the microfluid in these zones is regulated and it distributes the nutrients and other metabolites within the biofilm appropriately. The grain pore complexes utilize the architectural plasticity to their advantage and also contribute to their carrying capacity within the porous organization. The two architectural zones, i.e., base biofilm and streamer protruding space, exhibit different carrying capacity because there is a different mechanism for the movements of the microfluid in these zones. The tortuosity or fractality is more in base biofilm than

the streamer protruding space and this feature enhances the carrying capacity in this zone consequently enlarges the interface at the topography of the biofilm. This parameter enhances the mass transfer of the resources, nutrients, and metabolites. The relationship between the topography of biofilm and the growth of biofilm is similar to the benthic biofilms as these have easy access to open-channel flow. The biofilms are of microbial and ecological significance because this relationship and the porous architectural structure are advantageous, specifically in the stagnant region of porous environs. In these cases, the mass transfer processes regulate the delivery, distribution of oxygen, carbon, nutrients, and help the interaction between the porous biofilms and the physical aspects of the environment. The degree of architectural plasticity is different in the two zones of biofilm and the grain-pore complexes use this feature to their advantage. Overall, these architectural setups enhance the carrying capacity of the microfluid in the biofilms and ensure their success in any type of ambient environment or any ecological niche or anthropological site. Thus, these biofilms are the appropriate examples of successful competition and coordination among the microbial communities that form aggregation such as biofilm [33,46-48]. Biofilms mediate and ensure adherence between host or substratum and pathogens or the other organisms involved. The organisms are bacteria, fungi, and phototrophic prokaryotes, cyanobacteria. The substrate can be rocks, sandy shores, biomedical devices and tools, used during the medical procedures. The biochemical components like proteins, lipids, and exopolysaccharides play essential roles in establishing the behavior of biofilm; it is more so in the cases of host immune defense and antifungal therapy [49,50].

This specific coordinated communication among the microbes present in biofilm, and it involves characteristic the quorum sensing. Possibly, the ion-channel based cell to cell signaling is another option of communication within the biofilm and with the ambient environment. The microbes (*B. subtilis*) create electrical waves; these waves travel through the matrix of biofilm and beyond and carry K^+ signals. The microfluidic concept helps to understand this possibility of extension of electric waves beyond biofilm. Most likely, during this process, there is a change in the membrane potential of distant motile microbe (cell). Thus, biofilm sends signals that reach the motile cell and affects its membrane potential. The tumbling frequency of the membrane of the distant cell modulates and induces response. In response to this induction, there is a species independent attraction to words the source of electric wave, i.e., the biofilm. As a result, there is an incorporation of diverse microbial species in biofilm [51-53].

Biofilms experience environmental stress and the regulatory transcription process involves LexA type of proteins. The members of this protein family coordinate structurally and functionally with the operational aspects of the RecA recombinase coprotease enzyme. This enzyme plays a significant coopted role during the sustenance of the environmental stress responses in biofilms. The combined actions of genetic and biophysical aspects of biofilms trigger and stimulate the formation of the surface lectin. This lectin causes remodeling of the viscoelastic features of biofilm during the period of environmental stress. The fluctuations in the ambient external environment and the anti-biofilm agents initiate specific adaptive mechanisms in biofilms. These efforts ensure the survival integrity of the microbial communities of biofilm and such adaptations have a significant role during the noxious recalcitrance of biofilms [54]. There are different genotoxic factors like antibiotic mitomycin C, withheld DNA replication, and various DNA damages result in single-stranded DNA segmentation, initiate the activation of RecA recombinase enzyme, and create Mg^+ /ATP-binding form. This interplay induces genotoxic stress as RecA gets accumulated in the nucleoproteins of single-stranded DNA fraction. RecA also stimulates endogenous LexA peptidase autocleavage process and it acts as a coprotease for LexA. It is an SOS response repressor. The LexA brings about proteolytic degradation and causes a decline in DNA control for the synthesis of the required proteins to reduce the bad effects of DNA damage. Thus, RecA and LexA act as regulatory parameters during the mechanism related to prokaryotic stress [54-57].

Mechanics and the different microbes of biofilms play a significant role in its physical aspects. There appears to be some correlation between the mechanosensing process and fluctuations in the mechanical features of biofilms. Sheer stress on biofilm influences these correlations and the formation of a major component of biofilm the exopolysaccharides. This stress enhances the level of cyclic di-GMP; this, in turn, relates to the expression of type IV pili and regulation of change from the planktonic form into the lifestyle of biofilm in microbes like *Bacillus cereus* and *Pseudomonas fluorescens*. The parameters like chemical stimuli, pH, nutrients, surfactants, and the physical features influence the behavior of biofilms interdependently. The processes like a break-up, seeding, and dispersion of biofilms are related to the mechanics of biofilms [58-63].

Treatment of Biofilm is based on Targeting Strategies

It is essential and beneficial to target the obnoxious biofilms. Targeting is either specific or non-specific depending on nature, type, location, quantum, and severity of biofilms. The specific targeting depends on the indented ligands which selectively link with the concerned molecule within the biofilm. The non-specific targeting aims at

charge based interactions and hydrogen bonding present on the nanovehicles. The liposome drug delivery system and polymer, lipid-polymer hybrid nanoparticles, and immunoliposomes are among the common modes of the targeting process. Immunoliposome technique basically involves peripheral covalently linked antibodies.

Interaction between Nanomaterials and Biofilms

While accounting for the management of the risk factor of nanomaterials in the environment, the transport, persistence, and bioavailability of nanomaterials play a crucial role. The naturally present components of environment such as microorganisms, colloids, inorganic and organic matter, temperature, and sunlight, oxidant and reductant, etc., complicate the behavior of the nanomaterials released in the environment. Thus, the behavior of such nanomaterials is likely to be different than that exhibited in the laboratory during experimentation. Nanoparticles can influence the formation of biofilm either during its growth and cause derogative impacts on matured biofilms or exhibit abnormal growth behavior on the nanoparticle-coated surface [64]. There are possibilities that such nanomaterials exhibit agglomeration, sedimentation, become the components of the food chain once micro, meso, meio, and macrofauna, and flora ingest them either directly or indirectly. The formation of biofilms is one of the common phenomena among microbes, fungi, and algae under natural and anthropogenic systems. The cellular microbes are enveloped in a slimy matrix consisting of extracellular polymeric substance. Biofilms are one of the prime modes of portioning and probable transformation of nanoparticles under natural and engineered set-ups. These structural and functional modes behave as effective sponges for the nanoparticles [65]. Like any natural and engineered systems biofilms associate with bacteria, these microbes attache and enveloped within its extracellular polymeric substance. The aquatic biofilms are the site of interaction and accumulation of nanoparticles; these also act as a sorptive sponge for nanoparticles. The accumulation and interaction of nanoparticles depend on their biophysicochemical processes and play a crucial role in the environmental fate and biogeochemical cycles. Parameters like the ionic strength of the aquatic medium, and the pore size of the matrix of biofilm influence the interactions between nanoparticles and biofilms [66] (Figure 1).

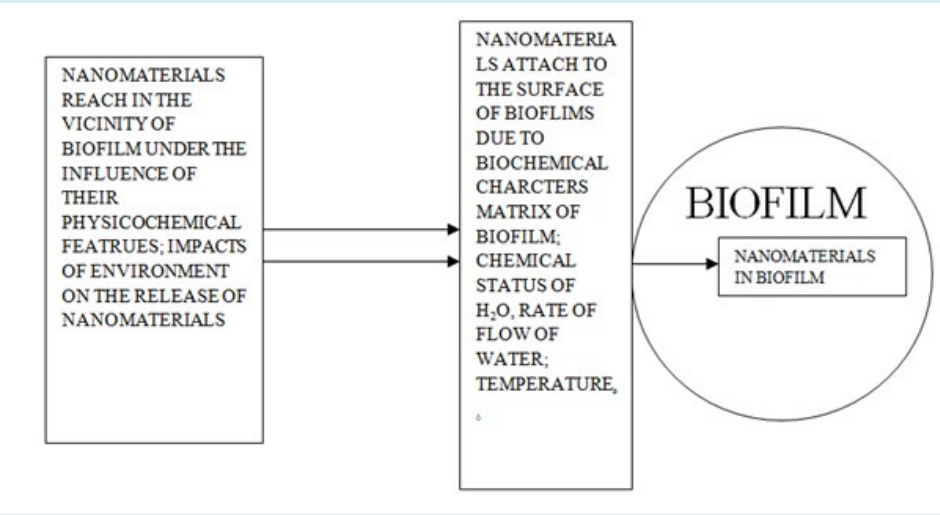

Figure 1: Flow Chart Indicating the Probable Mode of Entry of Nanomaterials in a Biofilm.

Under natural and engineered setups, the biofilms produce nanoparticles and as a result, participate in the bio-geo-chemical cycles in the environment. This production takes place during the detoxification of metals, in this process the metals precipitate as nanoparticles [67]. The biofilms that are rich in sulfate-reducing bacteria exhibit zinc sulfide nanoparticles [68]. An electrochemically active biofilm behaves like a catalyst and helps in the production of monodispersible crystalline silver nanoparticles [69]. Thus, biofilms are the favorite source for the production of nanoparticles using them as clean, non-toxic, and eco-friendly sources [65].

During the initial stages of formation of biofilm, the microbes aggregate and get in contact with the substratum as a random and interchangeable pattern; the Brownian movements, gravitational forces, and the ambient hydrodynamic forces help in establishing the initial contact.

Within this alcove, the microbes face specific attractive or repulsive forces because of the ionic strength, pH, and the ambient temperature. The velocity and the direction of these forces relate to the parameters like cell-surface composition, and characteristics of the medium (Figure 2).

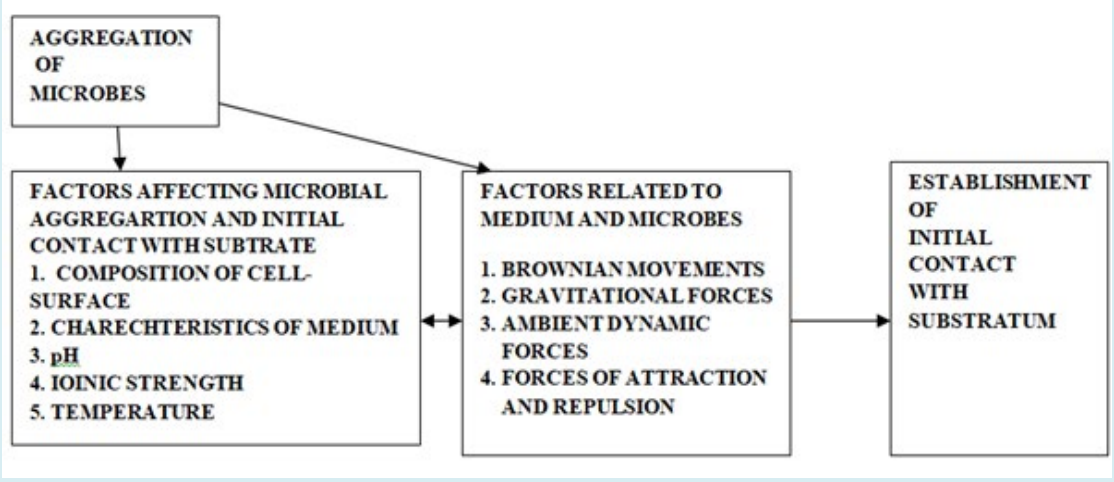

Figure 2: Events Occurring during Initial Stages of Biofilm Formation.

The motile microbes get the advantage of the hydrodynamic and repulsive forces because of the flagella, it is more so in pathogenic microbes like Vibrio cholerae, Listeria monocytogenes, and *E. coli*. During the initial stages of attachment, chemotaxis in response to the composition of the available nutrients helps in the direction of the formation of biofilms. The mutations in the CheR1 methyltransferase enzyme causes changes in its amino acid sequence and as a result, there is an impairment and maturation of biofilm in the case of P. aeruginosa but this is not so in the case of *E. coli* [70,71]. The methyl-accepting chemotaxis protein-II (tar) causes defects in the formation of biofilms in the case of uropathogenic *Escherichia coli* [72,73]. The repulsive and hydrodynamic forces, a force due to the sloughing off the surface, and the quantum of available nutrients play a significant role during the microbial adherence within a biofilm. The adherence involves the matrix, adhesin proteins secreted, and the microbes within a biofilm; these microbes can attach or detach themselves as per the need [40]. The microbes maintain a constant hold on the surface and withstand shear forces [71]. Cellular appendages, like pili of type-1, curli fibers, and antigens 43 help in the attachment of biofilm on abiotic surfaces along with the interbacterial interactions. Specifically, the curli fibers help in binding between the components of matrix-like laminin, fibronectin, plasminogen, and eukaryotic extracellular matrix [37].

Nanoparticles are one of the effective agents suitable to control and diffuse the problematic impacts of biofilms. These wonder materials are very useful carriers for antimicrobial agents and the dispersion agents of the biofilm matrix. To ascertain a check on the biofilm infection there is a need to have an in-depth understanding of the spatial, chemical variations, and complex nature of these slimy microbial aggregates [73]. The physicochemical properties of nanoparticles and properties of the aquatic medium affect the primary *three stages of* formation of biofilm; these are reaching of nanoparticles within the vicinity of biofilm, attachment of nanoparticles on the surface of the biofilm, and settling of nanoparticles in the deeper zones of biofilm. This complete process is referred to as bulk phase transport (Figure 1). Among the physicochemical features of nanoparticles, the surface modifications influence the interaction between biofilms and nanoparticles, it is more so in the case of specifically engineered and ligand capped nanoparticles. To counteract the antibiotic and other derogative impacts of biofilm, nanomaterials are the feasible options because these nanosized materials have the ability to disrupt and reduce the resistive effects of biofilms. To make this more result-oriented exercise one must consider parameters like type and concentration of nanomaterials, straining of microbes inhibiting biofilm, nature, quantification, and the ambient environment of the biofilms [74].

The functionalized nanoparticles with the organic ligand are basically applied for target accomplishments. Such functionalized nanoparticles come in contact with the biomolecules and get further modified due to the passive sorption within a biosystem. The protein corona formation involves organic biomolecules due to their temporary association with nanoparticles [75]. There are more mediated interactions between proteins and other

biomolecules when these nanoparticles with protein corona come in contact with the reacting surface [76]. The natural organic matter forms corona like coating on the surface of nanoparticles that have been released in the environment. Such coated nanoparticles along with other types of coatings affect the interaction between nanoparticles and biofilms in the environment. Silver nanoparticles having natural organic matter corona diminishes the degree of toxicity due to silver nanoparticles. Silver nanoparticles, pre-treated with natural organic matter (silver nanoparticles with corona) maintain a higher degree of cellular viability after incubation with the biofilms formed by Pseudomonas aeruginosa than the untreated silver nanoparticles with natural organic matter (silver nanoparticle without natural organic matter corona). Polyethylene glycol coated cadmium selenide quantum dots enter readily in the biofilm formed by Pseudomonas aeruginosa in comparison to the quantum dots coated with a carboxyl group (-COOH) [77].

The size and the charge on nanoparticles modulate the transfer of nanoparticles within the biofilm formed by P. fluorescens; the self-diffusion coefficients decline as the size of nanoparticles and the negative charge increases [78]. The density of the biofilm and the size of nanoparticles are also functionally related; self-diffusion of nanoparticles having a larger size than 50 nm becomes restricted in the case of biofilms having a higher density. It is the electrostatic force, not the steric force that controls the rate of diffusion of latex beads that are negatively and positively charged and are bigger than 28 nm in the cases of biofilms that have fewer numbers of Lactococcus lactis but higher numbers of Stenotrophomonas maltophilis extracellular polymeric substances [79]. The size, charge, and surface chemistry of nanoparticles collectively influence their mode and degree of transfer within porous biofilms [80]. Metal nanoparticles like silver, gold, and copper prevent the progress of biofilms. Some antimicrobial agents coated with polymeric materials, silica, and polysaccharide nanomaterials intercept the progress of biofilms. Some nanomaterials cause disruption and inhibition of biofilms. Such nanomaterials are silver nanoparticles coated with antibiotics, photosensitizers along with nanoparticles, antibiotics cocooned in polymeric nanomaterials, and silica or polysaccharide nanomaterials. Superparamagnetic iron oxide nanoparticles with or without an alternating magnetic field or external magnetic field also disrupt and inhibit biofilms. During the treatment of biofilms, metabolites of biofilms along with nanoparticles are administered to increase the efficiency of antibiotics, i.e., there are disruptions and inhibition of biofilms. Disturbance in the metabolic signaling system of biofilm causes its dispersion. Magnetic hypothermia kills the microbes of biofilms and instigates its premature dispersion [74]. The engineered nanoparticles partition within biofilms and affect it overall specifically, interrupting the quorum sensing adversely [81].

Quantification of Biofilm *in vitro*

It is essential to know about biofilm quantification if one has to reduce its harmful impacts. The quantification of biofilms is one mode to do so and this can be accomplished using simple techniques. There is a specific amount of light scattering by a given population of the organisms in a given sample. This principle is utilized in measuring the bacterial growth rate in biofilm by measuring optical density at 590 nm. Bacterial viability test is suitable to evaluate the dead and alive staining technique. The fluorescent green (SYTO 9) and fluorescent red (propidium) stains help to identify intact and dead bacterial cells, thus, evaluate the microbial cell viability of the biofilm bacteria [82]. Thereafter, treatment of a biofilm with nanoparticles and evaluation of the cellular viability using an alternating magnetic field, metabolic assays, such as the XTT assay and Alamar Blue assay help to the quantification of biofilm [83-a]. Staining the treated biofilm with crystal violet and measuring its optical density (absorbance) give the impact of nanomaterials on the biofilm under consideration [83-b]. The alternative method for the study of the biomass of biofilm is fluorescent labeling and using confocal microscopy [74].

Metal, Metal Oxide Nanoparticles, and Biofilms

Biomineralization is a ubiquitous process among microbes, unicellular protists, fungi, plants, and animals. Minerals associated with biological environ exhibit uncommon strength, degree hydration. These biominerals are the active participants in biomineralization and biogeochemical cycles [84]. The natural aquatic biofilms like Lake Biofilms, exhibit phase of adsorption, and have minimum masking of adsorption. The adsorption involves the suspended materials present in the aquatic environment containing sufficient oxygen for the biotic components (oxic environment). Metals like lead, iron, and manganese oxyhydroxides get readily adsorbed on natural biofilm and represent biogeochemical phases. There is a possibility that the insoluble minerals present in clay may contribute to the binding of metals, like lead and other transitional metals like cadmium or copper but iron and manganese oxyhydroxides play a regulatory role in the process of adsorption. The degree of adsorption depends on the surface characteristics of iron and manganese coating and subsequent binding of the trace metals. The biological materials involving deposits of iron oxyhydroxides can also act as a source of lead metals for the biofilms. The adsorption of trace metals can occur due to the adsorptive scavenging process and the composition of the aqueous phase and the reactive inorganic and organic components present at inter reactive surfaces [85]. In natural and industrial setups, most of the microbial biofilms exist in

coating forms on the surface of metal-oxides. These slimes affect the transport and fate of metals and metal oxides because of their partitioning behavior and speciation.

Silver nanoparticles inhibit the microbial synthesis of extracellular polysaccharide substances present in biofilm and these nanoparticles are an antibiofilm agent [86]. Microbes develop drug-resistance because of antibiotic drug associates with a specific chemical target. This tendency narrows down among the species of microbes. As a result, there is an increase in the number of infections. In such cases, silver nanoparticles act as a suitable option to fight infection due to a wide range of Gram-positive and Gram-negative bacteria and vancomycin-resistant strains. It is of common observation that microbes do not or may not develop resistance to silver nanoparticles, at least among hospital flora. Generally, such bacteria do not get a chance to undergo three discrete mutations in three microbial systems [87,88]. Silver nanoparticles exhibit increased active surface area due to the surface to mass ratio; the silver nanoparticles release more of oxidative Ag^+ and partially oxidized silver nanoparticles, i.e., the partially oxidized silver nanoparticles undergo chemosorbtion, Ag (I), and these bind to the surface. These oxidative and partially oxidized silver nanoparticles exhibit greater antibacterial activity in comparison to the silver ions [89]. The silver nanoparticles attach to the bacterial cell wall, penetrate, and elevate the permeability of the microbial cell. Finally, the microbial cell dies [90]. There are possibilities that positive Ag ions present on the silver nanoparticles form reactive free radicals that damage the microbial membrane and possibly cause apoptosis in bacterial cells [91]. A multilayered core or shell formulations of gold and silver metal nanoparticles exhibit synergistic antibacterial impacts depending on the thickness of the shell or core. The carbon nanostructures conjugated with metals like Pb, Co, and Ni, either inhibit or exterminate the biofilms [92,93].

The sizes of the silver nanoparticles having 1to 100 nm contain 20 to 15000 atoms of silver [94]. Smaller sizes of silver nanoparticles with 10 nm and 20 nm show a higher degree of reduction in the biomass and biofilm cell viability in comparison to the larger sizes of silver nanoparticles (100 nm). These smaller silver nanoparticles did not change the morphology of the biofilm matrix and of the cells present [95]. The inhibitory concentration of the smaller silver nanoparticles (8.3 nm sizes) does not influence the biofilm of the *E. coli* and the bacteria present, but in higher concentrations (100-150 g/ml) of same sizes silver nanoparticles, most of the inhibiting bacterial cells perish [96]. The varying sizes of chitosan-coated silver nanoparticles between 55 nm to 275 nm exhibit species-dependent impact; no disruption in 24 hours old biofilm of *P. aeruginosa* and *Staphylococcus aureus* but 65% reduction in the formation of biofilm of *P. aeruginosa* [97]. Chemically synthesized silver nanoparticles with 20nm-30nm also show inhibitory impacts on the *P. aeruginosa* biofilm [98].

There have been attempts to develop strategies in which conjugated form of antibiotics and silver nanoparticles to deal with biofilm-related complications. The aztreonam, a common antibiotic could not prevent or eradicate biofilms but induced post-treatment growth [95]. The formation of biofilm in the lungs and the airways is one of the serious problems in the medical field. If the amalgamated form of antibiotics, like cationic antimicrobial peptides and silver nanoparticles, probably acts as a good option to treat such ailments. An amalgamation of nanoparticles of poly (Lactide-co-glycolide) (PLGA) having model cationic antimicrobial peptides and colistin can be prepared using emulsion/solvent diffusion technique The engineered nanoparticles functionalized with chitosan and poly (vinyl alcohol) changes the surface properties, show better penetrating ability, which in turn helps its transport through the biofilm or mucus. These nanomaterials when sprayed on carriers like lactose and mannitol can regulate properties like bulk and flow properties, and degree of aerosolization. This complete product acts as a performance inducer and as a breath-actuated inhaler in dry powder form. This conjugated engineered product destroys the biofilm of *P. aeruginosa* [99]. It shows relatively extended efficacy in eradicating biofilm in comparison to colistin alone. The conjugated forms of silver nanoparticles having 10 nm size and aztreonam cause damage of the cell wall of the microbes and reduce the biomass, the thickness of biofilms up to 98%, and 50% respectively [95]. An amalgamation of silver nanoparticles and antibiotics forms more reactive oxygen species rather individually on administration in a biosystem [74,100]. These observations indicate the useful and remedial applications of a combination of silver nanoparticles and antibiotics towards handling the problems relating to biofilms.

Gold particles do not exhibit innate antimicrobial feature but gold nanoparticles are readily functionalized. This concept forms the bases of gold nanoparticles as remedial agents to treat biofilm infection [101,102]. Gold-silver nanoparticles (~93 nm) show more disruption and inhibitory activities towards the biofilm formation of *S. aureus, Acinetobacter baumannii,* in comparison to the gold and silver nanoparticles individually [101]. Bacteriogenically synthesized gold-silver nanoparticles exhibit antibacterial and antibiofilm properties against Gram-positive bacteria, like *Enterococcus faecalis* and *Staphylococcus aureus,* Gram-negative bacteria, like *Escherichia coli* and *Pseudomonas aeruginosa* and their biofilm phase. These bimetallic nanoparticles (209 nm) are more biocompatible and have better bactericidal activities than individual counterparts. These formulations at 10μM concentration show complete inhibition of biofilm formation. The smaller sized bimetallic

nanoparticles and having the layering of bio-organic matter show a higher degree of biocompatibility and internalization [102].

The light activates photosensitizer complexes like methylene blue conjugated with gold nanoparticles (~26 post conjugation size) and release ROS which damages microbes. The electrostatic interaction readily results in the conjugation of negatively charges gold nanoparticles and positively charged methylene blue (1:1 by volume). This conjugated form of gold nanoparticles causes 82.2% inhibition in *Candia albicans* biofilm under laser exposure at 38.2J/cm^2 for 40 seconds every 6 hours for 24 hours treatment. The 2, 3-Bis (2-methoxy-4-nitro-5-sulfophenyl)-5-(phenyl aminocarbonyl)-2H-tetrazolium hydroxide (XTT) biofilm reduction assay and scanning electron microscopy are effective techniques to evaluate the damage of the biofilm [74]. The gold synthesized from plant sources exhibit better antibiofilm performance of the methylene blue- a photosensitizer; there is a possibility of the similar impact of such gold nanoparticles with other photosensitizers like Rose Bengal and erythrosine. The gold nanoparticles that are chemically synthesized did not elicit similar interactions in the case biofilms of *A. baumannii* and *E. coli*. There is neither inhibition nor disruption in these biofilms [101]. There is a possibility of the effect of the synthesis of antimicrobial plant extract and this needs further investigation. Although, the bimetallic gold/silver nanoparticles show better performance in either inhibiting or disrupting biofilms in comparison to the gold and silver nanoparticles individually. The bimetallic gold and silver nanoparticles involving antimicrobial plant extract synthesis technique (may be regarded as a combinational concept) offer improved antibiofilm impact than chemically synthesized bimetallic gold/silver nanoparticles [74,101]. Magnetic iron nanoparticles are fairly biocompatible and cost-effective. These nanoparticles are very useful tools in the medical field as magnetic resonance imaging and targeted drug delivery agents, and treatment based on hyperthermia. The magnetic iron nanoparticles can be readily synthesized using the coprecipitation and thermal decomposition techniques, although, synthesizing magnetic iron nanoparticles is tedious because of the interference of parameters like pH, salts involved, concentration and the temperature [74,103]. Specifically, the functionalized superparamagnetic iron oxide nanoparticles (SPIONs) are effective against the biofilms of *S. aureus*, this microbe, and the biofilm is resistant to methicillin. The SPIONs coated with dimercaptosuccinic acid (this elevates the degree of solubility and metallic ion conjugation) followed by chelation with either Fe^{3+} form $FeCl_3$ or Zn^{2+} from $ZnCl_2$ acts as a good antimicrobial and antibiofilm agent. During this process, three forms of functionalized SPIONs namely dimercaptosuccinic acid-coated SPIONs, iron-coated SPIONs, and zinc-coated SPIONs are formed. On incubating these three functionalized SPIONs impact the growth of twenty-four hours old *S. aureus* biofilm; SPIONs (1mg/ml) show maximum inhibition; zinc/iron SPIONs (0.01 mg/ml) show a lower degree of inhibition (~20%) while plain $ZnCl_2$ stimulate the growth of the said biofilm. These observations reflect the possibilities that SPIONs help to increase the concentrations of the Fe^{3+} and Zn^{2+} in biofilms and these ions cause the death of the microbes present in biofilm [74,104,105]. The presence of fructose affects the efficacy of conjugated SPIONs in destroying the biofilm of *S. aureus.* Possibly the increase in the antibiofilm activity of fructose treated SPIONs depends on the amount of SPIONs deposition in biofilm. The antibiofilm activity increases in accordance with the amount of SPIONs deposited in biofilm as this condition leads to the elevated rate of ROS generation. The 1m M of fructose results in 99.9999% killing in comparison to the antibiofilm activity of vancomycin. The fluorescence microscopy imaging of dead and living microbes technique is very suitable for such studies. The fructose treated SPIONs and vancomycin also cause a 50% reduction in the biomass of the biofilm under study. Fructose also lowers the degree of toxicity of SPIONs. Similar observations are seen in the case of nanoparticles synthesized using plant extract [74,101, 105].

SPIONs under the influence of the external magnetic field penetrate the biofilm and accumulate at its bottom terminating all the intra-biofilm microbes. The functionalized SPIONs with carboxyethylsilanetriol (~13 nm and with zeta potential ranging from +43m V to −15 m v) terminate the microbes (gentamicin-resistant *Staphylococcus epidermidis* present in its biofilm. The functionalized SPIONs with carboxyethylsilanetriol result in 83% mortality of microbes under the influence of the magnetic field while the mortality is around 9% in its absence. The gentamicin did not influence this behavior of functionalized SPIONs with carboxyethylsilanetriol. The higher rate of microbial mortality is probably due to the production of ROS due to very small nanoparticles and not because of electrostatic interaction among SPIONs and cell wall of biofilm microbes [74,106]. Kim, et al. suggest highly effective and a safe novel antimicrobial thermo therapeutic technique; this technique involves excited antibody-targeted magnetic nanoparticles with high amplitude and high frequency. This technique rapidly clears 99.9% of the pathogen and the biofilm at a lower temperature (43ºC) which otherwise requires high temperature *in vivo* and *in vitro* in mice [107]. The size and chemical composition of magnetic nanoparticles and their formulation impact the antibiofilm activity. The iron oxide (Fe_2O_3) nanoparticles (2 nm) induce the formation of biofilm but Fe_3O_4 inhibits this activity. Even though Fe_2O_3 nanoparticles obstruct antimicrobial peptide function and reduce the activity of ciprofloxacin, but there is a possibility that the very small nanoparticles of Fe_2O_3 (2 to 540 nm (2 ±

1, 43 ± 6, 85 ± 25 and 540 ± 90 nm) may behave like growth inducers for the biofilms [108,109]. There is a need to delve more into the mechanism while studying their antibiofilm effect.

The response of biofilm to temperature is another interesting aspect that requires attention because of cold storing, refrigeration, or environmental conditions. The temperature can affect the process of biofilm formation, growth, and dispersion. The *Staphylococcus aureus* is relatively more thermotolerant than *Listeria sp.,* this calls for redesigning the thermal treatment and pasteurization in food technology [110]. The process of dispersal of biofilm is an essential phase and exhibits recalcitrance and infection due to antimicrobial tolerant biofilms. The temperature parameter influences the dispersion of biofilm. A rise of 5ºC in temperature helps the detachment in the case of the biofilm formed by b*acteria, Pseudomonas aeruginosa. The thermal up-shift declines the biomass of biofilm and a rise in the number of viable suspected cells. The secondary messenger cyclic di-GMP plays a prominent role during the change from sessile microbial form to motile form and involves a genetic network. The* Poly (oligo (ethylene glycol) methyl ether acrylate)-*block*-poly (monoacryloxy ethyl phosphate)-stabilized iron oxide nanoparticles (POEGA-*b*-PMAEP@IONPs) cause local hyperthermia in the established biofilms under the magnetic field. These [(POEGA-*b*-PMAEP@IONPs)] functionalized nanoparticles are not toxic to the microbes present in biofilm. This increase in heat (hyperthermia) results in the detachment of biofilm cells and the antibiotic gentamicin also reduced the microbial colonies [111]. The iron oxide nanoparticles, with restrictions, have a wide range of applications in clinical and industrial setups [111,112].

Copper nanoparticles are another suitable option as an antifungal and antimicrobial agent and are applicable in industries as a fungicide, for purifying water, and against fouling. The copper nanoparticles are cost-effective. Copper nanoparticle coating on biomedical devices is in use to prevent the growth of pathogens and to prevent their spreading. The copper nanoparticles are effective inhibitors of the formation of biofilms and the pathogens [74,113,114]. The copper metal and its nanoparticles form a surface oxide layer during their synthesis and under normal conditions [114]. Cupric oxide nanoparticle (40 nm) and 50μg/ml) reduced the EPS biomass of oral (dental) biofilm to around 61% [74,115]. The copper nanoparticles (100 ng ml) cause 94% decline in the biomass, 89% reduction in hydrophobicity of cell surface, and 92% decrease in exopolysaccharides in the biofilms of *Pseudomonas aeruginosa* [116].

The biogenic TiO_2 nanoparticles (10-30 nm) are better photocatalyst and form H_2O_2 and ROS at the interface between TiO_2 and the aquatic biofilm; these nanoparticles suppress the growth of the biofilm [117]. The dissolved ZnO nanoparticles (50 mg/L) interrupt the activity of biofilms; these nanoparticles adsorb on the surface of the biofilm and generated zinc ions. These zinc ions bring inhibition of the aerobic respiration and cause no damage to the microbial cell membrane [118]. The zinc oxide nanoparticles (35 nm; 53 μg m^{-1} and 76 μg m^{-1}) show antibacterial and antibiofilm activity and prevent the growth of oral biofilms of *Rothia dentocariosa* and *Rothia mucilaginosa* [119]. Selenium nanoparticles synthesized using the laser ablation technique adheres to the surface of biofilm of *Candida albicans* because of the electrostatic forces between negatively charged selenium nanoparticles and a positive charge on the biofilm. The selenium nanoparticles substitute sulfur groups of amino acids in the cell wall of *Candida albicans*. This substitution results in the cytological and morphological changes in this fungus. Due to the disoriented protein structure and the disintegration, the biofilm disorients and loses normal structural and functional integrity [120].

Silica Nanomaterials and Biofilms

In habitats rich in silica hot biofilms, the bacteria act as the spots for silica nucleation in the case of cyanobacterial biofilms. The extracellular proteins and carbohydrates of biofilms help the silicification of cells and results in the formation of an amorphous layer of silica. This amorphous layer facilitates the microbial survival at 3 mm depth. The extracellular polymeric matrix acts as a molecular site for the nucleation for silica mostly in a silica-rich substrate [121]. Biomineralization among biotic components of the environment is of common occurrence; it may involve specific cell, tissue, organs, and at a specific scale and this phenomenon is associated with an organic matrix with a self-directed deposition of crystals of the minerals [122].

Normally biodegradable materials are in use to transfer drugs in biosystems. The release of drugs may or may not be regulated due to the weaker physicochemical properties of the carrier involved. To avoid such malfunctioning, there is a need for robust and biocompatible but not biodegradable readily carrier. Silica nanoparticles meet such requirements. In normal practice, nitric oxide associated silica nanoparticles are suitable that carry NO within the biofilm and release it as endogenous free radical in the matrix. Nitric oxide and or its byproducts are the probable agents to inhibit the growth of microbial biofilms and their antibacterial drug resistance and cause less degree of toxicity at experimental concentrations [74]. Inorganic-organically functionalized silica nanoparticles are synthesizes using tetramethoxysilane, tetraethoxysilane, and different aminoalkoxysilanes (N-(6-aminohexyl) aminopropyltrimethoxysilane, N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, and (aminoethylamino-ethyl) phenethyltrimethoxysilane; these formulations of silica

effectively release nitric oxide within biofilms. The size of these nanoformulations is in relation to the concentration of N-(2-aminoethyl)-3-aminopropyltrimethoxysilane and can be regulated. The rate of release of nitric oxide is higher while using smaller functionalized silica nanoparticles as the distance declines or diffusion in order to donate nitric oxide, and their half-life is around 12 h. The rate of release of nitric oxide depends on the method of synthesis of hybrid silica nanoparticles [74,123].

The N-(6-aminohexyl) aminopropyltrimethoxysilane silica nanoparticles provide more nitric oxide radicals and cause 99% of the destruction of *P. aeruginosa* biofilm, and the microbes inhabiting. This destructive impact is concentration-dependent, and the rate of diffusion of nitric oxide. The functionalized silica nanoparticles are also effective 99.99% on the biofilms of E. *coli*, *S. aureus*, *S. epidermidis*, and *C. albicans,* Gram-negative microbes, 99.9% in case of the myco-biofilms, and 99.0% in the cases of biofilms of Gram-positive microbes. The primary cause of the destruction of biofilm is the electrostatic interaction among nitric oxide radicals and biofilms [124]. The shape of these nanomaterials also influences the degree dispersion of biofilms. The rod-like nanoparticles disperse the major portion of the biofilm and kill the microbes within 15 minutes while spherical nanoparticles remain restricted to a limited region of biofilm and did not kill any bacteria even after 60 minutes of treatment [123]. Silica nanoparticles functionalized with chlorohexidine show antibiofilm activity against mono-species biofilms such as *S. mutans*, *Streptococcus sobrinus*, *Fusobacterium nucleatum*, *A. actinomycetemcomitans*, and *E. faecalis* [125].

Liposomes and Biofilms

Liposomes are uni and multilamellar, spherical vesicles, and biocompatible nano entities. The lamellae are amphiphilic phospholipids in nature. The liposomes can encapsulate hydrophobic and hydrophilic chemicals. The synthesis methods like detergent depletion, ether/ethanol injection reverse-phase evaporation, emulsion, and the Bengham techniques help to regulate the degree of encapsulation of the liposomes [74, 126]. Liposomes exhibit a higher degree of permeability, loading, and cellular uptake because of their bilipid membrane. This membrane also facilitates the safety of hydrophilic antimicrobial agents, avoidance of the deactivation of their loads *in vivo* conditions present in the experimental model [74]. These liposomal nanoentities are one of the most suitable means against resistant biofilm in the medical field and food technology. There is a decline in the clinical minimal inhibitory concentration (MIC) and the minimal biofilm inhibitory concentration (MBIC) when an antibiotic drug embodies in liposome in comparison to the free form of antibiotic [127]. The composition of liposome and the features of the outer microbial membrane-like proteins, lipopolysaccharides, hydrophobicity, and membrane electrostatic potential, influence the degree of fusogenicity between the two. The positive and negative surface charges on the liposomal formulation function as antibiofilm and antibacterial features. The positively charged liposomal formulations destroy the biofilm at relatively low concentrations in comparison to the liposomal formulation having a negative surface charge. There is a possibility of establishing a stronger electrostatic attraction between the liposomal membrane and the negative charge on the outer microbial membrane [127].

Although liposomes appear good options to treat the biofilm-related infection because of their weak physicochemical features weaken their efficacy in clinical applications. The leakage, probable declined release of antibiotics, and unfavorable harsh conditions of biofilms may pose hindrances during their use in treating infection due to biofilms [74]. The size and class of liposomes influence the degree of penetration in biofilms; cationic, unilamellar small-sized liposomes (128 ±2.3 nm – 141 nm) infiltrate deeper in the matrix of biofilms of both *P. aeruginosa* and *S. aureus* in comparison to the multilamellar liposomes (having larger size 141 ±1.3 nm). Anionic unilamellar and multilamellar liposomes exhibit a very low degree of penetration. The blank cationic liposomes nano entities disrupt the electrostatic equilibrium of the biofilms of the two microbes and result in antibiofilm impact [128]. The neutral and anionic liposomes functionalized using 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol liposomes (426.3 ±26.4) a mixture of 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1, 2-dipalmitoyl-sn-glycero-3-phosphoglycerol sodium salt (DPPG) (228.5 ±34.9) to carry tobramycin; these configurations did not show superior impact than the impact of free tobramycin [129]. There appears to be the need for more conformational experimentation for considering liposomes to be suitable as drug carriers to treat the infection due to microbial biofilms.

The interaction between liposome and the surface polymer of the bacterial glycocalyx requires lipids that have polyhydroxy head groups like phosphatidylinositol. The lattice model concept is the functional basis of this interaction. It also involves the potential energy of the interaction at the interface between the bacterial surface and the surface of the liposome. The energy of interaction which is less than that of a single hydrogen bond can bring about an interaction between polyhydroxy head groups of liposome and polymer residue present on the surface of bacteria; this energy potentiates the interactions between repulsive and attractive dispersion forces at the interface. [The energy of a hydrogen bond depends on the nature of the donor and receptors atoms that participate in hydrogen bond

formation; this energy varies within 1 to 40 kcal/mol range]. This concept predicts the optimal liposomal composition needed for the adsorption of liposomes at the surface of the bacterium. This theory regulates the process of adsorption of polyhydroxy lipid liposomes on the surface of varied microbial oral and dermal biofilms. The lattice model concept explains the very fine changes in the liposomal configuration and the microbial surface; this process incorporates vary less energy of the interactions and impacts the interaction in the process [130]. The membrane of liposomes has an ability to merge with phospholipid membrane, thus these deliver their cargo directly to the targeted cell or microbe. The membrane of fusogenic liposomes is lipid in nature and this feature renders the bilipid layer more fluidic in texture and this state is likely to destabilize the biological membrane. Thus, the overall impact of this process favors the delivery of cargo or drugs comfortably at the desired site [127]. The microbes of a biofilm have differentiated phenotypes and form matrix consisting of extracellular polymeric substances rich in polysaccharides. This form of framework acts as a strong defense mode and impedes the process of penetration of antibiotics and decline the microbial susceptibility against exogenous compounds [131].

Because of the microbial resistance towards vancomycin, its efficacy as an agent to treat infection due to methicillin-resistant nature of *Staphylococcus aureus* is very low. When vancomycin is loaded in the liposome its efficiency enhances. This formulation exhibits sustained release, increases more intense interaction with the molecules of vancomycin with the bacterial cells, ameliorate pharmacokinetics, declines toxicity, widens the antimicrobial activity against Gram-negative microbes, and elevates the concentration of the drug by enhancing the degree of penetration in the matrix of a biofilm. There is a need to affirm the inhibitory impacts of such liposomal formulations [131-133]. The liposomal peptide (Lys-Val-Asp-His-Phe-Pro-Leu; origin: rice bran protein) is a spherical nanoentity (average diameter >200 nm) shows better antibiofilm activity. Its thermodynamic studies indicate an efficient, instantaneous, smooth, and exothermic delivery of liposomal peptide to the biofilms of *Listeria monocytogenes* [134].

Liposomes do not exhibit a minimum inhibitory antimicrobial biofilm effect. Liposomes with cationic surface charge inflict greater antimicrobial biofilm impact and raised retention in a biofilm of *Pseudomonas aeruginosa* but liposomes with anionic surface charge exhibit elevated permeability. Liposomes functionalized with polymethyl glycol (PEG) cause greater antimicrobial biofilm effect but the extent of retention declines. Thus, appropriate manipulation of surface charge and modification of polymethyl glycol help to regulate antimicrobial biofilm impact and the permeability to meet the required target [135].

Polymeric Nanomaterials and Biofilms

Polymeric nanomaterials or polymeric nanocomposites are made of either polymeric or copolymeric matrix in which nanoparticles or nanofillers like carbon nanotubes, graphene, etc., are inter spread. The functionality of such polymers depends on the concentration of the nanofillers. Parameters like reinforcement of nanostructures, alignment, type of dispersion, type of interfacial bonds between nanofillers and the polymers, affect the final product. These polymeric nanomaterials have one dimensional (yarn), two dimensional (sheet), and three dimensional (foam). These materials exhibit elevated functions that can be regulated [136]. Polymeric materials show high adaptability, specificity, and are cost-effective. These materials are suitably used in different applications as structural materials, coatings, health care, packaging, communication, energy, transportation, agro-food industries. The polymeric materials are lightweight, hard, strong, flexible, and have very extraordinary thermal, electrical, and optical features; their molecular structure and composition are easily formulated. All these properties make them suitable for innovative materials with targeted physicochemical and biological features. As a result, these polymeric materials have changed the scenario of materials science [137-141]. The fabricated polymeric nanoparticles are convenient agents to regulate the drug delivery time and its rate. This feature is helpful in establishing an elevated degree of antibiotic activity and its impact on biofilms. The contact and the aggregation (to increase the concentration) of the antibiofilm formulation can be controlled if one can suitably functionalize nanomaterials that are acting as a carrier involving specific ligand targeting.

The surface-immobilized quaternary ammonium containing resin acid-derived compounds and polycationic formulation act as antimicrobial and antibiofilm agents. Polymeric formulations like copper-catalyzed azide-alkyne 1, 3-dipolar cycloaddition, and surface-initiated atom transfer radical polymerized derivatives show strong antibacterial and anti-biofilm activities against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* [142]. It is quite convenient to structure polymeric chains, hydrogels to use as superhydrophobic surfaces with increased anti-adhesive ability. Binding antimicrobial peptides, antibiotics, chitosan, and enzymes with spacer molecules, biodegradable matrices, nanomaterials, and quaternary ammonium formulations and these formulations act as suitable antimicrobial and anti-biofilm agents [143]. The efficacy of the formulation of the polymeric surface coating increases when an end-to-end chain reaction helps the release of antimicrobial agents and this release gets activated in the presence of

bacteria and bacterial biofilm. This process depends on the physicochemical features of the surface of the material to be used like composition, charge, topography, porosity, and hydrophobicity. These features and other aspects that influence the functionality include mode of application on the surface, quantity to be mixed/used, nature of the polymer antiadhesive material. The product should suitably reduce the force of adhesion between the bacteria and the substrate, and decrease the surface for the attachment of microbes to the specific surface. These steps result in the preservation of the resistance between the material used and the bacterial colony. These steps are not very convenient because they only modifying surface chemistry or surface structure. Factors like fast degradation, desorption, non-biocompatibility interfere with the formation and functionality of such products [141].

There have been efforts to improvise polymeric formulations that are effective against biofilm formation and safe for human health and medical devices. The modulated polymeric compounds function in a specific mode like the interfaces have a sensing ability to retain the planktonic microbes and be able to isolate them prior to the formation of a biofilm. The modulated compounds should have the ability to disrupt the cell to cell communication and the physical structural aspects of biofilm. These actions disrupt the matrix or the planktonic state of biofilm to ensure the dispersal. Further, these impacts decline the degree of virulence of a biofilm, selection pressure, restrict their ability of drug-resistance, and reinstate the effectiveness of the antimicrobial and antibiofilm agents [142, 144-146].

Poly lactic-co-glycolic acid (PLGA) is one of the biodegradable and biocompatible polymeric materials. In an aqueous medium, the ester linkage present in PLGA hydrolyzes forming lactic acid and glycolic acid; both of these are natural bio-byproducts of biointeractions [147]. Polylactic-co-glycolic acid nanomaterials are known to release therapeutic agents in a sustained mode. The release of cargo relates to the rate of its degradation and the diffusion of the cargo molecules. If one changes the ratio of polylactic acid and polyglycolic acid, its rate of degradation gets controlled along with its hydrophobicity and crystallinity. If polyglycolic acid is more in the product, it shows a higher level of crystallinity and more amount of polylactic acid results in elevated the hydrophobicity of the formulated product [148]. The virgin COOH capped poly-co-glycolic acid exhibit negative zeta potential, because, the carboxyl group (COOH-) dissociates and releases hydrogen in an aqueous medium. The poly-co-glycolic acid capped with OH, this OH group undergoes deprotonation in basic conditions; thus, mostly, this formation is not preferred while considering its biological uses. The extended duration of sonication of fabricated poly-co-glycolic acid nanoparticles regulates their usable size as per the application [149].

The extracellular DNA is a structural and functional component of biofilm. Its degradation is an essential step during the earlier phase of biofilm formation. The deoxyribonuclease 1 (DNase1) functionalized poly-co-glycolic acid (PGLA) deteriorates this constituent of a biofilm, and active DNase1 placed on the surface of PGLA nanomaterials enhances the particles mobility and their rate of diffusion towards extracellular polymer [150]. The three nano-formulations of PGLA namely, PGLA-PL-CPX-DNase-1, PGLA-CPX, PGLA-PL-CPX and free CPX (Ciprofloxacin) show antimicrobial and antibiofilm activities; out of these four functionalized molecules, only PGLA-PL-CPX-DNase-1nanomaterials (as surface coatings) exhibit maximum efficacy in increasing the nanoparticle mobility and their diffusion into the extracellular polymeric substance of the biofilm [150]. Other polymeric nanomaterial lipid nanomaterials polymer containing pectin sulfate, is an antibiofilm agent that does not allow the adhesion of the biofilm of *Helicobacter pylori* [151].

The *Pseudomonas aeruginosa* produces rhamnolipid (RHL) - bacterial surfactant; it disintegrates extracellular polymeric substance of the biofilm and also intermingles with it [74]. Phosphatidylcholine (PC) lipid polymer functionalized with rhamnolipid (size 181 nm and negative zeta potential) is a suitable carrier for Amoxicillin (AMX) for treating the *Helicobacter pylori* biofilms [152]. The resin composite of quaternary ammonium polyethyleneimine nanoparticles interacts with the cell wall of biofilm bacteria and causes electrostatic disturbance on the cell wall of the bacteria. This leads to an apoptosis-like process in the affected cell [153].

Chitosan and Biofilms

Chitosan is among the most common, (next to cellulose) nontoxic biomaterials. It exhibits a higher degree of biocompatibility, bioactivity, and convenient physical moldability. Chitosan is derived for chitin- a structural component of the exoskeleton of most of the arthropods and fungal cell walls. This polysaccharide has an amino group that undergoes protonation. This feature ensures its solubility in dilute acidic solutions and plays a significant role during its biomedical applications [154]. The chemical structure of chitosan consists of several hundred to more than a thousand β-(1-4) linked d-glucose units and this configuration is similar to the structure of cellulose. In chitosan structure, an acetamide group replaces the hydroxyl group at position C-2 of cellulose. Chitosan, [β-(1-4) linked 2-amino-2-deoxy-β-D-glucopyranose] is an N-deacetylated derivative of chitin, in which the acetamide groups is transformed into

a primary amino group [155]. The process of deacetylation remains incomplete (mostly) and the resultant product still possesses some of the acetamide groups (5-8% nitrogen as primary aliphatic amine group) in its structure and helps in the typical reactions of amines. Chitosan shows higher activity in comparison to chitin because of the presence of primary and secondary hydroxyl groups in every repeat unit and each deacetylated unit with an amine group. These chemical groups undergo chemical alterations readily, and as a result, cause changes in the physical and chemical properties of chitosan [155]. Commonly, chitin undergoes deacetylation and changes into chitosan using an excess of sodium hydroxide in an aqueous medium.

The backbone of chitosan is positively charged and very reactive with the cell membrane; it reorganizes the proteins of intercellular junctions and opens them. Such interactions elevate the permeability of chitosan (polysaccharide). The chitosan molecule is polycationic in nature and it executes analgesic impact also. The molecule of chitosan is polymer having amino groups and is a polysaccharide having glycosidic bonds; this biochemical structural configuration facilitates its enzymatic biodegradation involving proteases and lysozymes, within a biosystem [154]. Chitosan also exhibits an antitumor, hemostatic, hypocholesterolemic, antimicrobial, and antioxidant nature [155].

The carboxymethyl chitosan inhibits biofilm formation in the case of Gram-positive and Gram-negative microbes about 74.6% and 81.6% respectively. This formulation prevents bacterial adhesion more than 90% and the dynamic status of the biofilm [156]. Chitosan dispersion (size $d_{50;}$ 5.61 ± 0.57 μm) and chitosan microparticles (size d_{50} 4.15 ± 0.20 μm) having narrow size distribution, polydispersity (2.22 ± 0.19) and exhibit high positive zeta potential (+58.7 ± 3.7). Chitosan microparticles (1%) increased the microbial viability and the degree of acidogenecity in comparison to the chitosan dispersion in the case of matured biofilms of *Streptococcus mutans* [157]. Microbial cell wall and the surface of extracellular polymeric substance have a negative charge while chitosan nanoparticles have positive surface charge; this condition is very conducive for interaction between these two entities, either as drug carrier or to disrupt the matrix of biofilm. The interactions between chitosan solution, chitosan semi-microparticles, and six different strains of *Pseudomonas aeruginosa* from clinical biofilms, depend on the strain susceptibility difference in phenotypes, and their natural antioxidant efficacies; of the two forms of chitosan particles, chitosan solution shows more antimicrobial effect than the semi-microparticles of chitosan against *Pseudomonas aeruginosa* [158]. Chitosan can be easily modulated; the amine group of chitosan in acetic acid solution interacts with sodium tripolyphosphate-a polyanion, and this step results in the formation of chitosan nanoparticles involving cross-linking process. Chelated chitosan nanoparticles exhibit higher antibiofilm activity and inhibit microbial recolonization on dentin [159]. Chavez de Pa,z et al. prepared chitosan nanoparticles using neutral pH, and chitosan with different molecular weights. The nanocomplexes derived for higher molecular weight exhibit lower degree of antimicrobial action (20-25%) but those nanocomplexes formulated form chitosan having lower molecular weight induce more than 95% antimicrobial effect [160]. Chitosan associated with Rose Bengal (a photosensitizer) show a significant degree of antimicrobial impact but it is very slightly toxic to fibroblast. This formulated chitosan complex adheres to the surface of bacteria and increases cellular permeability resulting in the loss of bacterial cells due to photodynamics. Photoactivated chitosan Rose Bengal complex disintegrates matrix and reduces the viability of the biofilms of *Enterococcus faecalis.* This interaction involves photo-cross-linking in the chitosan Rose Bengal complex as a result it increases the mechanical strength of dentin-collagen tissue and reduces degradation [161] Chitosan associated with linoleic acid immobilizes enzymes, like β-N-acetylglucosaminidase (DspB); this enzyme degrades a major polysaccharide called poly-β (1, 6)-N-acetyl-glucosamine (PNAG), present in the biofilms matrix (extracellular polymeric substance) of *S. epidermidis*, *S. aureus*, and *Actinobacillus actinomycetemcomitans.* The immobilized **β**-N-acetylglucosaminidase enzyme inhibits and disintegrates the biofilms *S. epidermidis*, *S. aureus*, and *Actinobacillus actinomycetemcomitans,* and remains functional for longer duration and a wider range of temperature [162].

Quantum Dots and Biofilms

The quantum dots are colloidal semiconductor nanomaterials. These consist of the core and corona layer. Their core is made of either one or more heavy metals like cadmium, tellurium, or zinc. The quantum dots exhibit fluorescent properties; these absorb photons at a specific lower wavelength and emit a relatively higher wavelength. Their photoluminescence emission is directly related to their size. The specific features of quantum dots like the smaller size, resistance to metabolic degradation in biosystems, photostability, fluorescent nature, and their faculty to conjugate with ligands or biomolecules, etc., ensure them to be a preferred option as fluorophore in the field of biological sciences [163]. Graphene quantum dots are cytobiocompatible, produce lactate dehydrogenase enzymes, and reactive oxygen species. The modified quantum dots (100 mM) also exhibit antimicrobial impact and antibiofilm activity against biofilms of S*. aureus.* This nano quantum dots readily disintegrate well-formed biofilm and prevent the

formation of new biofilm [164]. PEGylated silver graphene quantum dot nanocomposites exhibit synergistic impact and do not enhance the growth of *S. aureus* and *P. aeruginosa* [165].

The fluorescent faculty of quantum dots makes them suitable visualizing agents and this technique effective, least toxic, and cost-effective as compared to SEM, AFM, MRI, and Raman spectroscopic techniques. The synthetic complexes may be toxic and interfere with the analysis [163]. The physical scaffolding of microbial biofilm is extracellular polymeric substance and it plays an important role during its development and virulent nature. The synthesized amphiphilic carbon dots bind with this extracellular polymeric substance in the case of *Pseudomonas aeruginosa*, this feature facilitates the microscopic visualization of the related structural aspects, growth kinetics, biofilm disruption, and impacts of external parameters like temperature [166].

Dendrimers and Biofilms

Generally, dendrimers are biocompatible and show fairly good biodispersibility. Hence, these multi-branched nanomaterials are the good options as drug carriers within the biosystem, even to treat infections due to biofilms. The degree of penetration and accumulation of dendrimers depends on the electrostatic double-layer interactions between the peripheral compositions of the dendrimer and surface of the biofilm [167]. Dendrimers having peripheral pH-responsive NH_3^+ (Ph 7.0) marked with Red fluorescent dye; exhibit a higher rate of penetration in the biofilm of *P. aeruginosa (ambient acidic medium) in comparison to the dendrimer that has peripheral* OH^- *or* COO^-*. Regarding their distribution, dendrimers with peripheral* NH_3^+ groups exhibit electrostatic double-layer attraction (surface of biofilm shows negative charge) and these accumulate at the top zone of the biofilm while dendrons with peripheral OH^- *or* COO^- groups get uniformly dispersed within the matrix of the biofilm. This duration of exposure influences such distributions [167]. Glycopeptide dendrimers show affinity to lectins and bind with them, the lectins like LecA and LecB play significant roles during the development of antibiotic resistance towards the matrix of biofilm of *P. aeruginosa*. Specifically modulated glycopeptide dendrimers react with LecB like FD2 and D-FD2 and LecA like GalAG2 and GalBG2 and result in preventing the formation of biofilm of *P. aeruginosa* and increase its destructive dispersal [168]. Carbosilane metallodendrimers containing copper (II), ruthenium (II), ligands (chloride and nitrate), and generations (generations 0, 1, and 2) show biocidal impact towards Gram-positive, *Staphylococcus aureus*, and Gram-negative, *Escherichia coli*). The first-generation dendrimers Cu (II) metallodendrimer with nitrate) exhibit maximum biocidal and antibiofilm activity. This interaction is concentration-dependent [169]. Single-chain polymeric nanoparticles (1.4 μM) exhibit strong antibacterial (>99.99%) towards Gram-positive *Pseudomonas aeruginosa,* and its biofilm [112]. Peptide dendrimers severely inactivate the biofilm of *E. coli* –RP437, on polystyrene plates. The rate and extent of inactivation depend on the optimum concentration and the dose; maximum inhibition (93.5%) is at 40 μM concentration [170,171].

Carbon Nanomaterials and Biofilms

Carbon nanomaterials are very small in size, show increased surface area, photostability, fairly good biocompatibility, and can be easily modified for the set targets. These wonder nanoparticles exhibit specialized physicochemical abilities like mechanical, electrical tensility, the conductance of electrons, and heat. The carbon-based nanomaterials demonstrate broad-range one-photon property, biodistribution, and least toxic nature, these features attribute to their manifold diagnostic applications. The carbon nanoparticles and their modified forms are potential carriers for inter and intracellular drugs, diagnostic, therapeutic, bioimaging tools [172-174], (Figures 3 & 4).

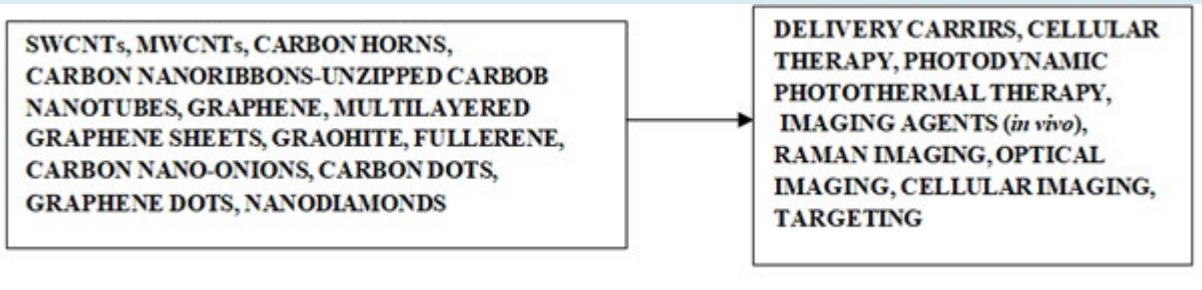

Figure 3: Forms of Carbon Nanomaterials and their Applications.

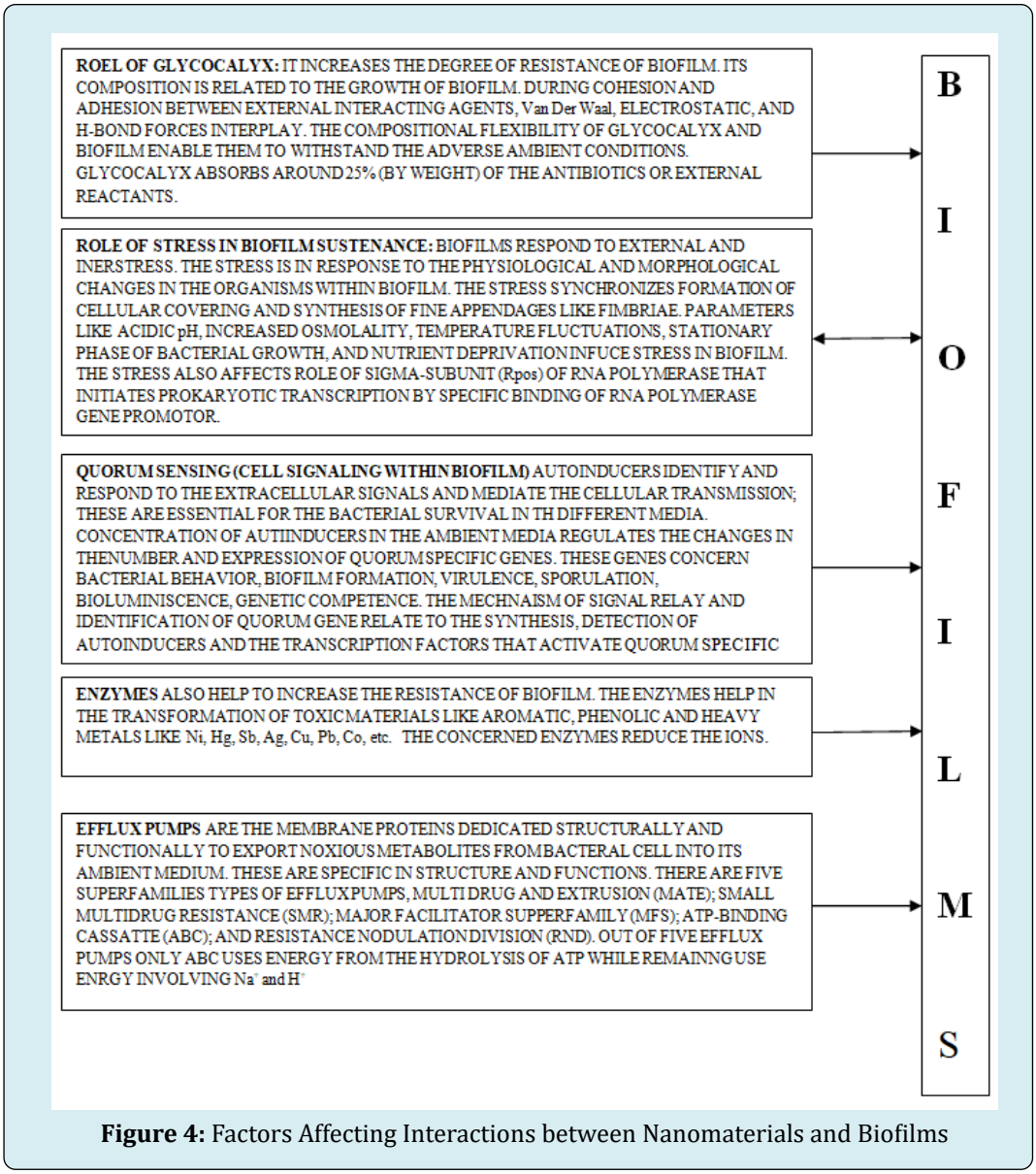

Figure 4: Factors Affecting Interactions between Nanomaterials and Biofilms

The single-walled carbon nanotubes disrupt and inhibit the biofilm formation in the case of *E. coli*. In the matured biofilms the inhabiting microbes are not affected during the presence of single-walled carbon nanotubes. The extracellular polymeric substance of biofilm mitigates the toxic impact of single-walled carbon nanotubes but in the absence of extracellular polymeric substance, these nanomaterials result in the detachment of microbes in the biofilms. At the very high concentration (around 10 times) of single-walled carbon nanotubes, there is inhibition and disruption of the biofilm of *E. coli*. The E. coli microbe does not colonize and produce biomass on the surfaces coated with single-walled carbon nanotubes [175].

The TiO_2/multi-walled carbon nanotubes and *Bacillus subtilis* have a negative charge on their surfaces; hence, there is an anti-adhesion impact because of the electrostatic repulsion between the two interfaces and diminished strength of the contact. There exists another correlation between the surface of negatively charged microbes and the nanosized surface; because of this parameter, the bacteria tend to settle within the narrow spaces between nanoparticles [176]. The carbon nanomaterials, like fullerene (C_{60}), absorb organic contaminants, this feature may help to restore or restrict the degree of toxicity to biofilms in the aquatic environment. Pristine fullerene is nontoxic to biofilms but when conjugated with triclosan, diuron, or venlafaxine, organic micro-contaminants inflict harmful impacts and these depend on their concentrations on the heterotrophic and phototrophic components present

in the biofilms. There is competition among C_{60} and organic micro-contaminants to hinder the receptors present on the microbial or other biological membranes [177]. The structural aspects of graphene indicate its ability of chemical transformation; these transformed forms are effective during loading and transport of drugs [178]. Graphene oxide is an efficient antibiofilm during the early stages of formation and also on matured biofilms of *Staphylococcus aureus* PECHA 10, *Pseudomonas aeruginosa* PECHA 4, and *Candida albicans* X3. These organisms are some of the most common wound pathogens. Graphene oxide retards bacterial growth, significantly reduces and inhibits biomass, and exhibits bacteriostatic effect in these organisms. This carbon nanomaterial also declines the degree of microbial adhesion and biomass. These interactions involve strong covalent bonding between graphene oxide and microbes in addition to electrostatic binding [179]. The graphene oxide nanoparticles (85µg/mL and 8.5µg/mL) cause a decline in viability in the case of 48-h biofilm and the detachment but not well pronounced in 24-h and 72-h biofilm of *Pseudomonas putida.* These impacts are age-based because of the physiological changes occurring in the microbe and the concerned biofilm [180].

The single-walled carbon nanotubes having OH^-, -COOH group, pristine form, multi-walled carbon nanotubes, and fullerene show different degrees of impacts on biofilms. Of these carbon nanomaterials, fullerene exhibits the least antibiofilm effects. These nanoparticles integrate within developing microbial riverine communities in the patchy pattern as indicated during scanning transmission X-ray microscopic studies. The extracellular polymeric substances of these biofilms act as the sink for the interacting carbon nanoparticles studied and protect or interfere with the said interaction. The carbon nanotubes exposure did not change parameters like biomass with reference to its thickness, chlorophyll-a, bacteria of the biofilms, and the mass of cyanobacteria in the biofilms under investigation.

Conclusion

Engineered and natural nanoparticles are the potential participants in multifaceted aspects of industries, medical and biological fields as analytic tools, carriers for drugs, imaging agents, technological agents, etc. These wonderful and multiutility materials reach the aerial, aquatic, and land components of the environment during their synthesis, functionalization, applications, storage, transportation, and distribution either unintentionally or intentionally. These become the potential threat to the varied components of the environment. Biofilms are the significant ecological alcoves that sustain micro flora and fauna. The research reported on biofilms confirms the role of biofilms as major sinks for these nanomaterials. The biofilms are not only in the environment but also in the biosystems, biomedical analytical tools, life-supporting implants, water and wastewater treatment, and tools used in day to day life. Such biofilms have their own uses and abuses in their ambient environment. Thus, it is imperative to device some novel strategies to control this component of the environment to make the best use in interdisciplinary applications.

Declaration

Author declares that there is no conflict of interest.

Contribution

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References

1. Hall-Stoodley L, Costerton JW, Paul-Stoodley (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews Microbiology 2: 95-108.

2. Baigorri R, Marta F, Gustova GG, Jose M, Garcia M, et al. (2009) Complementary multi-analytical approach to study the distinctive structural features of the a main humic fraction in solution: grey humic acid, brown humic acid, and fulvic acid. Journal of Agricultural and Food Chemistry 57(8): 3266-3272.

3. Hayes MHB, Mylotte R, Swift RS (2017) Humin: its composition and importance in soil organic Matter. Advances in agronomy 143: 47-138.

4. Flemming HC, Wingender J (2010) The biofilm matrix, Nature Review: Microbiology 8: 623-633.

5. Sigolene L, Stocker R, Rusconi R (2015) Focus on the physics of Biofilms. New Journal of Physics.

6. Pepper IL, Gerba CB, Gentry TJ (2015) Environmental Microbiology, Academic Press, Elsevier.

7. Ramirez-Mora T, Retana-Lobo C, Valle-Bourrouet G (2018) Biochemical characterization of extracellular polymeric substances from endodontic biofilms. PLoS One 13(11).

8. Stolze JF (2003) Structure of biofilms, in Fossil and recent biofilms (Eds.), Krumbein WE, et al. Springer Dordrecht, pp: 65-76.

9. Serra DO, Hengge R (2019) Cellulose in Bacterial Biofilms. In: Cohen E, et al. (Eds.), extracellular sugar-based biopolymers matrices, biologically-inspired systems, Vol 12, Springer, Cham.

10. Kaoru I, Decho AW, Lau BLT (2015) When nanoparticles meet biofilms: interactions guiding the environmental fate and accumulation of nanoparticles. Front Microbiol 6: 591.

11. Saleh NB, Chambers B, Aich B, Jaime PT, Phung-Ngoc HN, et al. (2015) Mechanistic lesson learned from studies of planktonic bacteria with metallic nanomaterials: Implications of interactions between nanomaterials and biofilm bacteria. Front Microbiol 6: 677.

12. Evans E, Brown MR, Gilbert P (1994) Iron chelator, exopolysaccharide, and protease production in *Staphylococcus epidermidis*: a comparative study of the effects of specific growth rate in biofilm and planktonic culture. Microbiology 140(1): 153-157.

13. Tang Y, Zhao H, Marcus AK, Krajmalnik-Brown R, Rittmann BE (2011) A steady-state biofilm model for simultaneous reduction of nitrate and perchlorate, Part 1: model development and the numerical solution. Environmental Science Technology 46(3): 1598-1607.

14. Stewart PS, (2002) Mechanisms of antibiotic resistance in bacterial biofilms. International journal of Medical Microbiology 292(2): 107-113.

15. Dade WB, Davis JD, Nichols PD, Nowell ARM, Thistle D, et al. (1990) Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrobiology J 8(1): 1-16.

16. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick L, et al. (2001) Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. Journal of Bacteriol 183(18): 5385-5394.

17. de Brouwer, JFC, Wolf stein K, Ruddy GK, Jones TER, Stal LJ, (2005) Biogenic stabilization of intertidal sediments: the importance of extracellular polymeric substances produced by benthic diatoms. Microbiol Ecol 49: 501-512.

18. Baillie GS, Douglas LJ (2000) Matrix polymers of *Candida* biofilms and their possible role in biofilm resistance to antifungal agents. J of Antimicrob Chemotherapy 46(3): 397-403.

19. Domozych DS, Kort S, Benton S, Yu T (2005) The extracellular polymeric substance of the green alga *Penium margaritaceum* and its role in biofilm formation. Biofilms 2(2): 129-144.

20. Aggarwal S, Steward PS, Hozalski RM (2016) Biofilm cohesive strength as bases of biofilm Recalcitrant: are biofilms overdesigned? SAGE Journal Microbiology Insights 8(Suppl 2): 29-32.

21. Padmavathi AR, Bakkiyaraj D, Pandian SK (2017) Biochemical and molecular mechanisms in biofilms formation of plant-associated bacteria, in Biofilm on Plants and Soil Health, (Eds.), Ahmed I, et al. John Wiley and Sons Ltd.

22. Tseng BS, Majerczyk CD, Passos da Silva D, Chandler JR, Greenberg EP, et al. (2016) Quorum sensing influences *Burkholderia thailandensis* biofilm development and matrix production. J Bacteriol 198(19): 2643-50.

23. Jain A, Enrico M, Bhonsle NP (2011) The biofilm returns: Microbial life at the interface. In the book Microbes and Microbial technology, Ahmad F, et al. (Eds.), Springer, New York, pp: 59-85.

24. Marban DL, Brdtker G, Kundan K, Pop IS, Radu FA (2020) Unscale model for permeable biofilm in a thin channel and tube, Transport in porous media 132: 83-110.

25. Decho AW (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28: 73-153.

26. Decho AW (2000) Microbial biofilms in intertidal systems: an overview. Continental Shelf Research 20(10-11): 1257-1273.

27. Flemming HC, Wingender J (2002) in Encyclopedia of Environmental Microbiology, pp: 1223-1231.

28. Ortega-Morales BO, Chan-Bacab MJ, Moppert X, Miranda-Tello E, Fardeau ML, et al. (2006) Characterization of extracellular polymers synthesized by tropical intertidal biofilm bacteria. J Appl Microbiol 102(1): 254-264.

29. Sutherland IW (2001) The biofilm matrix-an immobilized but dynamic microbial environment. Trends Microbiol 9(5): 222-227.

30. Cobine PA, Cruz LF, Navarrete F, Duncan D, Tygart M, et al. (2013) *Xylella fastidiosa* differentially accumulates mineral elements in biofilm and planktonic cells. PLoS One 8(1).

31. Navarrete F, De La Fuente L (2014) Response of *Xylella fastidiosa* to zinc: decreased culturability, increased

exopolysaccharides production, and formation of resilient biofilms under flow conditions. Applied Environmental Microbiology 80: 1097-1107.

32. Cruz LF, Cobine PA, De La Fuente L (2011) Calcium increases *Xylella fastidiosa* surface attachment, biofilm formation, and twitching motility. Applied Environmental Microbiology 78: 1321-1331.

33. Battin TJ, von Der Kammer F, Weilhartner A, Ottofuelling S, Hofmann T (2009) Nanostructured TiO_2: transport behavior and effects on aquatic microbial communities under environmental conditions. Environmental Science Technology 43(21): 8098-8104.

34. Ferry JL, Craig P, Hexel C, Sisco P, Frey R, et al. (2009) Transfer of gold nanoparticles from the water column to the estuarine food web. Nature Nanotechnology 4: 441-444.

35. Hawari AH, Mulligan CN (2006) Biosorption of lead (II), cadmium (II), copper (II) and nickel (II) by anaerobic granular biomass. Bioresource Technology 97(4): 692-700.

36. Biarnsholt T, Alhede M, Alhede M, Eickhardt-Soenen R, Moser C, et al. (2013) The in vivo biofilm. Trends in Microbiology 21(9): 466-474.

37. Maria K, Maria H, Hultgren SJ (2013) Bacterial biofilms: development, dispersal and therapeutic strategies in the dawn of the post-antibiotic era. Cold Spring Harbor Perspective in Medicine 3(4).

38. Paulson LK, Ballard G, Stahl DA (1993) Use of r-RNA fluorescence in situ hybridization for measuring the activity of a single cell in young and established biofilms. Applied and Environmental Microbiology 59(5): 1354-1360.

39. Dortch Q, Roberts TL, Clayton JR, Ahmed I (1983) RNA/DNA ratio and DNA concentration as indicators of growth rate and biomass in planktonic organisms. Marine Ecology Progress Series 13: 61-71.

40. Wo Y, Otten FW (2009) IscR controls iron-dependent biofilm formation. Journal of Bacteriology 191(4): 1248-1257.

41. Newman JR, Rodriguez C, and Lewis RJ (2013) Molecular basis of the activity of SinR protein, the master regulator of biofilm formation in *Bacillus subtilis*. Journal of Biological Chemistry 288(15): 10766-10778.

42. Drescher K, Dunkel J, Luis HC, Ganguly S, Goldstein RE (2011) Fluid dynamics and noise in bacterial cell-cell and cell-surface scattering. Proc Natl Acad Sci USA 108(27): 10940-10945.

43. Wensink HH, Dunkel J, Sebastian H, Drescher K, Goldstein RE, et al. (2012) Mesoscale turbulence in living fluids. Proc Natl Acad Sci USA 109(36): 14308-14313.

44. Dunkel J, Sebastian H, Drescher K, Wensink HH, Markus B, et al. (2013) Fluid Dynamics of Bacterial Turbulence. Physical Review Letters.

45. Pattem J, Davrandi M, Aguayo S, Allan E, Spratt D, et al. (2018) A multi-scale a biophysical approach to develop the structure-property relationship in oral biofilms. Scientific Reports 8(5691).

46. Ghoul M, Mitri S (2016) The ecology and evolution of microbial competition. Trends in Microbiology 24(10): 833-845.

47. Nadell CD, Drecher K, Forter KR (2016) Spatial structure, cooperation and completion in biofilms. Nature Reviews Microbiology 14: 589-600.

48. Scheidweiler D, Peter H, Paraskevi P, Pietro d-A, Battin TE (2019) Unraveling the biophysical under pinning to the success of multispecies biofilms in porous environment. ISME J 13(7): 1700-1710.

49. Lopez D, Vlamakis H, Koltor R (2010) Biofilms. Cold Spring Harb Perspective in Biology 12(7): 1-11.

50. Sheppard D, Howell PL (2016) Biofilm exopolysaccharides of pathogenic fungi: lesson form bacteria. J Biol Chem 291(24): 12529-12533.

51. Prindle A, Liu J, Asally M, Ly S, Garcia-Ojalvo J, et al. (2015) Ion-channels enable electrical communication in bacterial community. Nature 527(7576): 59-63.

52. Liu J, Prindle A, Humphries J, Gabalda-Sagarra M, Asally M, et al. (2015) Metabolic co-dependence gives rise to collective oscillation within biofilm. Nature 523(7562): 550-554.

53. Humphries J, Xiong L, Liu J, Prindle A, Yuan F, et al. (2017) Species independent attraction to biofilm through electrical signaling. Cell 168(1-2): 200-209.

54. Marx P, San Y, Qin H, Wang Q, Guo R, et al. (2020) Environmental stress perception activates structural remodeling extent *Streptococcus mutans* biofilm. npj Biofilm microbiomes 6: 77.

55. Kelley WL (2006) Lex marks the spot: the virulent side of SOS and closer look at the LexA Regulon. Molecular Microbiology 62(5): 1228-1238.

56. Schlacher F, Goodman MF (2007) Lessons from 50 years of SOS DNA damage-induced mutagenesis. Nature Reviewers Molecular Cell Biology 8: 587-594.

57. Butala M, Zgur-Bertok D, Busby SJ (2009) The bacterial LexA transcriptional repressor. Cellular Molecular Life Science 66(1): 82-93.

58. Patrick SC, Boni L, Fuller GG, Inglis RF, Fischer P (2013) In-situ quantification of the interfacial rheological response of bacterial biofilms to environmental stimuli. PLoS One.

59. Chew SC, Kundukad B, Sevlour T, van der Marrel JRC, Yang I, et al. (2014) Dynamic modeling of microbial biofilm by functionally distinct exopolysaccharides. mBio.

60. Tierra G, Pavissich JP, Nenenberg R, Xu Z, Alber MS (2015) Multi-component model of deformation and detachment of a biofilm under fluid flow. J R Society Interface 12(106): 1-13.

61. Ghanbari A, Dehghany J, Schwebs T, Mathias M, Sussane H, et al. (2016) Interlocular density and nutrition level determine the formation of mushroom-shaped structure in *Pseudomonas aeruginosa* biofilms. Science Reports.

62. Rodesney CA, Brian R, Dhamani N, Benjamin JC, Parag K, et al. (2017) Mechanosensing of sheer by *Pseudomonas aeruginosa* leads to an increased level of cyclic di-ATP signal initiating biofilms development. Proc Natl Acad Sci USA 114(23): 5906-5911.

63. Boudarel H, Jean-Denis M, Blaysat B, Grediac M (2018) Towards standardized mechanical characterization of microbial biofilms, analysis, and critical review. npj Biofilm Microbiomes.

64. Rodriguez DF, Elimelech M (2010) Toxic effects of single-walled carbon nanotubes in the development of biofilm of E. coli. Environmental Science and Technology 44(12): 4583-4589.

65. Kauro I, Madden AS, Decho AW, Lau BLT (2014) Deposition of nanoparticles onto polysaccharide-coated surfaces: implications for nanoparticle-biofilm interactions. Environ Sci Nano 1(2): 117-122.

66. Kauro I, Shi Z, Walker AV, Lau BL (2016) Effects of protein species and surface physicochemical features on the deposition of nanoparticles on to protein coated planer surfaces. RSC Advances 6(79): 75491-75498.

67. Reith F, Fairbrother L, Nolze G, Wilhelmi O, Clode PL, et al. (2010) Nanoparticles factories: Biofilms hold the key gold dispersion and nugget formation. Geology 38(9): 843-846.

68. Labrenz M, Benfield JF (2004) Sulfate-reducing bacteria dominated biofilms that precipitate ZnS in a subsurface circum-neutral pH mine draining system. Microbiology Ecology 47: 205-217.

69. Kalathil S, Lee J, Cho MH (2011) Electro chemically active biofilms mediated synthesis of silver nanoparticles in water. Green Chemistry 13: 1482-1485.

70. Schmidt J, Musken M, Becker T, Magnowska Z, Bertinetti D, et al. (2011) The Pseudomonas aeruginosa chemotaxis methyl transferase CheR1 impacts on bacterial surface sampling. PLoS One.

71. Pratt LA, Kolter R (1998) Genetic analysis of Escherichia coli, biofilm formation: role of flagella, motility, chemotaxis and type 1pili. Molecular Microbiology 30(2): 285-193.

72. Hadjifrangiskou M, Gu AP, Pinker JS, Kostakioti M, Zhang EW, et al. (2012) Transposon mutagenesis identifies uropathic Escherichia coli biofilm factors. Journal of Bacteriology 194(22): 6195-6205.

73. Stephanie F, Vitale S, Quinn L, Casey E (2019) Nanoparticles-Biofilms interactions: the role of EPS matrix. Trends in Microbiology 27(11): P915-922.

74. Han C, Romero N, Fischer S, Dookran J, Berger A, et al. (2017) Recent developments in the use of nanoparticles for the treatment of biofilms. Nanotechnology Review 6(5): 383-404.

75. Welczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA (2010) What the cell sees in nanobioscience. Journal of American Chemical Society 132(16): 5761-5768.

76. Monopoli MP, Aberg C, Salvati A, Dawson KA (2012) Biomolecule corona provide biological identity of nanosized matters. Nature Nanotechnology 7: 779-786.

77. Morrow JB, Arango PC, Holbrook RD (2010) Association of quantum dot nanoparticles with Pseudomonas aeruginosa biofilm. J Environ Qual 39: 1934-1941.

78. Peulen TO, Wilkinson KJ (2011) Diffusion of nanoparticles in a biofilm. Environ Sci Technol 45(8): 3367-3373.

79. Guiot E, Georges P, Brun A, Fontaine-Aupart MP, Bellon-Fontaine MN, et al. (2002) Heterogeneity of diffusion inside microbial biofilms determined by fluorescence correlation spectroscopy under two-photon excitation. Photochemistry Photobiology 75(6): 570-578.

80. Tripathi S, Champagne D, Tufenkji N (2012) Transport behavior of selected nanoparticles with different surface coatings in granular porous media coated with

Pseudomonas aeruginosa biofilm. Environ Sci Technol 46(13): 6942-6949.

81. Joo SH, Agarwal S (2018) Factors impacting the interactions of engineered nanoparticles with bacterial cells and biofilms: mechanistic insight and state of knowledge. Journal of Environmental Management 225(1): 62-74.

82. Berney M, Hammes F, Bosshard F, Weilenmann HU, Egli T (2007) Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry. Applied Environmental Microbiology 73(10): 3283-3290.

83. Welch K, Cai Y, Strømme M (2012) A method for the quantitative determination of biofilm Viability. Journal of Functional Biomaterial 3(2): 418-431.

b) Peeters E, Nelis HJ, Coenye T, (2008) Comparison of multiple methods for quantification of microbial biofilms grown in microtitre plates 72(2): 157-165.

84. Perry CC (2009) An overview of silica in biology: its chemistry, and recent technological advances In Muller WEG, et al. (Eds.), Biosilica in Evolution, Morphogenesis, and Nanobiotechnology, pp 295-313.

85. Nelson YM, Lion LW, Shuler ML, Ghirose WC (1999) Lead binding to metal oxide and organic phase of natural aquatic biofilms. Limnology and Oceanography 44(7): 1715-1729.

86. Kalishwaralal KS, Barath Mani Kanth SRK, Pandian VD, Gurunathan S (2010) Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis,* Colloids and Surfaces B: Biointerfaces 79(2): 340-344.

87. van de Belt H, Neut D, Schenk W, van Horn JR, van der Mei HC, et al. (2001) Infection of orthopedic implants and the use of antibiotic-loaded bone cements. Acta Orthopaedica Scandinavica 72(6): 557-571.

88. Lok CN, Ho CM, Chen R, He QY, Yu WY, et al. (2007) Silver nanoparticles: partial oxidation and antibacterial activities. Journal of Biological Inorganic Chemistry 12(4): 527-534.

89. Sotiriou GA, Pratsinis SE (2010) Antibacterial activity of nano-silver ions and particles. Environmental Science and Technology 44(14): 5649-5654.

90. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, et al. (2007) Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology. Biology and Medicine 3(1): 95-101.

91. Lee W, Kim KJ, Lee DG (2014) A novel mechanism for the antibacterial effect of silver nanoparticles on Escherichia coli. Biometals 27(6): 1191-1201.

92. Yah CS, Simate GS (2015) Nanoparticles as potential new generation broad-spectrum antimicrobial agents. Daru.

93. Yang LW, Wang YH, Zhuang H, and Zhang J, (2017) Shell thickness-dependent antibacterial activity and biocompatibility of gold@silver core-shell nanoparticles. Royal Society of Chemistry Advances 7: 11355-11361.

94. Williams DF (2008) On the mechanisms of biocompatibility. Biomaterials 29(20): 2941-2953.

95. Habash MB, Park AJ, Vis EC, Harris RJ, Khursigara CM (2014) Synergy of silver nanoparticles and aztreonam against *Pseudomonas aeruginosa* PAO1 biofilms. Antimicrobial Agents and Chemotherapy 58(10): 5818-5830.

96. Radzig MA, Nadtochenko VA, Koksharova OA, Kiwi J, Lipasova VA, et al. (2013) Antibacterial effects of silver nanoparticles on gram-negative bacteria: influence on the growth and biofilms formation, mechanisms of action. Colloids Surf B Biointerfaces 102(1): 300-306.

97. Jena P, Mohanty S, Mallick R, Jacob B, Sonawane A (2012) Toxicity and antibacterial assessment of chitosan-coated silver nanoparticles on human pathogens and macrophage cells. International Journal of Nanomedicine 7: 1805-1818.

98. Palaniswamy NK, Ferina N, Amirulhusni AN, Mohd-Zain Z, Hussaini J, et al. (2014) Antibiofilm properties of chemically synthesized silver nanoparticles found against *Pseudomonas aeruginosa.* Journal of Nanobiotechnology.

99. d'Angelo I, Casciaro B, Miro A, Quaglia F, Mangoni ML, et al. (2015) Overcoming barriers in Pseudomonas aeruginosa lung infections: engineered nanoparticles for local delivery of a cationic antimicrobial peptide. Colloids Surf B Biointerfaces 135(1): 717-725.

100. Gurunathan S, Han JW, Kwon DN, Kim JH (2014) Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale Res Lett 9(1): 373.

101. Salunke GR, Ghosh S, Santosh Kumar RJ, Khade S, et al. (2014) Rapid efficient synthesis and characterization of silver, gold, and bimetallic nanoparticles from the medicinal plant *Plumbago zeylanica* and their application in biofilm control. Int J Nanomedicine 9: 2635-2653.

102. Ramasamy M, Lee JH, Lee J (2016) Potent antimicrobial and antibiofilm activities of

bacteriogenically synthesized gold-silver nanoparticles against pathogenic bacteria and their physiochemical characterizations. Journal of Biomater Applications 31(3): 366-378.

103. Lu AH, Salabas EL, Schuth F (2007) Magnetic nanoparticles: synthesis, protection, functionalization, and application. Angewandte Chemie International Edition 46(8): 1222-1244.

104. Taylor EN, Kummer KM, Durmus NG, Leuba K, Tarquinio KM, et al. (2012) Superparamagnetic iron oxide nanoparticles (SPION) for the treatment of antibiotic-resistant biofilms. Small 8(19): 3016-3027.

105. Durmus NG, Taylor EN, Kummer KM, Webster TJ (2013) Enhanced efficacy of superparamagnetic iron oxide nanoparticles against antibiotic-resistant biofilms in the presence of metabolites. Adv Mater 25(40): 5706-5713.

106. Subbiahdoss G, Sharifi S, Grijpma DW, Laurent S, van der Mei HC, et al. (2012) Magnetic targeting of surface-modified superparamagnetic iron oxide nanoparticles Yields antibacterial efficacy against biofilms of gentamicin-resistant staphylococci. Acta Biomater 8(6): 2047-2055.

107. Kim MH, Yamayoshi I, Mathew S, Lin H, Nayfach J, et al. (2013) Magnetic nanoparticle targeted hyperthermia of cutaneous Staphylococcus aureus infection. Annals of Biomedical Engineering 41(3): 598-609.

108. Borcherding J, Baltrusaitis J, Chen H, Stebounova L, Wu CM, et al. (2014) Iron oxide nanoparticles induce *Pseudomonas aeruginosa* growths induce biofilm formation and inhibit antimicrobial peptide function. Environmental Science Nano 1(2): 123-132.

109. Masadeh MM, Karasneh GA, Al-Akhras MA, Albiss BA, Aljarah KM, et al. (2014) Cerium oxide and iron oxide nanoparticles abolish the antibacterial activity of ciprofloxacin against gram-positive and gram-negative biofilm bacteria. Cytotechnology 67(3): 427-435.

110. Kennedy J, Blair IS, McDowell DA, Bolton DJ (2005) An investigation of the thermal inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as a result of chilled storage. Journal of Applied Microbiology 99(5): 1229-1235.

111. Nguyen TK, Duong HTT, Selvanayagam R, Boyer C, Barraud N (2015) Iron oxide nanoparticle-mediated hyperthermia stimulates dispersal in bacterial biofilms and enhances antibiotic efficacy. Scientific Reports.

112. Nguyen TK, Lam SJ, Ho KKK, Naresh K, Oiao GG, et al. (2017) Rational design of single-chain polymer nanoparticles that kills planktonic and biofilm bacteria. ACS, Infection Diseases 3(3): 237-248.

113. Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman AA (2012) Synthesis and antimicrobial activity of copper nanoparticles. Material Letters 71: 114-116.

114. Kruk T, Szczepanowicz K, Stefanska J, Socha RP, Warszyński P (2015) Synthesis and Antimicrobial activity of monodisperse copper nanoparticles. Colloids Surf B Biointerfaces 128: 17-22.

115. Tabrez S, Khan, Ahamed M, Al-Khedhairy A, Musarrat J (2013) Biocidal effect of copper and zinc oxide nanoparticles on human oral microbiome and biofilm formation. Material Letters 97: 67-70.

116. Lewis Oscar F, Mubarak Ali D, Nithya C, Priyanka R, Gopinath V, et al. (2015) One-pot synthesis and anti-biofilm potential of copper nanoparticles (Cu NPs) against clinical strains of *Pseudomonas aeruginosa.* Biofouling 31(4): 379-391.

117. Dhandapani P, Maruthamuthu S, Rajagopal G (2012) Bio-mediated synthesis of TiO_2 nanoparticles and its photocatalytic effect on aquatic biofilm. Journal of Photochemistry Photobiology-B Biology 110: 43-49.

118. Hou J, Miao L, Wang C, Wang P, Ao Y, Qian J, et al. (2014) Inhibitory effects of ZnO nanoparticles on aerobic wastewater biofilms from oxygen concentration profiles determined by microelectrodes. Journal of Hazardous Materials 276: 164-170.

119. Khan ST, Ahamed M, Musarrat J, Al-Khedhairy AA (2014) Anti-biofilm and antibacterial activities of zinc oxide nanoparticles against the oral opportunistic pathogens *Rothia dentocariosa* and *Rothia mucilaginosa.* European Journal of Oral Science 122(6): 397-403.

120. Guisbiers G, Lara HH, Mendoza-Cruz R, Naranjo G, Vincent BA, et al. (2017) Inhibition of Candida albicans biofilm by pure selenium nanoparticles synthesized by pulse laser ablation in liquids. Nanomedicine 13(3): 1095-1103.

121. Jaroch DM, Zhang W, Shi J, Garland J, Banks MK, et al. (2013) Silica entrapment of biofilms in membrane bioreactors for water regeneration. Technical Reports.

122. Livingston B, Killian C, Wilt F, Cameron A, Landrum M, et al. (2006) A genome-wide analysis of biomineralization-related proteins in the sea urchin

Strongylocentrotus purpuratus. Developmental Biology 300(1): 335-348.

123. Slomberg DL, Lu Y, Broadnax AD, Hunter RA, Carpenter AW, et al. (2013) Role of size and shape on biofilm eradication for nitric oxide-releasing silica nanoparticles. ACS Applied Mater Interfaces 5(19): 9322-9329.

124. Hetrick EM, Shin JH, Paul HS, Schoenfisch MH (2009) Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. Biomaterials 30(14): 2782-2789.

125. Seneviratne CJ, Leung KC, Wong CH, Lee SF, Li X, et al. (2014) Nanoparticle-encapsulated chlorohexidine against oral bacterial biofilms. PLoS One.

126. Chang HI, Yeh MK (2012) Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. International Journal of Nanomedicine 7: 49-60.

127. Forier K, Raemdonck K, De Smedt SC, Demeester J, Coenye T, et al. (2014) Lipid and polymer nanoparticles for drug delivery to bacterial biofilms. Journal of Controlled Release 190: 607-623.

128. Dong D, Thomas N, Thierry B, Vreugde S, Prestidge CA, et al. (2015) Distribution and Inhibition of liposomes on *Staphylococcus aureus* and *Pseudomonas aeruginosa* Biofilm. PLoS One 10(6).

129. Messiaen AS, Forier K, Nelis H, Braeckmans K, Coenye T (2013) Transport of nanoparticles and tobramycin-loaded liposomes in Burkholderia cepacia complex biofilms. PLoS One 8(11).

130. Jones MN, Kaszuba M (1994) Polyhydroxy mediated interactions between liposomes and bacterial biofilms. Biochimica et Biophysica Acta (BBA)-Biomembrane 1193(1): 48-54.

131. Moghadas-Sharif N, Fazly Bazzaz BS, Khameneh B, Malaekeh-Nikouei B (2015) The effect of nanoliposomal formulations on Staphylococcus epidermidis biofilm. Drug Dev Ind Pharm 41(3): 445-450.

132. Lankalapalli S, Tenneti VK, Nimmali SK (2015) Design and development of vancomycin liposomes. Indian Journal of Pharm Education Research 49: 208-215.

133. Scriboni AB, Couto VM, Riberio LNd-M, Freires IA, Groppo FC, et al. (2019) Fusogenic liposomes increase the antimicrobial activity of vancomycin against *Staphylococcus aureus* biofilm. Frontiers in Pharmacology 10: 1401.

134. Pu C, Tang W (2017) The antibacterial and antibiofilm efficiencies of liposomal peptide originating from rice bran protein against Listeria monocytogenes. Food and Function 8(11): 4159-4169.

135. Ibaraki H, Kanazawa T, Chien WY, Nakaminami H, Aoki M, et al. (2020) The effects of surface properties of liposomes on their activity against Pseudomonas aeruginosa PAO-1 biofilm. Journal of Drug Delivery Science and Technology 57: 101754.

136. Harito C, Bavykin DV, Yullarto B, Dipojono HK, Walsh FC (2019) Polymer composites having high filler contents: synthesis, structures, properties, and applications. Nanoscale 11(11): 4653-4682.

137. Teo AJT, Mishra A, Park I, Kim YJ, Park WT, et al. (2016) Polymeric biomaterials for medical implants and devices. ACS Biomaterial Science and Engineering 2(4): 454-472.

138. Haque SKM, Ardila-Rey JA, Umar Y, Rahman H, Mas'ud AA, et al. (2018) Polymeric materials for conversion of electromagnetic waves from the sun to electric power. Polymers 10(3): 307.

139. Prabhu TN, Prashantha KA (2018) Review on the present status and future challenges of starch-based polymer films and their composites in food packaging applications. Polymer Composites 39(7): 2499-2522.

140. Agrillo B, Balestrieri M, Gogliettino M, Palmieri G, Moretta R, et al. (2019) Functionalized polymeric materials with bio-derived antimicrobial peptides for "active" packaging. International Journal of Molecular Science 20(3): 601.

141. Catto C, Cappitelli F (2019) Testing antibiofilm polymeric surfaces; where to start? International Journal of Molecular Science 20(15): 3794.

142. Genewatta MS, Miller KP, Singleton SP, Pegah MB, Chen PY, et al. (2015) Antibacterial and antibiofilm-disrupting coatings from resin acid-derived materials. Biomolecules (ACS publication) 16(10): 3336-3344.

143. Swartjes JJTM, Sharma PK, van Korten TG, vander Meitie, Muhamudi M, et al. (2015) Current developments in antimicrobial surface coatings for biomedical applications. Current Medical Chemistry 22(8): 2116-2129.

144. Villa F, Cappitelli F (2013) Plant-derived bioactive compounds at sub-lethal concentrations: towards smart biocide-free antibiofilm strategies. Photochemistry Reviews 12: 245-254.

145. Kostakioti M, Hadjifrangiskou M, Hultgren SJ (2013) Bacterial biofilms: Development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harbor Perspectives Med 3(4).

146. Remy B, Mion S, Plener L, Elias M, Chabrière E et al. (2018) Interference in Bacterial Quorum Sensing: A Biopharmaceutical Perspective. Frontier of Pharmacology 9: 203.

147. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM (2008) Nano/microtechnologies for delivering macromolecular therapeutics using poly(d,l-lactide-co-glycolide) and its derivatives. Journal of Controlled Release 125(3): 193-209.

148. Jain RA (2000) The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide) (PLGA) devices. Biomaterials 21(23): 2475-2490.

149. Dinarvand R, Sepehri N, Manoochehri S, Rouhani H, Atyabi F (2011) Polylactide-co-glycolide nanoparticles for controlled delivery of anticancer agents. Int J Nanomedicine 6: 877-895.

150. Baelo A, Levato R, Julian E, Crespo A, Astola J, et al. (2015) Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections. J Controlled Release 209: 150-158.

151. Cai J, Huang H, Song W, Hu H, Chen J, et al. (2015) Preparation and evaluation of lipid polymer nanoparticles for eradicating H. pylori biofilm and impairing antibacterial resistance *in vitro*. International Journal of Pharmacology 495(2): 728-737.

152. Cheow WS, Handinoto K (2012) Lipid polymer hybrid nanoparticles with rhamnolipid triggered the release capabilities of as antibiofilm by drug delivery vehicles. Particuology Science and Technology of Particles 10(3): 327-333.

153. Beyth N, Yudovin-Farber I, Perez-Davidi M, Domb AJ, Weiss EI (2010) Polyethyleneimine nanoparticles incorporated into resin composite cause cell death and trigger biofilm stress *in vivo.* Proc Natl Acad Sci USA 107(51): 22038-22043.

154. Ibrahim HM, El-Zairy EMR (2015) Chitosan as a Biomaterial-Structure, Properties, and Electrospun Nanofibers, Concepts, Compounds and the Alternatives of Antibacterials, Varaprasad Bobbarala. Intech Open.

155. Islam S, Bhuiyan MAR, Islam MN (2017) Chitin and Chitosan: Structure, Properties and Applications in Biomedical Engineering. Journal of Polymer and Environment 25: 854-866.

156. Tan Y, Han F, Ma S, Yu W (2011) Carboxymethyl chitosan prevents the formation of a broad-spectrum of biofilm. Carbohydrate Polymers 84(2): 1365-1370.

157. Kawakita ERH, Re ACS, Peixoto MPG, Ferreira MP, Ricomini-Filho AP, et al. (2019) Effect of chitosan dispersion and microparticles on older Streptococcus mutans biofilms. Molecules 24(9): 1808.

158. Machul A, Mikołajczyk D, Regiel-Futyra A, Heczko PB, Strus M, et al. (2015) Study on the inhibitory activity of chitosan-based materials against biofilm- producing *Pseudomonas aeruginosa* strains. Journal of Biomaterials Applications 30(3): 269-278.

159. Del Carpio-Perochena A, Bramante CM, Duarte MAH, de Moura MR, Aouada FA, et al. (2015) Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restorative Dentistry and Endodontics 40(3): 195-201.

160. Chavez de Paz LE, Resin A, Howard KA, Sutherland DS, Wejse PL (2011) Antimicrobial effect of chitosan nanoparticles on Streptococcus mutans biofilms. Applied Environmental Microbiology 77(11): 3892-3895.

161. Shrestha A, Hamblin MR, Kishen A (2014) Photoactivated Rose Bengal functionalized chitosan nanoparticles produce antibacterial/biofilm activity and stabilize dentin-collagen. Nanomedicine Nanotechnology Biol Med, 10(3): 491-501.

162. Tan Y, Ma S, Liu C, Yu W, Han F (2015) Enhancing the stability and antibiofilm activity of DspB by immobilization on carboxymethyl chitosan nanoparticles. Microbiology Research 178: 35-41.

163. Priyadarshini E, Rawat K, Bohidar HB (2017) Quantum dots-based nano-coatings for inhibition of microbial biofilms: A mini-review, in Non-magnetic and magnetic quantum dots. (Eds.), Stavrou VN.

164. Sun H, Gao N, Dong K (2014) Graphene quantum dot-ban-aid used for wound disinfection. American Chemical Society-Nano 8(6): 6202-6210.

165. Habiba K, Bracho-Rincon DP, Gonzalez-Feliciano JA, Villalobos-Santos JC, Makarov VI, et al. (2015) Synergistic antibacterial activity of PEGylated silver-graphene quantum dot nanocomposites. Applied Materials Today 1(2): 80-87.

166. Ritenberg M, Nandi S, Kolusheva S, Dandela R,

Meijler MM, and Julin K, (2016) Imaging *Pseudomonas aeruginosa* biofilm extracellular polymer scaffolds with amphiphilic carbon dots. ACS Chemical Biology 11(5): 1265-1270.

167. Rozenbaum RT, Andren OCJ, van der Mei AC, Woudstra W, Busscher HJ, et al. (2019) Penetration and accumulation of dendrons with different peripheral composition in *Pseudomonas aeruginosa* biofilm. Nano Letters 19(7): 4327-4333.

168. Reymond JL, Bergmann M, Darbre T (2013) Glycopeptide dendrimers as *Pseudomonas aeruginosa* biofilm inhibition. Chemical Society Reviews, 42(11): 4814-4822.

169. Llamazares C, Sanz Del Olmo N, Ortega P, Gomez R, Soliveri J, et al. (2019) Antibacterial Effect of Carbosilane Metallodendrimers in Planktonic Cells of Gram-Positive and Gram-Negative Bacteria and *Staphylococcus aureus* Biofilm. Biomolecules 9(9): 405.

170. Hou SZ, Zhou C, Liu Z, Young AW, Shi Z, et al. (2009) Antimicrobial dendrimers active against *Escherichia coli* biofilms. Bioorg Med Chem Lett 19(18): 5478-5481.

171. Zareena AS, Gopal S (2013) Dendrimers-a new dimension in targeting biofilms. Mini Rev Med Chem 13(10): 1448-1461.

172. Lahir YK (2017) Impacts of fullerene on biological systems. Clinical Immunology Endocrinology and Metabolic drugs 4(1): 47-58.

173. d´Amora M, Gioddani S (2018) Carbon nanomaterials for nanomedicine, pp: 103-113.

174. Patel KD, Singh RK, Kim HW (2019) Carbon-based nanomaterials as an emerging platform for Theranostics. Materials Horizons 6(3): 434-469.

175. Raie DS, Mhatre E, Desouki DS, Labena A, El-Ghannam G, et al. (2018) Effect of novel quercetin TiO_2 -decorated multi-walled carbon nanotube-composite on *Bacillus subtilis* biofilm. Materials 11(1): 157.

176. Freixa A, Acuna V, Gutierrez M, Sanchis J, Santos LHMLM, et al. (2018) Fullerenes Influence the Toxicity of Organic Micro-Contaminants to River Biofilms. Frontiers of Microbiology 9: 1426.

177. Lahir YK (2019) Graphene and graphene-based nanomaterials are suitable for drug delivery, Chapter 7 in Applications of Targeted Nano-Drugs and Delivery Systems (Eds.), Mohapatra S, et al. Elsevier; 50 Hampshire Street, Cambridge, MA 02139; Elsevier Publications, pp: 157-190.

178. Di-Giulio M, Zappacosta R, Di-Lodovico S, Di-Campli E, Siani G, et al. (2018) Antimicrobial and antibiofilm efficacy of graphene oxide against chronic wound microorganisms. Antimicrobial Agents Chemotherapy.

179. Fallatah H, El-haneid M, Hanene AB, Overton TW, El-Kadri H, et al. (2019) Antibacterial effect of graphene oxide (GO) nanoparticles against *Pseudomonas putida* biofilm of variable age. Environmental Science and Pollution Research 26: 25057-25070.

180. Lawrence JR, Waiser MJ, Swerhone GDW, Roy J, Tumber V, et al. (2016) Effects of fullerene (C60), multi-wall carbon nanotubes (MWCNT), single-wall carbon nanotubes (SWCNT) and hydroxyl and carboxyl modified single-wall carbon nanotubes on riverine microbial communities. Environmental Science and Pollution Research 23(10): 10090-10102.