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Biofilm Inhibitory Potential of Chitosan based Nano-Encapsulated Phytochemicals: An Improved Antibiofilm Drug Delivery System for Antimicrobial Therapy

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Abstract

The production of biofilm by bacteria is the survival tactic in inappropriate environmental conditions. Biofilms also encourage pathogenic bacterial strains to develop antibiotic resistance. Exopolymeric substances (EPS) are the main constituent of biofilm that restricts the diffusion of plenty of antimicrobial compounds. Therefore, plant-derived compounds 'phytochemicals (flavonoids, tannins, alkaloids, terpenes, etc.) provide alternative options by showing the capability to inhibit biofilm produced by bacteria. However, the low stability, improper absorption, poor aqueous solubility, and easy degradability are few unfavorable features of phytochemicals that limit their use for further medical applications. However, the advancement in nanotechnology revolutionized antimicrobial chemotherapy, as various nanocarriers were shown their effectiveness in delivering bioactive compounds. Chitosan, derived from chitin, acts as bio carrier for a wide range of phytochemicals and improve their delivery and stability by exhibiting excellent antimicrobial and antibiofilm activities. The chitosan-based nanoparticles provided a feasible options to provide stability for encapsulated 'phytochemical/antibiofilm drug, and ensure its slow and proper release at the target site. Various methods such as ionic gelation, spray-drying' and 'emulsification' are used for encapsulating the natural bioactive components such as phytochemicals. The chitosan-based nanoparticles encapsulated with a number of phytochemicals namely ferulic acid, curcumin, cinnamaldehyde, chrysin, quercetin , and baicalein showed inhibitory potential against biofilm. This review presents a concise view of nano encapsulated phytochemicals for antibiofilm application, and development of efficient chitosan nanoparticles based antimicrobial therapeutic approach through effective drug (especially phytochemicals) delivery.

Keywords: Biofilm; Phytochemicals; Chitosan nanoparticles; Drug delivery.

Introduction

Biofilm formation is the unique trait in bacteria which assist their survival in different environmental settings. The clinical pathogens were also determined to possess the ability of biofilm formation and thereby engender a risk of antibiotic resistance (Høiby et. al., 2010). Extracellular polysaccharides are the main architectural component of the biofilm, and besides, biofilm also possesses extracellular proteins and DNA. These components serve as a blockade for the antimicrobial compounds given during the treatment (Khan et al., 2020a; Limoli et al., 2015). Therefore, a higher dose of the combination of antibiotics is administered to treat the microbial biofilm infection, but it also results in the development of antibiotic resistance pattern in bacteria and also induction of formation of recalcitrant biofilm (Khan et al., 2020b).

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So, there is a need to explore novel antimicrobial compounds and the development of effective strategies to combat bacterial infections (Römling et al., 2012). 'Herbal plants', the eminent gift endowed by nature, are the hub of 'phytochemicals' possess a ample range of medicinal values (Gupta et al., 2017). Numerous phytochemicals was assayed for their antibiofilm trait against several clinical pathogens (Ta et al., 2015). But the low bioavailability, poor stability, and nonsoluble nature in aqueous solution limit the potential of phytochemicals for using as biofilm inhibitory agents (Akolade et al., 2017; Gopalakrishnan et al., 2014). So, the advancement in nanotechnology further illustrated the nano-encapsulation of phytochemicals or other natural bioactive components for further development of antimicrobial therapy (Mu et al., 2016).

The natural polymer especially chitosan is potentially used as a bio-carrier in order to get desirable effects of loaded phytochemicals. The chitosan-based nanoparticles also exhibited the antibiofilm effect as it can easily diffuse into the biofilm composite structure (Chávez de Paz et al., 2011). 'Ionic gelation', 'spray-drying', and 'emulsification' are the three key methods employed in the chitosan-based nanoencapsulation of an antibiofilm agent (phytochemicals, herbal extract, antimicrobial drug) (Akbari-Alavijeh et al., 2020; Detsi et al., 2020; Khan et al., 2020b). 'Chitosan nanoparticles', exhibited the excellent potential for the delivery of numerous phytochemicals and other drugs for inhibiting biofilm formed by bacterial pathogens (Ilk et al., 2016; Khan et al., 2020b). The main benefits of nanoencapsulation are to slow and constant release of the particular embedded phytochemical or drugs at the target location. Moreover, chitosan based nanoparticles present numerous benefits in terms of better effectiveness, economically feasible, eco-friendly, augmented absorptibility (Ilk et al., 2017; Pattnaik et al., 2018a).

Biofilm formation: an unique mechanism of pathogenic bacteria

The drug resistance mechanism in bacteria is well known, and the formation of biofilm is a major factor which engenders the pattern of antibiotic resistance in clinical pathogens (Høiby et al., 2010; Wu et al., 2015). Bacteria establish biofilm on biotic and as well as abiotic surfaces through secreting extracellular polymeric substances. The biofilm matrix, besides the exopolymeric substances, also contains other components including carbohydrate-binding proteins, extracellular DNA, pilli, adhesive fibers, and flagella (Kostakioti et al., 2013). The biofilm matrix contains some unique features that provide protection to bacterial cells from numerous external environmental factors. The bacterial cells show tolerance or resistance pattern towards antibiotics and cells in biofilm may exhibit a thousand times antibiotic resistance ability than the 'planktonic cells' (Khan et al., 2020a). Formation of biofilm is the tactic adopted by bacteria for their survival in hostile conditions (Wu et al., 2015). There are the following main three stages for the development of biofilm on surfaces including a) attachment of microbial cells to an abiotic/biotic surface, b) aggregation of microbial cells to construct microcolonies, and finally, c) differentiation of biofilm into full-grown structural components (Roy et al., 2018).

In the clinical settings, biofilm-producing bacterial cells are usually the source of nosocomial, chronic, and medical/ implantable device-associated infections (Khatoon et al., 2018). Bacterial produced biofilms are coupled with human ailments and also responsible for a number of biofilm-associated infections such as chronic obstructive pulmonary diseases, lung infections of Cystic Fibrosis) patients, otitis media, chronic wound infections, rhinosinusitis, endocarditis, vaginosis, urinary tract infections, inflammatory bowel disease, prostatitis, cancer, etc. (van Tilburg Bernardes et al., 2015; Vestby et al., 2020). A number of clinical isolates such as 'Pseudomonas aeruginosa', 'Burkholderia cepacia', 'Listeria monocytogenes', 'Staphylococcus aureus' and 'Candida albicans', as causal agents of nosocomial infections in immune-compromised people were reported to form the biofilm structure (Ta et al., 2015).

Phytochemicals: as effective antibiofilm agents

'Phyto-therapy' employed the use of herbal plants for combating against several ailments. A number of parts such as leaves, flowers, seeds, root, rhizome, etc are used for remedial purposes in various ethnic communities (Kumar et al., 2017). Plants are the ultimate hub of numerous compounds commonly known as 'phytochemicals'. Particularly, the health benefit effects are ascribed due to the phytochemicals (Mohanraj et al., 2018). In the last few decades, the emergence of antibiotic resistance in pathogenic bacteria posing problems, and therefore there is a requisite to search for other approaches such as using phytochemicals for combating microbial infections (Gupta et al., 2017).

Biofilm formation by bacteria also decreases the effect of antibiotic and presents the major mechanism of antibiotic resistance. Therefore, phytochemicals from the herbal sources may provide an alternative

solution for inhibiting biofilm formation in bacteria. Various phytochemicals such as plant phenolics (including 'benzoates', 'phenylpropanoids', 'stilbenes', 'flavonoids', 'gallotannins', 'proanthocyanidins' and 'coumarins'), terpenes, alkaloids, quinones, and organosulfur compounds were showed remarkable antibiofilm activity against clinical pathogens (Ta et. al., 2015). Eugenol, a 'phenylpropene' showed antibiofilm potential against one clinical pathogenic strain of Pseudomonas aeruginosa and reduced biofilm formation (43% at 400 µM) (Zhou et al., 2013). Tannins were also tested for their anti-biofilm and quorum sensing inhibitory properties. 'Punicalagin' an example of tannin (at the concentration 15.6 µg/ml) was reported to down-regulate the expression of motility and QS related genes in Salmonella typhimurium (Li et al., 2014).

Tannic acids were determined for biofilm inhibitory effect against Pseudomonas and Staphylococcus aureus (Ta et al., 2015). Flavonoids such a quercetin (at 1 µg/ml concentration) inhibited biofilm production in MRSA (>80%) and MSSA (>50%) strains and also declined the expression of genes participated in QS and virulence of Staphylococcus aureus (10 µg/ mL) (Lee et al., 2013). Other flavonoids namely 'catechins' isolated Camellia sinensis L. also inhibited biofilm production in Eikenella corrodens (Matsunaga et al., 2010). Various kinds of terpenes (such as 'monoterpenes', 'limonoids', and 'triterpenes') were also been found to contain anti-biofilm and anti-quorum sensing activities (Ta et al., 2015). Monoterpenes namely thymol and carvacrol had shown efficacy against biofilms of Listeria monocytogenes (Upadhyay et al., 2013), while the 'sesquiterpenoids' (such as salvipisone and acanthospermolides) reduced biofilm formation in Pseudomonas aeruginosa (Cartagena et a., 2007). Moreover, allicin (sulfur containing phytochemical) inhibited biofilm formation in Staphylococcus epidermidis strains (at 4 mg/ml) (Pérez-Giraldo et al., 2003). At a concentration of63.5 µg/ml, 'berberine' (an example of alkaloids) reduced biofilm growth in different clinical isolates of Klebsiella pneumoniae (Magesh et al., 2013). Coumarins also exhibited QS and biofilm distorting activities, as 'aesculetin', at the level of 500 µM, was found to inhibit QS in C. violaceum, E. coli and P. aeruginosa (Brackman et al., 2009).

Chitosan-nanoparticles as effective antimicrobial drug delivery tool

Chitosan is an example of a linear polysaccharide comprised of 'D-glucosamine (GlcN)' and 'N-acetyl glucosamine (GlcNAc)' connected by β-1, 4-glycosidic bond. It is synthesized from the chitin (second huge polymer after cellulose, exist in the body of crustacean, mollusks, and insects) after the partial alkaline deacetylation (Jung et al., 2014). Either homogenous or heterogeneous deacetylation methodologies are applied for deriving the chitosan from the chitin (Aam et al., 2010). On the contrary, chitooligosaccharides and other derivatives of chitosan are produced either by enzymatic or acid hydrolysis from chitosan (Khan et al., 2020). Molecular weight (MW) and extent of deacetylation of chitosan and chitooligosaccharides are two key factors for determining their biological activities (Foster et al., 2015). Furthermore, chitosan and chitooligosaccharides can be used as 'bio-carriers' for the phytochemicals or other natural compound showed antibiofilm activity.

As a carrier molecule, the chitosan/ chitooligosaccharides can improve the drug delivery and maintain the stability of encapsulated plant-derived compounds. Numerous studies exhibited limitations of antibiofilm drugs (including phytochemicals) such as poor stability, low bioavailability, degradation, and water insolubility (Dos Santos Ramos et al., 2018). In order to fight such challenges, the controlled release strategy is emerging where the antibiofilm compounds are loaded to the various preparations of chitosan as 'carrier molecules' (e.g. 'nanocomposites', 'microspheres', 'nanofibers', 'hydrogels', and nanoparticles) (Bilal et al., 2019). The benefits of such carrier molecules are to facilitate the controlled release of phytochemicals having antibiofilm potential such as quercetin (Omwenga et al., 2018), cinnamaldehyde (Pattnaik et al., 2018a), ferulic acid (Dasagrandhi et al., 2018), caffeic acid (Kim et al., 2018), and kaempferol (Ilk et al., 2016) which had resulted in extended effectiveness and the slightest cytotoxicity effects. Moreover, the natural antimicrobial agents such as phytochemicals, and essential oils encapsulated within chitosan nanoparticles were more stabilized. Encapsulated antibiofilm agents were also capable of protecting themselves from environmental degradation (Khan et al., 2020).

Need of phytochemical encapsulated chitosan nanoparticles

Using phytochemicals for their wide spectrum of pharmacological applications is widely accepted as they are effective and safe. But some inappropriate behaviors such as low bioavailability, degradation,

poor stability, and adsorption limit the uses of phytochemicals (Gopalakrishnan et al., 2014). Often phytochemicals showed potential bio-activities under laboratory or in vitro conditions becomes fail in terms of activities and efficiency under in vivo studies and clinical trials (Akolade et al., 2017). Khan et al, (2014) illustrated the low absorption of phytochemical especially polyphenols after ingestion from the gastrointestinal tract. Other phytochemicals, for instance 'catechin', depicts unstable nature in gastrointestinal solutions (Dube et al., 2011). Some bioactive compounds experience the process of enzymatic oxidation and show the nature of degradability in plenty of food processes and storage and may form components bearing harmful effects (Detsi et al., 2020). Reduced aqueous solubility of numerous phytochemicals is a further drawback (Akolade et al., 2017; Detsi et al., 2020). Additionally, numerous agents (either the surfactants or solubilizing agents) used in the process of extraction of the bioactive components might reduce their concentration (Akolade et al., 2017). The augmented dosage of phytochemicals requisite for the preferred curative effectiveness is also posing a strong limitation of their utilization (Yadav et al., 2012). This frequently results in selective or functional toxicological complications because of loss, inactivation, and/or degradation at the period of transportation of the molecule from the site of administration to the target location (Akolade et al., 2017).

Therefore, it becomes imperative to seek a carrier-based system for the phytochemicals which could abolish most of abovementioned limitations with the aim of improving the clinical results (Khan et al., 2014). So, 'nanoencapsulation' is a proficient tactic to conquer these limits through following such as a) increasing the targetability, b) prolonging the rate of release of the encapsulated bioactive components and, c) improving the stability of the encapsulated material. After ingestion, nanoparticles containing 'phytochemicals' may possibly adhere to the mucosa of the gastrointestinal tract, because of their 'mucoadhesive traits' (Khan et al., 2014), and then be transported through the circulation to various organs-targets extending their remedial effects (Detsi et al., 2020). The Barros and Casey, (2020) advocated the use of nano encapsulated plant extracts, essential oils and isolated pure phytochemicals as they become more effective weapons against biofilm.

Preparation of phytochemical encapsulated chitosan nanoparticles

There are three important methods namely 'ionic gelation', 'spray-drying' and 'emulsification' used in the chitosan-based nanoencapsulation of natural products or phytochemicals (Akbari-Alavijeh et al., 2020). The method of ionic gelation is simple which presents an organic solvent-free strategy for the development of stable nanoparticles (Detsi et al., 2020). It is based on the interaction amid oppositely charged macromolecules and non-hazardous and multivalent material in order to provide the charge density. Ionic gelation results owing to cross-linking of the 'polycationic chitosan' (both inter and intra cross liking) via an anionic cross-linker such as tripolyphosphate. For this, the chitosan firstly dissolves in an aqueous solution of acetic acid followed by the dropwise addition of tripolyphosphate. The formation of nanoparticles occurs at room temperature (under mechanical stirring) (Desai et al., 2016; Detsi et al., 2020; Wang et al., 2016). The elevated loading ability is determined as the benefit; but, the huge particle sizes, pH sensitivity, and higher polydispersity are the major downsides of this technique (Das et al., 2019; Shetta et al., 2019).

The second approach is the spray drying method which is carried out in the following steps a) dissolving of chitosan in acetic acid solution to form the matrix, b) homogenization of compound to be encapsulated with matrix, c) addition of selected cross-linking agent such as tripolyphosphate (Oliveira et al., 2005) or d, l-glyceraldehyde (Ravi Kumar et al., 2000; Wang et al., 2016), e) atomizing mixture in a chamber by a nozzle or spinning wheel, f) evaporation of water by hot air contacting the atomized material, and collection of material fall to the bottom of the dryer (Detsi et al., 2020; Gibbs et al., 1999). This approach is considered to be simple, fast, and economically feasible (Detsi et al., 2020).

Furthermore, the third approach is illustrated as the 'emulsification–solvent diffusion technique'. This method is relied on the cross-linking of functional amines groups of 'chitosan' and 'aldehyde' (for instance 'glutaraldehyde', 'formaldehyde') or even 'vanillin' as an eco-friendly substitute. The precipitation of chitosan takes place upon the organic solvent diffusion into water (Detsi et al., 2020; Wang et al., 2016). Various phytochemicals such as flavonoids (e.g., genistein) (Rahmani et al., 2020), tea polyphenols (Dube et al., 2011; Liang et al., 2017), curcumin (Almutairi et

al., 2020), baicalein (Ahmed et al., 2020), quercetin (Omwenga et al., 2018), kaempferol (Ilk et al., 2017), phenolic compounds such as ferulic acid (Panwar et al., 2016) were encapsulated in chitosan nanoparticles, and also determined tested for therapeutic approaches.

Antibiofilm effect of chitosan nanoparticles loaded with the phytochemicals

Nano-encapsulated phytochemicals were potentially determined for their antimicrobial activity against pathogenic bacteria (both Gram-negative and Gram-positive) and fungi. But very few reports are reported on using nano encapsulated photochemical as 'antibiofilm agents' where nanocarrier improved the delivery of phytochemicals for distorting bacterial produced biofilm. Two flavonoids, quercetin, and baicalein, in associated with chitosan-based nanocapsules exerted inhibiting potential against quorum sensing in bacteria and biofilm formation (Omwenga et al., 2018). 'Ferulic acid grafted chitosan nanoparticles (FA-CSNPs)' prepared by employing ionotropic gelation method exerted potential antibiofilm activity against Candida albicans (Panwar et al., 2016). The study of Jahanizadeh et al., (2017) showed the role of novel bio nanocomposite of 'Carboxymethyl Starch (CMS)-Chitosan (CS)-Montmorillonite (MMT)' for the delivery of curcumin, and illustrated the effective role of curcumin loaded bio-nanocomposite against biofilm on dental models formed by Streptococcus mutans.

Kaempferol encapsulated chitosan nanoparticles were found to inhibit quorum sensing (QR) related processes (as this encapsulated material inhibited the production of a pigment 'violacein' in bacterial strain namely Chromobacterium violaceum CV026) and thus provoked for developing antimicrobial therapy as stable quorum sensing (QS)-based antibiofilm agent (Ilk et al., 2017). 'Cinnamaldehyde encapsulated chitosan nanoparticles' was reported to down-regulate the quorum sensing linked virulence and biofilm production in Pseudomonas aeruginosa PAO1 (Pattnaik et al., 2018a). Moreover, Pattnaik et al, (2018b) exhibited the potential antibiofilm property of 'ferulic acid encapsulated chitosan-tripolyphosphate nanoparticles (FANPs)' against Pseudomonas aeruginosa in comparison of 'nonencapsulated ferulic acid'. The study also illustrated the promising features of nano encapsulated ferulic acid such as anti-quorum sensing activity and slow and constant release of ferulic acid at the targeted location. Furthermore, Dasagrandhi et al., (2018) showed antibacterial and antibiofilm the activity of 'ferulic acid-grafted chitosan (CFA)' against Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus. The antibiofilm ability of chitosan implanted with phenolics (such as caffeic acid, ferulic, and sinapic acid) were determined against foodborne pathogens such as Pseudomonas aeruginosa and Listeria monocytogenes (Kim et al., 2018). 'Chrysin-encapsulated chitosan nanoparticles (CCNPs)', prepared by emloying the ionic gelation methodology exhibited excellent anti-biofilm activity against Staphylococcus aureus (Siddhardha et al., 2020).

Conclusion

The nano-encapsulated phytochemicals are being used in current researches to explore their antimicrobial and antibiofilm activities. Using chitosan-based nanoparticles offers a better strategy to maintain the stability of embedded phytochemicals, and thereby show excellent antibiofilm activity through improvement in delivering phytochemicals at the target site. However, very few studies have been done for decoding antibiofilm activity in terms of chitosan-based nano encapsulated pure phytochemicals. More investigations are still required for encapsulating wide arrays of phytochemicals through chitosan-based nanoparticles in clinical trials.

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