ISSN 2816-573X

REVIEW ARTICLE

The Two Dimensional Nanomaterials Functionalized with Antimicrobial Peptides as a Novel Strategy to Combat Biofilms and its Associated Infections

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Boddety C, Modali S, Pentakota G, et al. The Two Dimensional Nanomaterials Functionalized with Antimicrobial Peptides as a Novel Strategy to Combat Biofilms and its Associated Infections. Int J Adv Nano Comput Anal. 2023;2(2):97-117.

Abstract

The resilient and adaptive nature of biofilms and its associated infections pose a serious threat in the current state of play aiming the need for a promising strategy. The two-dimensional nanomaterials functionalized with antimicrobial peptides serve as a novel approach to combat biofilms and their related infections. This review article explains the current landscape of research in this field focusing on classification and physiochemical properties of two-dimensional (2D) nanomaterials and their exploitation as antimicrobial peptide delivery system. The review also offers insights into their potential application in various settings such as medical devices wound healing and water treatment. Additionally we discuss the challenges and future directions in the development and implementation of this innovative strategy, emphasizing the need for multidisciplinary approach that bridges the gap between fundamental research and practical applications. Through a comprehensive synthesis of current literature, this review aims to provide researchers, clinicians and industry professionals with a thorough understanding of promises and challenges, which aim in the development of advanced materials and strategies for combating microbial biofilms and improving industrial control measures.

Key Words: *2D nanomaterials; Antimicrobial peptides; Biofilm; Targeted drug delivery*

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Received: December 19, 2023, Accepted: December 28, 2023, Published: December 30, 2023

Introduction

Antimicrobial resistance (AMR) and novel viral infections have emerged to be the two principle health crisis in the current state of play posing a serious growing concern not only to humans, animals, and environment health but also creating run down predicament to global economy. The failure of development (or) discovery of new antibiotics and non-judicious use of currently available antibiotics has led to the emergence, spread, and persistence of multidrug-resistant (MDR) bacteria or "superbugs causing antibiotic resistance. [1,2]. The recent outbreak of pandemic Coronavirus disease 2019(CoVID-19) caused by Severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) also possess antimicrobial resistance as a hidden threat [3]. This creates a pressing need for the requirement of more potent approaches that can tackle AMR and viral infections in a promising way.

The major factor contributed to antibiotic resistance is the ability of microorganisms to form sessile communities on both biotic and abiotic surfaces as biofilms [4]. It is estimated that more than 65% of microbial infections are caused by microorganisms when they grow in biofilms [5]. The possible reason is that bacterial cells during their transition from planktonic to sessile state undergo extensive changes (e. g. , behavior, structure and physiology) which are reflected in the phenotypic and metabolic characteristics of biofilm cells [6] conferring furthermore resistance to antibioticsdue to their poor penetration, enzymatic inactivation , disabling by acidic (or) anaerobic conditions produced by inhabitants of biofilm.

The WHO announced that the COVID -19 outbreak was the highest level of health emergency with negatively affecting global economy growing on a scale that has not been experienced since at last the global financial crisis of 2008-2009 [7]. Many studies have found the development of secondary bacterial co-infections in hospitalized COVID-19 patients [8]. According to the analysts of Research and Development Corporation (US), a worst-case scenario may evolve in the coming future where the world might be left without any potent antimicrobial agent to treat bacterial infections. In this situation, the global economic burden would be about $120 trillion ($3 trillion per annum), which is approximately equal to the total existing annual budget of the US health care. In general, the world population would be hugely affected: as of the year 2050, about 444 million people would succumb to infections and birthrates would rapidly decline in this scenario. This makes a compelling necessity worldwide in creation of new antimicrobials and designing new strategies that combat resistance against pathogenic microorganisms [9].

Antimicrobial peptides (AMPs) have become a promising candidate and have received notable attention as a novel class of antibiotics [10]. They are peptides naturally produced by all organisms including prokaryotes to human beings in response to foreign microbes and have a role in innate specific defense system and provide instant non-specific defense against infections. They have a broad spectrum of activity against a wide range of microorganism including bacteria, fungi, parasites and viruses [11,12]. However, their certain limitations need to be overcome prior to its therapeutic usage like physical stability under physiological conditions, *invivo* degradation by proteases and susceptibility to serum, salt, and pH in host fluids [13]. To surmount these limitations of AMP many studies have been carried out to explore application of various nanostructures in drug delivery.

The two-dimensional (2D) nanomaterials provides with attractive and unique properties in nanomedicine as it has high surface area

for desired drug-loading capacity, efficient cell uptake, attractive biocompatibility, and established chemical functionalization routes, layered pattern which can be significantly exploited to function as intelligent drug delivery platforms in modern medicine [14]. The functionalized 2D Nano graphene oxide against Coronavirus has been recently reported as a helpful therapeutic approach [15]. Experimental studies have shown graphene family 2d nanomaterials to possess strong antibacterial, antifungal and antiviral activity [16,17].

In this review, we highlight how functionalization of external surfaces of 2D nanomaterial integrated with stimuli responsive characterization provides a precise control over the AMP release from the delivery system to the target site thus contributing to improve therapeutic efficacy with diminished side effects. The 2D nanomaterials offer promising biomedical applications like bio sensing, image guided therapy, efficient drug delivery platform that are critical limitations of conventional delivery systems. We offer a brief forward-looking perspective where we can expect to see next significant development in utilizing 2D nanomaterials as AMP delivery system to combat against antibiotic resistance in biofilm associated chronic infections and novel viral infections as a fundamental work progress.

Emergence of AMR by Bacteria Related Biofilms and Novel Viral Infections

Antibiotic resistance specifies bacteria that shows impedance to previously susceptible antibiotics and contributes a significant part in antimicrobial resistance (AMR). Most of the microbial infections are associated with bacterial biofilms, which are resistant to antibiotics in chronic (persistent), nosocomial (hospital acquired) and medical devices related infections. Biofilm refers to aggregates of microbial cells embedded in self-produced matrix of extra polymeric substances adherent to each other and/or surface (living or non-living). This biofilm mode of bacterial lifecycle is widely distributed and exhibits successful mode of life due to favorable emergent properties unlike their planktonic (free-living) counterpart that possess in general susceptibility towards antibiotics. This complex system has high cell density ranging from 10^8 to 10^{11} cells g^{-1} wet weight comprising either single or many species of microbes [18].

Biofilm formation involves the sequential occurrence of following events:

- **Attachment:** In human body, the solid liquid interface between tissue surface and aqueous medium (water, blood) provides an ideal environment for attachment and growth of microorganisms through biofilms. It begins with adsorption of organic or inorganic molecules to a solid surface forming a thin conditioning layer where initially planktonic bacteria attach by reversible weak Vander Waals forces and then irreversible attachment promoted by fimbriae, flagella or piliof bacterial cells thus forming a monolayer. This monolayer produces extracellular matrix comprising polysaccharides, proteins, lipids, extracellular DNA (eDNA) collectively called as extracellular polymeric substances (EPS) as a protective barrier against surrounding stressful conditions.
- **Microcolony formation:** The bacteria within biofilm undergoes growth and division forming small aggregate colonies called microcolonies.
- **Maturation:** At this stage, the biofilm is multilayered with microbial colonies present at different concentration gradients of nutrients, pH, oxygen, temperature with minute pores and water channels connecting the colonies for circulating water along with nutrients. Cell to cell interaction within matrix is either

by electrical communication using nanowires orchemical communicationthrough quorum sensingwhich producessignaling molecules called autoinducers that controls their coordinated behaviorand overall gene expression. This gene expression increases the production of more virulence factors by bacteria leading to infection and also EPS production is substantially increased thus recruiting different components like exopolysaccharides, DNA, proteins forming a thick layer that shields the microbes from host immune responses and external antimicrobial agent.

- **Dispersion:** On maturation the microbial cells undergo detachment from biofilm colonies, translocate to a favorable location followed by its surface attachment in new location.

Emergent properties are common in both monospecies and multispecies biofilm that possess self-produced EPS matrix to continuously remodel biofilm in response to environmental changes such as hydrodynamic stress and streamers providing transient storage and increased nutrients cycling. These properties includes increased pathogen survival through Tolerance and Resistance to antimicrobials, habitat diversity through localized gradients of pH, oxygen and nutrients, resources capture through sorption and their recycling, cooperation through synergistic micro consortia, facilitation of external digestion system through enzyme retention, inherent protection from desiccation [19].

The pathogenic bacteria within biofilms utilizes both resistance and tolerance as survival mechanisms against antimicrobial agents. Tolerance is a non-heritable trait of bacteria to survive without further growth in presence of drug (antibiotic). This is granted by cumulative effect of slow growth rate and dormancy as “persistors” and favorable matrix properties that act as diffusion barrier to antibiotics through inactivation or entrapment. This property is lost when cells in biofilm are dispersed into planktonic form. Resistance is a genetically heritable permanent trait that allows the bacteria to survive and multiply in the presence of drug (antibiotic) that would normally kill them. This phenotypic trait develops over certain period due to consistent exposure of bacteria to multiple antibiotics. Intrinsic resistance pertains to natural innate ability of bacteria to resist a class of antibiotics due to lack of metabolic process or target site, which is always expressed in that species. One such example is natural resistance of gram negative bacteria to vancomycin drug Acquired resistance is attained through genetic means as a result of spontaneous mutations of existing genes present in chromosome or by acquisition of resistance genes from the same species or different microbial species through horizontal gene transfer mediated by extrachromosomal vectors like plasmids, transposons, integrons. The emergence of multidrug resistance in a biofilm is mainly contributed by acquired resistance phenomenon.

The main biochemical strategies of bacterial resistance are:

- Reduction of penetration into cell thus preventing drug (antibiotics) from reaching its target.
- Removal of the taken antibiotics by utilization of general or specific efflux pumps.
- Inactivation of drug through modification or degradation.
- Modification of the drug target structure by bacteria [20].

There also seem to exist a significant correlation of bacteria-virus interaction in microbial infections progression. Human viruses often interact with bacteria either directly or

indirectly imparting disease pathogenesis. Direct interactions involves a specific bacterium or bacterial products like mucus or enzymatic secretions to cause virus attachment following viral infection. This interaction suggest that bacterial population may aid in viral infections and is commonly seen in enteric viruses.

Contrarily indirect interactions causes primary viral infections to produce favorable conditions for bacterial colonization by employing the following mechanisms.

- Host immune suppression
- Increase of bacterial cell receptors concentration
- Displacement of commensal bacteria
- Disruption of host epithelial layers for bacterial colonization.

Here the virus indirectly promotes harmless bacteria to become opportunistically pathogenic [21]. This interaction is commonly seen in respiratory viruses which often lead to secondary bacterial infections involving *Streptococcus pneumonia, Staphylococcus aureus, Hemophilus influenza , Streptococcus pyogenes*. Such co-infections are problematic during influenza pandemic, swine-flu pandemic and recent Covid -19 pandemic [22]. Matthew, et. al work reported how Respiratory syncytial virus(RSV) induces *Pseudomonas aeruginosa* biofilm formation through a mechanism of dysregulated iron homeostasis through airway of respiratory tract epithelium in cystic fibrosis and chronic obstructive pulmonary disease patients [23].

These serious problems encountered in current situation urges the development of new antimicrobial strategy that could act as antimicrobial resistance (AMR) breakers and antibiofilm agent with antiviral property. This characteristic property is possessed by some antimicrobial peptides as well as by nanomaterials having remarkable and tunable properties, thus their convergent exploration can serve as a promising strategy providing synergistic effect in combating multidrug resistance by biofilms and also other novel viral infections.

Antimicrobial Peptides

Antimicrobial peptides (AMP) are ubiquitously distributed and naturally produced host defense molecules that provides immune response against pathogenic microbes. They comprise less than 100 amino acids residues with cationic nature, hydrophobicity as favorable aspects of specificity towards targeted microbes. AMPs were discovered in 1939 where Dubos extracted Gramicidin as antimicrobial agent from soil Bacillus strain. The first animal originated AMP is Defensing isolated in 1956 from rabbit leukocytes. The current molecular development has described more than 2000 AMPs in several databases that includes natural and pharmacologically designed synthetic AMPs.

The chemical structure and sequence diversity of AMPs classify them into four structural groups

- Linear structure alpha helix
- Beta sheet or strand (with 2 or more disulphide bridges)
- Mixed helical sheets
- Extended non-helical linear sheets (rich in amino acids Histidine, Tryptophan, lysine) with mostly adopted structure being alpha helix. Most peptides transform from flexible to fixed conformation when interacting with target cell membranes to show their activity. A single or two amino acids change can cause significant variation in their efficacy due to its considerable effect on secondary peptide structure.

The General Governing Characteristics Influencing AMPs Potential

- **Length:** The length of AMP influences its 3D structure for activity as well as cytotoxicity at least 7-8 amino acids are needed to form amphipathic structure with hydrophobic and hydrophilic residues facing opposite sides of a peptide molecule. The favorable size for AMP to transverse the lipid bilayer of bacteria in Barrel Stave model should be at least 22 amino acids for alpha helical AMP and 8 amino acids for beta sheeted AMP [24].
- **Net Charge:** The initial interaction of AMP with cell membranes having negative charge depends on the net charge (sum of all ionizable groups) on AMP, which varies from negative to positive. This feature also influences the property of selective killing and hemolytic activity.
- **Helicity:** It is the spin forming ability of AMP, which determines toxicity on eukaryotic cells. To lower the hemolytic effect helicity can be reduced by incorporating D-amino acids into the primary sequence with retained antimicrobial effect and enhanced peptide stability against proteolytic cleavage.
- **Hydrophobicity:** This is conferred by non-polar amino acid residues in AMP structure that significantly plays key role in determining the range of target cells, (gram positive and gram-negative bacteria). Natural AMP's possess 50% of hydrophobic residues in their primary structures. In most cases, increase in the hydrophobicity on positive charge side of AMP below a threshold can increase its antimicrobial activity and vice versa.
- **Amphilicity:** this ensures the activity and interaction with the microbial membranes due to strong partitioning effect in the membrane interface.
- **Solubility:** AMP's need to be soluble in aqueous environment in order to act or enter through the lipid membrane of target cell.

Key factors aiming amp specificity towards targeted cell membrane

The amphipathic phospholipid bilayer is present, as core constituent in all natural bio membranes. There exists a fundamental difference in cell membrane structure and composition between bacteria (prokaryotic) and mammalian (eukaryotic) cell membrane, which allows the AMP to selectively target only the bacteria membrane over the other.

- **Cell membrane composition and charge:** The chemical composition of the cell membrane phospholipid bilayer differs in both prokaryotic and eukaryotic cells. Bacteria cell membrane structure generally comprise of hydroxylated phospholipids like phosphatidyl glycerol(PG), Phosphatidyl serine(PS), Cardiolipin(CL) that possess net negative charge under physiological conditions while the mammalian membranes are abundant of Phosphatidyl choline(PC), Phosphatidyl ethanolamine(PE), Spingomyelin(SP) which are neutrally charged under physiological conditions along with unique presence of cholesterol(neutral) and other sterols.
- **Transmembrane potential:** Depends on the charge separation existing between outer and inner layers of cell membrane. A normal mammalian cell membrane has between -90mv and -110mv in range while pathogenic bacteria with -130mv to -150mv range. This facilitates the entry of AMP into the bacterial membrane since a high transmembrane potential will facilitate pore formation by drawing the positively charged peptide into the membrane [25].
- **Receptor mediated membrane**

interaction: The anionic components in bacteria cell membrane like cardiolipin, phosphatidylglycerol, lipopolysaccharides may serve as pseudo receptors that enable initial interaction between AMP and target cell thus allowing receptors to be an alternate interaction pathway for AMP action.

- **Asymmetry in cell membrane:** There exists an asymmetric charge distribution between the outer and inner leaflet with negative charged head groups such as phosphatidylglycerol, cardiolipin, phosphatidylserine in cell membrane of bacteria. The zwitterion phospholipids like phosphatidylcholine, sphingomyelin and other neutrally charged components like cholesterol are distributed in abundance within outer and inner leaflets of mammalian cell membrane. This significant difference allows AMP to distinguish between host (mammalian cell membrane) and target microbial membrane by electrostatic interaction.

Another significant aspect regarding the selective toxicity based on AMP design is that these peptides may undergo conformational transition, self –association (multimerization) only within the target pathogen membrane but not in host cell membrane that in turn increases cell specific toxicity.

Mechanism of Action of AMP

The initial electrostatic interaction is based on the positive charge abundance of lysine and arginine residues in AMP with negatively charge lipopolysaccharides (LPS) in cell wall of gram-negative bacteria and gram-positive bacteria with highly anionic structures like teichoic acid of cell wall. Followed by the initial binding the entry of the peptides into the membrane depends on the hydrophobic interactions, threshold concentrations and the confirmatory changes adopted by the AMP in order to show targeted cell toxicity.

In amphipathic peptides, the hydrophobic residues interact with non-polar hydrophobic core region of lipid bilayer driving the peptide deeper into the membrane where they simultaneously interact with hydrophobic residues of other peptides promoting multimerization as an attempt to protect the hydrophobic phase exposure to aqueous environment. The AMP insertion and permeability depends on P/L ratio (peptide: lipid ratio). Where at low P/L ratio, peptides are bound parallel to the lipid bilayer and as this ratio increases, they begin to orient perpendicular to membrane. At high P/L, ratio AMPs are oriented perpendicularly to form transmembrane pore by inserting themselves into the lipid bilayer. This multimerization of AMP promotes disintegration and permeabilization of cell membrane leading to cell lysis of target bacteria.

The models that explains the mechanism of action of AMP

- **Toroidal pore model:** AMPs on reaching critical concentration align perpendicularly and affect the local curvature of the bilayer structure forming toroidal pore with their hydrophobic regions associated with the central part of the lipid bilayer and hydrophilic region facing the pore.
- **Disordered toroidal pore:** It is a random pore-forming model with inward lipid distortion allowing few peptides to aggregate in the Centre of the pores with many untranslocated peptides in the peripheral region of pore.
- **Carpet model:** AMP's adsorb parallel to lipid bilayer and cover the surface of the membrane thereby, forming carpet. This leads to unfavorable interactions on lipid surface and consequently the fluidity and the membrane integrity is lost leading to osmotic imbalance and lysis of cell.

- **Detergent model:** AMPs at high concentration cause membrane disintegration due to micelle formation where the peptides intercalate into the membrane in a detergent like manner causing the bilayers to continuously bend so the water core is lined by both the inserted peptides and the lipid head groups.
- **Barrel stave model:** Firstly, the staves are formed by AMPs parallel to the cell membrane then inserted perpendicularly to the plane of membrane bilayer thus forming the barrel shaped pore solely by lateral peptide peptide interaction.
- **Charge lipid clustering model:** It is observed when AMPs induces clustering of anionic lipids thereby causing membrane depolarization and leakage of intracellular contents.
- **Oxidized phospholipid targeting model:** In this model, the AMPs intercalate more efficiently into membranes containing oxidized lipids such as phosphatidylcholine through Schiff's base formation leading to release of ROS (Reactive oxygen species) for phagocytosis.
- **Electroporation model:** It depicts the accumulation of AMPs on outer leaflet which increases the membrane potential above a threshold rendering to transient membrane permeability to various molecules including peptides.
- **Membrane thinning model:** Here AMPs intensively adsorb to membrane preferring parallel orientation relative to membrane and reduces membrane thickness that causes lowering the membrane conductance leading to ionic leakage.
- **Non-lytic membrane depolarization model:** AMP causes inhibition of ATP dependent multidrug efflux pumps by affecting proton motive force and transmembrane potential followed by inhibition of RNA and protein synthesis by localizing AMP into cytoplasm.
- **Anionic carrier model:** shows how AMP causes anionic selective leakage of zwitterion liposomes as these cationic AMPs operates a carrier of organic anions in lipid membrane disrupting their osmotic balance.
- **Sinking raft model:** depicts the perturbation of lipid bilayer in the form of mass imbalance caused directly by peptide binding to outer leaflet resulting in local curvature strain. This perturbation would allow small peptide aggregates to sink deeply into bilayer and cross in so-called sinking raft manner.

Disruption of cell membrane mode of action by AMP plays a major role in bacterial targeting as one third of the total proteins of a bacterial cell are associated with the membrane possessing many functions that are critical to the cell viability including active transport of nutrients, respiration, proton motive force, ATP generation, and intercellular communication [26]. The function of these proteins can be altered with AMP treatment even if complete cell lysis does not occur. Therefore, AMPs' rapid killing effect does not only come from membrane disruption but can also come from inhibition of these functional proteins.

Apart from acting on cell wall and cell membrane of bacteria AMPs also exhibit its activity on intracellular targets by inhibiting protein synthesis, enzyme activity, nucleic acid synthesis and cell organelles damage [27] the mechanism of intracellular targeting is energy dependent and takes place by direct penetration and endocytosis. The cellular uptake of AMPs through endocytosis includes micropinocytosis where the cell membrane folds inward and form vesicles with the help of dynamin proteins. These vesicles are called macropinosomes and they are like small cells with one membrane around

them [28]. In receptor-mediated endocytosis, a part of the membrane is coated with clathrin or caveolin proteins followed by pit formation that bud from the membrane to inner side of the cell and form vesicles. Both these mechanisms are dependent on receptor presence on the surface of target cell.

Amp as anti-biofilm agent

Most studies report that AMPs are more effective in inhibiting biofilm development at early phases by killing planktonic bacteria prior to their attachment and by inhibiting bacteria adhesion, which serves as critical step in biofilm formation. The eradication of preformed biofilm is also attributed by AMP as it has ability to disaggregate the biofilm matrix by diffusing deep into layers of biofilm to kill the bacteria within. It is reported that the functionalization of AMP poly lysine to graphene silver conjugate causes biofilm disruption and inhibition by "contact-kill-release "mode of action [29]. WLBU2, a cationic antimicrobial peptide has significantly reduced viability of *Pseudomonas aeruginosa* biofilms with simultaneous reduction of viral pathogen Respiratory syncytial virus (RSV) infectivity in airway epithelium of cystic fibrosis and chronic obstructive pulmonary disease patients thus expanding its potential applications as antimicrobial therapeutic [30].

AMP as antiviral in other viral infections

The various antiviral mechanisms of action by AMPs include

- Direct interaction.
- Blockage of host cell surface receptors.
- Inhibition of viral fusion to host cells.
- Inhibition of viral replication.
- Activation of adaptive immune response.

Several AMPs such as Defensin serves as a therapeutic tool against Human immunodeficiency virus (HIV), Influenza-A virus, Human Adenovirus, Severe acute respiratory syndrome corona virus (SARS-CoV), Human papilloma virus, Respiratory syncytial virus (RSV), Herpes simplex virus (HSV) by direct killing action on viral particles or indirect inhibition at various stages of virus cycle [31]. P9 from AMPdefensin-4 was tested and exhibits potent and broad spectrum antiviral effects against multiple respiratory viruses in vitro and in vivo, including influenza [31]. A virus H1N1, H3N2, H5N1, H7N7, H7N9, SARS-CoV and MERS-CoV [32].

Multifunctional therapeutic potential of amp's includes immunomodulatory action by promoting host cell response for efficient pathogen killing like recruitment of monocytes, neutrophils, mast cells, and other immune cells by chemo attraction, regulation of cytokines, chemokines, and histamine release stimulation of fibroblast growth, vascular endothelium proliferation, angiogenesis, wound repair, promotion of apoptosis and clearance of infected cells neutralization of bacterial LPS(lipopolysaccharide), LTA(lip teichoic acid), and endotoxins and the expression of genes related tocell proliferation and cell adhesion in macrophage cell line. AMPs when administered at early infection stage can guarantee efficient protection against polymicrobial infections or in case of host inability to skillfully distinguish pathogens at target site [33].

Limitations of AMP

The main limitation of AMP is its physical stability under physiological conditions due to degradation by proteases and susceptibility to serum proteins, salt, pH because of the human fluids carrying high salt concentration that neutralizes AMP and restrict their electrostatic interaction to bind with bacterial cell membrane [34]. Another major limitation is its high production cost that uses sophisticated

technology concerning synthesis and purification of AMP. These limitations need to be overcome prior to their therapeutic uses.

Several modification strategies have been used to overcome the limitations of AMP by covalent conversions like cyclization (linking C-terminal and N-terminal of peptides), reducing cationic residue content, aminoacid change that inturn changes the physicochemical properties of AMP , non-natural aminoacids or D-aminoacids introduction into peptide structure, end modification of N-terminal and C-terminal through amidation, acetylation, phosphorylation, glycosylation, formation of disulphide bridges and also computer assisted modification methods for production of synthetic peptides is followed [35]. One of the promising approachesas antibiofilm, antiviraland AMRbuster is through functionalizing AMP on a multifunctional and versatile platform such as 2D materials as a fruitful strategy in theranostics (integration of therapy and diagnostics.)

Opportunities for nanomaterials

Nanomaterials offer boundless applications in various fields including medicine marking their astounding utility as nanotechnology. Recent advances in nanomedicine have identified novel and promising opportunities as advanced drug delivery system, new therapies, *invivo* imaging for diagnostic purpose. Nanomaterials possess idealistic biological and physiochemical characteristics enhances their chemical reactivities and bioactivities, a property which is absent in bulk counterparts. The antibacterial activity of nanomaterials cause irreversible damage to pathogenic cell membrane, proteins, and nucleic acids by various mechanisms of action. The presence of long plasma life and high surface to volume ratio facilitate them as targeting entities and drug loading platforms.

The pre-requisite characteristics of drug delivery vehicles

- High shelf life and stable formulation.
- Efficient drug incorporation and release.
- Biocompatibility.
- Bio distribution and targeting.
- Functionality.

The 2D nanomaterials are actively being investigated for biological and biomedical applications toprovide huge opportunity in various research fields. These materials aim to provide holistic approach in theranostics by not only acting as a versatile platform for carrying therapeutic agent but also in diagnosis by reporting the location, identifying infection and provision of information regarding the treatment responses and also their exploration in tissue engineering and biosensor applications.

Exploitation of 2D Nanomaterials as AMP delivery system

The 2D Nanomaterials tend to revolutionize antibacterial applications as a delivery system for potential antimicrobial peptides. The functionalization of nanoparticle surface with AMPs renders more resistance to their degradation, facilitates efficient delivery and also the peptides form a protecting shell on nanoparticles which reduce their toxic effect that in turn increases their biocompatibility. Thus, this approach not only is able to overcome the drawbacks of AMP but also provide a combinational effect of AMPs antimicrobial activity and diagnostic feature of 2D nanomaterial in achieving infection control.

Fabrication of 2D nanomaterials

The synthesis of 2D nanomaterials is by two approaches

- Top down process refers to successive cutting of bulk material to get nano-sized particles.

- Bottom up process refers to build up of material from bottom that is -atom-by-atom, molecule by molecule, or cluster by cluster [37].

Top down includes

- **Micromechanical cleavage:** this is a conventional method of fabricating thin flakes by exfoliating the layered bulk crystals through application of mechanical force using scotch tape, the applied force weakens the Vander Waals interaction present in between layers of bulk crystal without breaking the covalent bonds of each layer producing single or few layers of 2D crystal. This is widely employed method for graphene synthesis.
- Mechanical force assisted liquid exfoliation involves the applying of mechanical force on layered bulkcrystal dispersed in liquid phase while using amide solvents such as N-methyl-2-pyrolidone, N-cyclohexyl-2-pyrolidone and in some cases alcohol such as isopropanol. Based on the nature of mechanical force it can be sonication assisted, where sound energy is used along with liquid solvents that includes exfoliating agents (proteins, polymers) and stabilizing agents (surfactants) to separate into layers. Shear assisted liquid exfoliation uses shear force apparatus to generate high shear ratesbyrapidrotation speed.
- Ion intercalation assisted exfoliation intercalates ions (cations or anions) to weaken the interlayer Vander Waals bonds of crystal structurethen through agitation (thermal, shear, or ultrasound) causing exfoliation in specific solvent. Layered metal oxides, dichalcogenides are produced by cation (alkali metal) exchange assisted liquid exfoliation and LDH (layered double hydroxides) by anion exchange assisted liquid exfoliation.
- Oxidation assisted liquid exfoliation includes the modified Hummers method that uses strong oxidizing agents(Potassium permanganate, Sulphuric acid) to oxidize graphite to graphite oxide which generates abundant oxygen containing carboxy, epoxy, hydroxyl functional groups on the surface of each graphene layer followed by exfoliation.
- Selective etching is commonly used for synthesis of Transition metal carbides and MXenes (2D Carbides and Nitrides).

The two bottom up approaches for 2D nanomaterial fabrication are

- CVD (chemical vapor deposition) which is based on the formation of thin film or layer of precursor vapor on suitable substrate. This method produces high quality 2D nanomaterials. The decomposition of gas is induced by various energy forms like chemicals, thermal or photon and reacted on temperature-controlled surface forming thin films.
- Wet chemical synthesis involves low cost and high yield production of 2D nanomaterials especially non-layeredforms like metal oxides, metal chalcogenides by chemical reaction in solution. It includes hydrothermal synthesis, 2d oriented attachment, interface mediated synthesis, surface synthesis [35].

Classification and Physiochemical Properties of 2D nanomaterials

2D materials are single or few-layered crystalline materials with nanometer thicknesses possessing high degree anisotropy and functionality with diverse physicochemical properties.

These can be classified as

- Carbon-based nanomaterials, (graphene, graphene oxide, reduced graphene oxide, graphene, fluorographene, graphyne, graphdiyne

- 2D clay materials (clay minerals, laponites, layered double hydroxides)
- Transition metal based that includes (transition metal dichalcogenides (TMDs), transition metal oxides (TMOs))
- Black phosphorous (BP)
- Other Inorganic 2D materials like hexagonal boron nitride (hBN, graphiticC3N4, elemental monolayers like silicene, germanene, arsenene and antimonene.

Physicochemical Properties

Carbon based 2D Nanomaterials Graphene

This is the most explored nanomaterial possessing unique characteristics and versatile applications. Graphene is an allotrope of carbon consisting of single layer of sp2 hybridized carbons in a honey comb structure that can offer as potential drug carriers since both the sides of the 2d plane sheet are available for drug binding. The hydrophobic nature of graphene confers formation of irreversible aggregates due to pi-pi stacking and Vander Waals interaction thus preventing its utility in multifaceted applications. This aggregation can be controlled by functionalization (surface attachment of molecules) for electric, optic and chemical properties by covalent or noncovalent modification with increased solubility, biocompatibility, targeted delivery and *invivo* imaging [36]. Graphene oxide (GO) consist of carboxylic groups that render negative charge to surface and also has oxygen atoms connected to carbon network making it hydrophilic and more water dispersible and less toxic. It also serves as a good drug delivery system due to formation of pi-pi stacking and improves solubility of insoluble drugs.

Reduced graphene oxide (RGO)

Reduced graphene oxide has similar mechanical, optical, electronic or conductive properties to pristine graphene because it possesses graphene-like basal plane with additionally decorated structural defects and populated areas containing oxidized chemical groups. The reduction of graphite oxide to reduced graphene oxide causes removal of oxygen atoms that are connected to carbon framework reducing hydrophilicity and increasing hydrophobicity due to pi-pi stacking and Vander Waals interactions between RGO. The graphene-like properties make reduced graphene oxide a highly desirable material to be used in sensors, biological, environmental or catalytic applications as well as optoelectronic and storage devices. Graphene is fully hydrogenated form of graphene consisting puckered carbon layer with sp^3hybridized carbon atoms. It can occur either in boat form where hydrogen atoms alternate in pairs or the chain form with hydrogen atoms occurring alternate on both sides of carbon layer. It has wide applications in bio sensing, spintronic and hydrogen storage.

- **Fluorinated graphene:** This single layer of graphene fluoride occurs in chain form and does not prefer boat form. Fluorinated graphene has wide band gap that stimulates wide range of applications in electronic gadgets. It exhibits luminescence in UV-Visible light with optical properties similar to diamond. Graphene consist of linear acetylene chain that is connected to hexagonal carbon chains with carbon atoms in sp2 or sp hybridization. It has a wide tunable band gap and is used in transistors, Nano fillers and sensors.
- **Graphdiyne:** These are the 2d carbon framework possessing sp2 and sp hybridized carbon skeleton with high pi conjugation and porous nature with uniform pore distribution that enables to absorb biomolecules. Their electronic structure and arrangement of atoms provide attractive chemical, mechanical, optical and electronic properties.

- **2D clay materials:** Based on the layering of tetrahedral and octahedral structural units, clay minerals are classified into 1:1 and 1:2 types. In 1:1 clay mineral type each octahedral layer sheet is linked to tetrahedral sheets with stacking each layer one above the other by Vander Waals forces and weak hydrogen bonding. In 2:1, compound single octahedral sheet layer is sandwiched between two tetrahedral layers. kaolite belongs to 1:1 clay mineral and is less reactive, non-swelling and electrically neutral clay mineral. Laponites are 2:1 clay minerals consisting of octahedral layers of magnesium atoms sandwiched between tetrahedral layers of silicon atoms bonded with sodium atoms. Their high surface area and cation exchange capabilities have huge applications in drug delivery and as ion exchanger. Different functional groups can be used to functionalize laponite surface that is hydrophilic in nature by thiols, amines, long carbon chains. The silanol group present at laponite sheet edges can be covalently attached to organic functional groups. Layered Double hydroxides (LDH) are also called as anionic clay material and possesses characteristics like interlattice space, pH sensitivity and biocompatibility making their utility in drug delivery or gene transfer. Hydrophilic nature of the surface can be modified by incorporation of anionic surfactants on the interlayer to confer hydrophobicity that enhances interlayer space and holds them intact [37].
- **Transition metal dichalcogenides (TMDs):** These layered structures have transition metal layer sandwiched in between layers of chalcogen atoms held by Vander Waals forces. Depending on the arrangement of atoms, TMDs are trigonal prismatic, defect structure and octahedral. They exhibit good semiconductor properties and have good mechanical, electrical, optical properties for biomedical applications. They show strong photoluminescence making them ideal for sensors. In Transition Metal Oxides (TMOs), the electrons of transition metals are held strongly by oxygen atoms where the d orbital electrons of transition metal influence the physical, chemical and structural properties. They are used in biomedical applications as nanoelectric devices, targeted drug delivery and MRI (magnetic resonance imaging).
- **Black phosphorous (BP):** It is also known as phosphorene and is thermodynamically most stable allotrope of phosphorous having puckered honey comb structure with interlayer held by Vander Waals forces and interlayers by covalent bonds. The high charge carrier mobility, anisotropy, tunable direct band gap in near infrared region favors optical, mechanical, electronics and biomedical applications especially in drug delivery with characteristic light induced biodegradability. The lone pair of electrons present in BP reacts with oxygen to form oxides that readily reacts with water to form phosphoric acid and this result in BP degradation. This problem can be overcome by passivating the BP surface through surface modification using suitable ligands or encapsulation or by doping [39].

Other inorganic 2D nanomaterials

Graphitic carbon Nitride: this is relatively new member of 2D materials, an organic semiconductor that consists of alternating carbon and nitrogen held by strong covalent bonding and vanderwaals interactions pertaining to a highly stable monolayer structure. Their fascinating properties are tunable band gap, thermal stability; strong photo adsorption, high photosensitivity and biocompatibility make them wonderful option for biosensors. Recent approaches for its functionalization include incorporation

of metals (to manipulate optical and electric properties) and organic molecules (to maximize higher light absorption).

- **Hexagonal boron nitride (HBN):** It is also called as white graphene because of its structural similarities with graphene. It consist of honeycomb like structure with boron and nitrogen insp2 hybridization arranged alternatively forming hexagonal lattice. Tunable bandwidth falling in ultraviolet region makes them reliable for laser devices and high thermal and chemical stability of their surface area favors them for drug delivery. HBN also acts as biological probe.
- **Arsenene and antimonene:** are the monolayer of elements arsenic and antimony respectively. The corresponding elements when transformed to 2d monolayer counterparts changes its nature from semi-metallic to semiconductor and has good optoelectronic applications.
- **Silicene and germanene:** These two nanosheets have high structural stability, compressibility, flexibility, high proton scattering ability for various ultrafast electronicapplications but their exploration is still in infancy.

Functionalization of AMP onto 2D nanomaterials

Principle interactions governing functionalization

Both covalent and non-covalent interactions facilitate the conjugation of peptide biomolecules with 2D Nanomaterials. AMPs bind preferentially at the sites containing functional groups either at edges (or) at planes thereby modifying the spatialfeatures of nanomaterials. Interactions between peptides and nanomaterials undergoes complicated reactions because the nature of charge on surface functional groups of peptides depends on environmental conditions such as pH, ionic strength of buffer. AMPS posses negative (or) positive charges likewise there is variation in functional groups possessed by nanomaterials.

Functionalization of AMP on nanomaterial surface depends on the physiochemical properties on planar surface, its morphology, charge, polarity, energy. The outer surface of the 2D Nanomaterial is dynamic in nature due to its contact with environment and their outer surface properties are different from its bulk materials thus the surface characteristics changes on interaction with biomolecules by hydrophobic (or) hydrophilic nature and surface charge. The main forces stabilizing peptides are non-covalent interactions like hydrophobic, electrostatic and hydrogen bonding alongwith other covalent interactions. The degree and type of interaction involved in stabilizing peptide structure are different at various levels of organization. Secondary structures represented by **α-**helix can be stabilized by hydrogen bonds alone while beta sheets with combination of hydrogen bonds and hydrophobic interactions. The tertiary structure involves all hydrophobic interactions that form core of peptide, which becomes more rigid due to disulphide bridges, hydrogen bonds, and electrostatic interactions between side chain residues of peptide with Vander Waals forces due to induced polarity thus, strengthening the further tertiary structure of peptide.

The properties that nanomaterial surface imparts to peptide is hydrophilicity (or) hydrophobicity and its degreedepends on the number of such sites offered for AMP interaction. At atomic level hydrophobicity is determined by of polar functional groups (or) uncoordinated metal on outer surface layer. If the nanomaterial surface is hydrophobic then the non-polar residues hidden within peptide becomes exposed without

any contact with water and if the nanomaterial surfaces the interaction takes place directly by hydrogen bonds [40].

The important property of a surface that determines its interaction with biomolecules is its charge. If a nanomaterial with a charged surface is placed into an aqueous solution, an electric double layer consisting of two layers of oppositely charged ions informed on the surface. The first layer consists of ions adsorbed directly on the surface due to electrostatic forces, hydrogen bonds, coordination bonds, and Vander Waals interactions. The second layer is called the diffuse layer that is weakly bound to the surface by electrostatic forces composed of free ions that move in the fluid because of electric attraction and thermal motion but are not rigidly attached to the surface is called the diffuse layer. The potential drop across the mobile second layer responsible for electrokineticphenomena is called the Zeta potential, which is quantitative characteristic of the charged surface. Thus, the electrostatic interaction, hydrophobic interactions, hydrogen bonding and covalent bonding are principle bonds involved in functionalization.

Delivery of amp by 2D nanomaterials

The nanomaterial based delivery system must possess a high drug loading capacity attained either by Incorporation method that requires AMP incorporation at the time of nanoparticle formulation or Adsorption/absorption method where the AMP is loaded after the nanoparticle formulation this is achieved by incubating nanocarrier (nanomaterial serving as delivery system) with concentrated AMP solution. The 2D nanomaterial serves as an intelligent loading platform for AMP as well as the functional groups on their external surface serves as interaction points with immediate biological environment facilitating stimuli driven response.

There are two main objectives of drug delivery systems:

- **Drug targeting:** to deliver a drug to the desired location in the body facilitated by specific cellular binding and intracellular uptake of drug carrying nanomaterial by targeted cells.
- **Controlled release:** to deliver a drug at a desired rate for a desired length of time in its active form. Many drug delivery systems attempt either controlled or targeted delivery; some drug delivery systems attempt both. This drug targeted and control release delivery systems has many advantages like increased efficacy, site specific delivery, reduced toxicity, better patient compliance and potential for prophylactic application.

Mechanisms to Control Drug Release

According to the mechanism by which a drug escapes a carrier, the drug release can be classified into four categories (diffusion, solvent, chemical reaction, and stimuli-controlled release) [41].

- **Diffusion-controlled release:** In this method drug, diffusion is driven by its concentration difference across the membrane in which the drug first dissolves in the core then diffuses through the membrane. Matrix-type nanospheres where drug molecules are dispersed throughout the polymer matrix show a diffusion-controlled release profile.
- **Degradation controlled release**: Drug carriers comprising biodegradable polymers such as polyesters, polyamides, polyaminoacids and polysaccharides undergo this mechanism and release drugs through hydrolytic or enzymatic degradation of ester, amide, and hydrazone bonds. In this method the rate of drug release is dependent on rate of polymeric matrix degradation
- **Solvent-controlled release:** The solvent-

controlled release includes osmosis-controlled release and swelling control release. Osmosis-controlled release occurs in a carrier covered with a semi-permeable polymeric membrane, through which water can flow from outside of the carrier (with a low drug concentration) to the drug-loaded core (with a high drug concentration). Swelling control release systems are utilized by hydrogels, which are 3D polymeric networks, and when placed in aqueous solution like body fluids undergoes swelling of the polymeric particles followed by drug release.

- **Stimuli-controlled release:** In this mechanism, the release of drug from nanocarrier is due to response to specific stimuli, which is either exogenous (variations in magnetic field, temperature, light or electric impulses, ultrasound intensity) or endogenous (changes in enzyme concentration, pH or redox gradients). At cellular level, pH sensitivity not only triggers the release of the transported drug into late endosomes or lysosomes but also promote the escape of the nano carriers from the lysosomes to the cell cytoplasm. The microenvironment-associated changes in pathological situations such as bacterial infections, tumors, inflammatory diseases can be utilized for stimuli responsive delivery at tissue level. The 2D nanomaterial based AMP delivery is based on this mechanism that responds to specific stimuli by hydrolytic cleavage and molecular conformational changes in its structure for its target cargo release. Drug targeting involves passive targeting where the prepared drug carrier complex circulates through the blood stream and is driven to target site by affinity as binding influenced by properties like pH, temperature. In active targeting moieties like antibody, peptides are coupled with drug delivery system to anchor them to receptor structure expressed at target site, followed by application of external stimulus to the Nanomaterial to drive the release of nanomaterial-associated cargo. These are further classified based on exactly where the activating stimuli emerges.

Endogenous stimuli responsive drug delivery system

- Redox: responsive materials play a potential role in extracellular and intracellular areas of diseased tissues or cells for redox species with variable concentrations. The concentration of a reducing agent such as glutathione is two times greater in cellular cytosol and nucleus as compared to theintercellular (endosomes) and extracellular fluids. drug release is triggered by endogenous thiol molecules including glutathione (GSH)which cleaves the disulphide bond through reduction or disulphide exchange reactions. The pH-responsive system releases the drug on sensing lower acidic pH in microenvironment of bacterial infection through cleavage of drug conjugated bond like ester bond, amide bond, hydrazine bond by the following enzymes.

- Esterases and hydrolases in acidic endosomes and lysosomes.

- Certain hydrolytic enzymes overexpressed in the extracellular membrane or plasma, and, c) to a lesser extent, acid- or base catalyzed hydrolysis. Thus, acid-responsive release occurs after internalization into the acidic environments of endosomes and lysosomes (pH 5−6) rather than in the extracellular environment (pH >6) [42].

- Enzyme responsive drug delivery system exploits the presence of enzymes in the extracellular environment. Enzyme based dysregulation in diseased cells/tissues also provokes the drug delivery system to identify

altered expression of specific enzymes such as proteases, phospholipases or glycosidase observed in pathological conditions and release drugs from drug loaded carriers through enzyme assisted degradation of nanomaterial.

Exogenous Stimuli-Responsive drug delivery system

- Temperature responsive system utilizes external stimuli like magnetic field, light, ultrasound in response tointernal stimulus originated in diseasedtissue having a higher temperature (40C-42C) than that of normal tissue (37C) . These drug carriersretain drug in normal physiological temperature and release drugupon exposure of higher temperature of diseased tissue. This external stimulus will stimulate a sensitive drugcarrier component to produce heat, which, in turn, alters temperature-sensitive material present incarriers that lead to a burst release of drugs at the targeted site. Xie. et. al proposed a PEGylated SnS-based dual-function theranostic platform where SnS nanosheet was used as a drug carrier and a photothermal agent which induces hyperthermia [43].

- Electrical-Responsive system uses a weak electric field applied over targeted tissue after administration of electro responsive drug carriers for controlled on-site drug release. Different mechanisms for controlling drug release by electrical stimulation includes oxidation-reduction reaction, disruption structure of carriers, and stimulation of thermo-responsive carrier through electrically-produced heat [44].

- Ultrasound responsive drug delivery system possess many advantages like safety, tissue penetration, non-invasiveness, and better spatiotemporal control. Ultrasound waves have been explored as an external stimulus where the thermal, mechanical effects and radiation forces produced by ultrasound waves are responsible for stimulating target control drug release from carriers.

- **Magnetic responsive drug delivery system:** The magnetic field can penetrate effectively in the body tissue and is commonly employed for body imaging MRI (magnetic resonance imaging). Apart from imaging, controlling drug release from magnetic field responsive carriers through external magnetic field stimulus have also been explored. The magnetic field induced hyperthermia for drug release and the magnetic field guided drug targeting.

- **Photo/light responsive system:** also controls drug release. However, due to poor penetration, visible and UV light are not considered suitable for in vivo applications pertaining to therapeutics while the NIR range is the potential source of light for controlling drug release due to safety and better tissue penetration. Three different mechanisms for drug release from NIR responsive systems are the photo-thermal effect, two-photon activation, and upconverting nanoparticles. In addition, Yuan *et al.* fabricated a functional MoS_2/PDA-arginine-glycine-aspartic acid (RGD) to inhibit bacterial infection *in situ* and improve Osseo integration and found that NIR-induced hyperthermia of MoS_2/PDA-RGD samples could efficiently increase GSH oxidation. Besides, the intrinsic ROS-independent oxidative stress of MoS_2 nanosheets could also destroy the integrity of the bacterial membrane, finally significantly killing bacteria. After NIR irradiation, the antibacterial efficiency of MoS_2/PDA-RGD reached 96.6% for *S. aureus* and 97.8% for *E. coli*, which is ascribed to the synergism between PTT (photothermal therapy) and oxidative stress [45].

Mode of Administration

The major parenteral routes for AMP-Nano conjugate delivery system is through intramuscular, intravenous or subcutaneous injection into the systemic circulation. However, in order to overcome limitations with needle-based administration and improve patient satisfaction and compliance there is a need for non-invasive methods like Mucosal (oral, nasal, pulmonary) and transdermal administration, which are generally painless and simpler than traditional injection.

Technologies

The oral delivery uses the oral route that is patient-friendly, as solid tablets or liquids can simply be non-invasively ingested, increasing patient compliance. Transdermal patches are utilized in transdermal delivery that offer painless alternative to parenteral injections, but their application is generally limited to small hydrophobic drugs such as AMPs this is because large or hydrophilic molecules are not easily absorbed into the skin because of the very low permeability of the stratum corneum. Nasal and Pulmonary routes delivery involves the respiratory tract which offers several advantages for peptide delivery because the local proteolytic activity in both administration routes is relatively low compared to the gastrointestinal tract and it is easy to elicit strong immune responses in both routes and also they require lower doses of drug compared to the oral route [46].

Current challenges and future perspectives

The delivery of AMP through 2D Nanomaterials can serve as a great stride promoting solution towards multidrug resistance not only to biofilm associated bacterial infections but also other novel viral infections that possesses antimicrobial resistance as a hidden threat. The multifunctional aspects of AMP and 2D Nanomaterials provides add on value and opens up room in the realm of nanotheranostics. The main challenges includes fabrication of 2D Nanomaterials in a controllable manner, which involves understanding their growth mechanism and development of reliable and effective characterization techniques. Their easy oxidation under ambient conditions, irreversible aggregation, and systemic toxicity concern raises questions for their advanced utility. The synthesis of natural AMP as well as synthetic peptides through computational approaches requires a high cost input and sophisticated technology. The functionalization of AMP to 2D Nanomaterials demands a deeper understanding on effects of structural modification on physiochemical characteristics to derive a structure function relation for their efficient activity towards targeted spectrum and to achieve improved pharmacokinetic profile. As the current US FDA Regulations are no longer appropriate to ensure safety and quality of the emerging 2D Nanomaterials there exists a desperate need of different regulatory framework in field of nano medicine to fill the potential gap between preclinical, clinical and post marketing phase. The correlation of *invitro* and *invivo* studies plays a significant role for translation of these nanomaterials in clinical trials, which is not easy, as it requires suitable animal models to simulate the original purpose of study. To surmount all these limitations the straightforward solution is promotion of extensive research in this understudied area by close collaboration of different scientific communities across the world.

The recent suggestion by WHO highlights the fact that antibiotics don't treat (or) prevent viruses as they work only against bacterial infections [47]. This opens new possibilities of AMP that exhibits broad range of activity against bacteria, fungi, protozoa, viruses as its utility can be explored against the recent pandemic Coronavirus disease 2019 (CoVID-19.) which

is caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [48]. It was first identified in December 2019 in Wuhan, China and the infection is spread primarily by small droplet transmission through coughing, sneezing or talking in close contact with infected people , affecting 212 countries and territories around the world.

It has been reported that AMP Defensin serves as a therapeutic tool against Human immunodeficiency virus(HIV), Influenza A virus, HumanAdenovirus, Severe acute respiratory syndrome corona virus (SARS-CoV), Human papilloma virus, Respiratory syncytial virus(RSV), Herpes simplex virus (HSV) by direct killing action on viral particles or indirect inhibition at various stages of virus cycle [49]. Thus, this novel promising approach is a powerful arsenal to harness wide range of microbial infections.

Conclusion

This review attempts to provide an insight about how the established biofilms and novel viral infections contribute to multidrug resistance with 2d Nanomaterials functionalized AMP as its powerful arsenal. It focuses mainly on the utilization of 2d Nanomaterials as delivery vehicles to achieve targeted action on bacterial biofilm associated infections and image guided therapy along with their mechanism of delivery. The different mode of actions of AMP along with their auxiliary roles in immunomodulation, angiogenesis, wound repair, promotion of apoptosis encourages its real utility. On the other hand, the 2D nanomaterials intrinsic antibacterial activity, favorable physiochemical properties, enhanced bacterial targeting and permeability increases their probability in biomedical applications. Therefore, this idea is unprecedent and relies in combining the strengths of these two major entities holding valid functionalization, optimized antibacterial activity and attractive biocompatibility acting as a prudent approach in combating multidrug resistance and biofilm related infections.

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