THERAPEUTIC POTENTIAL OF NANOSCALE ASSEMBLIES - A COMPREHENSIVE APPROACH

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Abstract

The current advancements in nanobiotechnology are reflected through the development of novel materials with diverse applications in many fields including health, pharmaceuticals and food, etc. This review article updates the potential of nanoscale biological assemblies in therapeutics and diagnosis. Nanoscale assemblies are considered the functional form of the molecules that are used in gene delivery and gene silencing, nanolithography, fabrication of conductive nanowires, cell targeting, bioimaging, drug delivery, purification, detection and proteins delivery, gene transfection, colorimetric detection of copper ions and melanoma therapy. The biomolecular assemblies also play an integral part through short-lived microscopic activities like guiding genetic code transcription, gene translation and signaling between and within cells to construct long-lived macroscopic structures like collagen networks and amyloid plaques.

Key words: Biological assemblies, Drug delivery, , Gene therapy, Nanobiotechnology, Nanoparticles

Introduction to biological assemblies:

Self-assembly is a random molecule organizing mechanism that exists abundantly in biological environments, promoting the creation of bio-macromolecular superstructures of sufficient size and complex roles. Their consistency, flexibility and flexibility in planning make such a self-assembly process desirable for nano-device manufacturing (Medina-Bailon *et al*., 2021). There have been significant attempts to build self-assembled nanoparticle superstructures. Also, nanoparticles can be arranged in a series and concurrently into ordered structures that provide them with new developments. The self-assembly process is ongoing for most nanoparticles, which form into the solutions, till all elements are depleted, culminating in a growing polydispersion of structure in the method. Furthermore, the method of self-assembly is controlled primarily through thermodynamics and is therefore incapable of manual interference. Therefore, it is not possible to measure the scale of the self-assembled superstructure. Besides that, physical forces, like van der Waals forces, electrostatic forces, hydrophobic forces, magnetic interactions and entropic influences push in several instances the self-assembly processes for nanoparticles. Due to providing a high degree of symmetry, accessibility for adjustment or structural homogeneity, protein nanoparticles are superior to the design of regulated self-assembled superstructures. The protein surface may be genetically engineered without disrupting their structural stability, with functional ligands (Men *et al*., 2015). Self-assembly is a powerful bottom-up approach (Gao *et al*., 2016). Examples of biological assemblies include liposomes, nucleic acid-based nano assemblies, virus-like particles, ferritin protein cages, protein-based organelles in bacteria and eukaryotic cells, designed protein nano assemblies (Yadav *et al.,* 2020).

Applications of biological assemblies:

Almost in every life cycle, biomolecular assembly plays an integral part, through short-lived microscopic activities like guiding genetic code

transcription, gene translation through proteins, and signalling between and within cells, to constructing long-lived macroscopic structures like collagen networks and amyloid plaques. These could be used for medicinal uses, like therapeutics, vaccines, bioimaging, and gene therapy (Siddique and Chow, 2020).

Gene delivery and gene silencing:

Cationic liposomes (CLs) in gene delivery and gene silencing therapies are synthetic vectors of nucleic acids. RNA molecules with two nuclear overhangs at end 3' (usually from 19 to 25bp) have proven complicated to carry RNAi downstream components to control sequence-specific gene silencing. The detection of RNAi pathways has already been defined by functional genomics, and medicine is quite interesting. A few of the key vectors for siRNA transmission remain cationic liposomes. While non-viral gene therapy becomes highly exciting, the difficulty of developing stable programmable supply vectors for *in vivo* applications still has major clinical consequences (Ewert *et al.,* 2021).

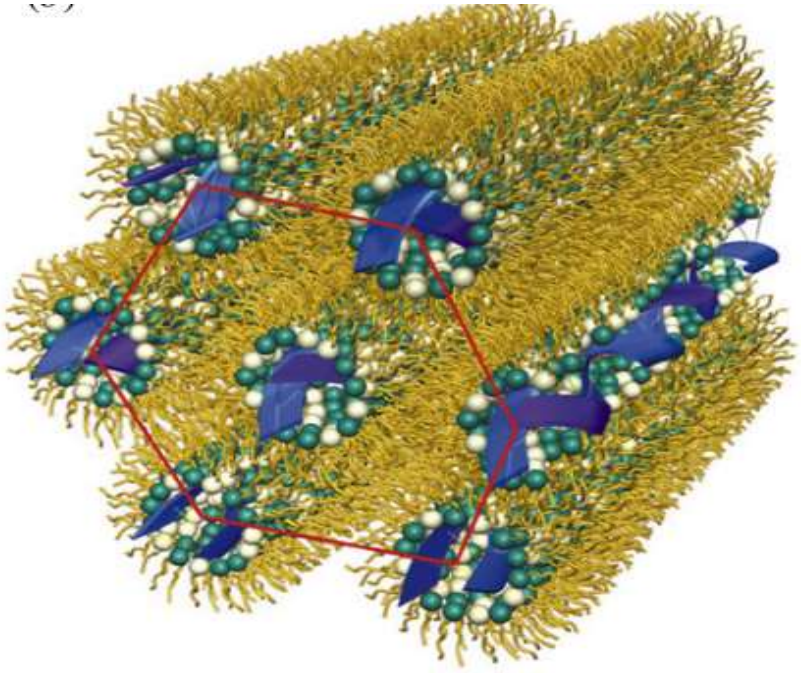

Fig 1**.** CL-DNA complexes are inverted hexagonal HC II forms, consisting of DNA injected into reverse lipid tubules organized on just a hexagonal lattice (Majzoub *et al.,* 2016).

The Use of S-layers for nanolithography:

Sleytr, Schuster, Egelseer, & Pum, 2014 have shown that S-layer subunits can be recrystallized onto diverse substrates appropriate for nanofabrication, like silicon and silicon oxide wafers, as well as on lipid membranes or even at the air-water interface. It has been shown that such fantastic self-assembled nano-structures are quite effective in lithographic purposes. Extracted basic elements of the S-layer readily reconfigure *in vitro* with well-ordered two-dimensional crystals. For efficient structures of nanoparticles, for example, semiconductors and metallic dots, as well as for biomolecules like enzymes and antibodies, S-layers are most often used as joining templates. As mentioned, the process is:

- The use of crystal-line array as layering medium for the Nano-scale lithography enabled such a method of assembly and molecular interaction.

- It has been shown that ultraviolet radiation can be used to model S-layers.
- S-layers placed on just a silicon wafer are immediately placed in direct contact with such a photomask or

subjected to ultraviolet irradiation. This lithographic layer layering, along with the unique layering of inorganic molecules and biomolecules, can help lab-on-a-chip to be generated (Liu *et al*., 2021).

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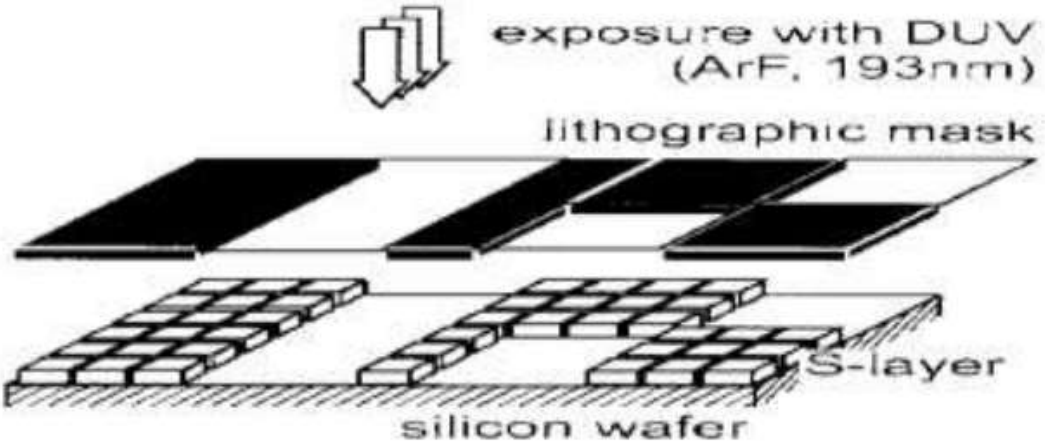

Fig 2. Schematic drawing of the partitioning of s-layer on the silicon wafer by using a Duv radiation and laser

The use of DNA for fabrication of conductive nanowires:

DNA has been used with unique identification details incorporated as just a nano-wire. To shape a smooth metallic wire on the double-stranded DNA template, the researchers were using a silver deposit and enhancing technique. The wire was roughly 100 nm in dimension and has also been found to have ohmic conductivity. The capacity of the DNA to also act as just a carrier of identifying data that helps guide the DNA strand to particular positions, which would be a critical aspect for both the accomplishment of self-assembled electronic circuit, is among the major benefits of DNA-based wires (Linko and Jussi Toppari, 2013).

RecA protein for "molecular lithography:

The RecA protein attaches the DNA uniquely to the particular sequence which protects the same area from being metalized.

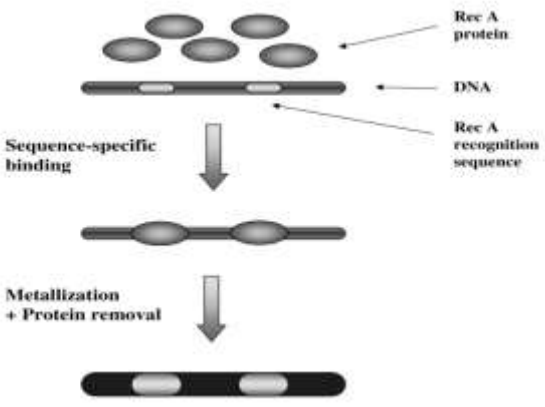

Fig 3. Application of RecA protein in "molecular lithography" (Keren *et al*., 2002).

Cell targeting:

The goal of cell targeting would be to achieve high therapeutic or other clinical reagent absorption in such a favorable position including a tumor as well as a higher blood / normal tissue tumor ratio. Throughout this manner, adverse health risks will be minimized and medicinal/prognostic performance increased, which are critical objectives in the care of various diseases. Another approach to achieve targeting would be to modify the nanoscale assemblies' physicochemical characteristics (surface topography and charge) to enable their intracellular distribution. Proteins, peptides, nuclear acids (aptamers), small molecules or vitamins or starch may also be used to attack proteins (primarily antibodies or substructures). Specificity for cell targeting is acquired by inserting targeting monomers through receptor-mediated endocytosis. For example, a Hepatocellular carcinoma (HCC) target peptide (SP94) was chemically bound to bacteriophage MS2 VLP. For this reason, the HCC is a target peptide. It has enabled selective delivery to HCC of nanoparticles, chemotherapy, siRNA cocktails and protein pollutants. A key obstacle in the cell-targeting area is the development of targeting ligands, that promote cellular uptake. To extract unique prostate cancer (Pc) internalizing aptamers, a novel tissue-uptake selection technique has recently been introduced. Aptamers are small RNA or DNA oligonucleotides which, with high binding affinity and specificity, assemble towards three-dimensional sequences (Zhu *et al.*, 2021).

Drug delivery:

Every available space within the nano assemblies is beneficial for encapsulation of chemotherapeutics, immunotherapeutics and nucleic acid, etc. Genetic and chemical variations are still at the center of efficient encapsulation for targeted drug delivery, whether the nano assembly is rod-shaped or spherical. Vault nanocapsules, for instance, include hollow cavities which can be used as carriers of the chemokine (C-C motif) ligand 21 (CCL21). These CCL21-carrying vaults suppress lung cancer development after a single intra-tumoural treatment in rodents (Kar *et al*., 2011). This should be remembered because when concerned with therapeutics, there are many other basic concerns to address, like the path of treatment, transportation of drugs in tissues and cells, body clearance, drug tolerance, the spread of doses and overall drug deposition to target tissues, toxicity and antigenicity and, ultimately, dosage, different doses as well as a timetable of administration. The drug's encapsulation inside the nano-container also influences the drug's absorption, delivery, and metabolism and may result in safer, more patient-friendly care protocols. For different distribution uses, liposomes may act as closed-caged nano-containers (Alqahtani *et al*., 2021).

Bioimaging:

Bioimaging strives to visualize specific molecular processes using bioimaging probes. Over the last few decades, a broad variety of imaging techniques were applied successfully in biomedical areas. Polymer-based bioimaging samples also dramatically improved reliability, decreased sensitivity and increased target precision, rendering them attractive competitors and in coming years towards selective bioimaging. Particularly, supramolecular-designed polymer bioimaging probes have shown significant benefits mostly in the treatment/medication of traditional polymer probe-compatible tumors (Li *et al*., 2021).

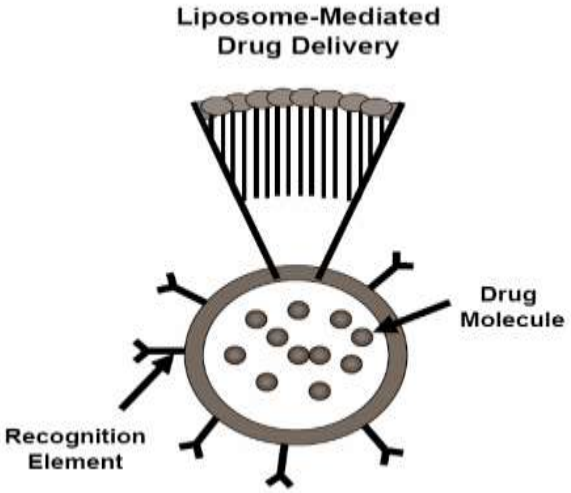

Fig 4. Application of the phospholipid liposome in drug delivery.

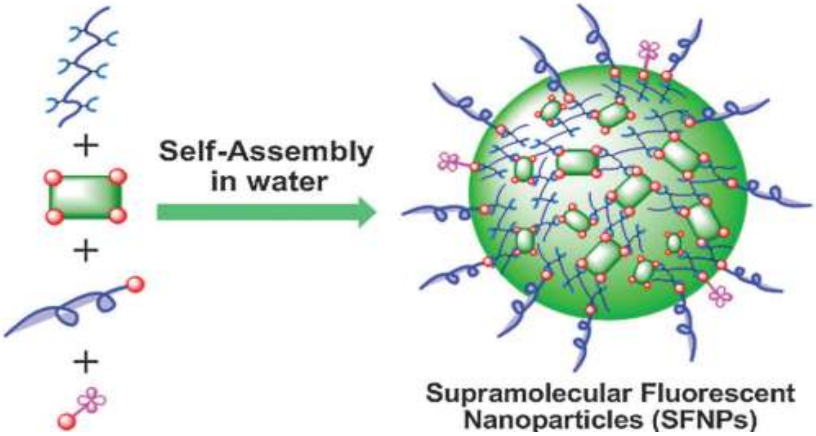

Fig 5. Fabrication of SFNPs (Wang *et al*., 2014).

Cationic antimicrobial peptides:

The antimicrobial cationic peptides can kill multi-drug-resistant bacteria, therefore, they are also of concern. Many peptides construct α-helices and β-sheet like configurations which could be injected onto negatively charged bacterial cell surfaces and eventually decompose them. The antimicrobial core-shell-shaped nanomaterials self-assembled mostly from amphiphilic peptide CG3R6TAT were engineered and synthesized by Liu *et al*. (2009) They also demonstrated its antimicrobial activities were greatly improved by the production of nanoparticles. Such nanoparticles have a wide variety of antimicrobial actions that effectively limit the formation of different forms of gram-positive and drug-resistant gram-positive, medium-minimum inhibitory concentrations (MIC) bacteria, fungi and yeast, but cause comparatively low hemolysis. Those who include, however, a high therapeutic index (50) against *Staphylococcus aureus* infection inside a mice form. In addition, such peptide nanoparticles will cross the blood-brain barrier (BBB) in the *S. aureus*-induced template of rabbit meningitis suppresses bacterial development within the brain. Importantly, such nanoparticles do not always induce serious toxicity to the main organs, suggesting that they could include an effective antimicrobial agent within the diagnosis of brain illnesses. They can also be used for certain bacterial infections, along with diseases infected

with MRSA and *C. albicans* infection of the brain (Liu *et al*., 2009).

Protein Purification, Detection and Delivery Through HBP Assemblies:

The hyperbranched polymers (HBPs) are a subclass of dendritic polymers. The core structure is globular dendritic units that are highly branched with a 3-D architecture. It is possible to deliver proteins using HBPs. For bovine serum albumin (BSA) delivery, Zhang and colleagues in 2009 utilized self-assembled HPG-star-PLA micelle. The results showed up to 23% and 86%, respectively were the loading capability and connection performance. A rapid impact accompanied by a sustained release of bovine serum albumin (BSA) was seen in the protein release profiles, as well as the emitted BSA should stay in its original structure for four days. To achieve protein cleansing and identification, HBP assemblies have also been used. When compared with the linear analogs, HBP assemblies provide unique advantages in supramolecular self-assembly and biomedical applications (Zhou *et al.*, 2010).

Gene Transfection of HBP/DNA Polyplexes:

Gene therapy provides a novel means of treating numerous human illnesses, like hereditary abnormalities and tumors, that is now one of mainstream molecular medicine's increasingly evolving guidelines. The injection of external nucleic acids into the genome of targeted cells is indeed a shared goal in gene therapy. Provided whether free oligonucleotides and DNA are quickly destroyed through serum nucleases throughout the blood while administered intravenously, it is also invaluable to shield genomic information against decay by self-assembling them via gene vectors. Polycationic vectors display many notable benefits compared with viruses and cationic liposomes, like high protection, low immunogenicity, unregulated trans-gene density, low synthesis expense and fast scale-up. The self-assembly in cationic polymers and genetic materials has, thus, drawn growing interest. Cationic HBPs incorporate several amino groups with such an extremely unusual branch stability and large molecular versatility between various cationic polymers, that significantly enhances the passage of genetic materials across different cellular obstacles as well as targets the nucleus in cells (Zhou, Huang, Liu, Zhu, & Yan, 2010).

Biomedical and electrical applications of Graphene-like 2-D nanosheets:

A novel bottom-up technique to turn lignosulfonate into graphene-like materials has been successfully developed (Wang *et al*., 2019a). Significantly, this method is simple to handle the output of 2-D graph-like flakes. During this process, 2-D nanosheets based on lignosulphonate were first developed by self-assembly in the water/acetone dual solvent system. The nano-size rod-like was made of amphiphilic lignosulfonate, powered by α-α interaction and hydrogen bonds by the addition of antisolvent, and these aggregates are spontaneously paralleled to form faulty flak. Due to stacking, eventually, the layers were constructed on Lignosulfonate with a smooth surface. After carbonization, assembled nano-sizing flakes were seen to be significant in the development of graphene-like designs and local defects were reduced in the graphitized lignosulfonate-based nanosheet structure with increasing carbonization temperature. This study opens up a promising area for the development of graphic materials in biomass-based lignin (Yao *et al*., 2020).

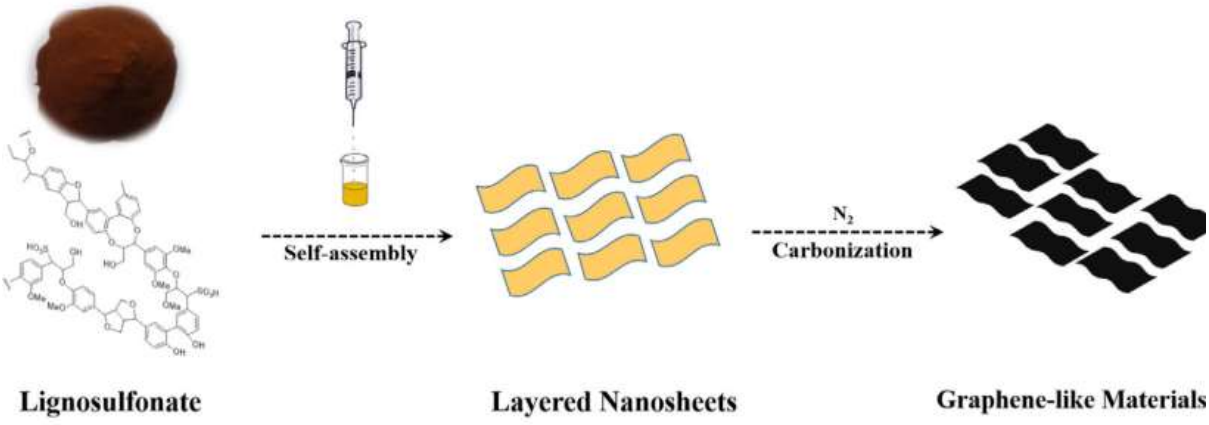

Fig 6. Manufacturing procedures of graphene-like nanosheets based on lignosulfonate (Wang *et al*., 2019b).

Colorimetric detection of copper ions:

Molecule-coated nanoparticles are composite materials with novel properties and can be engineered. The molecular coating of metal nanoparticles will help chemical flexibility and make it possible to mount nanoparticles necessary for applications such as biosensor systems. Recently, a colorimetric assay was used for the identification of metal ions, such as Pb^{2+}, Hg^{2+} and Cu^{2+}, using practical golden nanoparticles (GNPs). The ICPMS technique to detect metal ions has been developed, however, and the development of a new analytical instrument for cost-effective and easier detection of metal ions remains a challenge. It is advantageous to achieve this goal through the assembly of specific nanostructures that have been demonstrated to facilitate plasmon coupling. One of the nanostructures, which consists of a dielectric core and metal satellites, was registered as a composite form of raspberry. This central satellite nanostructure was improved by plasmon coupling between gold particles in the shell. The linear relationship between Cu^{2+} concentration and absorption has been achieved, further suggesting its ability to classify and quantitatively evaluate certain ions reliably and highly sensitively (Weng *et al*., 2013).

Peptide amphiphiles for skin care:

Peptide amphiphiles can serve as surfactants. They are often biological, natural and can be used directly as nutrients, such as anti-wrinkles, antimicrobial actions. So, they have great potential for aesthetic applications. The standard lipopeptide is Palmitoyl pentapeptide-3 or 4, with a C16 acyl chain as tail, and a KTTKS peptide sequence as a head. The small peptide is a structural replica of part of the type I collagen sequence. The fatty acid tail binding greatly strengthens its oil solubility and enhances skin insertion. The peptide sequence stimulates synthesis for important skin matrix components such as collagen, elastin and blood glucosaminoglycan when used in the culture of fibroblast cells. In several anti-ageing drugs, lipopeptide has been used and has proven efficacy against wrinkles without skin irritation. The addition of C16-GHK can probably promote the replenishment of the skin matrix and minimize wrinkles (Zhao *et al*., 2010).

Retinoid Nanoparticles for Melanoma Therapy:

Retinoic hydroxamic acid (RHA), an intermediate of histone deacetylase (HDAC) is an all-trans-retinoic acid (ATRA) fusion therapeutic agent and vorinostat with a synergistic effect on ATRA cancer care. While RHA was synthesized in 2005, its nanoscale self-assembly characteristics, anti-cancer function and potential related mechanism remain unknown.

The decreased proliferation is usually regarded as a result of cell cycle arrest and/or cell death. The alteration of cell cycles and apoptosis of A-375 treatment nanoparticles RHA have been identified explaining the proliferative inhibition process of A-375 induced RHA nanoparticles. A dose-dependent cell cycle arrest is shown by RHA nanoparticles. RHA nanoparticles, in comparison, have the property of low systemic toxicity (Liao *et al.,* 2019).

Future perspectives:

The research of nanoscale assemblies depends on scientists' experiences and coordination in a multitude of areas, from organic synthesis to protein engineering and beyond. The convergence of biology, physics and chemistry. The rich sizes and types that can be generated are amazing if the building block of a nanoscale assembly is a polymer or a biomolecule. This refers not only to the scientific interest and evidence of concepts but also to the potential application of nanoscale biomedical assemblies. For biomedical applications, there is a clear trend towards smart multifunctional nanoparticles (Aflori, 2021).

Conclusion:

Self-assembly is a random system wherein disorderly molecules via intramolecular and intermolecular noncovalent interactions are self-organized via higher-ordered two or three-dimensional stable nano to macrostructures (Mahata and Mandal, 2018). An important technique to create novel functional nanomaterials is the self-assembly of nanoparticles into broader superstructures, since these super-structures exhibit aggregate properties which are distinct from those exhibited through individual nanoparticles and bulk samples. Mostly on the nanometer scale, DNA nanotechnology has reshaped the capacity to impact and manipulate three-dimensional structures. Both for cross-disciplinary research and uses, designer sensors, nanopores and ion channels designed from DNA provide tremendous potential. DNA chemical functionality has started opening avenues to turn rigid DNA structures through dynamic nanomechanical sensors.

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