

Chapter 6

Benefits of Molecular Medicine from Self-Assembled Nanostructured Materials



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6.1 Introduction to Self-Assembled Nanostructured Materials

Democritus, a Greek philosopher in 400 BC proposed that the earth and solar system have emerged from atomic organization. A French philosopher Descartes hypothesized that as per the natural laws of physics, the universe had emerged from self-structure, i.e., organization of small components into large assemblages. Later in 1935, Langmuir and Blodgett developed a method to form a closely packed monolayer of amphiphilic molecules on solid and liquid surfaces. Bigelow observed the assembly of a monolayer of long alkylamine chains on a solid surface in 1946. Self-assembled monolayers (SAMs) were the initial terminology for self-assembly since 1983 when Nuzzo et al. developed well-ordered monolayers of alkanethiolate molecules chemisorbed on gold surfaces. Currently, nanostructures are fabricated via self-assembly (Castillo-León et al., 2011).

Self-assembly is the characteristic assembly of biological structures such as atoms, molecules, or nanoscale building blocks from their component parts into structured, non-covalent aggregates through random molecular movement. The individual components get organized without any human involvement, for example, the phospholipid bilayer of the cell membrane and the formation of bacteriophage

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and viral particles (Ariga et al., 2019). Molecular self-assembly occurs through weak noncovalent bonds like electrostatic interactions (ionic bonds), hydrogen bonds, hydrophobic and hydrophilic interactions, water-mediated hydrogen bonds, and van der Waals interactions resulting in structurally and chemically stable structures. These forces and their kinetic pathways are controlled by specific properties such as charge, binding affinity, and hydrophobicity. Best example of natural molecular self-assembly is a cell that is formed by biological molecules like proteins, peptides, nucleic acids, lipids, etc. with self-assembly of complementary properties (Wang et al., 2019). Molecular self-assembly is also a prominent feature of antigen–antibody recognition, amyloid fibril formation, and chromatin assembly. The orderly arrangement of the constructs in the self-assembly is dependent on the shape, size, and surface properties of the blocks (Zhang, 2002).

6.2 Description of Nanostructures Formed by Self-Assembly

Self-assembled peptide and lipid systems have been described extensively in the literature due to their wide bionanotechnology applications. The self-assembly of peptide and protein systems forms nanostructures such as nanofibers, nanotubes, vesicles, helical ribbons, and fibrous scaffolds. It is the most promising practical low-cost and high-throughput approach for nanofabrication (Delfi et al., 2021).

6.2.1 Peptide Nanostructures Formed by Self-Assembly

6.2.1.1 Nanofibers

Nanofibers as nanostructures have drawn great attention as building blocks for their application as the next-generation biosensors. They have been used along with different structures in a self-assembly configuration. Insoluble amyloid fibrils made of aggregates of beta sheets of amino acids are associated with rare pathologies such as amyloidosis. However, this insoluble property has been made use of in biosensor fabrication as this will ensure long-term stability of the sensor (Jeevanandam et al., 2018).

6.2.1.2 Nanotubes

Functionalization of peptide shell-coated metal nano-wires and drug-filled caviated structures can be performed using nanotubes. They can be formed using various types of monomers like linear and cyclic peptides, disc-shaped motifs, etc. (Gao & Matsui, 2005).

6.2.1.3 Nanoparticles

They cover structures such as solid structures as well as nanospheres with hollow cores. These hollow nanospheres can be a possible drug delivery system while the non-hollow nanospheres can be used as the biological counterpart of nanobeads.

6.2.1.4 Nano-tapes

Peptide beta sheets are stacked together to form peptide nano-tapes. Hydrogels are formed when their concentration increases above a certain limit (Lee et al., 2019).

6.2.1.5 Hydrogels

Hydrogels are materials with the properties of a gel depending on variables such as ionic strength, pH, salinity, and temperature.

6.2.1.6 Hydrophobic Dipeptides

They can be self-assembled to form nanofibers or nanotubes based on their condition during the formation process. It includes dipeptides such as diphenylalanine. Spherical nanostructures can also be formed when a protective group is added to the amino group of the dipeptide structure. These structures have hydrophobic side chains attached to both the amino groups of the dipeptide because of which they are named “hydrophobic dipeptides”. These interactions take place due to hydrophilic-hydrophobic reactions along with stabilization through hydrogen bonding in between the peptides (Ahmed, 2015).

6.2.2 Lipid-Based Self-Assembled Nanostructures

6.2.2.1 Liposomes

They are phospholipid vesicles that contain concentric bilayers of lipid which in turn contain aqueous spaces. Liposomes have been used as drug delivery agents since the mid-twentieth century in both pharmacy and medicine. The commonly used liposomes are cholesterol and phosphatidylcholine. They can encapsulate compounds like glucose, peptides, or proteins. Hydrophobic compounds are stored in the bilayers of the vesicle whereas the hydrophilic compounds are stored in the aqueous compartment. Liposomes require vital geometrical analysis for self-assembly and aggregation. Based on their size and the number of bilayers present, they can be classified into multi-lamellar liposomes (MLV) and large and small

unilamellar vesicles (LUV and SUV). The quantity of the drug loaded, the permeability of liposomes, and their half-life can be varied by modifying these parameters. Another classification of liposomes includes first, second, and third generation of liposomes. The first one consists of a liposome with no modifications, like cholesterol and phospholipids. But, one of their disadvantages includes accelerated elimination and hence hampers their bioavailability. In the second generation of liposomes, the liposome group is coated with unreactive polymeric molecules like glycoproteins, oligosaccharides, PEG, etc. This coating increases their half-life and bioavailability, due to their hydrophilic nature. In the third group, antibodies or peptides are attached to the liposome or PEG compounds, which in turn improves the efficacy of the drug. Various techniques to characterize the preparation of liposomes in the laboratory have been developed which include hydrating a previously dried lipid film with an aqueous solution, reverse phase evaporation method, solvent injection technique, sonication, and homogenization. Liposomes have made a huge impact on medical care, but regulatory and clinical limitations are still a challenge. Some of the regularly used liposomes include Ambisome (injectable liposomal formulation of amphotericin B), Doxil (liposomal doxycycline), Marqibo (liposomal formulation of vincristine), Visudyne (lyophilized formulation of liposomal verteporfin), etc. (Tan et al., 2019).

6.2.2.2 Solid Lipid Nanoparticles (SLN)

They are nanoparticles that are formed from a lipid matrix that stays in the solid state at room and body temperature. They provide protection of the incorporated drug from degradation, physical stability, and controlled release with low cytotoxicity, they do not require the use of organic solvents for their assembly, and the synthesis process is cost-effective and readily performed on a large scale. A few of their limitations include drug expulsion during storage and drug-loading capacity. Formulation techniques for encapsulation of drug solutions into SLN can be done using high-pressure microemulsion formation, homogenization, ultrasonication, solvent injection (or solvent displacement), phase inversion, emulsification/solvent evaporation (precipitation), multiple emulsion technique, and the membrane contractor technique. The readily used formulations of SLN includes Acyclovir-loaded, SLN as carriers of glibenclamide, SLN-based stearic acid, and injectable soya lecithin, etc. In general, SLNs act as carriers of poorly water-soluble drugs to improve their bioavailability and therapeutic potential (Scioli Montoto et al., 2020).

6.2.2.3 Lipid Nano-capsules (LNC)

Lipid nano-capsules have the advantageous properties of both lipid delivery systems and colloidal particles. They contain three principal constituents which include an oily phase, an aqueous phase, and a non-ionic surfactant. These constituents can

be varied which in turn changes the stability and formulations of LNC. They are a hybrid between liposomes and polymeric nano-capsules because their structure is similar to lipoproteins. LNCs have good stability in contrast to liposomes which are unstable and leaky in body fluids. The formulation technique needs soft energy process and is free of organic solvents. LNCs can be used for targeted drug delivery as a therapeutic and diagnostic cancer agent (Huynh et al., 2009).

6.2.2.4 Microemulsions

They contain solutions with self-assembly systems dispersed disorderly. The internal structure contains both hydrophilic and hydrophobic regions. These microemulsions are consisting of water, oil, and amphiphiles (surfactant, usually in combination with a cosurfactant) optically transparent and isotropic nanostructured solutions, thermodynamically stable systems. They can be classified, according to Winsor, into, Winsor I (oil-in-water) (o/w) ME; Winsor II (water-in-oil) (w/o) ME; Winsor III (bi-continuous or middle self-assembled nanomaterials 75 phases ME); and Winsor IV (simple phase ME or pure ME). Properties like oil-surfactant ratio, surfactant mixture, and temperature change the formation of each type. The use of ME as a drug delivery agent has been shown to improve the bioavailability at the site of action of various drugs (Suhail et al. 2021).

6.2.2.5 Self-Microemulsifying Drug Delivery Systems (SMEDDS)

SMEDDS are pre-concentrates of microemulsions which contain oil, surfactant, and drugs.

6.3 Types of Self Assembled Systems

6.3.1 *Discrete System Versus Continuum System*

Self-assembled systems can be classified based on the building blocks into two categories: Discrete and continuum.

A system that uses prefabricated building blocks with fixed sizes and shapes is a discrete system. A bond forms when two particles are close to each other with matching orientations. A self-assembled chain of alternating structures is formed due to selective bonding when there are two bonding sites. When there are multiple bonding sites they self-assemble into a network of alternating particle chains. Nanoparticles and nanorods can be assembled into various configurations by modifying the electric and magnetic fields which induces a dipole-type interaction or shear forces which utilize hydrodynamic interactions.

Continuum system makes use of the formation of nanoscale domains in the form of patterns such as binary monolayers, block polymers, and organic molecular adsorbates on metal surfaces. A binary monolayer is 1–10 nm in size, stable on annealing, and can be self-assembled into triangular lattices of dots, parallel stripes, or serpentine stripes patterns in an orderly fashion. Polymer systems develop by phase separation and consist of two immiscible polymer fragments joined by a covalent bond. The size ranges from 10 to 100 nm. The advantages of a continuum system are presynthesized building blocks that are not required as the domains and their patterns self-assemble simultaneously, it provides significant flexibility and control of the assembly process and this approach can be used for other different systems (Lu, 2012).

6.3.2 *Static Self-Assembly Versus Dynamic Self-Assembly*

The types of self-assembly categorized based on the nature of interactions are static and dynamic. The process involving the formation of static structures using energy minimization leads to static self-assembly. Microphase separation and colloidal crystals are classic examples of this process. When the self-assembly system dissipates energy, dynamic type is observed with the formation of structures away from the thermodynamic equilibrium. However, if the influx of energy stops, structural disintegration occurs. The most typical example of dynamic self-assembly is a living organism (Subramani & Ahmed, 2018).

6.4 Applications of Self-Assembled Nanostructures

There are various applications of self-assembled nanostructures. The films can be used as membranes, sensors, insulators, electronic, and magnetic optical devices such as the construction of Bragg diffraction devices for Raman spectrometers (Chen et al., 2018). The incorporation of nanoparticles into self-assembled structures has been employed in optoelectronic devices, plasmon waveguides, focusing lenses, light generators, optical switches, nanoscale thermometers, plasmon rulers, and pH meters (Nie et al., 2010). Pharmacological, biological, and medical fields demonstrate the use of polymersomes, liposomes, or polymer capsules incorporated self-assembled nanoparticles as carriers for delivering biologically active species (Puri et al., 2009). The self-assembled nanostructures have also been used to stimulate pre-osteoblast cell attachment and growth. They are incorporated in extracellular matrix for the growth of human fibroblasts. They possess therapeutic applications as wound dressing materials, detection of neurotoxins, antiviral agents, and as immunosensors (Habibi et al., 2016).

6.5 Techniques for Characterization of Self-Assembled Nanostructures

Self-assembled nanostructures play a vital role in various biologic processes and has led to a lot of research due to their potential use in diagnosis and biosensing or designing biocompatible products. The designing and development of artificial nanostructures through different techniques for self-assembly is a method of analysing the basis of different functional structures with superior and desirable properties. These factors have made the characterization of self-assembled nanostructures more challenging. Significant research is being done on polymer self-assembly for similar reasons.

Researchers consider nanostructures as dynamic systems with continuously evolving and improving properties and structures, as they interact with the environment. Hence, the characterization of these nanostructures becomes very important to utilize their properties in various applications (Kasotakis et al., 2009).

This section gives an overview of the different techniques for characterizing self-assembled nanostructures based on their properties.

6.5.1 Atomic Force Microscopy

Atomic force microscopy (AFM) is a method of characterization which creates 3-D images of the surface of a nanostructure at high magnification. This method was introduced by 'Gerard Binnig and Heinrich Rohrer' in the year 1986. AFM has been a topic of interest because it is simple to perform and is very flexible. The main principle of AFM is measuring the force between a fine probe of the microscope and the sample. The apparatus contains a cantilever, which has a probe attached at the tip, made of silicon. As AFM scans the sample, the attraction or repulsion involving the tip and the surface of the sample may lead the cantilever to deflect. This deflection can be computed with the help of a laser beam. The final calculation is done by adding the values obtained through laser alteration and the previously established cantilever stiffness. AFM tips can also be used to bring about surface reactions by carrying different catalysts. AFM tips can also cause direct local oxidation on surfaces.

The three types of AFM-based nanofabrication techniques include nano-shaving, nano-grafting, and nano-pen reader and writer (NPRW). In nano-shaving, the AFM tip applies excessive localized pressure at the point of contact of the sample. This pressure leads to the production of high shear force while scanning, which in turn leads to the displacement of self-assembled monolayer adsorbates. In nano-grafting, the self-assembled monolayer and the AFM cantilever are dipped in a solution with a thiol group. These thiol groups adsorb to the exposed surface of the sample. In NPRW, the required molecules and groups are pre-coated to the exposed surface of

the sample under high force. Following this, the nanostructures are characterized by decreased force.

AFM can function with three types of modes based on the proximity between the probe and the surface of the sample, into 'contact', 'non-contact', and 'tapping'. Tapping is the most commonly used mode for characterization of nanostructures. Using AFM an 'edge resolution' of 1 nm can be achieved, high spatial resolution characterization of nanostructures can be done in situ, complicated patterns can be produced automatically, and various constituents can be produced by modifying the components of the tip or the solution. The fabricated patterns can be modified in situ without modifying the process of fabrication and the height of the nanostructure can be analysed and visualized. In addition, the resolution of images produced by AFM is comparable to SEM and TEM. It is cost-effective and requires minimum laboratory equipment and space. Nevertheless, AFM displays slower scanning times than any electron microscope.

6.5.2 Electron Microscopy

6.5.2.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is a dominant and among the most popular technique used for material characterization and imaging of the surfaces of various materials. SEM can provide a resolution of up to 1 nm, highly focused, and a fairly straightforward image interpretation, making the imaging of three-dimensional nano-structures feasible.

SEM works on a voltage range of 100 V–30,000 V with the electron probe with a diameter of 1–3 nm and a field emission source. As the electron beam and the sample interact, it produces secondary electrons. The efficiency of emission of these secondary electrons varies based on the surface topography and chemical properties of the sample.

Undesirable excitation of electrons is inevitably involved in this interaction which makes radiation damage one of the most important set back of SEM imaging low-voltage SEM has been used for imaging such beam radiation-sensitive specimens (Zuccheri & Samorì, 2011).

6.5.2.2 Transmission Electron Microscopy

Transmission electron microscopy (TEM) has been utilized in the characterization of nanostructures in the form of a tool that can be used to analyse the spatial arrangement at high resolution and their chemical nature. A modern transmission electron microscope makes imaging atoms in crystalline samples directly, possible. TEM utilizes the interaction between the electron beam with a homogenous current density and the sample to be investigated, to determine the physical properties of the

nanostructure. The extent of the interaction depends on various properties including the density, size, and elemental composition of the sample. The transmitted electrons, in turn, form an image by utilising the information assimilated from their interaction with the sample. They can be analysed at resolutions close to 0.1 nm, which is lower than inter-atomic distance.

One of the most valuable applications of TEM is to determine the shape of nanostructures. Electron diffusion along with high-resolution lattice imaging can be used to analyse the facets and structure of nanocrystals. The 3D structure of a nanocrystal can be identified and analysed by using a minimum of two TEM images which are captured from different orientations. Simple nanostructures can be determined directly from TEM images while some of the more complex nanocrystals may require the analysis of experimental and theoretically obtained images.

TEM has been used as a method of characterization of nanostructures for various bio-medical purposes such as:

- (1) Diagnostics and bio-sensing, where the aggregation changes based on the presence or absence of the concerned biomarkers.
- (2) Therapy, where the aggregation enhances the therapeutic effect of the nanostructures.
- (3) Imaging, in which, the aggregation enhances the response received from the tissue being visualised, in the form of signals.

TEM also aids in the identification and differentiation between hollow or solid pathologies which makes it a vital tool in diagnosis. In TEM, high-energy electrons interact with the concerned tissue so that the intensity of this interaction can be recorded in the image. The centre of hollow structures appears lighter than its capsule or wall in the image recorded.

TEM is one of the most readily used techniques to analyse the shape and size of various nanostructures because it provides the accurate valuation of nanostructure homogeneity and direct images of the sample. A few limitations of this technique include inaccurate images due to flawed orientations and the inability to quantify more particles.

6.5.2.3 High-Resolution Transmission Electron Microscopy

High-resolution TEM (HRTEM) is a technique that uses both scattered and transmitted electrons to produce an image, known as 'phase-contrast imaging'. Phase-contrast imaging produces images with the highest resolution among others which aids in the identification of various atoms in crystalline structures. While conventional electron microscopy can only aid in the statistical characterization of nanostructures, HRTEM can produce images of a single particle crystal structure. Therefore, HRTEM is constantly being utilized to characterize the internal structure of nanostructures. This ability of HRTEM can also be used to assess the structural defects of these nanocrystals which can explain some of their atypical properties.

The characterization of all the nanostructures is not always possible using HRTEM. The random and haphazard orientation of nanocrystals can result in the formation of complex images that may be inaccurate.

6.5.2.4 Liquid Transmission Electron Microscopy

A vacuum system is used in TEM which protects the filament, used in the apparatus, along with the reduction of electron beam scattering. Traditional TEM imaging is not used to assess liquids since it can compromise this vacuum system. Therefore, liquid systems characterization has not been explored as much as solid systems. Due to these limitations, the other imaging techniques are only able to produce data in a single time frame, most commonly after the completion of nanoparticle growth and not during the growth process. Liquid TEM gives the opportunity to track and analyse the nanoparticles. It permits the tracking of the course of nanoparticles while they are growing and hence provide a direct outlook on the evolution of nanoparticles. Liquid TEM also enables researchers to analyse the movement of nanoparticles with the liquid, which may be due to Brownian motion or some other parameters contributing to this movement.

6.5.2.5 Cryo-Electron Microscopy

Cryo-electron microscopy (Cryo-EM) is an effective method of characterization used to analyse the structure of polymer and protein self-assembly nanostructures.

The sample preparation in traditional TEM comprises drying and staining steps which might end up affecting their morphology and structure. In contrast, cryo-EM samples are processed and stored in a hydrated and frozen state, which is close to their inherent form, by vitrifying them and studying at cryogenic temperature using a TEM. Liquid nitrogen is commonly used to freeze-dry the nanostructure samples (Mourdikoudis et al., 2018).

Cryo-TEM can be used to view nanostructures at sub-nanomolar resolutions. It has been utilised to study the complicated mechanism of nanoparticle aggregation. Cryo-TEM imaging is vital to observe the atypical form of nanostructures because the rapid plunge freezing prevents the particles from rearranging during the preparation process of the sample and its characterization can be done.

6.5.3 Focused Ion Beam

Focused ion-beam (FIB) bombardment is a fascinating and alternative method used to prepare self-assembly nanostructures. This technique permits in-situ analysis of the sample using ion-beam or scanning electron beam imaging. FIB enables locating the desired spot for deposition or removal of materials using 'high energy ion beam

sputtering' and 'ion-assisted chemical vapor deposition' (Kochovski et al., 2020). The merits of the FIB technique are high resolution, mask-less processing, provide prompt prototyping, and flexibility to adapt to different materials and structures. One of its major drawbacks is the time consumed to analyse nanostructures. A single ion beam is utilised to analyse various structures, making it inefficient for large-scale production characterization of nanostructures. The FIB is a vital tool in defect characterization, design modification, failure analysis, and process control for various applications.

6.5.4 Fourier Transformed Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is the method that measures the absorption of electromagnetic (EM) radiation with wavelengths in the range of the infrared spectrum. FT-IR spectroscopy gives important information regarding the various functional groups which are present in a system through the frequency of vibrations of chemical bonds present in the nanocrystals. It recognizes the composition of different microorganisms by observing the difference in the functional groups in their structure. It can produce data about the kind of interaction that takes place between microorganisms and metal ions.

Biochemical bonds are identified depending on their molecular degree of rotation and the type of movement, like twisting, stretching, or bending.

The mechanism of FTIR includes an infrared source emitting radiation through an interferometer consisting of a moving and a fixed mirror along with a beam splitter. The interferometer gauges the wavelength of the emitted light. IR spectra are found by exposing infrared radiation to the nanostructure sample and quantifying the intensity of radiation at a particular wavenumber.

Some of the types of FTIR techniques include attenuated total reflectance (ATR-FTIR), transmittance FTIR, and micro-spectroscopy FTIR (Stephen et al., 2009).

The advantages of FTIR include time efficiency and both solid and liquid samples can be analysed using this technique. The quantity of samples required for analysis is also small and is a cost-efficient alternative for the identification of microorganisms.

However, there are a few demerits such as the need for numerous sample and background scans in order to avoid artefacts. Processing of the sample prior to analysis is generally needed to avoid peaks due to overlapping. Even after processing, the information and data require further analysis.

6.5.5 X-Ray Diffraction

X-ray diffraction (XRD) is one of the most commonly used characterization methods to determine the formation of crystalline self-assembly, as long as there is a sufficient quantity of the sample. Traditionally, XRD gives information about the crystalline structure, lattice parameters, phase, and crystalline grain size. The

spectrum of diffraction can directly be correlated to the atomic structure of the nanocrystals at high angle regions, while at low angle regions, the spectrum is linked to the organised arrangement of nanocrystals. This analysis is done assuming that every particle in the nanostructure is similar in shape, size, and orientation so that the principles of diffraction physics can be used for the analysis. But practically, this assumption cannot be made because there is evident variability in their shape, size, and orientation. XRD is one of the most popular method used to measure the average inter-particle distance, and it is one of the few techniques that can be used to study the ‘in situ temperature as well as pressure induced phase transformation’ in nanostructures (Zuccheri & Samorì, 2011). XRD can be used to study the powder form of samples, once their colloidal forms are dried. It leads to statistically averaged volume values. The intensity and position of the peaks correspond to the composition of particles, which can be compared with the already available data for reference. However, XRD peaks are extremely wide and broad for particles with a size less than 3 nm and hence is not suitable for amorphous nanostructures.

6.5.6 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a technique used in thermal analysis that is readily used by researchers because it is simple to use, easily available, and gives rapid results. In this technique, a nanostructure sample and a control for reference are placed in the DSC apparatus. Heaters either gradually raise the temperature at a specific pace or hold the DSC at a previously determined temperature. This instrument evaluates the variation in the heat flow among the nanostructure sample and the control reference.

Modern DSC apparatuses use software that helps the user in characterizing different physical properties of nanostructures like melting points, heat capacity values, and glass transition temperatures (Koshy, 2017).

The quantitative applications of DSC include the assessment of fusion heat and the degree of crystallization for nanocrystals. DSC is used for quality control applications because the melting points of different materials correlate with their purity. One of the major applications of DSC is glass transition temperature (T_g) determination. At T_g , the polymer undergoes changes in its physical characteristics like volume and expansion, heat capacity, and heat flow.

6.5.7 Thermogravimetric Analysis

Thermal gravimetric analysis (TGA) helps in the analysis of information about the composition and mass of nanostructures. In this method of characterization, a nanomaterial sample is heated which makes its constituents degrade at different temperatures and end up decomposing and vaporising, which at the end leads to

variation of mass. This can also help in determining the quantity and type of nanoparticle organic ligands present in the nanocrystal sample.

TGA is used for analysing a few thermal processes like adsorption, absorption, desorption, sublimation, vaporization, oxidation, decomposition, and reduction. TGA can also be used to study volatile or gaseous components which are lost during such thermal reactions for samples such as nanomaterials. TGA can also be utilised to analyse the kinetics of chemical reactions of nanostructures under various conditions.

The thermobalance is regarded as the functional unit of TGA. The subunits of TGA include a sample holder, electronic microbalance, temperature programmer, recorder, and a furnace. It consists of a clamp that is used to stabilize the electron microbalance.

Some of the advantages of TGA are its simplicity and no requirement for special sample preparation. A disadvantage of TGA is the requirement of few milligrams of a sample, which in turn leads to increased cost and lab production feasibility problems (Table 6.1).

Table 6.1 Characterization techniques corresponding to specific parameters to be analysed (Zuccheri & Samorì, 2011)

Parameter	Characterization techniques suitable for analysis
Size	Transmission electron microscopy, X-ray diffraction, high-resolution transmission electron microscopy, scanning electron microscopy, atomic force microscopy, differential scanning calorimetry
Shape	Transmission electron microscopy, high-resolution transmission electron microscopy, atomic force microscopy
Chemical and elemental composition	X-ray diffraction
Crystalline structure	X-ray diffraction, high-resolution transmission electron microscopy
Size distribution	Differential scanning calorimetry, scanning electron microscopy
Growth kinetics	Transmission electron microscopy, cryo-transmission electron microscopy, liquid-transmission electron microscopy
Composition/ligand binding/density/surface composition/arrangement/mass	Fourier transform infrared spectroscopy, thermal gravimetric analysis
Concentration	Differential scanning calorimetry
Agglomeration state	Differential scanning calorimetry, scanning electron microscopy, cryo-transmission electron microscopy, transmission electron microscopy
Density	Differential scanning calorimetry
Properties of Single particle properties	High-resolution transmission electron microscopy, liquid transmission electron microscopy
Three-Dimensional visualization	Atomic force microscopy, scanning electron microscopy
Dispersion of nanoparticles	Scanning electron microscopy, Atomic force microscopy, Transmission electron microscopy
Defects in the structure	High-resolution transmission electron microscopy
Detection of nanoparticles	Transmission electron microscopy, scanning electron microscopy

6.6 Advantages of Self-Assembled Nanostructured Materials

6.6.1 Synthesis

Self-assembled nanostructures are synthesised under non-harsh conditions, at room temperature without specialized equipment. This reduces the cost of these nanostructures.

They can be used to synthesize structures like nanotubes, nanoparticles, or nanofibers, by changing the initial building block used to fabricate these nanostructures. Hence, it can be used to obtain structures of varying shapes.

6.6.2 Functionalization

Self-assembled nanostructures can be used as agents used in contrast imaging or as biosensors. They can perform such specific functions because they are combined with suitable functional molecules which give them desired properties. Some of these functional groups include functional antibodies, enzymes, magnetic or metallic particles, fluorescent compounds, etc.

6.6.3 Biocompatibility

Studies have shown that these nanostructures can be used as a cell culture medium. They simplify the loading and handling of the culture medium. The matrix density of these cultures can be fully structured by tailoring it chemically. It has been seen that cell growth in these media is unhindered (Pignatello, 2011).

6.7 Mechanisms of Molecular Self-Assembly

Molecular self-assembly is the assembly and arrangement of molecules without manipulation from an external source. By definition, molecular self-assembly is, “the spontaneous organization of molecules under near-thermodynamic equilibrium conditions into structurally well-defined and stable arrangements through non-covalent interactions”. This is largely regulated by weak noncovalent bonds like hydrogen bonds, electrostatic interactions (ionic bonds), water-mediated H-bonds, van der Waals forces, and hydrophilic and hydrophobic interactions. The collective interaction between these weak bonds produces chemically and structurally stable nanostructures (Li, 2017).



Fig. 6.1 The forces acting in the process of molecular self-assembly

Molecular self-assembly depends on two factors: structural compatibility and chemical complementarity. The molecular constituents require complementary properties like specific surface charge, surface characteristics, polarizability, surface functionalities, and mass, in order to self-assemble to give rise to various physiological forms. Biological components such as lipids, nucleic acids, and proteins, among other cellular components, also self-assemble to form a cell. Some of the examples of molecular self-assembly include antigen–antibody recognition, phospholipid membrane, chromatin assembly, and amyloid fibril formation.

Self-assembly involves maintaining a balance between three types of forces. They include ‘driving force (attractive in nature)’, ‘opposition force (repulsive in nature)’, and ‘directional force’. A non-hierarchical self-assembly is observed in most of the nanostructures. The process becomes directional because of the supplementary 1 directional forces (Fig. 6.1).

6.8 Benefits of Molecular Medicine from Self-Assembled Nanostructured Materials

Studies done previously have shown that self-assembled nanostructures can be used to manufacture various molecular medicines which have properties which are superior to the ones made conventionally. The benefits of using these nanostructures for the assembly of molecular medicines for tissue engineering include:

1. Designing using active biological peptides.
2. Incorporation of different functional groups and their combinations with nanostructures can help to study cell behaviour.
3. Good biocompatibility since it degrades in the body by natural enzymes.
4. Molecules can be altered at the level of single amino acids based on the requirement in a cost effective way using conventional methods.
5. They can be used to analyse cell signalling processes and controlled gene expression.

Thus, these self-assembled nanostructures derived molecular medicines are being used readily for 'bionanotechnological applications'.

Nanoparticles can be used as potential vessels for drug targeting because their pharmacodynamics and pharmacokinetics in the body are controlled by their physicochemical properties, such as hydrophobicity, particle size, and surface charge. Colloidal particles can overcome the setback of bringing a hydrophobic substance into the aqueous blood medium. Another advantage of colloidal carrier systems is their protective action towards sensitive drugs, such as specific peptides and synthetic vaccines (Li, 2017).

Gene therapy is another therapeutic technique that can benefit from nanotechnology since it also has the problem of drug delivery given that it faces the same problems of delivering the gene to the target cell.

In the future, nanostructures can also be used to construct well-defined two-dimensional and three-dimensional structures. The fabrication of these nanostructures with different substrates can be explored.

6.9 Challenges When Using Self-Assembled Nanostructures in Molecular Medicine

Even though self-assembled nanostructures have many advantages, a few challenges regarding the manipulation, size control, stability in liquids, and immobilization have to be considered.

6.9.1 Size Control

Size control becomes a very important parameter when it comes to applications like drug-delivery systems and biosensors. Due to the process of self-assembly, size control during the synthesis process is very challenging.

6.9.2 *Stability of Nanostructures in Liquid Environment*

The biomedical applications of self-assembled peptide nanostructures, where they come in direct contact with a liquid environment are numerous, such as drug-delivery systems, contrast imaging agents, and biosensors. They have to be highly stable in the solution it is interacting with, in the body. It has been noted that nanostructures are unstable when dissolved in water, methanol, or phosphate buffer. This limits their application in various biomedical processes.

6.9.3 *Manipulation*

Manipulation of nanostructures is a very critical process because of their dimensions. It is a challenge to link nanostructures, such as nanofibers, nanotubes, and nanoparticles, with macroscopic applications.

It is also very vital to avoid damage to nanostructures when they interact with the object being manipulated and the instrument. Any alteration in the structure of the self-assembled nanostructure, during processing and manipulation, can cause a change in the behaviour required for particular medicine applications (Pignatello, 2011).

6.9.4 *Conductivity*

Another factor that makes the application of self-assembled nanostructures challenging is their low conductivity limits. Their use in the production of biosensors or diagnostic aids omitting the functionalization step incorporates molecules and compounds to improve conductivity.

6.10 Conclusion

Self-assembled nanostructures are biomaterials with various merits, which makes them an excellent candidate for biomedical applications. Nanostructures used in molecular medicine are developed under mild environments, in a rapid and cost-effective way. It can also be readily functionalized with various other functional groups like enzymes, fluorescent molecules, and antibodies. Nevertheless, it is notable to acknowledge the intricacies involved while studying self-assembled nanostructures. Multiple ways to overcome problems associated with manipulation, size control, stability, and low conductivity have been continuously explored. In spite of these limitations, nanostructures play a very important role

in biomedical applications. Until now, the size and shape of nanoparticles have been characterized. Some of the other parameters that can also be characterized and explored include ‘surface area’, ‘degree of aggregation’, ‘surface charge’, and ‘size distribution’, and also appraise their ‘surface chemistry’. These parameters can affect the properties and applications of nanostructures. Reliable and accurate characterization techniques for nanostructures will significantly have an impact on the use of these materials, commercially. The next step in self-assembled nanostructures can be aimed at a comprehensive analysis of the immunogenicity and biocompatibility of these nanostructures with the purpose of understanding the outcome of their use.

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