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Nanotechnology in Modern Medicine

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Chapter 1
Introduction to Nanotechnology

Mayuri Napagoda, Darsha Jayathunga, and Sanjeeva Witharana

Abstract In principle, nanomaterials are materials with at least one dimension of 100 nm or less. Although nanotechnology; the science of the nanoscale, has emerged as a scientific discipline in the late twentieth century, human exposure to nanomaterials has begun in the times of ancient civilizations. In comparison to their bulk counterparts, nanomaterials exhibit unique physical and chemical properties hence having enormous applications in different fields such as agriculture, engineering, medicine and biomedical sciences. The main approaches for the synthesis of nanomaterials are bottom-up and top-down approaches which utilize various physical, chemical or biological synthesis procedures. This chapter gives a general overview of the history of nanotechnology, the properties of nanomaterials, their classification as well as the synthesis and characterization of nanomaterials.

Keywords Bottom-up approach · Characterization · Nanomaterial · Top-down approach

1.1 Nanotechnology—Historical Perspective

Nanotechnology is a multidisciplinary field which involves the design, synthesis, characterization and application of materials in the dimensions of the nanometer scale (1×10^{-9} m). The prefix 'nano' is derived from the Greek word 'nanos' which means 'a dwarf' (Whatmore 2006). Over the past few decades, nanotechnology has revolutionized many scientific areas including agriculture, food industry, engineering, medicine, pharmaceutical industry and biomedical sciences.

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1.1.1 Pre-epoch of Nanotechnology

Although nanotechnology started to emerge as a scientific discipline in the late twentieth century, it has a long history of humans synthesizing and utilizing nanoparticles without understanding the reasons for their specific features that differ from bulk material. The world's oldest use of nanomaterials is attributed to the use of carbon nanotubes (CNTs) as a covering for inner walls of pottery shards recovered at Keeladi, Tamilnadu, India, around the sixth to third century BC (Kokarneswaran et al. 2020). Passing more milestones in the nanotechnological evolution between the fourth to eighteenth centuries AD includes the coloured glass by the Romans, Lycurgus cup, vibrant stained glass windows in European cathedrals, Egyptian cosmetics and hair dyes, and Islamic potteries that have used copper and silver nanoparticles to improve lustre, renaissance Mediterranean pottery, and "Damascus" sabre blades (Bayda et al. 2019; Nanotechnology Timeline 2022). Later on, in 1857, Michael Faraday unveiled the colloidal "ruby" gold that created a range of colours depending on the illumination conditions (Tolochko 2018).

1.1.2 Modern Epoch of Nanotechnology

The invention and characterization of the nanometer by Richard Zsigmondy marked the beginning of nanotechnology's notion in 1925. Later he was able to measure the size of gold nanoparticles using an ultra-microscope. The initiation of modernistic nanotechnology commenced with the concept of "manipulating matter at the atomic level" by Nobel laureate Richard Feynman in 1959 (Toumey 2008). Fifteen years later, the word "nanotechnology" was introduced by Norio Taniguchi in commentating on nanoscale precision machining in semiconductor manufacturing. This was the next paramount phenomenon that contributed to the subject area. He demonstrated that nanotechnology is the consolidation of four material mechanisms at the atomic or molecular level: processing, separation, consolidation, and deformation (Guttula et al. 2020). Modern nanotechnology attained its next level with the finding of fullerenes by Kroto, Smalley, and Curld in the 1980s and the introduction of molecular manufacturing by Eric Drexler. Drexler conceptually defined nanotechnology as an approach to manipulating individual atoms and molecules that is deterministic rather than stochastic and promoted the significance of nanotechnology through his speeches and publications. In 1981, he published a scientific paper in the Proceedings of the National Academy of Sciences titled "Molecular Engineering: An Approach to the Development of General Capabilities for Molecular Manipulation" (Hulla et al. 2015). Intervention of the scanning tunneling microscope (STM), atomic force microscope (AFM), nano layer-base metal–semiconductor junction transistor, which is the smallest nanoelectronic device in the world, buckyballs and the origination of cluster science have taken place in the same era furnishing the discipline (Mansoori and Soelaiman 2005; Guttula et al. 2020).

A turning point in nanoscale manufacturing was Don Eigler's and Erhard Schweizer's manipulation of the IBM logo using 35 atoms of xenon in 1989 (Eigler and Schweizer 1990). This is illustrated in Fig. 1.1. Commercialization of nanotechnology was evident since the early 1990s, with Nanophase Technologies and Helix Energy Solutions Group companies being the first to enter the industry.

The synthesis of carbon nanotubes (CNT) (Fig. 1.2) by Iijima further accelerated the nanotechnological evolution (Iijima 1991). Extraordinary properties of single-walled CNTs led it to be a part of a variety of applications in the fields of photonics, electronics, fabrics, biology, energy and communication etc. (Bayda et al. 2019). In 1992, catalytic nanomaterials of MCM-41 and MCM-48 were discovered and have since been used widely in crude oil refining, drug delivery, and water purification (Pradeep 2007). Further, the introduction of dip-pen nanolithography (DPN), the controlled nanocrystal synthesis method and the design of quantum dots synthesizing methods have contributed to the advancement of nanoscience (Ginger et al. 2004; Alivisatos 2008; Li and Zhu 2013).

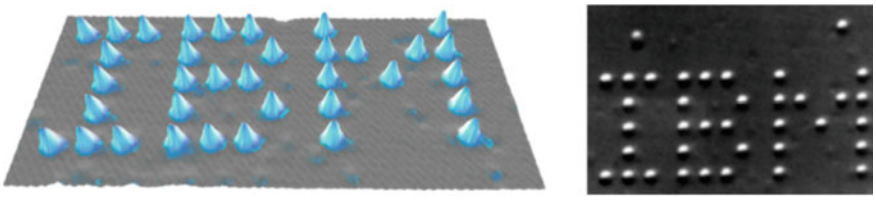

Fig. 1.1 The IBM logo created by positioning Xenon atoms on a nickel substrate (adapted with permission from Bayda et al. 2019)

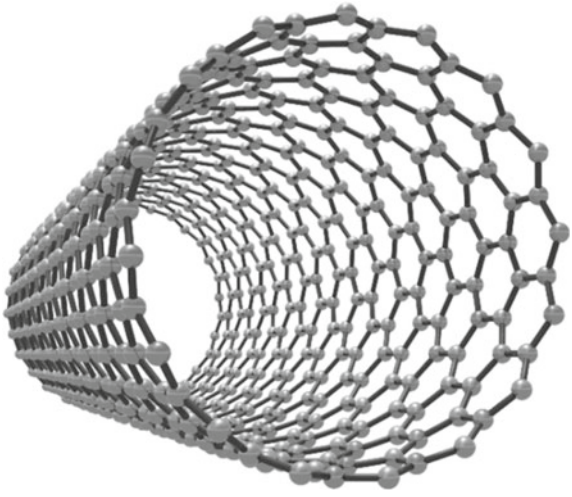

Fig. 1.2 Carbon nanotubes (adapted with permission from Bayda et al. 2019)

1.1.3 Recent-Epoch of Nanotechnology

From the beginning of the 2000s, nanotechnology-based consumer products began to appear in the market which further boosted research and development. In 2003, the researchers at Rice University developed gold nano-shells that could be used in breast cancer treatments as a substitute for existing invasive treatment methods (Young et al. 2012). The sector expanded further through remarkable inventions that include carbon dots (2004), theories for DNA-based computation and "algorithmic self-assembly" (2005), the nanoscale car (2007), a non-harmful virus-based lithium-ion battery (2007), robotic DNA nanodevices (2009–2010), nanoscale 3D world map (2010), carbon nanotube computer (2014), tic-tac-toe game DNA nano board (2018) and objects shrinking to the nanoscale (2018). These opened up new interdisciplinary opportunities at low cost, low complexity and low environmental impact.

As of today, nanotechnology has contributed to over 5000 commercially available products (The Nanodatabase 2022), and over 22,000 patents where IBM Corporation, Samsung Electronics, Intel Corporation, BOE Technology Group and Taiwan Semiconductor Manufacturing are the leaders in holding intellectual property rights (Statnano 2022). Furthermore, China, USA, India, Iran and South Korea have become the top five countries in nanotechnology research publications (Zhu et al. 2017; Zhao et al. 2021; NBIC+ 2022). Nanotechnology has recently ramped up multi-disciplinary research activities in conjunction with machine-learning algorithms, predictive analytics, and nanoinformatics, all of which are helping to shape its future path. The regulatory framework also has improved since its initiation in the 1900s, and a growing number of international organizations are actively attempting to enhance it in order to safeguard the safety, health, and environmental friendliness of nanotechnology and its outcomes as it develops (Thomas et al. 2006).

1.2 Properties of Nanomaterials

The high surface area over volume ratio phenomenon is mainly accountable for the exceptional properties of the nanomaterials (Asha and Narain 2020). As the size of the particle decreases, the ratio of surface area to the volume increases and as a result, the percentage of atoms residing on the surface of a material increases. For example, for a particle of 1 μm in diameter, only about 0.15% of the atoms are on the surface, however, this percentage increases up to about 20% when the diameter of the particle decreases to 6 nm (Issa et al. 2013).

The properties of nanomaterials differ substantially from those of their bulk counterparts. Due to the negligible number of surface atoms, the properties of bulk materials are almost entirely determined by the interior atoms. However, the properties of nanomaterials are significantly affected by surface atoms which possess exotic localized electronic states (Asha and Narain 2020). Therefore, size-dependent effects become more prominent at the nanoscale, for example, gold nanoparticles (AuNPs)

show characteristic colours depending on the size and the shape (Fig. 1.3), although the solution appears in yellow when in the bulk (Njoki et al. 2007; Baig et al. 2021).

The electronic structure of the nanomaterial is largely dependent on the surface atoms and as a result, optical properties like reflection, transmission, absorption, and light emission differ depending on the size, composition and arrangement (Asha and Narain 2020). The size-dependent optical properties of nanomaterials are mainly due to the change in the energy band gap that influences the surface plasmon resonance of the nanomaterials (Malhotra and Ali 2018; Asha and Narain 2020).

In comparison to the bulk materials, nanomaterials possess different electronic properties. An example is boron allotropes. In the bulk form, it is not considered a

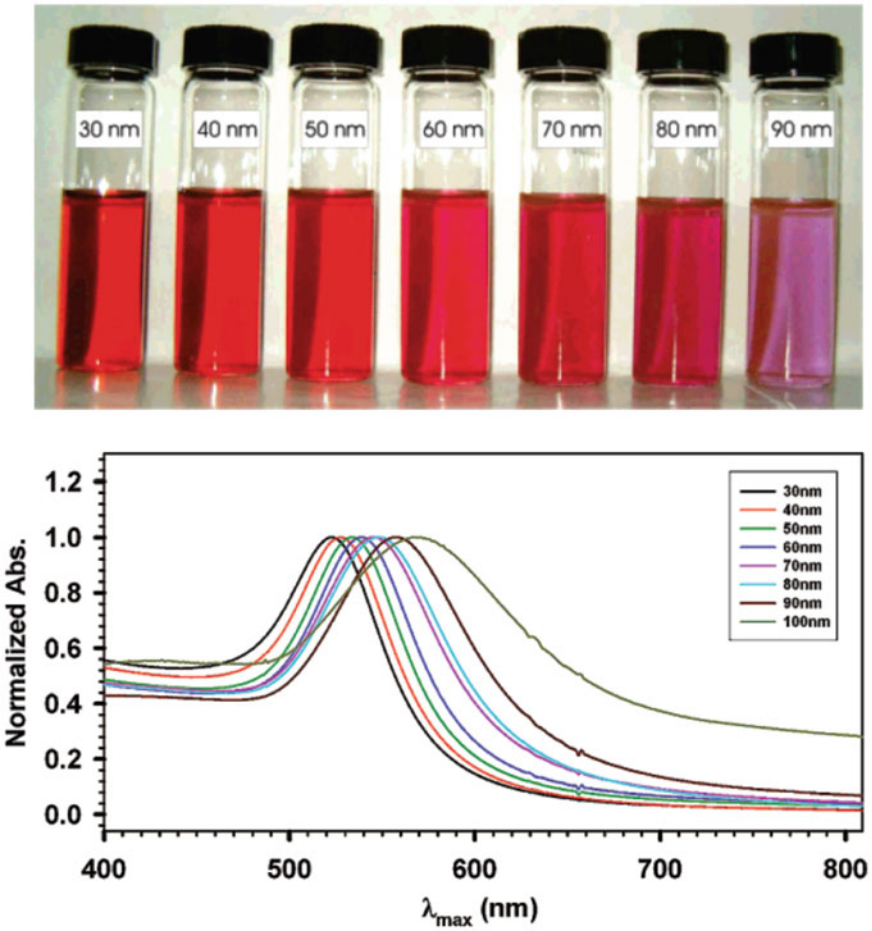

Fig. 1.3 A photo showing the colours of gold nanoparticles with different particle sizes and the UV–vis spectra (normalized) for Au nanoparticles with different particle sizes in an aqueous solution (adapted with permission from Njoki et al. 2007)

metal, however, borophene sheets (crystalline two-dimensional network of boron) are metallic with anisotropic electronic properties (Mannix et al. 2015; Baig et al. 2021). Moreover, quantum effects become more prominent at the nanoscale level. However, the size at which these effects will appear strongly depends upon the nature of the semiconductor material (Geoffrion and Guisbiers 2020; Baig et al. 2021). The magnetic behaviour of elements may also change at the nanolevel, therefore, it is possible to control the magnetic characteristics of a nanomaterial to a certain extent by altering the size, shape, composition and structure (Gubin et al. 2005). An example is the superparamagnetic-like behaviour of Fe_3O_4 nanoparticles in comparison to the ferromagnetic behaviour of bulk iron oxide (Fe_3O_4) (Asha and Narain 2020).

Compared to their macroscopic counterparts, an enhancement of the mechanical properties are observable in nanomaterials mainly due to the increased crystal perfection or reduced crystallographic defects (Baig et al. 2021). Mechanical properties of metals generally consist of brittleness, strength, plasticity, hardness, toughness, fatigue strength, elasticity, ductility, rigidity and yield stress. When nanoparticles are added to a bulk material, they will refine the grain to a certain extent, forming an intragranular or an intergranular structure, which thereby improves the grain boundary, promoting the mechanical properties of materials (Wu et al. 2020). For instance, the addition of 4 vol% of Al_2O_3 nanoparticles to ceramic tool materials has enhanced the fracture toughness and hardness by 6 and 5.4% respectively (Yi et al. 2014), while, nano-TiN in cermet had exhibited nearly a 25% higher bending strength (Xie et al. 2004). When 0.05 vol% of nanographene oxide was added, the compressive strength and flexural strength of cement had increased by 33 and 41%, respectively (Pan et al. 2015). Type of nanomaterial and the nanoparticle concentration played a significant role in determining the degree of enhancement.

Similarly, the addition of nanoparticles has shown substantial enhancement in thermal and electrical properties of the respective bulk materials. This behaviour is often attributed to the size, shape and material of nanoparticles. For thermal conductivity, a 3 wt% of CuO nanoparticles in neopentyl glycol showed upto 408% enhancement (Praveen and Suresh 2018); 1 wt% of Al_2O_3 nanoparticles in pentaerythritol showed 51% enhancement (Venkitaraj et al. 2017); 10 wt% of graphite nano-powder in Paraffin showed 1000% enhancement (Bahiraei et al. 2017) etc.

There were fewer investigations conducted on electrical conductivity of nanocomposites. For example, 7 wt% of exfoliated graphite nanoplatelets in paraffin exhibited (xGnP-15) 10^{16} S/m enhancement (Kim and Drzal 2009); 4 wt% of graphene in paraffin wax exhibited 6×10^{16} S/m enhancement (Mhiri et al. 2019); 95 wt% of single wall carbon nanotubes in poly(3,4-ethylene dioxythiophene):poly(styrenesulfonate) exhibited 1.9×10^6 S/m enhancement (Moriarty et al. 2013).

Nanomaterials exhibit excellent catalytic properties due to the high surface-to-volume ratio. Moreover, functionalization substantially enhances the surface activity of nanomaterials and thereby their catalytic activity. Therefore nanomaterials have emerged as promising heterogeneous catalysts (Karak 2019; Asha and Narain 2020).

1.3 Classification of Nanomaterials

Nanomaterials can be classified according to their geometry, morphology, chemical composition, uniformity as well as agglomeration. This chapter focuses on geometrical classification and the classification according to the chemical composition (Saleh and Gupta 2016).

1.3.1 Geometrical Classification of Nanomaterials

According to this classification, nanomaterials are categorized into four groups such as zero dimension, one dimension, two dimensions, and three dimensions (Fig. 1.4).

1.3.1.1 Zero Dimension (0-D)

Nanomaterials with all dimensions (x, y, z) at the nanoscale (1–100 nm) are included in this group. Most of these materials are spherical while some possess cubical and polygonal shapes. In addition, nanomaterials like clusters, fullerenes, rings, metallocarbohedrenes (metcarbs), particles, and grains are also included in this category. E.g.:- metal nanoparticles, quantum dots with uniform particle arrays, hollow spheres, and nano lenses (Saleh and Gupta 2016).

1.3.1.2 One Dimension (1-D)

This group consists of materials with two dimensions (x, y) at the nanoscale and the other dimension is larger than 100 nm, i.e. outside the nanoscale E.g.:- Nanowires, nanorods, nanofibers, nanotubes of metals or metal oxides, filaments, spirals, belts, springs, columns, needles, bundles, ropes, and pillars (Saleh and Gupta 2016).

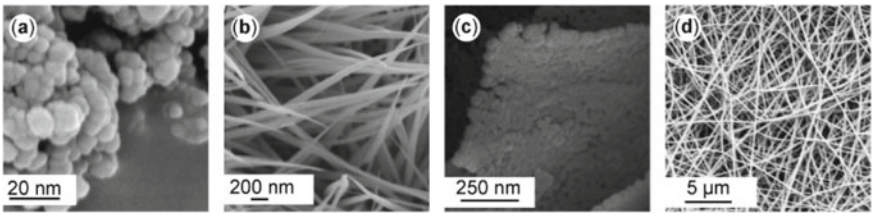

Fig. 1.4 Different material structures. **a** 0D: SiO_2 nanoparticles; **b** 1D: CoO nanowires; **c** 2D: ZnO–NiO nanosheets; and **d** 3D: PA6 nonwoven structure (adapted with permission from Hornak 2021)

1.3.1.3 Two Dimensions (2-D)

Materials with one dimension at the nanoscale and the other two dimensions beyond the nanoscale are included in this group. Usually, these nanomaterials are plate-like structures. E.g.:- Nanosheets, nanowalls, nanocoatings, nanostraw, nanofilms, fullerene films, nanolayers and fullereno-powders, bridges, and graphenes (Saleh and Gupta 2016).

1.3.1.4 Three Dimensions (3-D)

Materials that are having all three dimensions beyond the nanoscale are classified under this category. These materials are bulk and composed of individual blocks, however, possess a nanocrystalline structure or features at the nanoscale. E.g.:- Fullerites (Saleh and Gupta 2016).

1.3.2 Classification According to the Chemical Composition

According to the chemical composition nanomaterials can be classified as below (Saleh and Gupta 2016).

1. Metal-based nanomaterials. E.g.:- silver nanoparticles, copper nanoparticles
2. Metal oxide nanomaterials. These are made up of a combination of oxygen and metal. E.g.:- silica, titania
3. Carbon-based nanomaterials. E.g.:- nanotubes, fullerenes
4. Dendrimers which are highly branched nanomaterials
5. Nanocomposites

Quantum dots. These are autofluorescent semiconductor nanocrystals. E.g.:- Binary metal complexes like CdSe, CdS, CdZn.

1.3.2.1 Metal-Based Nanoparticles

Nanoparticles synthesized by either the destructive or constructive method from their metals are known as metal-based nanoparticles. Aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn) are the metals that are widely used. These nanoparticles are 1–100 nm in size and are usually spherical or cylindrical in shape (Salavati-niasari et al. 2008).

1.3.2.2 Metal Oxide-Based Nanoparticles

Metal oxide nanoparticles are modified by their respective metal nanoparticles. E.g.:- iron oxide nanoparticles are produced from iron nanoparticles by oxidizing. Metal oxide nanoparticles have higher reactivity and efficiency than metal-based nanoparticles (Tai et al. 2007). Silica is also considered as a metal oxide nanoparticle. The average pore size of those particles is usually between 2–50 nm. The shape of the porous is mainly important in the fusion of both large and small particles such as DNA attachment and gene transformation. Those silicon-based systems are used in biological imaging, treatments as well as in diagnosis (He et al. 2017).

1.3.2.3 Carbon-Based Nanomaterials

These nanoparticles are only made up of carbon atoms (Bhaviripudi et al. 2007). E.g.:- Fullerenes, carbon nanotubes, carbon nanofibers, carbon black, and graphene (Ealias and Saravanakumar 2017). Fullerenes are also known as buckyballs and are made up of only carbon atoms. These materials are normally about 1 nm in diameter and have a natural hollow core (Liang et al. 2010). Buckminster Fullerene (C60) was identified in 1985 for the first time and interestingly all fullerenes contain an even number of carbon atoms in the cage. Each carbon atom is connected with the other three neighbouring carbon atoms and the remaining p electrons are localized both inside and outside resulting in a π electron cloud. Hence, chemical properties are enhanced in these materials than in other allotropes of carbon. Fullerenes are also important in medicine as these have been employed in photodynamic therapy while antiviral activity was observed against certain viruses (Klupp et al. 2016).

Another form of carbon-based nanomaterials is graphene, an allotrope of carbon usually having a 1 nm thickness. It has a hexagonal network structure (Ealias and Saravanakumar 2017).

Carbon nanotubes (CNT) are graphene nano foils that can be seen as hollow cylindrical structures. The diameter of CNT is usually lesser than 0.7 nm for a single-layered one while 100 nm for a multi-layered one. The length of these structures is usually in the range of a few micrometres to few millimetres (Ealias and Saravanakumar 2017). These are made up of hexagonal carbon rings which are having sp^2 hybridization and the average distance between the two carbon atoms is nearly 1.4°A. Two types of CNT are available; single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) (Zhang et al. 2011). CNTs are much important in biomedicine due to their characteristic features such as higher external surface area and cellular internalization (Cirillo et al. 2014). The drug immobilization can be achieved by encapsulation inside the carbon nanotube as well as by attaching the drug molecule to the nanotube by either a covalent bond or non-covalent bond (Niemirowicz and Car 2012).

Another type of carbon-based nanomaterials is known as carbon black. Carbon black is an amorphous solid material and spherical in shape. The diameter varies

from 20 to 70 nm. Due to the high interaction, agglomerates are usually formed (Ealias and Saravanakumar 2017).

1.3.2.4 Dendrimers

The first scientific publication about dendrimers was published in the late 1970s and the name was introduced by Tomalia (Tomalia and Frechet 2002). These are highly branched tree-like structures with three different regions; core moiety, branching units, and closely packed surface (Nikalje 2015). The branch structures can be found on the surface of the dendrimer and are called dendrons. These dendrons can be modified by substituting or attaching different functional groups which may result in changes in the chemical and physical properties of the whole dendrimer structure (Sękowski et al. 2008). Dendrimers are formed by adding polymer layers around the central core. This synthesis is known as the divergent synthesis method (Niemirowicz and Car 2012). The size of the dendrimer is normally less than 10 nm. (Nikalje 2015). Due to the small size of the dendrimer, they can penetrate easily. In addition to that, there are two major ways that can be seen in the immobilization of the substances on dendrimer. One is drug molecules are covalently bound to the surface of the dendrimer by the electrostatic forces and the other one is incorporating the drug molecule into the cavity (core) of the dendrimer (Niemirowicz and Car 2012). Because of the above-mentioned features, they are widely used to deliver bioactive materials (Nikalje 2015). Hence, Dendrimers are very important in the field of medicine.

1.3.2.5 Nanocomposites

Nanocomposites are multiphase materials where at least one of the phases with dimensions in the nanoscale or structures have a nanoscale repetition distance between phases (Saleh 2020). The formation of a composite requires, materials such as matrix, filler and resin, as well as a reinforcement, like fibres. Nanocomposites can be classified based on the dispersed matrix and dispersed phase materials. Broadly these materials can be classified as polymer-based, metal-based, carbon-based and ceramic-based nanocomposites. Colloids, carbon–metal oxides, gels, and copolymers are some examples of nanocomposites (Saleh and Gupta 2016).

1.3.2.6 Quantum Dots

Quantum dots are semiconductor nanocrystals that are highly resistant to chemical degradation apart from their high thermal stability and optical properties (Fang et al. 2012). Quantum dots are of much importance in the field of medicine, particularly employed in cancer imaging and cellular tracking (Zhang et al. 2008).

Apart from the aforementioned categories of nanomaterials, lipid-based nanomaterials like solid-lipid nanoparticles, liposomes and micelles are also widely used in

the field of medicine. Solid–lipid nanoparticles are submicron-sized lipid emulsions in which the liquid lipid is substituted by a solid-lipid (Mukherjee et al. 2009). Liposomes are bio-compatible structures of usually 50–200 nm in size and are widely used as novel drug carriers, particularly to deliver genes, peptides and also proteins. Further, liposomes possess good entrapment efficiency (Nikalje 2015). Liposomes and bilayer structures are amphiphilic possessing both hydrophobic and hydrophilic features (De Jong and Borm 2008). Micelles are also amphiphilic nanomaterials that are arranged in a way that hydrophilic parts are at the exterior surface while hydrophobic parts are at the interior core in aqueous media. These materials can trap hydrophobic drugs by their interior core and protect them from the outside environment while capable of absorbing polar drugs by their exterior surface (Shubhika 2013).

1.4 Synthesis and Characterization of Nanomaterials

Different techniques have been adopted in the preparation of nanomaterials which include physical, chemical, biological, and also hybrid techniques. The selection of a synthesis method usually depends on the type, size and desired quantity of the nanomaterial. The synthesis can be achieved by a bottom-up or a top-down approach (Fig. 1.5) (Malhotra and Ali 2018; Khanna et al. 2019). The bottom-up synthesis mostly involves chemical and biological methods while the top-down approach utilizes physical (e.g.: mechanical) or chemical methods for the production of nanoparticles (Khanna et al. 2019).

The bottom-up approach exploits the self-assembled properties of atoms or molecules to construct complex conformations at the nanoscale. The nanomaterials obtained from this approach are less defective and are more uniform with consistent chemical composition (Malhotra and Ali 2018; Karak 2019). Chemical vapour deposition (CVD), chemical reduction, laser pyrolysis, solvothermal and hydrothermal methods, sol–gel method, soft and hard templating methods, and reverse micelle methods are some examples of the bottom-up approaches in the synthesis of nanomaterials (Khanna et al. 2019). On the other hand, the top-down approach involves the breakage of bulk materials into nanoscale dimensional materials. This approach involves methods like mechanical milling, electrospinning, lithography, laser ablation, etching, sputtering, electro-explosion and arc discharge method. However, the top-down approach usually results in somewhat defective and contaminated nanomaterials with variable compositions (Malhotra and Ali 2018; Karak 2019).

Various Spectroscopic and diffractographic techniques are employed in the determination of the size, shape, distribution, surface morphology, surface area, chemical composition etc. of the synthesized nanomaterials. These characterization techniques include UV–visible spectroscopy (UV–vis), scanning electron microscopy (SEM), transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HR-TEM), dynamic lights scattering (DLS), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy

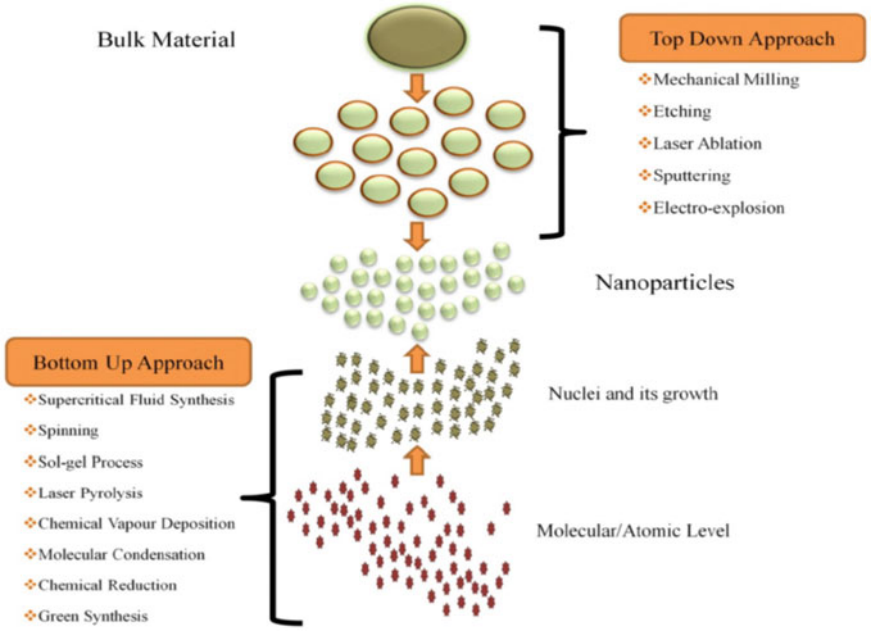

Fig. 1.5 Synthesis of nanomaterials via bottom-up and top-down approaches (adapted with permission from Khanna et al. 2019)

(FT-IR), X-ray photo-electron spectroscopy (XPS), atomic force microscopy (AFM) and Raman spectroscopy (Khanna et al. 2019).

UV–visible spectroscopy is normally employed to confirm the formation of a nanomaterial and it is based on the phenomenon named surface plasmon resonance. Surface electrons of the metal and metal oxide nanoparticles interact with the incident UV radiation resulting in specific surface plasmon resonance peaks in the UV–visible region (Karak 2019). Dynamic light scattering (DLS)/ photon correlation spectroscopy/quasielastic scattering is a tool used in the determination of the size of nanoparticles in colloidal suspension/solution. The scattered light from dispersed nanomaterials in solution is measured to determine the hydrodynamic diameter of the hydrated nanoparticles and it also allows for the monitoring of real-time variation in the size of the hydrated nanoparticles (Mourdikoudis et al. 2018; Karak 2019).

Beams of highly energetic electrons are used as a source of illumination in high-resolution microscopy techniques which are widely employed to examine and visualize various structural details of nanomaterials. Scanning electron microscopy (SEM) is a surface-imaging technique that produces high-resolution and high magnification images of nanoscale materials. On the other hand, transmission electron microscopy (TEM) offers a higher resolution than SEM and nowadays, high-resolution TEM (HRTEM) has become one of the most popular techniques to characterize the internal structure of nanoparticles, particularly to visualize crystalline structures (Rao and Biswas 2009; Mourdikoudis et al. 2018; Kaliva and Vamvakaki

2020). The electron microscopy can be coupled to an energy dispersive X-ray (EDX) spectrophotometer to identify the elemental composition of the nanomaterial. Similar to SEM, atomic force microscopy (AFM) can also be utilized to determine the size, distribution, shape, surface area and morphology of nanostructural materials (Karak 2019; Kaliva and Vamvakaki 2020).

X-ray diffraction (XRD) is extensively utilized to obtain information on the crystallographic structure of the nanomaterial. This technique is based on the constructive interference of monochromatic X-rays and a crystalline sample and measures the average spacing between layers or rows of atoms providing information on the atomic arrangement, crystallite grain size, lattice parameters and imperfections (Mourdikoudis et al. 2018; Kaliva and Vamvakaki 2020). X-ray photo-electron spectroscopy (XPS) is another technique that provides details on the elemental composition, empirical formula, and chemical and electronic state of the atoms within a nanomaterial (Rao and Biswas 2009; Kaliva and Vamvakaki 2020).

Fourier-transform infrared spectroscopy (FTIR) is a very common technique used to identify the presence of different chemical functional groups in nanomaterials and nanoparticle–stabilizer interactions. This is based on the measurement of the absorption of electromagnetic radiation in the mid-infrared region (4000–400 cm^{-1}) (Mourdikoudis et al. 2018; Karak 2019). The structural characteristics of nanomaterials can be studied by nuclear magnetic resonance (NMR) spectroscopy. Although it provides important information on the interactions/coordination between the ligand and the surface of diamagnetic or antiferromagnetic nanoparticles, this technique is not suitable to characterize ferri- or ferromagnetic nanomaterials (Mourdikoudis et al. 2018). Apart from these techniques, Raman spectroscopy which is based on the inelastic scattering of a high-frequency monochromatic light by matter is often employed to characterize carbon-based nanomaterials (Rao and Biswas 2009) while mass spectrometry is used to obtain information on the elemental and molecular composition, the structure of the nanomaterial and their bioconjugation to target biomolecules (Mourdikoudis et al. 2018).

The surface charge of a nanoparticle is a key parameter of a nanoparticle which determines the dispersion stability and aggregation state. Zeta potential (ζ-potential) measurements determine the surface charge of nanoparticles in a colloidal solution. Usually, nanoparticles with highly positive or negative surface charges tend to repel each other, thus forming stable colloidal dispersion. The nanoparticles with low surface charge often tend to agglomerate and precipitate (Mourdikoudis et al. 2018; Kaliva and Vamvakaki 2020).

There are several other techniques employed in the characterization of nanomaterials, for instance; Brunauer–Emmett–Teller (BET) technique which is based on the physical adsorption of a gas on a solid surface is used in the determination of the surface area of nanostructures; Thermal gravimetric analysis (TGA) provides information on the mass and composition of the stabilizers in the nanomaterial; Photoluminescence (PL) spectroscopy for the characterization of fluorescent nanoparticles; Vibrating sample magnetometer (VSM) and superconducting quantum interference device (SQUID) for the magnetic characterization of nanomaterials (Rao and Biswas 2009; Mourdikoudis et al. 2018; Kaliva and Vamvakaki 2020) etc.

1.5 Conclusion

In principle, materials with at least one external dimension measuring 1–100 nm are considered nanomaterials. The high surface area and nanoscale size are accountable for their unique physical and chemical properties. Nanomaterials can be classified based on their geometry, morphology, chemical composition, uniformity or agglomeration. The synthesis of nanomaterials can be achieved by either top-down or bottom-up approaches utilizing various chemical, physical, and biological methods. Several spectroscopic and diffractographic techniques are available for the determination of the size, shape, distribution, surface morphology, surface area, and chemical composition of the synthesized nanomaterials.

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Chapter 2
Biological Applications of Nanofluids: Antimicrobial Activity and Drug Delivery

Ziyuan Wang, Zichen Wang, Meng Zhang, Jiandong Cui, Ming Xie, and Yunhong Jiang

Abstract Recently, nanofluids have attained a vast field of application in the engineering industry and biomedical research due to unique physicochemical properties such as small and controllable size, large surface area to mass ratio, high reactivity and functionalize structure. Numerous antimicrobial drugs using nanofluids have been prescribed to kill or inhibit the growth of bacteria, fungi, and viruses. Nanofluids can also increase the mass and heat transfer through different media to repair or damage the cells, human organs and tissues based on different technologies such as magnetic hyperthermia or active coating. In this chapter, we highlight the recent progress in the development of biological applications of nanofluids in drug delivery systems and antibacterial activities for the treatment of microbial disease. For example, nanofluids could use as a potential antimicrobial agent to target antibiotic resistance. The current progress and challenges in using nanofluids as antimicrobial agents and the interactions between nanofluids and bacterial cells are presented in this chapter. The overview of the effect of particle size, morphology, surface charge and colloidal stability on the biological impact is discussed. This article could be useful

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for presenting the unite shared interest between nanoengineers and microbiologists in developing nanofluids for success in current medical and biological practice.

Keywords Antibacterial · Drug delivery · Nanofluids · Zinc oxide

2.1 Introduction and Background

Nanofluids are defined as a relatively new class of fluids that consist of a base fluid with nano-sized particles (1–100 nm) suspended within them (Saidur et al. 2011; Pauzi et al. 2020). There are two phase systems with one (solid phase) in another (liquid phase). The nanoparticles used in nanofluids are typically made of metal, oxides, carbides, and/or carbon nanotubes (Yang et al. 2020). Common base fluids include water, ethylene glycol and oil. Although human manipulation of nanoparticles is considered a relatively new technology, nanoparticles have existed in our world for aeons, such as volcanoes and sea spray. There are human-made sources and natural things which are depicted in Fig. 2.1.

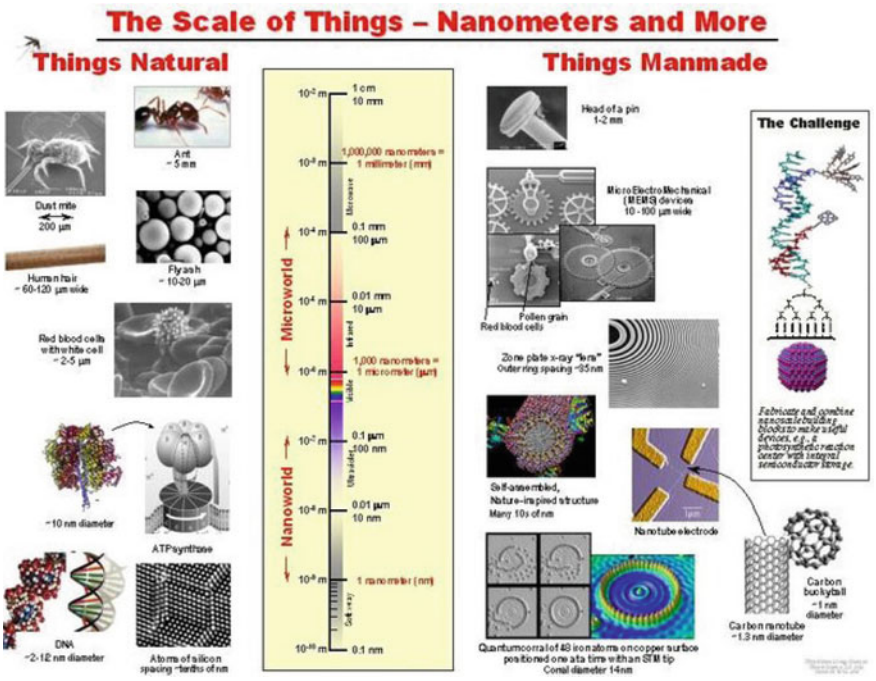

Fig. 2.1 Nanometer materials (Natural and Manmade). *Source* Office of Basic Energy Science, Office of Science, U.S. Department of Energy (2012, "The Scale of Things - Nanometers and More," https://nanohub.org/resources/13842)

Many publications on nanofluids are increasing exponentially in the past few years covering theoretical, experimental and numerical aspects of formulation, characterization, flow behaviour and thermal behaviour of nanofluids (Yu and Xie 2012). Owing to their special properties, nanofluids can be used in a wide range of engineering applications ranging from the energy industry to the engineering industry to use in material and device systems as well as biological application (Röcker et al. 2009; Masood et al. 2014; Pauzi et al. 2020). However, there are some important challenges we have to face. One of the most important issues is the stability of nanofluids, and it remains a big challenge to achieve desired stability of nanofluids (Pauzi et al. 2020; Qamar et al. 2021). The fact that nanoparticles exist in the biological cells can be considered as a kind of nanofluid. Nanoparticles usually form a core to exist in the cell. The size and size distribution might be important in some cases, for example, if penetration through a pore structure of a cellular membrane is required. The physicochemical properties, i.e. size and surface charge are the two main determining factors that affect the fate of the nanoparticle in the body. Some reports indicate that the behaviour of nanoparticles in the body does not only depend on their chemical composition, but also it is of the decisive importance of how they interact with biological molecules (Röcker et al. 2009). With the decrease in the dimensions of the materials to the atomic level, over the last decade, nanofluids have been extensively exploited in their potential antimicrobial and biological applications due to their unique physio-chemical properties and high surface area to volume ratio. There is an increase in the use of nanofluids in the biomedical industry for various purposes, such as drug delivery, antimicrobial, molecular detection and imaging (Allen et al. 1999; Stoimenov et al. 2002; Lok et al. 2007; De Jong and Borm 2008; Jones et al. 2008; Rai et al. 2009; Wong and De Leon 2007; Masood et al. 2014). A list of some of the applications of nanomaterials to biology is given below: fluorescent biological labels, drug and gene delivery, bio-detection of pathogens, detection of proteins and tissue engineering and so on. Therefore, understanding biological processes on the nanoscale level is very important to develop nanotechnology. The aim of this chapter is first to give the reader a review of the application of nanofluids as antimicrobial agents. The application of ZnO nanofluids as antimicrobial agents is mainly discussed and the mechanisms of action have also been deeply discussed. Secondly, this review tries to overview the latest developments in the field of nanofluids as drug delivery.

2.1.1 Antimicrobial Applications

Recently, nanofluids can be found in different industrial, biomedical, drug delivery, optical and electronic fields. The preparation of uniform nanosized drug particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products. Silver and highly ionic metal oxide nanofluids such as magnesium oxide, calcium oxide, copper oxide, titanium dioxide and zinc oxide have attracted lots of attention as they showed good

antibacterial properties. Silver has been used in the treatment of a wide variety of diseases, including venereal diseases and dysentery, throughout the centuries. The Greeks, Romans, Phoenicians, Macedonians and ancient Chinese are all known to have used silver for hygiene and medicinal purposes. The advantage of using these inorganic oxides nanofluids as antibacterial agents is that they are not only stable under harsh process conditions but also generally considered as essential mineral elements to humans and animals at a lower concentration. Inorganic antimicrobials such as silver and copper were used since ancient times to treat microbial infections. The preliminary studies have shown that silver, magnesium oxide, calcium oxide and zinc oxide exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria (Sawai et al. 1995c, 1998b; Sawai 2003; Sondi and Salopek-Sondi 2004; Brayner et al. 2006; Jones et al. 2008; Rai et al. 2009; da Silva et al. 2019a). Thus, the preparation, characterization, surface modification and functionalization of nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials. Recently, ZnO nanofluid has attracted a lot of interest from researchers as an antibacterial agent. In this chapter, this topic will be discussed in detail and the most recent developments in this field will be overviewed. Finally, the possible antimicrobial mechanisms of this nanofluid will be discussed here. Several studies indicated that among the metal oxide nanoparticles such as MgO, TiO_2 and CuO, ZnO nanosuspensions with relatively small particle sizes have shown the highest antibacterial activity on various microorganisms including major pathogens (Sawai et al. 1996a; Yamamoto 2001; Dodd et al. 2006; Zhang et al. 2007; Simon-Deckers et al. 2009; Qi et al. 2020; Naser et al. 2021). It was reported that ZnO nanoparticles possessed a high efficacy as antibacterial materials against both Gram-positive and Gram-negative bacteria (Sawai et al. 1998b; Yamamoto et al. 1998, 2000; Liu and Yang 2003; Sawai and Yoshikawa, 2004; Adams et al. 2006; Fang et al. 2006; Franklin et al. 2007; Reddy et al. 2007; Wang et al. 2007; Huang et al. 2008; Padmavathy and Vijayaraghavan 2008; Tam et al. 2008; Zhang et al. 2008, 2010; Applerot et al. 2009a; Hu et al. 2009; Jiang et al. 2009a, b; Liu et al. 2009; Wu et al. 2010). In addition, several researchers have coated ZnO nanofluids on special substrates such as glass, paper and fibres and so on for antimicrobial food packaging and antimicrobial healthcare materials (Li et al. 2006, 2007, 2008, 2009a, b; Ghule et al. 2006; Applerot et al., 2009b, 2010; Gittard et al. 2009; Ji and Zhang 2009; Perelshtein et al. 2009; Patil et al. 2019; Valerini et al. 2020). Figure 2.2 shows a graph detailing the increasing interest in ZnO nanofluids as an antibacterial agent in recent years.

Although there are lots of publications on the antibacterial and toxicity of ZnO nanoparticles, the interactions of ZnO nanoparticles with microorganisms and biomolecules are largely unclear. Here we are going to review the antimicrobial action of ZnO nanofluids against bacteria and critically analyse the results in the published papers. The ultimate goal is to understand the interactions between bacterial cells and ZnO nanoparticles.

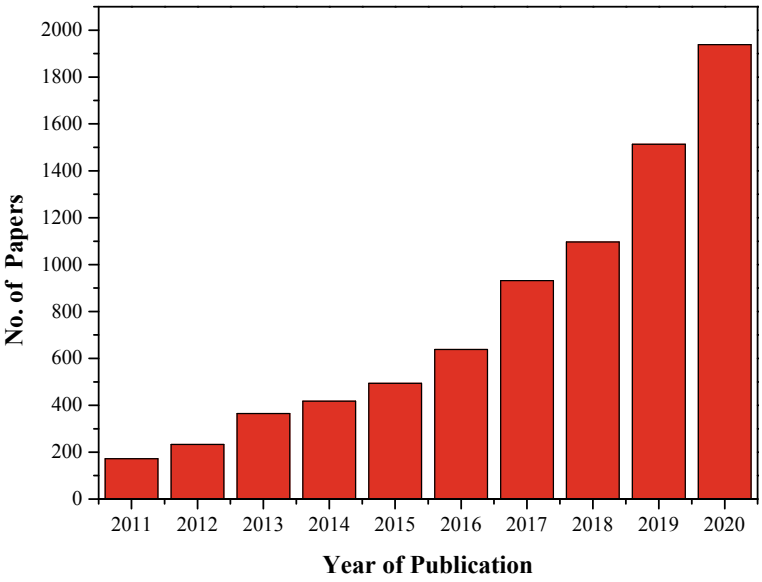

Fig. 2.2 A breakdown of the number of papers published per year from 2011 to 2020 on ZnO as an antibacterial agent. Only papers that are included by ScienceDirect have been used in the diagram (June 2021)

2.2 Antibacterial Activity of ZnO Nanofluids

The literature reported the inhibition of the growth of Gram-negative and Gram-positive bacteria by ceramic powders such as ZnO, CaO and MgO (Sawai et al. 1995a, b, 1996a, 1997; Mubina et al. 2019; Singh et al. 2019). It was found that ZnO powder slurry inhibited the growth of Gram-positive bacteria stronger than Gram-negative bacteria (da Silva et al. 2019b; Yusof et al. 2019). The ZnO powder (2.6 μm) exhibited a bacteriostatic action against both the spores of *Bacillus subtilis* and the vegetative cells over 1.6 g/L. In contrast, the effect of ZnO against Gram-negative *Escherichia coli* was bacteriostatic action at a slurry concentration of up to 100 g/L and a bactericidal action over 100 g/L. Sawai et al. also reported that ZnO powder exhibited a very weak antifungal activity against *Saccharomyces cerevisiae* NBRC1950 by an indirect conductance assay. The growth inhibition was observed at a concentration of 100 g/L (Sawai 2003). Yamamoto et al. reported the effects of shape, concentration and specific surface area of ZnO powder on antibacterial activity against *E. coli* and *Staphylococcus aureus* (Yamamoto et al. 1999, 2001, 2002a, 2004). It was clarified that the antibacterial activity increased with the increase of powder concentration ranging from 1.6 to 100 g/L in the physiological saline. Meanwhile, Brayner et al. studied biocidal effects and cellular internalization of ZnO nanoparticles on *E. coli*. They found that the presence of ZnO nanoparticles at a concentration between 0.25 and 1.63 g/L caused 100% inhibition of bacterial growth and concentrations between

0.12 and 0.25 g/L inhibited bacterial growth by 85% (Brayner et al. 2006). In 2019, Yusof et al. reported that ZnO nanoparticles displayed an inhibition zone of 13 and 16 mm against *E coli* and *S. aureus* respectively and concluded that Gram-negative bacteria were more resistant to ZnO nanoparticles than Gram-positive bacteria (Yusof et al. 2019). Adams et al. reported the potential eco-toxicity of ZnO water suspensions against Gram-positive *B. subtilis* and Gram-negative *E. coli*. ZnO water suspensions exhibited antibacterial properties toward *B. subtilis* and to a lesser extent to *E. coli*. At 0.01 g/L, ZnO resulted in a 90% growth reduction of *B. subtilis* but only a 48% growth reduction in *E. coli* has resulted at 1 g/L (Adams et al. 2006). Similar results were reported by Reddy et al. in (2007) where ZnO (13 nm) showed complete inhibition of *S. aureus* growth at concentrations ≥0.081 g/L, whereas growth of *E.coli* was completely inhibited for ≥0.28 g/L (Reddy et al. 2007). Akiyama et al. showed that the attachment of *S. aureus* to plastic tissue-culture coverslips after 24 h was weaker in rabbit plasma with 5% ZnO (no particle size information) than in the control rabbit plasma without ZnO (Akiyama et al. 1998). It was also found that the minimum inhibitory concentration (MIC) of hinokitiol against *S. aureus* peaked at a MIC distribution of 0.016–0.032 g/L. The peak shifted to below 0.001 g/L with the addition of 5% ZnO in the agar plate method.

By comparing the above results, it can be concluded that ZnO nanofluids have a significant antibacterial effect against both Gram-positive and Gram-negative bacteria. The effective antibacterial concentration of ZnO nanofluids against *E. coli* ranged from 0.25 to 1.63 g/L regarding the particle size. The effective antimicrobial concentration of ZnO nanofluids against Gram-positive bacteria (*S. aureus* and *B. subtilis*) ranged from 0.016 to 0.081 g/L. Da Silva et al. found that ZnO nanofluids were more effective in inhibiting the growth of *S. aureu*s, as compared with the effect on *E. coli* while holes in the cell wall of *S. aureu*s incubated with ZnO nanofluids were also observed (da Silva et al. 2019a). It means that ZnO nanoparticles showed strong antibacterial activity against bacteria and zinc oxide nanofluids inhibited the growth of Gram-positive bacteria more effectively than Gram-negative bacteria. The main reason for this is the difference in the cell wall structures of Gram-negative and Gram-positive bacteria. The Gram-positive cell walls are in the form of a very thick peptidoglycan layer, which is responsible for the retention of crystal violet dyes during the Gram staining procedure. Peptidoglycan makes up as much as 90% of the cell wall. The structural principle of the rigid layer is identical among Gram-positive and Gram-negative bacteria, though this layer is much thicker in the case of the former. The cell wall of Gram-negative bacteria is more chemically complex, thinner and less compact in structure than that of Gram-positive bacteria. Peptidoglycan makes up only 5–20% of the cell wall and is not the outermost layer. It lies between the plasma membrane and an outer membrane. The outermost layer is a membrane that is less permeable and composed of lipopolysaccharides (LPS) (Kashef et al. 2017). The structural unit of the out layer is unique to the Gram-negative bacteria. It is an effective penetration barrier due to the lack of an outer membrane. The cell wall of Gram-positive bacteria is much more penetrable than that of Gram-negatives. However, the effective concentration of ZnO nanofluids against bacteria shows a wide range. Regarding the biological impacts of nanofluids, factors such

as particle size, morphology, crystallinity, surface charge, surface chemistry and colloidal stability are important in determining the biological impact. Covering all aspects of nanofluids characterization, exposure and toxicity assessment, a standard testing strategy is required for an adequate assessment for the impact of nanofluids on bacteria.

2.2.1 *Overview of the Effect of Size on the Antibacterial Activity of ZnO Nanoparticles*

Theoretical considerations suggest that smaller particles with a higher specific surface area should be more toxic (Huang et al. 2017; Liu et al. 2017). The particle size should play a major role in the determination of the antibacterial activity of ZnO nanoparticles due to the large surface area and the ability to produce more chemical species are considered to be related to their toxicity against bacteria cells. However, previous studies of the effect of ZnO nanoparticle size on antibacterial have reported variable results, from little significant effect to increasing toxicity with decreasing particle size. Some reported that the antibacterial efficacy of the ZnO nanoparticles depends on the size of the nanoparticles (da Silva et al. 2019b). This can be confirmed by studying the inhibition of bacterial growth by differentially particle size nanoparticles. Sawai et al. showed that an increase in particle size of the ZnO (from 30 to 560 μm) reduced their antibacterial activity against both Gram-positive and Gram-negative bacteria due to the decrease in powder surface area available for contact with bacteria (Sawai et al. 1996a). Yamamoto studied the influence of particles on the antibacterial activity of ZnO ranging from 100 nm (Specific surface area 26 m^2/g) to 800 nm (Specific surface area 0.85 m^2/g). It was concluded that the antibacterial activity of ZnO nanofluids increased with decreasing particle size and increasing powder concentration as the concentration of H_2O_2 generated from the surface has increased with decreasing particle size and increasing concentration (Yamamoto 2001). Zhang et al. also reported that smaller particles (actual size 200 nm) have a much better bacteriostatic activity compared with large particles (actual size 2400 nm) under dark conditions (Zhang et al. 2007). Jones et al. reported that for ZnO ultrafine powder (>1000 nm) and ZnO nanopowder (50–70 nm), the reduced growth rates were smaller (50%). However, ZnO nanoparticles (8 nm) were able to reduce 99% of growth at the colloidal suspension concentration of 0.16 g/L and the MIC for ZnO nanoparticles was observed at 0.08 g/L against *S. aureus*. However, for the large particles of ZnO, the MIC was observed at 1.2 g/L (Jones et al. 2008). Similar results were also reported by Padmavathy and Vijayaraghavan (2008). The results by Joe and his co-workers also showed the dependency of the size of ZnO on the antibacterial activity (Joe et al. 2017). The improvement of the antibacterial activity of ZnO/cellulose nanocomposite was also reported. The enhanced antibacterial activity was found to be due to the smaller crystal size of ZnO in the composite (Lefatshe et al. 2017). Interestingly, it was found that the MIC of ZnO powders against

rod shaped-bacteria markedly varied. On the other hand, there was a small variation of the MIC against spherical-shaped- bacteria. It was also reported that the influence of the variation of particle size on *S. aureus* was relatively smaller than those on other bacteria (Sawai et al. 1996a). The influence of particle size on *S. aureus* was less than that on *E. coli* which could be due to the different cell wall structures and the chemical compositions (Yamamoto 2001). Nair et al. reported similar results on the effect of the size of ZnO particles on the antibacterial activity against bacteria. The size effects of ZnO were less toward Gram-positive than the Gram-negative bacteria (Nair et al. 2009). Seil and Webster et al. found that ZnO nanoparticles with a diameter of 60 nm did not reduce *S. aureus* populations as effectively as compared with 20 nm particles. At a concentration of 500 μg/mL, 20 nm ZnO nanoparticles alone reduced the bacteria viability by approximately four orders of magnitude after 8 h and 24 h compared to controls, while 60 nm ZnO nanoparticles alone reduced bacteria viability by about three orders of magnitude compared to controls (Seil and Webster 2012). However, Adams et al. reported that the advertised size of nanoparticles used to prepare the suspensions did not significantly affect antibacterial activity because the advertised particle size is a poor indicator of the true particle size in suspension. For example, while the advertised particle size is around 67 nm, the actual mean particle size in suspension is about 480 nm ranging from 420 to 640 nm (Adams et al. 2006). Compared with the published studies, several external factors, including surface chemistry, particle morphology, light intensity, particle shape and bacterial concentration, can affect the results. In addition, advertised particle size did not correspond to the true particle size. However, the census from all the publications is that the particle size can affect the antibacterial activity of ZnO nanofluids. To study the ecotoxicology of nanofluids on aquatic organisms, the first step is to introduce the particles to an aquatic medium. It is important to know about the degree of agglomeration of the system as if the nanoparticles are strongly aggregated at the time of testing, then it is not actually a toxicity test on a material of nanoscale, as the dimensions of the aggregates will be far greater than that of the primary particle size.

2.2.2 Overview of the Effect of Lattice Constant on the Antibacterial Activity of ZnO Nanoparticles

Yamamoto et al. firstly reported that the antibacterial activity of ZnO increased with the increase of C_0 value in crystal structure due to the generation amount of H_2O_2 increasing with the increase of C_0 value (Yamamoto et al. 2004). Wang et al. also reported that at a given size of ZnO nanowire, inhibition of bacterial growth depends on the orientation of 1-D ZnO nanostructure (in Fig. 2.3).

The membrane of randomly oriented ZnO nanoarrays, in comparison to other surfaces that contain less (0002) plane, showed a higher antibacterial activity. In addition, the influence of orientation for *S. aureus* was less than for *E. coli* due to

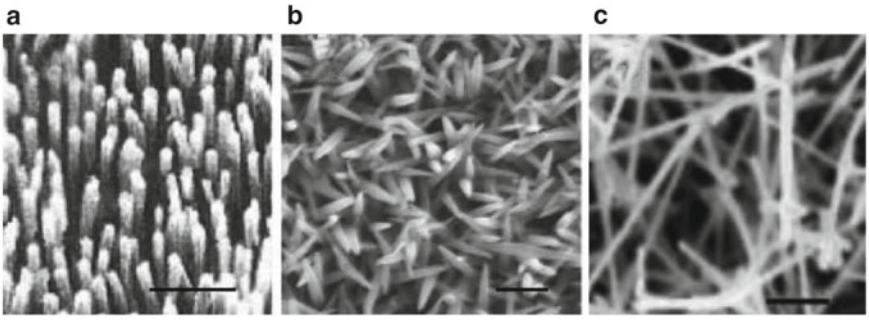

Fig. 2.3 SEM images of ZnO with various shapes. Adapted with permission from Wang et al. (2007) (Copyright 2007, The Royal Society of Chemistry)

the different structure and chemical composition of the outer membrane between Gram-positive and Gram-negative bacteria (Wang et al. 2007). However, Ohira et al. reported that, although the antibacterial activity in ZnO powder with crystallographic orientation was weaker than that in commercial ZnO powder without orientation at the same powder concentration, regarding specific surface area of powders, the crystallographic orientation of ZnO did not affect the antibacterial activity. In addition, irrespective of the kind of powders, the activity toward *S. aureus* was stronger than that toward *E. coli* (Ohira et al. 2008). Tam et al. also reported that the seed layer did not affect antibacterial activity. In the case of *S. aureus*, it was found that there was no difference in the activity according to the characteristics of the powders signifying that surface defects do not play a significant role in the antibacterial activity of ZnO (Tam et al. 2008). Joe et al. found the conventional ZnO nanoparticles were displaying the highest antibacterial activity towards both *S. aureus* KCTC No. 3881 and *Klebsiella pneumonia* KCTC No. 2246 as compared with those of nanoassemblies and nanoplates (Joe et al. 2017). Some parameters including particle size and surface area significantly affected the results of crystallographic effects and the antibacterial activity of ZnO nanoparticles. In the case of shape, it affected the particle size and surface area. It means that ZnO nanoparticles used in the experiments with different shapes have a significant difference in particle size and surface area. ZnO nanoparticles with flow-like structures have drawn great attention due to their superior antibacterial activity (Thakur and Mandal 2020). Studies indicated that the morphologies of ZnO nanoflowers also have a great effect on their antibacterial property. ZnO nanoflowers with different morphologies, namely rod flowers, fusiform flowers, and petal flowers, were synthesized and their antibacterial activities were investigated by Cai et al. (Fig. 2.4). The results showed that the antibacterial power was in an order of petal flowers > fusiform flowers > rod flowers, and was attributed to the different microscopic parameters such as pore size and specific surface area (Cai et al. 2016). Therefore based on the aforementioned results it is possible to mention that the particle size and shape have a significant effect on the antibacterial activity of ZnO.

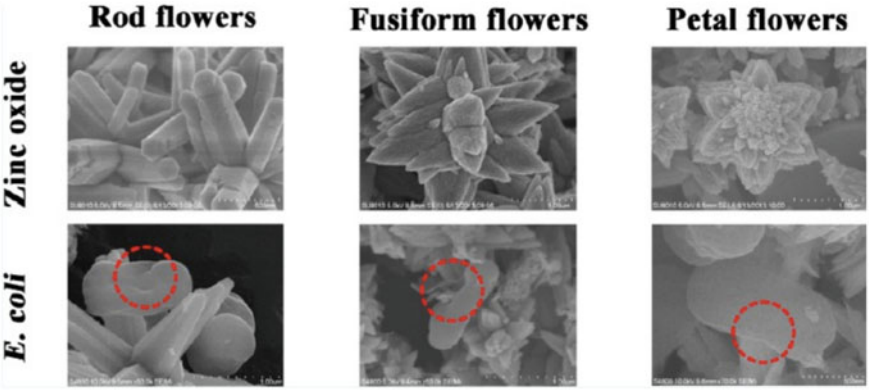

Fig. 2.4 SEM images of ZnO Nanoflowers of different morphologies and their antibacterial effect on *E. coli*. Adapted with permission from Cai et al. (2016) (Copyright 2016, American Chemical Society)

2.2.3 *Overview of the Effect of Light on the Antibacterial Activity of ZnO Nanoparticles*

ZnO is a semiconductor and can be excited by UV light. The presence of light was a significant factor under most conditions, presumably due to its role in promoting the generation of reactive oxygen species (ROS). Jones et al. reported that the antibacterial activity of ZnO nanoparticles in the dark is less than that in ambient laboratory conditions. In addition, the growth inhibition rate of ZnO with UV light at 254 nm for 30 min in a UV transilluminator did not differ significantly from that of ZnO in ambient laboratory conditions. It suggests that the ambient laboratory conditions are sufficient for the optimal biocidal activity of the ZnO nanoparticles (Jones et al. 2008). However, Adams et al. showed that illumination seemed not to enhance the antibacterial activity of ZnO. There was near complete inhibition of the growth of *B. subtilis* under both dark and illuminated conditions (Adams et al. 2006). Similar results were reported by Zhang et al. and Hirota et al. They both showed that ZnO nano-suspension has a strong antibacterial activity even under dark conditions (Hirota et al. 2010; Zhang et al. 2010). Before definitive conclusions can be drawn regarding the effect of light on toxicity, further studies should be performed. Joe et al. evaluated the antibacterial activity of ZnO nanoparticles with different sizes and oxygen defect sites under dark conditions. In the absence of a light source, the antibacterial activity of ZnO nanoparticles depended on the ZnO attachment to the bacterial cell wall and the amount of released Zn^{2+} in the bacterial cytoplasm by the attached nanoparticle, but not related to transfection of ZnO nanoparticles and the ROS generation (Joe et al. 2017).

2.3 Antimicrobial Mechanisms of ZnO Nanoparticles

The exact mechanism for the activity of ZnO is still under debate. Several possible mechanisms of ZnO nanoparticles have been suggested according to the morphological and structural changes found in the bacterial cells. The electrostatic interaction between nanoparticles and bacteria surfaces may be a reason suggested by Stoimenov and co-workers (Stoimenov et al. 2002). Some studies indicated that Zn^{2+} released by ZnO nanoparticles were electrostatically attracted to the negatively charged bacterial cell membrane surface leading to severe cell deformation, and finally resulting in bacterial lysis (Zhang et al. 2010). SEM analysis (in Fig. 2.5) was performed to investigate the morphological changes of *E. coli* (Zhang et al. 2007).

It was suggested that the interaction of nanoparticles and cell membrane could be a mechanism because the treatment with ZnO nanoparticles has caused damage to some *E. coli* and the damage has led to the breakdown of the membrane of the bacterial cells. Reddy et al. used flow cytometry-based assays and revealed that the growth inhibitory properties of ZnO nanoparticles were accompanied by a corresponding loss of cell viability (in Fig. 2.6).

Therefore the exposure of ZnO nanoparticles has led to bacterial cell death, mediated in part, by a loss in membrane integrity (Reddy et al. 2007). Padmavathy & Vijayaraghavan and Tam et al. believed that bacterial cell death is caused by the

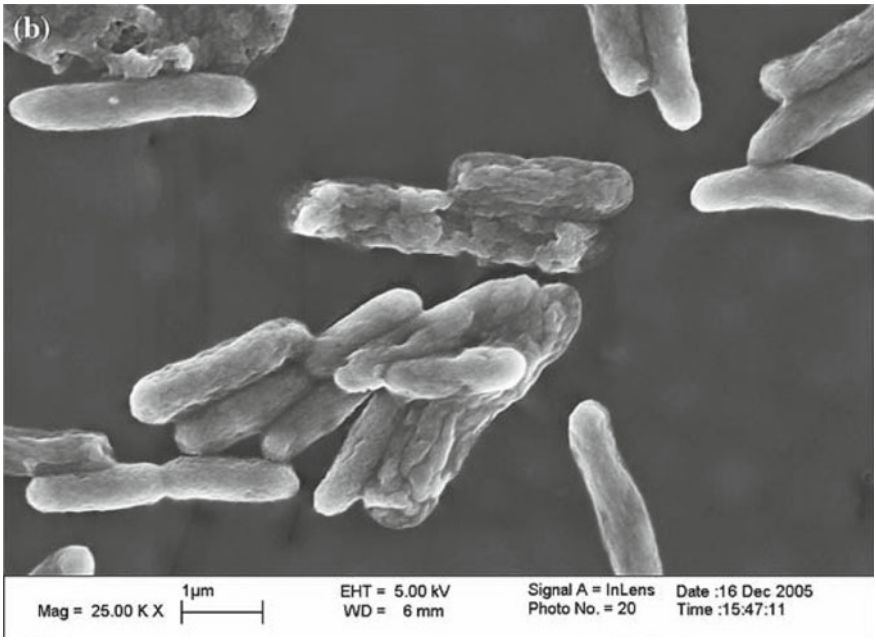

Fig. 2.5 SEM image of bacteria cultured with ZnO nanoparticles. Adapted with permission from Zhang et al. (2007) (Copyright 2007, Springer Nature)

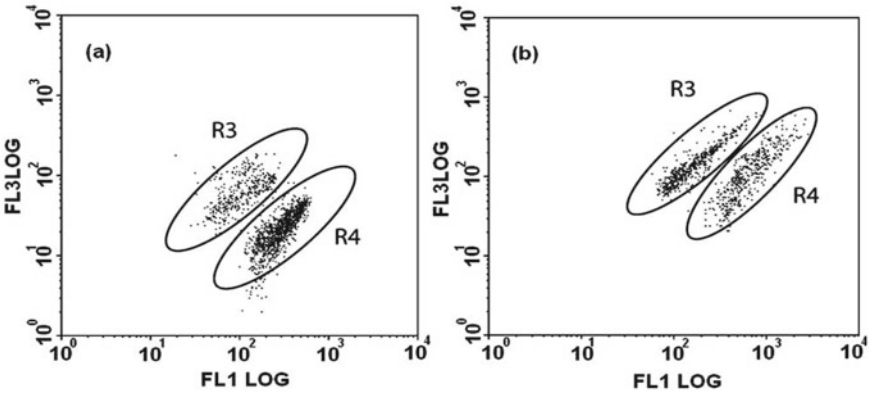

Fig. 2.6 Flow cytometry images of *E.coli* and *S. aureus* both treated with ZnO particles. Adapted with permission from Reddy et al. (2007) (Copyright 2007, AIP Publishing)

decomposition of the cell wall followed by the subsequent decomposition of the cell wall membrane (in Fig. 2.7).

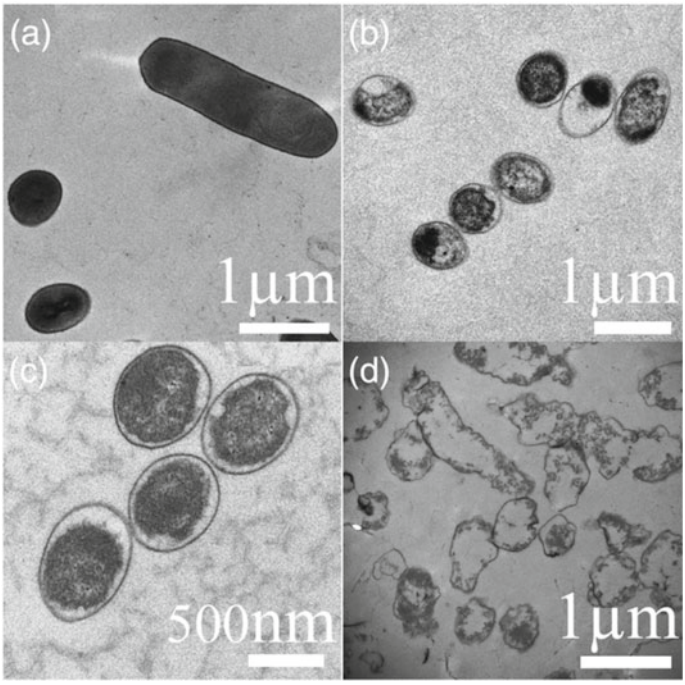

Fig. 2.7 TEM images of *E. coli* (a) control, (b) ZnO powders, (c) ZnO nanorods and (d) ZnO nanoparticles. Adapted with permission from Tam et al. (2008) (Copyright 2008, Elsevier)

The damage to the cell membrane directly leads to the leakage of minerals, proteins and genetic materials, causing cell death (Padmavathy and Vijayaraghavan 2008; Tam et al. 2008). Hewitt et al. also reported similar results in 2001 (Hewitt et al. 2001). Yang and Xie and Tam et al. reported that the release of Zn^{2+} ions as a result of ZnO decomposition may be responsible for the observed antibacterial activity (Yang and Xie 2006; Tam et al. 2008). Sevinç and Hanley also believed that the antimicrobial efficiency of the materials is a function of the types and quantities of metal ions as well as the strain of microorganisms. Metal ions can bond with thiol, imidazole, amino and carboxyl groups of membrane proteins in microorganisms causing structural changes. The changes in membrane structure exhibited a significant increase in permeability, disrupting the regulation of transport through the plasma membrane and ultimately leading to cell death (Sevinç and Hanley 2010). However, Sawai et al. suggested that eluted zinc ions are considered not to inhibit the bacteria growth because of the low solubility of ZnO. The antibacterial activity of the ZnO powder slurry originates from the powder surface (Sawai et al. 1995a). Zhang et al. also showed that the presence of ZnO nanoparticles leads to damage to the membrane wall of *E. coli* (Zhang et al. 2007). In the study on the antibacterial effect of ZnO nanorod-modified graphene nanosheets (ZNGs) against *Streptococcus mutans*, Zanni et al. observed that the amount of Zn^{2+} released was negligible and thus the high cell mortality was not due to Zn^{2+} release. That indicates the release of Zn^{2+} may not be the primary mechanism for the antibacterial activity of ZnO (Zanni et al. 2016). As a wide bandgap semiconductor material, under ultraviolet/visible light, ZnO nanoparticles can generate ROS due to the fact that electrons and H^+ undergo a series of redox reactions with oxygen and water on the surface of ZnO (Miao et al. 2017). The dominant mechanisms of the observed antibacterial behaviour of ZnO particles were the generation of ROS and the resulting interaction between ROS and the cell, but Zn^{2+} and H_2O_2 do not cause a significant level of damage to the lipid membrane. Similar results were also reported by some other researchers (Liu et al. 2009; Nair et al. 2009; Shrestha et al. 2010; Wahab et al. 2010). Zhang et al. also reported that a small amount of physical damage to the cell envelope might also be induced by ZnO (Zhang et al. 2010). It seems that active oxygen species generated in ZnO particles could be a reason reported by Sawai et al. (1996a, b, 1998a), Yamamoto et al. (2004), Li et al. (2007). Therefore the production of active oxygen species due to the presence of ZnO nanoparticles, damage to the membrane cell wall through adhesion on the cell membrane, penetration through the membrane cell wall and cellular internalisation of nanoparticles were some speculations for their observations. The generation of reactive oxygen from ZnO slurries (2.6 μm) was examined by oxygen electrode analysis and chemiluminescence analysis. The generation of H_2O_2 was detected from the ZnO powder slurry and increased linearly with increasing slurry concentration. The results also showed that there is slight or no generation of O_2^- from the ZnO powder slurry. It can be concluded that H_2O_2 generated from the ZnO powders might take some part in growth inhibition of bacteria (Yamamoto et al. 2003). Moreover, there was a higher efficiency of the antibacterial activity in the ZnO nanofluids with longer storage period because more amount of H_2O_2 were produced. As reported by Xu and Xie, holes carrying positive electricity (h^+) and moveable electrons (e^-) are

produced when ZnO nanoparticles are irradiated by sunshine, especially by UV light. This kind of a hole can activate oxygen in air and give active oxygen with very strong chemical activity (Xu and Xie 2003). The water molecule can be split into·OH and H^+ with the hole. A superoxide radical anion ($\cdot O_2^-$) transformed from the dissolved oxygen molecules reacts with H^+ to generate a hydrogen peroxide radical ($\cdot HO_2^-$). A molecule of H_2O_2 is produced when an electron collisions with a hydrogen ion (Liu and Yang 2003). Sawai et al. used penicillin C, chloramphenicol, nalidixic acid and rifampicin as the selective reagents in order to specify the damaged parts in *E. coli* which was mainly due to the different primary inhibitory actions of these agents against bacterial cells. The results suggested that the ZnO powder slurry enhanced the sensitivity of the *E. coli* to chloramphenicol. It meant that ZnO powder slurry exert some adverse influences on the ribosomes in the *E. coli* cells. They reported that H_2O_2 was one of the primary factors in the antibacterial mechanism of the ZnO powder slurry. It was assumed that hydrogen peroxide generated from the ZnO powder slurry penetrates the cell membrane of *E. coli*, produces some type of injury, and inhibits the growth of the cells or kills them (Sawai et al. 1998a; Yamamoto et al. 1998).

However, according to Tam et al., the release of H_2O_2 from ZnO may not contribute to the cell damage of *E. coli* and *Bacillus atrophaeus* (Tam et al. 2008). They investigated the effects of H_2O_2 (30 mg/L) on the *E. coli* and *B. atrophaeus* and revealed that the concentrations that cause damage to *E. coli* cells do not damage *B. atrophaeus* cells. It indicated that the release of H_2O_2 from ZnO may contribute to the cell damage of *E. coli* but the cell damage of *B. atrophaeus* is caused by a different mechanism since no effect of H_2O_2 is observed in this case. Thus, there is no correlation between antibacterial activity and H_2O_2 (Tam et al. 2008). This suggested that there are other mechanisms contributing to the antibacterial activity of ZnO nanoparticles. By using electron-spin resonance (ESR) measurement, Applerot et al. showed that aqueous suspensions of small nanoparticles of ZnO produce increased levels of reactive oxygen species, namely hydroxyl radicals. In addition, the smaller particles generate a larger amount of hydroxyl radicals in their aqueous suspensions, as assessed using ESR measurements (Applerot et al. 2009a). Similar ESR results were reported by Hirota et al. (Hirota et al. 2010). The generation of hydroxyl radicals and superoxide from ZnO suspension has been observed by ESR measurement. The authors suggested that the antibacterial activity of ZnO might be originated from the generation of superoxide. Zhang et al. also reported that, although the dominant mechanisms of the observed antibacterial behaviour of ZnO particles is the generation of ROS and the resulting interaction between ROS and the cell, Zn^{2+} and H_2O_2 does not give a significant level of damage to the lipid membrane (Zhang et al. 2007, 2008, 2010). Sawai reported that ZnO showed no significant effect on cytoplasmic membrane polarisation or permeability. The antibacterial mechanism of ZnO was studied by a mutagenicity test against *Salmonella typhimurium* TA 98 and TA 100. Mutagenicity is a process in which chemicals and stress act on DNA in cells and cause injury to the base sequences. If the injury is serious, the cells can be killed. However, a proper degree of injury may cause a change in the character of cells by the synthesis of proteins which have different amino acid sequences depending on

the injury. The results showed that the antibacterial mechanisms of ZnO powders do not depend on the DNA damage in bacterial cells (Sawai et al. 1995a, b, c). Up to our knowledge, the antibacterial mechanism of ZnO nanofluids is still not well known and must be further studied, while the antibacterial behaviour of ZnO nanofluids has been clearly reported in the literature.

Some uses of ZnO nanofluids for coating and impregnation as antibacterial agents are given below.

- **ZnO coated carbons**: Activated carbons have attracted much attention as one of the effective adsorbents. In addition, as ZnO is non-toxic and possesses antimicrobial properties it has encouraged researchers to use ZnO nanoparticles in activated carbons. Yamamoto et al. used activated carbon-containing ZnO as a novel antibacterial agent to remove the bacteria that are harmful to humans from water. An ion-exchange resin with a particle size of about 0.5 mm was treated for 24 h with an aqueous solution of $[Zn(NH_3)_4]^{2+}$ complex. The resin was carbonized for 10 min in nitrogen gas at a temperature ranging from 500 to 900 °C (Yamamoto and Sawai 2001; Yamamoto et al. 2001, 2002b; Özkal et al. 2007; Sawai et al. 2007). Similar results were obtained by using steam activation of pitch containing organometallics (Tamai et al. 2001). Enhanced antibacterial activity was observed for the mixture of GO-ZnO nanoparticles. The antibacterial activity of the composite was nearly four times and twice times as that of GO nanoparticles and ZnO nanoparticles respectively.
- **ZnO coated polymer films**: Xu and Xie used a direct dispersing method to form multi-functions of ZnO resin coatings. This composite with uniform distribution of nano-ZnO in the resin exhibits both anti-electrostatic and antibacterial functions. For the coating, ZnO was put in an acetone solution containing a surfactant and was ultrasonically dispersed. Then ZnO was filtration dried with a vacuum drier and mixed with acrylic resin with solvent and curing agents. The experimental results also showed that ZnO has a higher antibacterial effect on colibacillus and *S. aureus* with an increase in filling quantity, a decrease in particle size and an increase in specific superficial area (Xu and Xie 2003). Similar results were also reported by Li et al. in (2008). The hydroxyl-acrylic resin (HAR) and trimer of hexamethylene-1,6-diisocyanate (HDI trimer) were mixed with ZnO nanoparticles and then spread on a plastic substrate with roasting at 60 °C for 30 min (Zhang et al. 2010). Li et al. and Bajpai et al. coated ZnO nanoparticles on plastic films as a functional antibacterial material and suggested that this kind of material can be used in food packages to extend the storage time of food (Bajpai et al. 2010; Li et al. 2010). The antibacterial activity of ZnO nanoparticle coated low-density polyethylene film was evaluated. The coated films exhibited high antibacterial activity against both Gram-positive and Gram-negative bacteria and the antibacterial efficiency retained even after eight months (Rokbani et al. 2019).
- **ZnO coated paper and glass**: Ghule et al. reported a simple approach of ultrasound-assisted coating of paper with ZnO nanoparticles without the aid of a binder. For coating ZnO particles on the paper surface, ZnO particles were dispersed in deionized water using a fixed power sonicator. Then the paper surface

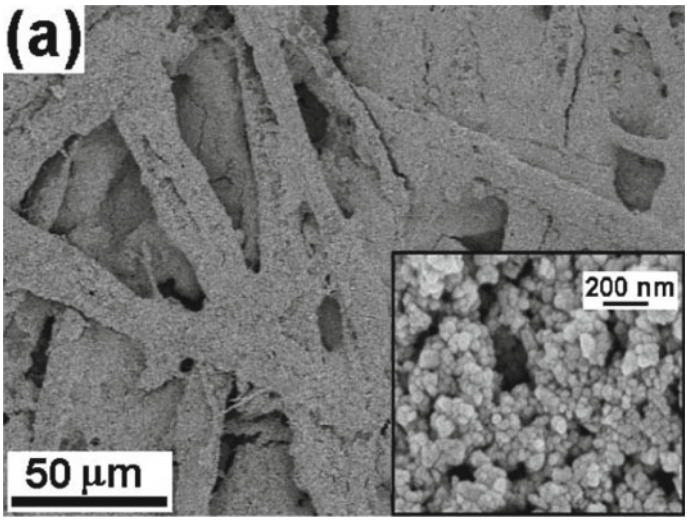

Fig. 2.8 SEM image of ZnO nanoparticles coated paper. Adapted with permission from Ghule et al. (2006) (Copyright 2006, The Royal Society of Chemistry)

to be coated were just touched with the dispersed solution and were sonicated for various time. The coating was visualized by SEM image as shown in Fig. 2.8.

This coated paper can be used to provide a good economic alternative for TLC plates in separation science (Ghule et al. 2006). Applerot et al. coated ZnO nanoparticles on the glass by using the ultrasonic irradiation method (Applerot et al. 2010).

– **ZnO coated fabrics**: In addition, ZnO nanoparticles were added into fabrics by a regular wet finishing process pad-dry method at room temperature as antibacterial cotton fabric (Li et al. 2007) and visualized under SEM. ZnO nanoparticles were used as a multi-component antibacterial agent and coated on the surface of cotton fabric with acacia and chalcone as blinders (Sivakumar et al. 2010). Similar functions were also reported by El Shafei et al. and Ugur et al. (El Shafei et al. 2010; Uğur et al. 2010). Yuvaraj et al. deposited ZnO nanoparticles on various substrates including polymer sheets, cotton fabrics and glass at room temperature by activated reactive evaporation (Yuvaraj et al. 2010).

2.4 Antibacterial Activity in Titanium Dioxide Nanofluids

Another non-toxic metal oxide of interest is titanium dioxide. It becomes a very potent oxidation photocatalyst when illuminated with UV light. Fu et al. reported good inhibition of TiO_2 nanoparticles on *E. coli* (Gram-negative) and *Bacillus megaterium* (Gram-positive), even under room light. A higher concentration of the TiO_2

suspension was required to kill *E. coli* than to kill *B. megaterium*, and this observation was related to the different cell wall structures and cell membranes in the two types of bacterial species studied (Fu et al. 2005). The conclusion from the literature reports is that as a very strong photocatalyst the titania nanofluids catalyze the catalytic oxidation and destruction of the cell wall by generating a high concentration of highly reactive inorganic radicals. It was observed that smaller nanoparticles perform better antibacterial behaviour (Chung et al. 2008; Liu et al. 2008; Muranyi et al. 2010).

2.5 Nanodrug Delivery

Among the various applications of nanofluids to biology, there is a huge potential for the application of nanofluids in the field of drug delivery as demonstrated by several publications over the last few years. The scheme of drug delivery is relatively simple. A drug is attached to a biocompatible magnetic nanoparticle carrier. These complexes are introduced into the circulatory system of the patient and an external magnetic field is used to concentrate the complex at a specific target site within the body. Once the drug/carrier is concentrated at the target site, either the carrier is taken up by the cells or the drug is released and taken up by the target cells. The release can be activated by physiological conditions (pH or temperature), via enzymatic reaction, or by an alternating magnetic field. Water-dispersed TiO_2@ZnO–GO with pH-dependent release properties were synthesized by a facile sono-chemical method to design a new drug delivery carrier (Zamani et al. 2018). Another possibility is to confer a local effect, such as irradiation or hyperthermia, using the particles (Kleinstreuer et al. 2008; Choi 2009). It is not surprising that nanofluids have implications in biological systems in general and as drug delivery systems in particular since most cells are 10,000–20,000 nm in diameter. They can be fabricated at different sizes and surface modifications, which determine their properties in biological systems. In general, nanofluids have the potential to improve drug delivery and provide tools for the controlled release of drugs, protect the active drug from alteration and inactivation, treat target cells using hyperthermia and prevent multi-drug resistance. The main issues in the search for appropriate carriers as drug delivery systems include drug incorporation and release, formulation stability, biocompatibility, particle size and surface area, and degradation properties. The main elements of nanofluids in drug delivery include site-specific delivery of drugs, controlled release of drugs, protection of drugs, treatment using hyperthermia and prevention of multi-drug resistance (Soppimath et al. 2001; Duncan, 2003; Panyam and Labhasetwar 2003; Fahmy et al. 2007; Liong et al. 2008). Europium (Eu) and gadolinium (Gd) doped ZnO nanoparticles can be used as photoinducers to increase the efficacy of X-rays within the cancer cell, and at the same time, act as an effective CT/MRI nanoprobe for simultaneous CT/MR imaging (Ghaemi et al. 2016). Potential problems associated with nanofluid use in drug delivery include the risk of agglomerating particles or their breakdown

products. The potential for the use of nanofluids in the task of drug delivery is huge and it may change the way of currently used drug delivery systems.

2.5.1 Preparation of Nanofluids for Drug Delivery

- **Surface modification:** The concept of surface modification seems promising (Sruthi et al. 2018; Sathishkumar et al. 2021). Through the coating with biodegrade matrices, nanoparticles become invisible to macrophages. The choice of the coating polymer can prolong the half-life of nanofluids in circulation. The choice of hydrophilic or hydrophobic matrices for coating determines the fate of nanofluids. Hydrophilic coatings are polyethylene glycol, polyethylene oxide and silicones. Lode et al. showed that polymethylmethacrylate nanospheres coated with polyoxamer 407 can increase retention and accumulation in B16 tumours in mice (Lode et al. 2001). ZnO nanoparticles coated with chitosan and loaded with paclitaxel formed a drug delivery system with high cytotoxicity against breast cancer cell line (MCF-7) but a low side effect on normal cell line (Akbarian et al. 2020). A ferrofluid cluster composed of magnetic nanoparticles was used to target and concentrate drugs in the drug delivery system (Nakano et al. 2008). The nanomagnetic fluids targeted and concentrated drugs using a ferrofluid cluster composed of magnetic nanoparticles. The potential of magnetic nanoparticles stems from the intrinsic properties of their magnetic cores combined with their drug-loading capability and the biochemical properties that can be bestowed on them by means of a suitable coating. The degree of surface coverage has been postulated to be the main parameter in cellular uptake.
- **Polymers for encapsulation:** One of the most important characteristics of nanofluids is their ability to encapsulate drugs. Nanofluids as drug carriers can be prepared in two different methods. One is as a drug reservoir, and the other one is nanospheres, where the drug is dispersed in a polymeric matrix. Encapsulation of the drug offers a solution for drug protection (LaVan et al. 2003). Functionalised nanohybrid hydrogel using L-histidine conjugated chitosan, ZnO nanoparticles and dialdehyde cellulose was synthesized as a sustained drug delivery carrier for the polyphenol drugs (George et al. 2020a). L-Cysteine was conjugated with chitosan followed by the incorporation of phyto-synthesised zinc oxide nanoparticles. The obtained nanohybrid hydrogel was then used as a carrier for the enhanced therapeutic delivery of naringenin (George et al. 2020b). The different approaches in SPION coating result in the different assembly of polymers (Veiseh et al. 2010) and are summarized in Fig. 2.9. In polysaccharide coating and coating with copolymers, the resulting particles are found as uniformly encapsulated cores. In another coating approach, polymer molecules are anchored to the magnetic particle surface resulting in a brush-like structure. Liposome and micelle-forming molecules result in a core–shell structure with magnetic particles in the core.

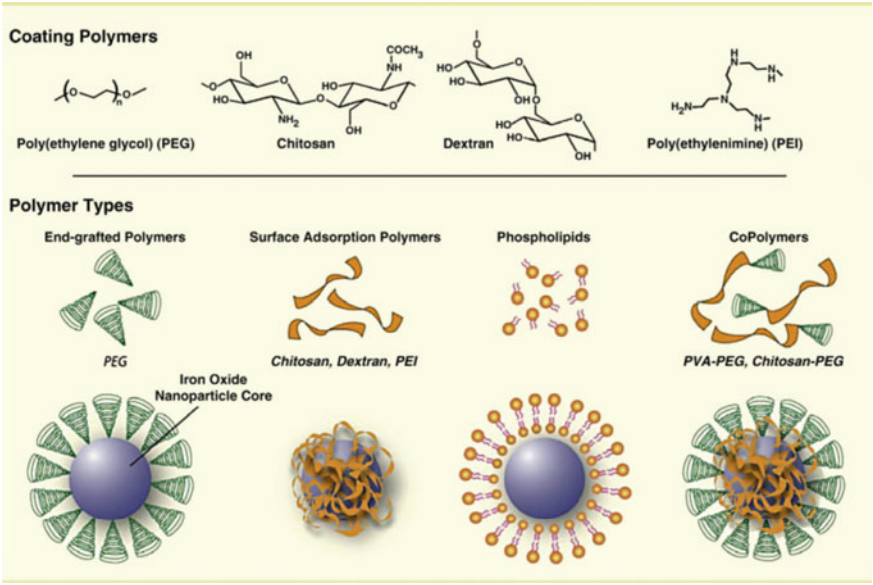

Fig. 2.9 Illustration depicting the assembly of polymers onto the surface of nanoparticles. Adapted with permission from Veiseh et al. 2010 (Copyright 2010, Elsevier)

2.5.2 Hyperthermia Therapy

Temperatures between 40 and 45 °C are generally referred to as hyperthermia. The tumour cells are more sensitive to temperatures above 42 °C than normal cells and can be destroyed by increasing the temperature locally to 41–42 °C. Thus, magnetic nanofluids can be used in hyperthermia cancer therapy due to their special physical properties. Hyperthermia may make some cancer cells more sensitive to radiation or harm other cancer cells that radiation cannot damage. When hyperthermia and radiation therapy are combined, they are often given within an hour of each other.

Clinical hyperthermia falls into three broad categories, namely localized hyperthermia, regional hyperthermia and whole-body hyperthermia. The challenge in this method is to restrict local heating of the tumour surrounding by self-control heating or by lowering the exposure time (Sharifi et al. 2012). However, the magnetic nanofluids are easy to achieve the temperature enhancement needed for a special application with as low as possible amounts of nanoparticles. The heating potential is directly correlated to the size of the magnetic particle, which can be controlled by an appropriate synthesis method. In clinical applications of magnetic nanoparticle hyperthermia for cancer treatment, it is very important to ensure maximum damage to the tumour while protecting the normal tissue (Brusentsov et al. 2001; Rosensweig 2002). By using a soft-chemical approach, multifunctional magnetic Fe_3O_4@ZnO core–shell nanoparticles were prepared as new nanoplatforms for cancer treatment to be used as a promising material for image-guided magnetic hyperthermia therapy (Gupta et al.

2021). It is important to select the synthesis method and ferrite chemistry for hyperthermia treatment. Sharifi et al. showed magnetic FeCo-graphite nanoparticles for target tumour cells with concomitant hyperthermia-based therapy. They found that FeCo/C MNPs have shown an excellent magnetization value, a high Curie temperature and high magnetic anisotropy energies, all of which are critical in order to enhance their potential for biomedical applications, such as MRI and hyperthermia (Sharifi et al. 2012).

2.6 Conclusion

Many interesting physical and chemical properties of nanofluids have been identified in the past decades. This chapter presented an overview of the recent developments in the antimicrobial application of nanofluids, especially ZnO nanofluids. These new properties and behaviour in biosystems can be put to good use as a new line of defence against biological threats. The potential for the use of nanofluids in the task of drug delivery and microbial protection is huge. However, the following key issues require greater attention in the future. The stability of the nanofluids is a crucial issue for both scientific research and practical applications. Up to now, there is a lack of agreement between experimental results from different groups. It is important to systematically identify the detailed and accurate physicochemical properties of nanofluids to explain the discrepancy in the experimental data. Further, the development and research in the field of nanofluids in the biological application requires the understanding and interaction between particles and biological cells by experts from physics, mathematics, the biomedical sciences, and from chemists and medical personnel.

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Chapter 3
Nanotechnology in Drug Delivery

Mayuri Napagoda and Sanjeeva Witharana

Abstract A biologically active substance (chemical compound, peptide, antibodies, gene-based drugs etc.) capable of producing a therapeutic effect can be administered to the body via different routes. However, the limited aqueous solubility, low permeability, non-specific distribution, poor bioavailability, rapid clearance and uncontrollable release of drugs are among the main challenges in achieving the therapeutic efficacy of these agents. In this respect, the properties of nanomaterials like small particle size, surface charge, shape etc. can be exploited for the development of effective drug delivery systems. These novel nano-drug delivery systems can circumvent the problems associated with conventional drug delivery approaches and allow targeted and site-specific delivery of therapeutic agents while mitigating the adverse effects. This chapter provides an overview of novel nano-drug delivery platforms developed against different disease conditions and signifies their potential in future medicine.

Keywords Nano-drug carriers · Nanoencapsulation · Targeted delivery

3.1 Introduction

The term drug delivery refers to the process involved in the administration of biologically active compounds to accomplish their therapeutic impact (Vega-Vásquez et al. 2020). Different routes can be used to introduce a drug into the body and those are usually named by the site at which the drug is being administered. These include buccal (through buccal mucosa), nasal, ocular, oral, pulmonary (via inhalation through the mouth), sublingual (under the tongue), transdermal, subcutaneous, vaginal, rectal, intravenous and intramuscular drug delivery (Sun et al. 2017).

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The potency and efficacy of these bioactive compounds/drugs largely depend on the prolonged availability of such molecules in the intended target site. The availability of a particular drug is mainly governed by the properties of the molecule itself such as solubility, pK_a, an affinity for the receptor, molecular weight etc. In addition, other extrinsic factors like the physiological stage of the receptor organism, enzymatic machinery, and external pH in the surrounding environment can also influence the availability. Moreover, during the delivery process, these compounds may encounter different substances that can interact with them and as a result, the potency of the drug can get altered or sometimes unexpected responses can be observed. The dynamic interactions between the drug and its new surrounding environment during the metabolism process may also cause physicochemical modifications thus altering the potency and efficacy of a drug. Therefore, to obtain the real therapeutic effect, a drug should overcome hostile physiological conditions during the distribution process and the proper amount of active compound should reach and enter the target site (Vega-Vásquez et al. 2020). In this respect, nanotechnology can be utilized to achieve targeted and site-specific delivery of drugs particularly the ones having poor solubility and absorption (Mirza and Siddiqui 2014).

3.2 Nano-Drug Carriers

Nanoparticles of size <100 nm at least in one dimension are usually employed as nanocarriers. This includes various biodegradable materials like synthetic or natural polymers, lipids, or metals (Suri et al. 2007; Sánchez et al. 2020). Some examples of polymeric nanocarriers are given in Fig. 3.1. These diversified nano-drug delivery systems not only assist in the targeted biomolecular interaction of drugs but also are capable of reducing various side effects, thus resulting in effective treatment and management of disease (Chandrasekaran et al. 2021). An ideal drug carrier should possess features like biocompatibility, absence of side effects, stability and loss-free transit to the affected part and biodegradability.

The nano-drug carriers are designed either to target a drug to a particular site or to achieve a controlled release of the drug at a particular location. These nanocarriers deliver drugs via two modes;

(i) Passive delivery

In the passive delivery mode, drugs are normally associated with nanostructures by either physical encapsulation or chemical conjugation. In the physical encapsulation method, the drug is encapsulated in the inner cavity of the nanostructure and is stabilized by noncovalent interactions (eg: hydrophobic effect) between the drug and the nanocarrier. Once the nanostructures are disassembled at the target site, the intended amount of the drug is released in consequence. In the chemical conjugation method, drugs are attached to the nanostructures by direct chemical conjugation and this conjugation should be cleavable at the target site to achieve good control over the triggered release of the therapeutics (Lu et al. 2016).

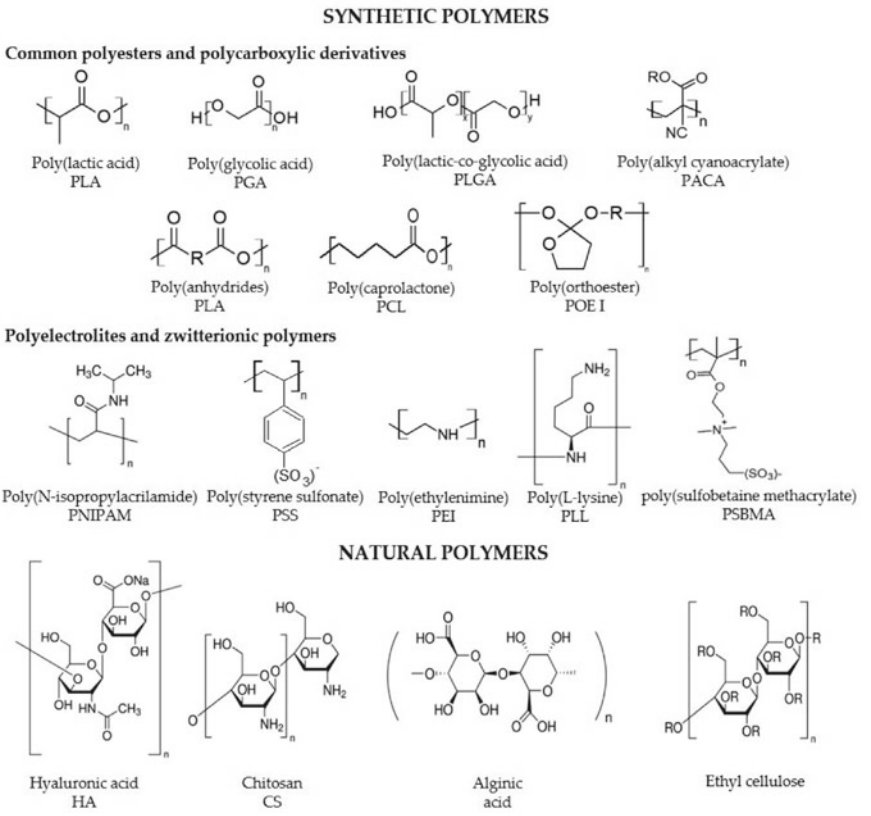

Fig. 3.1 Polymers commonly used to manufacture polymeric nanocarriers. The use of polymeric nanocarriers has the potential to reduce the dose and adverse side effects, providing better efficiency and effectiveness of therapeutic regimens, especially in drugs having high toxicity, low solubility in the physiological environment and low bioavailability (Adapted with permission from Sánchez et al. 2020)

(ii) Self-delivery

In this approach, well-defined nanostructures with a high and fixed content of drug molecules as building units are created. For example, the conjugation of hydrophobic drug molecules to hydrophilic polymers may form amphiphilic prodrugs that can spontaneously self-assemble into stable nanostructures providing a sustained drug release (Lu et al. 2016).

Targeting of drugs is another important aspect in the development of nano-drug carriers and it can be identified as passive or active targeting. In passive targeting, the drug carrier complex is capable of avoiding different mechanisms in the body like metabolism, excretion, opsonisation, and phagocytosis which may result in the removal of the drug. This drug carrier complex circulates through the bloodstream and is driven to its target site by properties like pH, temperature, molecular size, or

shape. On the other hand, in active targeting, antibodies, antibody fragments, peptides and various other moieties are coupled to drugs and delivery systems to anchor them to the receptor structures expressed at the target site (Khanna 2012; Patra et al. 2018). Receptors on cell membranes, lipid components of cell membranes and antigens or proteins on cell surfaces are some examples of the drug targets (Khanna 2012).

Nano-drug carrier systems developed against different diseases are discussed in the following sections.

3.3 Nano-Drug Carriers to Treat Different Disease Conditions

3.3.1 Cancer

Cancer is one of the leading causes of death worldwide. According to International Agency for Research on Cancer, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths have occurred in 2020 (Sung et al. 2021). Chemotherapy is widely used in cancer treatment. It is based on the inhibition of the division of vigorously growing cells which may include both cancerous cells as well as normal cells with rapid proliferation rates (hair follicles, bone marrow and gastrointestinal tract cells). Due to this indiscriminate destruction of normal cells, chemotherapy often results in various side effects like hair loss, bone marrow suppression, gastrointestinal reactions etc. In addition, the development of drug resistance is also observed with conventional chemotherapeutic agents (Pérez-Herrero and Fernández-Medarde 2015; Yao et al. 2020). Therefore, many attempts have been made over recent years to develop nano-carriers for the delivery of chemotherapeutic agents to molecular targets overexpressed on the surface of tumour cells (Pérez-Herrero and Fernández-Medarde 2015).

Chemotherapy can be followed by multiple drug resistance (MDR) where cancer cells develop resistance to structurally and mechanistically unrelated drugs. This has become a major obstacle in the treatment of cancers. It is usually associated with the overexpression of ATP-binding cassette (ABC) transporters resulting in the increased efflux of drugs from the cancer cells. Ultimately this would lead to the decreased intracellular concentration of the drug of interest (Sun et al. 2012). Therefore, multifunctional nanoparticles can be employed as important delivery platforms in treating MDR cancers (Iyer et al. 2013).

The direct pulmonary delivery of anti-cancer drugs via the inhalation route is highly beneficial as it enables the targeting of drugs to the tumour sites at high concentrations and thereby increasing their local availability in the lungs. Moreover, this non-invasive approach reduces the adverse systemic side effects while improving patient compliance vs. injections. However, a considerable amount of residual drugs may remain in the mouth, nose, throat or upper respiratory areas resulting in local toxicity. Similarly, frequent administration of such drugs is often required thus it may

cause a burden to patients with a weak inspiration capacity. Kim et al. (2013) fabricated an inhalable highly porous large PLGA microparticles containing doxorubicin and tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) called TRAIL/Dox PLGAMP to obtain an efficient anti-tumour therapeutic with a low dose of doxorubicin. TRAIL is a transmembrane protein and is considered a non-toxic anticancer agent capable of inducing apoptosis in some cancer cells. It can specifically bind to the death receptors (DR4/TRAIL-R1, DR5/TRAIL-R2) that are expressed in some cancer cells but usually not expressed by normal cells. A gradual release of TRAIL and doxorubicin and the deposition of TRAIL/Dox PLGA MP in mouse lungs were observed in this study. The antitumour activity of TRAIL/Dox PLGA MP was determined using the BALB/c nu/nu mice mouse model of H226 cell metastasis. Interestingly, the tumours in H226-implanted mice subjected to TRAIL/Dox PLGA MP treatment were found to be significantly smaller and fewer in number in comparison to the mice treated with TRAIL or Dox PLGA MP alone. It was speculated that the synergistic apoptotic effects of TRAIL and doxorubicin were responsible for the improved performance. The results of this study suggested that TRAIL/Dox PLGA MP system that utilizes very low doxorubicin dosages has great promise as an inhalable anti-lung cancer agent with sustained release and long-acting properties. Later Choi et al. (2015) fabricated inhalable self-assembled TRAIL/Dox HSA-NP nanoparticles by conjugating human serum albumin (HSA) with doxorubicin and octyl aldehyde and then adsorbed with TRAIL. A gradual release of TRAIL and doxorubicin-HSA was observed for over 3 days. Compared to HSA-NP comprised of TRAIL or Dox alone, synergistic cytotoxic and apoptotic activities were observed in H226 lung cancer cells treated with TRAIL/Dox HSA-NP. The improved antitumour efficacy was assigned to the synergistic apoptotic effects of TRAIL and doxorubicin (Choi et al. 2015).

Branched polymers (eg: star-shaped, dendrimers) that are characterized by the presence of several branches extending from a single point can be more useful than linear polymers, in the preparation of nanoparticle-based drug delivery systems. A small hydrodynamic radius, low solution viscosity, high loading content and enhanced encapsulation efficiency are often observed in the nano-drug carrier systems based on star-shaped polymers. Therefore, Tao et al. introduced PLGA, D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and mannitol-based star-shaped block copolymer nanoparticles (M-PLGA-TPGS NPs) having six branch arms as a carrier of the anti-cancer drug docetaxel. These docetaxel-loaded M-PLGA-TPGS NP exhibited a very high in vitro cytotoxicity against human breast carcinoma cells (MCF-7) as well as high anti-tumour activity in in vivo experiments. Further M-PLGA-TPGS NP system was capable of carrying higher levels of drug than linear polymer (Tao et al. 2013). Similarly, a star-like copolymer M-PLA-TPGS nanoparticle system was developed by a modified nano-precipitation method to deliver paclitaxel for prostate cancer treatment. The M-PLA-TPGS NPs were found to be stable and displayed a biphasic drug releasing pattern that can be described as an initial burst release which was followed by slow and continuous release. Higher antitumour activity was observed in both in vitro and in vivo experiments conducted with paclitaxel-loaded M-PLA-TPGS nanoparticles when

compared with linear PLA-TPGS nanoparticles and PLGA nanoparticles (Wang et al. 2014).

Thereafter, Zhang et al. introduced doxorubicin-loaded M-PLGA-b-TPGS NPs and studied the stability of these nanoformulations in vitro. The in vivo assessment of the pharmacodynamics of these formulations was carried out using the H1975 Xenograft BALB/c nude mice tumour model. The developed M-PLGA-b-TPGS NPs were around 110 nm in size and demonstrated fast drug release speed along with a high cellular uptake efficiency. The developed nanoformulations displayed high cytotoxicity against human lung carcinoma cells (H1975 and A549 A549) and higher anti-tumour activity than linear DOX-loaded PLGA-b-TPGS NPs and free doxorubicin (Zhang et al. 2015). Similarly, a star-shaped block copolymer M-PLGA-TPGS nanoparticle system with three branch arms was developed for sustained and controlled delivery of the modelled drug molecule genistein for liver cancer treatment. The therapeutic effect of this newly developed nanoparticle system was evaluated using the liver cancer cell line and hepatoma-tumour-bearing nude mice. The particle size of the genistein-loaded M-PLGA-TPGS nanoparticles was around 220 nm in diameter and the particle size and surface charge remained unchanged during the 90-day storage of its aqueous solution. Both in vitro and in vivo experiments revealed a higher antitumour activity in genistein-loaded M-PLGA-TPGS nanoparticles in comparison to the linear PLGA-TPGS nanoparticles and PLGA nanoparticles. In addition, a faster drug release and a higher cellular uptake were observed in star-shaped M-PLGA-TPGS nanoparticles in comparison to linear PLGA nanoparticles and linear PLGA-TPGS nanoparticles (Wu et al. 2016). Based on the above observations, the star-like copolymer M-PLA-TPGS nanoparticle system appears as a potential and promising biomaterial for the delivery of various anti-cancer agents.

In another experiment, Zeng et al. developed cholic acid functionalized PLGA-*b*-TPGS nanoparticle system (CA-PLGA-*b*-TPGS) for the sustained and controlled delivery of anti-cancer drug docetaxel for the treatment of cervical cancer. The docetaxel-loaded star-shaped CA-PLGA-*b*-TPGS nanoparticle system developed by a modified nano-precipitation method outclassed docetaxel-loaded PLGA nanoparticles and the linear PLGA-*b*-TPGS copolymer in terms of cellular uptake efficiency and antitumour activity (Zeng et al. 2013).

Albumin is a natural endogenous protein containing a number of chemical moieties to functionalize drugs or for ligand attachment. Over the recent years, it has drawn increased attention as a carrier protein in nano-drug delivery systems. Particularly albumin nanoparticles appeared to be highly effective in loading water-insoluble drugs used in cancer therapy. Kim et al. developed an albumin nanoparticle formulation (PTX/CCM Alb-NPs) for the co-loading of paclitaxel (PTX) and curcumin (CCM) by high-pressure homogenization technique. Although the particle size of PTX/CCM Alb-NPs was slightly higher (~250 nm) than that of plain PTX Alb-NPs (~234 nm) and CCM Alb-NPs (~134 nm), the zeta potential of the above three formulations were in the same range. A gradual release of PTX and CCM from the PTX/CCM Alb-NPs was observed over a period of 24 h. Further, this newly formulated nanoparticle system got efficiently internalized into human pancreatic cancer

cell line MIA PaCa-2 and resulted in a significant decrease in cell viability. These observations indicate the potential of PTX/CCM Alb-NPs system as an anti-cancer agent (Kim et al. 2016).

Sorafenib (SFB) is a multikinase inhibitor that is used as an antiangiogenic drug for advanced hepatocellular carcinoma (HCC). However, the very low solubility in water, short in vivo half-life and drug resistance has restricted its use in HCC therapy. Gan et al. developed an SFB-loaded polymeric nanoparticle referred to as NP-SFB-Ab from the self-assembly of block copolymers TPGS-*b*-PCL [comprised of D-α-tocopheryl polyethylene glycol 1000 succinate and poly(caprolactone)], pluronic P123 and sorafenib and this was followed by conjugating the anti- Glypican-3 (GPC3) antibody. NP-SFB-Ab showed higher cellular uptake and cytotoxicity than non-targeted NP- SFB and free SFB while significantly inhibiting the tumour growth in the in vivo experiment (Gan et al. 2018). Later, Li et al. designed sorafenib-encapsulated dendritic polymeric nanoparticles (NP-TPGS-SFB) using biodegradable poly(amidoamine)-poly(γ-benzyl-L-Glutamate)-b-D-α-tocopheryl polyethylene glycol 1000 succinate. The efficacy of this novel nanoformulation was evaluated by in vitro and in vivo assays. NP-TPGS-SFB displayed good stability and acid-responsive release of sorafenib. Furthermore, NP-TPGS-SFB showed significantly higher cellular uptake efficiency in HepG2 human liver cells and cytotoxicity than PEG-conjugated NP (NP-PEG-SFB). The in vivo experiments conducted with mice bearing HepG2 xenografts revealed that NP-TPGS-SFB was highly effective in inhibiting tumour growth with negligible side effects, suggesting its potential in the enhanced therapy of hepatocellular carcinoma (Li et al. 2020).

Cancer metastasis is a multistep process that results in the spread of tumour cells from the original (primary) tumour site to distant organs or tissues in the body (Adjei et al. 2018, Fig. 3.2). Metastasis to the bone is usually accompanied by bone pain and bone breakages and is common in patients diagnosed with prostate, breast or lung cancers.

It is believed that the glycophosphoproteins included in the SIBLINGs (small integrin-binding ligand N-linked glycoproteins family, ie: bone sialoprotein (BSP),

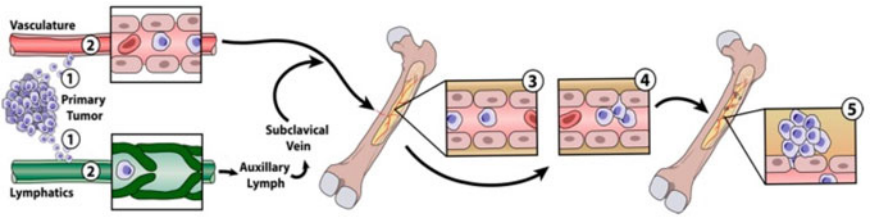

Fig. 3.2 Mechanism of bone metastasis. (1) Cancer cells from the primary tumour detach and invade surrounding tissue before (2) intravasation into the circulatory and lymphatic systems. Lymph, and the cancer cells within it, ultimately enter the bloodstream through the subclavian vein, via the thoracic ducts. Cancer cells that evade the immune system (3) translocate through capillaries in the bone where they (4) extravasate into the bone marrow to (5) establish metastatic sites (Adapted with permission from Adjei et al. 2018)

osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE), dentin matrix protein 1 (DMP1), and dentin sialophosphoprotein (DSPP), are involved in several processes required for bone metastasis (Kruger et al. 2014). Therefore, OPN and BSP have been identified as potential targets for antisense oligonucleotides therapy which is aimed at preventing or reducing lytic skeletal metastasis (Elazar et al. 2010). Antisense oligonucleotides are short, synthetic, single-stranded oligodeoxynucleotides that are capable of altering mRNA expression and thereby reducing, restoring, or modifying protein expression (Rinaldi and Wood 2018). However, the permeability of antisense oligonucleotides is restricted by their large molecular size and high negative charge density. Therefore, Elazar et al. attempted to encapsulate antisense oligonucleotides designed against OPN and BSP-II in PLGA nanoparticles. The therapeutic activity of this novel delivery system was determined in vitro, and in a breast cancer bone metastasis animal model. Interestingly, a sustained release of antisense oligonucleotides and a prolonged action were observed along with a significant reduction in tumour bone metastasis incidence as well as in the size of the lesions. These observations suggested that the delivery of antisense oligonucleotides by biocompatible polymeric nanoparticles could be a promising therapeutic approach (Elazar et al. 2020).

The delivery of therapeutic oligonucleotides into tumours is one of the novel strategies employed to influence the regulation of the cancer genome. Nanoparticles are widely employed in these situations to protect the therapeutic oligonucleotides from nuclease-mediated degradation, to enhance their accumulation at tumour sites and cellular uptake efficiency and thereby to increase the therapeutic index (Wu et al. 2021).

RNA interference (RNAi) therapeutics have emerged over recent years as a new class of pharmacologic inhibitors for silencing gene expression. RNAi is a well-conserved, endogenous cellular mechanism that occurs to protect organisms from aberrant transcription and employs small non-coding RNAs to silence gene expression. Here the target mRNA undergoes enzymatic degradation resulting in a decreased abundance of the corresponding protein (Bumcrot et al. 2006; Dykxhoorn and Lieberman 2006). Once small double-stranded RNAs known as small/short-interfering RNA (siRNA) molecules are introduced into cells, these siRNAs bind to the endogenous RNAi machinery and interrupt the expression of specific mRNAs (Dykxhoorn and Lieberman 2006). Although harnessing RNAi holds great promise for the treatment of various diseases, the delivery of functional siRNA to specific target organs was identified as one of the major obstacles. As a result, various lipid nanoparticle-based systems have been developed to enhance the delivery of RNAi therapeutics (Schultheis et al. 2014).

Schultheis et al. introduced Atu027 as a novel liposomal RNAi therapeutic containing a chemically stabilized siRNA that silences the expression of protein kinase N3 in the vascular endothelium. It was capable of reducing tumour growth, inhibiting local tumour invasion and metastasis in various mouse cancer models. The phase I clinical trial conducted using 34 patients with advanced solid tumours revealed that Atu027 was safe in patients and the disease stabilization was observed

in 41% of patients recruited for the study. Therefore, further clinical trials are ongoing to evaluate the effectiveness of this treatment (Schultheis et al. 2014).

B-cell lymphoma 2 (BCL2) is a member of the BCL-2 family of regulator proteins that involves in the regulation of apoptosis by either promoting or inhibiting apoptosis. Apart from the above function, BCL2 proteins play a critical role in many processes taking place in a normal healthy cell. The derangement of BCL2-regulated control mechanisms can lead to the evasion of apoptosis and extended survival of malignant cells. This may result in conditions like B-cell non-Hodgkin lymphomas (NHL) and chronic lymphocytic leukaemia (Tolcher et al. 2014; Adams et al. 2019; Klanova and Klener 2020). Therefore, several attempts were made to block the transcription of BCL2 gene via DNA interference (DNAi). As a result, Tolcher et al. designed a single-stranded, 24-base phosphodiester DNA oligodeoxynucleotide (PNT100) which is specific to the regulation of the BCL2 gene (Fig. 3.3). The interaction of PNT100 with the target DNA was capable of silencing BCL2 transcription, provoking initiation of the cell death pathway and pro-apoptotic events that ultimately lead to the death of cancer cells. A pilot phase 1 clinical trial was conducted with the use of PNT2258 system in which the DNA oligonucleotide was encapsulated within a liposomal nanoparticle. The average diameter of the nanoparticle was determined as 130 nm. Patients with advanced solid tumours were treated with PNT2258 and it was found to be safe and well tolerated up to the dose of 150 mg/m^2, the highest tested dose in the experiment. The treatment with PNT2258 resulted in a clinically manageable reduction in lymphocyte and platelet concentration (Tolcher et al. 2014).

Administration of drugs via the rectal route (in the form of suppositories, enemas, creams, ointments etc.) is practised for patients who are under post-operative treatments as well as paediatric, geriatric, and comatose patients who are vomiting or unable to swallow. The recent development in nanotechnology offers a more controlled delivery of drugs by rectal route for local or systemic actions (Purohit et al. 2018). A novel docetaxel (DCT)-loaded liquid suppository using thermosensitive and bioadhesive nanomicelles was developed by Seo et al. to enhance the bioavailability of DCT and its anti-tumour efficacy. This liquid suppository displayed sufficient bioadhesive force and gelation strength required to keep in contact with the rectal mucosa for a longer duration. In comparison to the oral administration of DCT, a high bioavailability was observed with the rectal administration. Furthermore, a significant decrease in the tumour volume along with a reduced toxicity profile was evident upon the treatment of rats with a DCT-loaded liquid suppository compared to the administration of DCT as an oral solution (Seo et al. 2013).

3.3.2 Microbial Diseases

Nanomedicine shows great potential in the treatment of bacterial infections. The nanoparticles themselves can function as antibacterial agents while the loading of antibiotics into nanocarriers has enhanced the bioavailability and effectiveness of

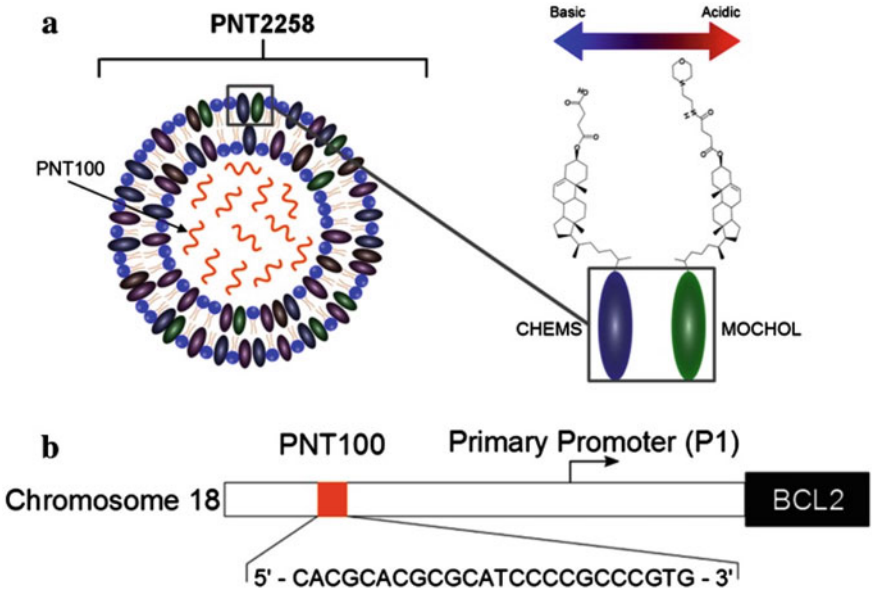

Fig. 3.3 **a** A representation of the PNT2258 molecule. PNT2258, the anti-BCL2 experimental therapeutic consists of a protective liposomal formulation composed of four lipids encapsulating a 24-base, chemically unmodified DNA oligonucleotide called PNT100, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesteryl hemisuccinate (CHEMS), and cholesteryl-4-([2-(4-mor-pholinyl)ethyl]amino)-4-oxoburanoate (MOCHOL). **b** Diagrammatic representation of the DNA "target" for PNT100 binding located on chromosome 18. The PNT100 oligonucleotide sequence is designed to hybridize to a region 5′ upstream of the BCL2 gene start site (Adapted with permission from Tolcher et al. 2014)

antibiotics. Diverse nanomaterials like zinc oxide/iron oxide/gold/silver nanoparticles, carbon nanotubes etc. have been developed for anti-infection applications, especially for direct use as biomedical devices (Taylor and Webster 2011). On the other hand, encapsulation of antibiotic agents can allow the co-delivery of multiple anti-infectious agents in a single nano-based system thus improving the efficacy of the treatment (Walvekar et al. 2019; Yeh et al. 2020).

Allicin (diallyl thiosulfinate) is an organosulfur compound present in garlic. Sharifi-Rad et al. evaluated the effects of allicin, silver nanoparticles (AgNPs) and their combination against skin infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) by an in vivo experiment. The initial in vitro assay revealed that the minimum inhibitory concentration (MIC) of allicin and Ag NPs on MRSA strains were 2.2 and 5.6 mg/mL respectively and when used in combination the MIC was determined as 0.4 mg/mL. The in vivo experiments on skin infection also indicated a synergistic activity with a significant reduction of bacteria in the allicin-AgNp combination (0 CFU/mL) in comparison to the control (377 × 108 CFU/mL), Ag NPs (80 × 106 CFU/mL) and allicin (43 × 105 CFU/mL) (Sharifi-Rad et al. 2014).

Tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis* is a leading cause of death among adults worldwide. The incidence of *Mycobacterium* strains that are resistant to many first-line and second-line drugs has been reported in many countries over the recent years urging the development of novel treatment modalities. *M. tuberculosis* resides primarily inside alveolar macrophages, hence making them a preferred target for TB therapy. Tenland et al. designed an experiment to load antimicrobial peptide NZX into mesoporous silica particles and study the therapeutic potential in a murine infection model. The NZX-loaded mesoporous silica particles were easily taken up by primary macrophages (Fig. 3.4) as well as a gradual release of the functional NZX from mesoporous silica particles into simulated lung fluid was observed in this study. Moreover, The NZX-loaded mesoporous silica particles exert good antimycobacterial activity and were able to control the infection in vivo in a murine model (Tenland et al. 2019).

In another experiment, rifampicin and levofloxacin which have been widely used against tuberculosis were complexed with cyclodextrin and then conjugated to curdlan nanoparticles to achieve simultaneous sustained release of both drugs for a longer duration (Yunus Basha et al. 2019).

Individuals who are immunocompromised or with lung dysfunction are highly prone to acquire pulmonary bacterial infections caused by *Haemophilus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* etc. Although activated macrophages play a major role in the defence against these invasive bacterial species, some pathogens can escape this macrophage-mediated defence mechanism. Therefore, targeted delivery of antibiotics to infected pulmonary tissues and maintaining adequate concentrations of therapeutics is an innovative strategy to eradicate residual bacteria. Wang et al. employed 4-(hydroxymethyl) phenylboronic acid pinacol ester-modified α-cyclodextrin (Oxi-αCD) to load the broad spectrum antibiotic moxifloxacin (MXF) and thereby to develop core–shell nanoparticles (MXF/Oxi-αCD NPs). The surface of the nanoparticles was coated with DSPE-PEG-folic acid and DSPE-PEG to improve the targeting capacity (Fig. 3.5). The results indicated that the release of MXF from the nanoparticles was accelerated in the presence of H_2O_2 while the antibacterial efficacy against *P. aeruginosa* was higher in MXF/Oxi-αCD NPs when compared with MXF. Moreover, folic acid-modified MXF/Oxi-αCD NPs were found to be superior to non-targeted MXF/Oxi-αCD NPs in terms of the ability to eradicate resident bacteria in macrophages and antibacterial efficacy against *P. aeruginosa* (Wang et al. 2019).

3.3.3 Neurological Disorders

The diseases affecting the central nervous system (CNS) and peripheral nervous system (PNS) are called neurological disorders. Alzheimer's disease, Parkinson's disease, and Huntington's disease are some examples of neurological conditions affecting the CNS. The main challenges in the discovery of new therapeutics for these diseases include the limited permeability of blood–brain barrier (BBB) and

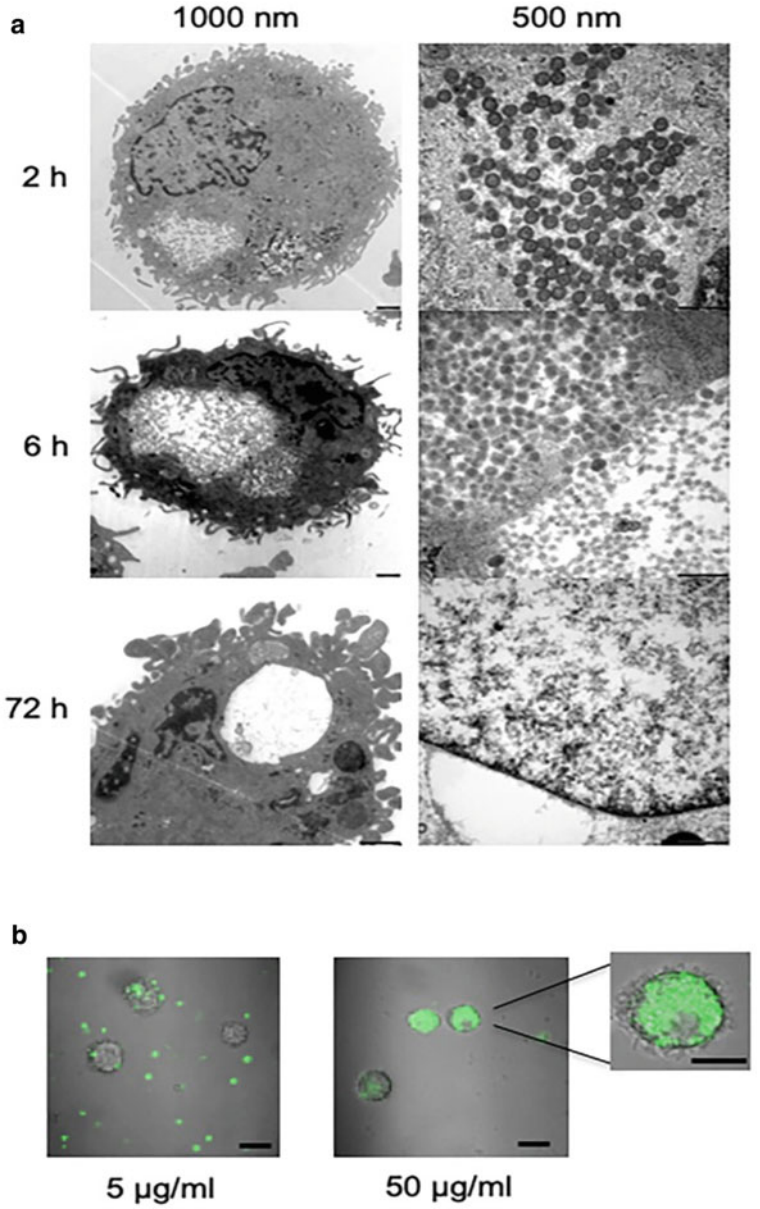

Fig. 3.4 Macrophages store mesoporous silica particles (MSPs) in vesicles TEM visualization of MSPs inside primary macrophages. Macrophages were observed to concentrate the internalised MSPs in vesicle-like structures. Over time, degradation of the internalized particles was observed. **b** Confocal microscopy of Atto-488 conjugated MSPs in primary macrophages showing concentration-dependent uptake to the cytosol after 2 h (Adapted with permission from Tenland et al. 2019)

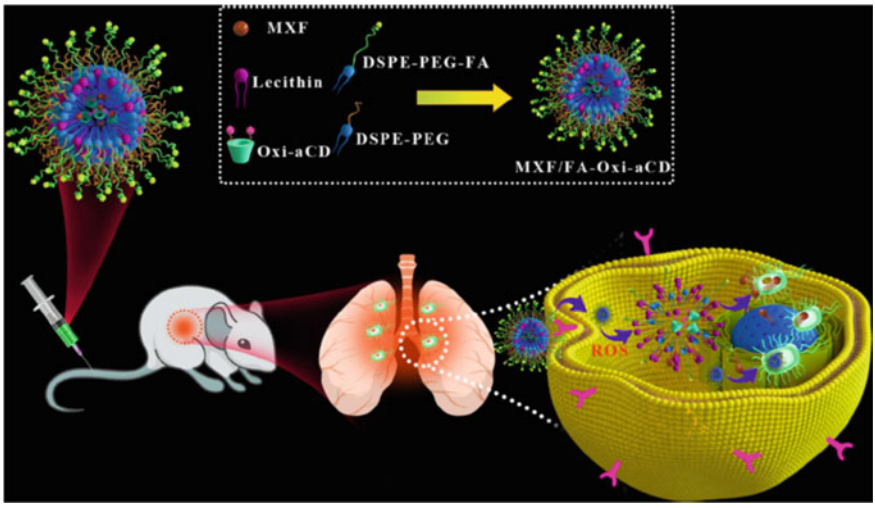

Fig. 3.5 Schematic illustration of the fabrication of ROS responsive MXF/FA-Oxi-αCD NPs and their application for targeted treatment of pulmonary *P. aeruginosa* infection (Adapted with permission from Wang et al. 2019)

undesired neuroimmune activities that occur due to the untargeted drugs. Therefore the development of nanoscale drug carriers capable of penetrating across the BBB could be a promising strategy in the treatment of disorders in CNS (Kang et al. 2018).

Alzheimer's disease is a neurodegenerative disease in which certain proteins; amyloid-β (Aβ) plaques and tau tangles accumulate within the brain resulting in a progressive loss of brain function. Aβ peptide occurs in isoforms of different lengths. The 40-residue peptide Aβ(1–40) is the most abundant Aβ isoform in the brain. Another isoform is 42-residue Aβ(1–42) which shows a significant increase with certain forms of AD (Schmidt et al. 2009).

In an earlier attempt, Gao et al. demonstrated the inhibition of Aβ aggregation by AuNPs@POMD-pep nanocomposite which was capable of dissociating Aβ fibrils, diminishing Aβ-mediated peroxidase activity and Aβ-induced cytotoxicity. This nanocomposite was made of gold nanoparticles (AuNPs) as a vehicle with the ability to cross BBB along with polyoxometalates with Wells–Dawson structure (POMD) + LPFFD peptides having the potential of inhibiting Aβ aggregation (Gao et al. 2015).

The pathogenesis of this Alzheimer's disease is promoted by metal ions (specially Zn^{2+} and Cu^{2+}) by accelerating the Aβ aggregation and inducing the formation of H_2O_2 which is neurotoxic. These effects can be overwhelmed by metal chelators, however, their inability to cross BBB and the non-specific interactions with metal ions essential for normal cellular activities limit their use as a therapeutic option for Alzheimer's disease. Therefore, Yang et al. introduced a mesoporous silica system (MSN-CQ-AuNPs) for the targeted delivery of the metal chelator clioquinol (CQ) and AuNPs (an Aβ inhibitor). In this system, the release of CQ has taken place

only upon exposure to high concentrations of H_2O_2, a condition that exists in Aβ aggregates. Interestingly, the MSN-CQ-AuNPs system was capable of inhibiting Cu^{2+}-induced $A\beta_{40}$ aggregation as well as reducing the cell membrane disruption, microtubular defects and ROS-mediated apoptosis, thus demonstrating the potential as a novel therapeutic strategy (Yang et al. 2016).

Ali et al. investigated the neuroprotective potential in anthocyanin-loaded polyethylene glycol-gold nanoparticles (PEG-AuNPs) using $A\beta_{1\text{-}42}$ mouse model of Alzheimer's disease. Although both anthocyanin-loaded PEG-AuNPs and free anthocyanins were capable of ameliorating memory impairments in the $A\beta_{1\text{-}42}$-injected mice, the effect was more pronounced in anthocyanin-loaded PEG-AuNPs. Anthocyanin-loaded PEG-AuNPs prevented the hyperphosphorylation of tau protein and inhibited apoptosis and neurodegeneration in the experimental mice (Ali et al. 2017). Similarly, the surface-functionalization of AuNPs with mimosine, a plant-based non-protein amino acid (Mimo-AuNPs) was found to suppress $A\beta_{1\text{-}42}$ aggregation. Mimo-AuNPs were capable of crossing BBB and triggered the disassembly of matured $A\beta_{1\text{-}42}$ fibres while increasing neuronal viability via reducing the phosphorylation of tau protein and the production of oxyradicals (Anand et al. 2021).

Parkinson's disease is characterized by tremors at rest, rigidity, bradykinesia/hypokinesia/akinesia and postural instability. These symptoms are primarily related to the death of dopaminergic neurons in the substantia nigra and the accumulation of Lewy bodies. The over-expression of α-synuclein (SNCA), a presynaptic neuronal protein, is believed to be a reason for the death of dopaminergic neurons. Therefore, suppressing the over-expression of SNCA via RNA interference can be a useful strategy to protect dopaminergic neurons. In this respect, gold nanoparticles (GNP) composites (CTS@GNP-pDNA-NGF) were developed (Fig. 3.6) by Hu et al. and transfected into cells via NGF receptor-mediated endocytosis (Fig. 3.7). The in vitro and in vivo experiments revealed that CTS@GNP-pDNA-NGF nanocomposite was capable of suppressing the expression of SNCA, thus exhibiting good therapeutic effects against Parkinson's disease (Hu et al. 2018a).

Excess iron deposition in the brain may be a reason for oxidative stress-related impairments and necrosis of dopaminergic neurons in the substantia nigra. Therefore the use of iron-chelates like deferoxamine (DFO) could be effective in inhibiting nigrostriatal degeneration, however, DFO has a very short half-life under in vivo conditions and hardly crosses the BBB. You et al. introduced a DFO-based nanosystem for the intracerebral delivery of DFO. It was speculated that the nanoparticle system was capable of penetrating BBB via receptor-mediated endocytosis triggered by the RVG29 component (a 29 amino acid peptide derived from rabies virus glycoprotein) of the nanosystem. The administration of the DFO-based nanosystem caused a significant reduction in the cellular iron content and the iron-related oxidative stress levels in substantia nigra (You et al. 2018).

The specific receptor-mediated transport mechanisms operating in the BBB, for example, transferrin receptors, insulin receptors etc. can be exploited as a means to target therapeutic agents to the brain. As a result, lactoferrin (Lf), a single-chain iron-binding glycoprotein in the transferrin family has been investigated by Huang et al.

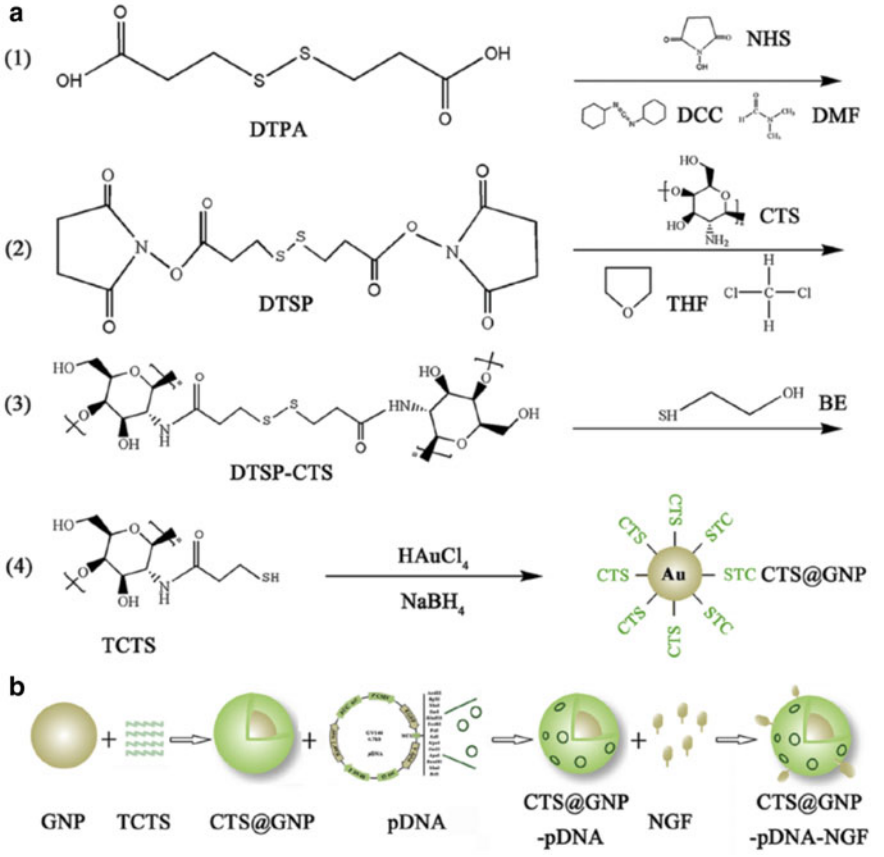

Fig. 3.6 Schematic illustration of **a** the preparation of CTS@GNP-pDNA-NGF the synthesis of CTS@GNP-pDNA-NGF (Adapted with permission from Hu et al. 2018a)

as a novel brain-targeting ligand. Here, the human glial cell line-derived neurotrophic factor gene (hGDNF) was selected as the therapeutic gene and it was encapsulated in Lf-modified brain-targeting nanoparticles. The in vivo experiments conducted on rotenone-induced Parkinson's disease rats with Lf-modified nanoparticles showed potent neuroprotective effects by improving locomotor activity, reducing the loss of dopaminergic neurons and enhancing monoamine neurotransmitter levels in the experimental animals (Huang et al. 2010).

Moreover, the detection of dopamine at a nanometric scale is useful in the monitoring of patients with impaired release of dopamine. For this purpose, Xu et al. developed an aptamer biosensor for this neurotransmitter based on a gold electrode modified with carbon nanoparticles and thionine-labelled gold nanoparticles as a probe. This biosensor displayed ultrasensitivity, high selectivity, and good reusability (Xu et al. 2015).

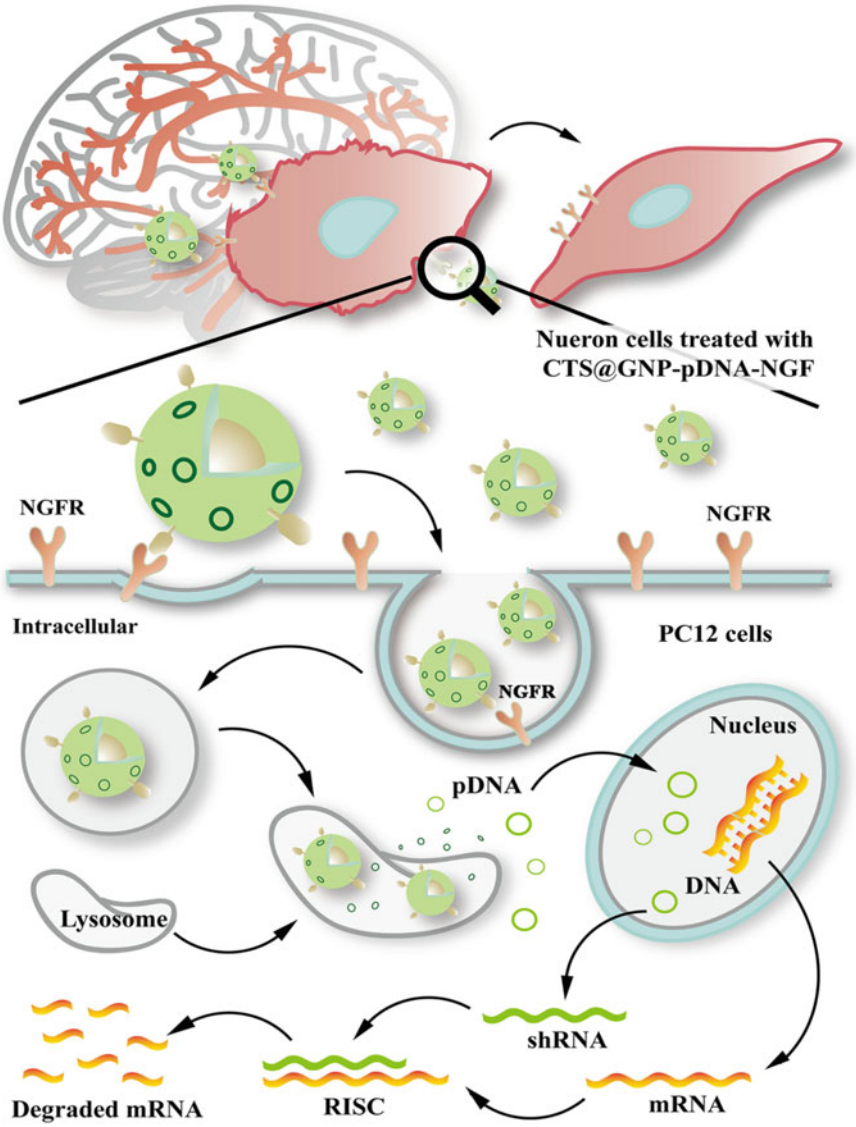

Fig. 3.7 Schematic diagram showing the process of GNP composites treatment GNP composites were internalized by PC12 cells and that the pDNA escaped from the lysosome and expressed shRNA which then associated with RNA-interfering silencing complex (RISC) to suppress SNCA expression via mRNA degradation (Adapted with permission from Hu et al. 2018a)

3.3.4 Liver Diseases

Liver fibrosis results from chronic damage to the liver in conjunction with the accumulation of extracellular matrix proteins and is a characteristic of most types of chronic liver diseases (Bataller and Brenner 2005). Activation of hepatic stellate cells (HSCs) is established as a major mechanism for liver fibrosis. Various nanoparticle systems targeting HSC have been evaluated in the treatment of liver fibrosis (Poilil Surendran et al. 2017).

Lin et al. developed a nanoformulation from a mixture of PEG-PLGA copolymers with PLGA for the systemic delivery of sorafenib, a tyrosine kinase inhibitor. The antifibrotic potential in this nanoformulation was evaluated using a CCl_4-induced fibrosis mouse model. The results of the study revealed that the systemic administration of the nanoformulation containing sorafenib can decrease α-smooth muscle actin content and collagen production, and thereby ameliorate liver fibrosis in the livers of CCl_4-treated mice (Lin et al. 2016). Similarly, dexamethasone-loaded liposomes significantly reduced liver injury and liver fibrosis when compared with the free drug (Bartneck et al. 2015).

In another study, the therapeutic effects of curcumin-encapsulated hyaluronic acid-polylactide nanoparticles (CEHPNPs) on liver fibrosis amelioration were determined. CEHPNPs were capable of binding to the CD44 receptor (the receptor for hyaluronic acid, which is upregulated on activated HSCs) and getting internalized through endocytosis to release the liver protective agent curcumin. This system induced significant cell death in activated HSCs, however, did not affect quiescent HSCs, hepatic epithelial or parenchymal cells. Moreover, treatment with CEHPNPs led to a significant reduction in serum aspartate transaminase/alanine transaminase, as well as attenuation of tissue collagen production and cell proliferation (Chen et al. 2016).

3.3.5 Cardiovascular Diseases

Cardiovascular diseases are considered as one of the leading causes of high morbidity and mortality around the globe. In this respect, nanoparticle-based cardiovascular drug carriers could be useful in the provision of effective treatment along with a few adverse effects on the non-target tissues (Pala et al. 2020).

Boarescu et al. compared the effect of curcumin nanoparticles (nC) and conventional curcumin (Cs) on blood pressure, electrocardiogram, and biological changes in vivo. Their observations revealed that nC was more efficient than Cs in terms of the antioxidative effects, the capacity to prevent cardiomyocyte damage, and electrocardiogram alterations (Boarescu et al. 2019). In another study, the effect of PEG-coated gold nanoparticles (AuNPs) on acute myocardial infarction (MI) was studied and it revealed that PEG-coated AuNPs possess cardioprotective effects, hence there is a

potential to employ in drug delivery systems to treat cardiac diseases (Tian et al. 2018).

Atherosclerosis, a condition characterized by the thickening of the arterial wall and inflammation of plaques is identified as the primary cause of heart disease and stroke (Pala et al. 2020). These plaques can become increasingly complex and eventually it may lead to complications like acute occlusion due to the thrombus formation resulting in MI or stroke (Lusis 2000). Thrombin, a protease enzyme of the coagulation cascade, contributes to the pathology of atherosclerosis. Although drugs that could inhibit thrombin and thrombin-related signalling are clinically available, the side effects like increased bleeding tendencies limit their application. Therefore, Palekar et al. introduced perfluorocarbon nanoparticles carrying the thrombin inhibitor D-phenylalanyl-L-prolyl-L-arginyl chloromethylketone (PPACK-NP). The fat-fed apolipoprotein E-null mice treated with PPACK-NPs for one month were observed with reduced vascular procoagulant activity and restored vascular endothelial barrier integrity. Further, this treatment has led to a retardation of atherosclerotic plaque progression in lesion-prone areas Palekar et al. (2016a). In follow-up work, Palekar et al. employed a static model of stent thrombosis to evaluate the potential of PPACK-NPs in inhibiting stent thrombosis. The results of this study revealed that the marked antithrombin activity in PPACK-NPs could prevent intravascular stent thrombosis and occlusion (Palekar et al. 2016b).

Early reperfusion therapy which is aimed at restoring blood flow to the ischemic region is a standard practice to limit infarct size in patients with ST-segment elevation acute MI. However, the studies have revealed that the reperfusion of coronary arteries paradoxically induces myocardial ischemia–reperfusion (IR) injury. Therefore, Nakano et al. introduced angiotensin II type 1 receptor blocker irbesartan incorporated PLGA nanoparticles as a therapeutic agent for IR injury. Interestingly, Irbesartan-NP inhibited the recruitment of inflammatory monocytes to the IR heart and reduced the infarct size (Nakano et al. 2016).

3.3.6 Lung Inflammation

Exposure to toxins, pollutants, irritants/allergens as well as pathogens is usually causative of lung inflammation. During inflammation, different types of inflammatory cells get activated triggering the release of cytokines and mediators which eventually leads to a progression of inflammation. Pneumonia and acute respiratory distress syndrome (ARDS) are some examples of acute lung inflammation while asthma and chronic obstructive pulmonary disease are considered chronic inflammatory conditions (Moldoveanu et al. 2009).

Asthma is characterized by respiratory difficulty and is associated with airway hyper-responsiveness, airway inflammation and airflow obstruction. Although dexamethasone (DEX) has been widely used to treat asthma, long-term and frequent

usage of DEX has many side effects. Thus, Lee et al. introduced DEX-loaded H_2O_2-activatable boronate maltodextrin (DEX-BM) nanoparticles hypothesizing a synergistic activity of DEX and BM that could reduce the dose of DEX to avoid its adverse effects. The experiments conducted using a murine allergic asthma model revealed that DEX-BM nanoparticles can effectively inhibit the inflammatory cell infiltration and airway inflammation more than equivalent DEX and BM nanoparticles (Lee et al. 2020). Similarly, Camara et al. developed hyaluronic acid-DEX nanoparticles (Fig. 3.8) for pulmonary drug delivery. The core–shell type structure of the nanoparticles comprised of dexamethasone nanocrystals inside and hyaluronic acid chains on the surface provided stability and muco-inert properties (Camara et al. 2021). In another study, the cationic lipid dioleoyltrimethylammoniumpropane (DOTAP) modified PLGA nanoparticles have been introduced for the pulmonary delivery of siRNA to treat lung diseases (Jensen et al. 2012).

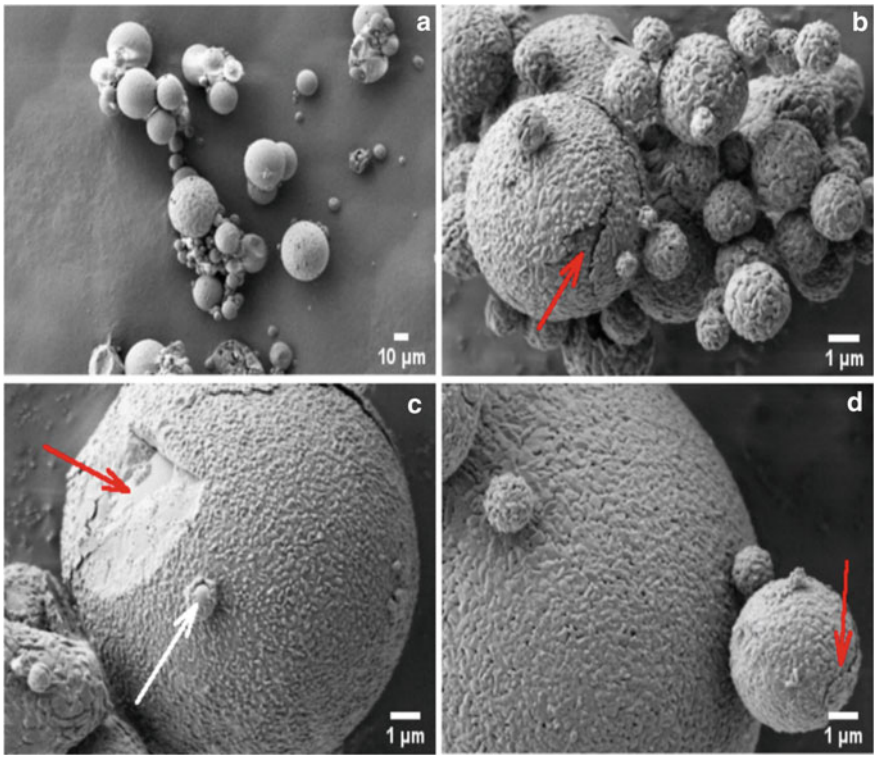

Fig. 3.8 SEM picture of HYA–DEX microparticles taken at **a** 1000 × and **b–d** 20,000 × magnifications. Red arrows: **b**, **d** particle cracks, **c** wrinkled surface. White arrow: small and smooth particles, partially surrounded by the wrinkled coating (Adapted with permission from Camara et al. 2021)

3.4 Reproductive Healthcare

Although vaginal drug administration is important in the treatment of various disease conditions associated with the female reproductive tract, for example, sexually transmitted diseases, microbial infections, and cancer, it is somewhat challenging to achieve a sustained local drug concentration in the vagina. The factors like high permeability of the epithelium of the vagina, vaginal peristaltic activity and dilution of drugs by vaginal secretions limit the efficiency of vaginal drug delivery (Ensign et al. 2014; Osmałeket al. 2021). In this respect, nanoparticle-based drug delivery may offer sustained release and cellular targeting, thus, improving the potency and efficacy of prophylactic/therapeutic modalities (Ensign et al. 2014).

Many viral and bacterial pathogens responsible for sexually transmitted infections, and inflammatory and neoplastic diseases use female genital mucosa as the port of entry. Therefore, topical application of microbicide agents is considered an effective strategy to prevent the transmission of these pathogens. Agents capable of activating the RNAi pathway by delivering siRNA or short-hairpin RNA targeting specific viral/bacterial pathogens appear as promising microbicides. Hence, biodegradable polymer nanoparticles were employed as delivery vehicles for siRNA to the vaginal mucosa to provide protection against infectious diseases (Woodrow et al. 2009). Similarly, Steinbach et al. demonstrated that the intravaginal administration of PLGA nanoparticles encapsulated with siRNA molecules was an effective strategy to prevent genital HSV-2 infections. This was achieved by siRNA-mediated knockdown of the host cell protein known as nectin. The nanoparticles were characterized in vitro to evaluate the optimal formulation based on siRNA loading, controlled release profile as well as mRNA knockdown. The in vivo experiments conducted with mice revealed that the topical application of nanoparticles containing siRNA could improve survival after HSV-2 infection (Steinbach et al. 2012).

Jøraholmen et al. introduced PEGylated liposomes encompassing interferon alpha-2b (IFN α-2b) as a localized therapy against human papilloma virus (HPV) vaginal infections. These PEGylated liposomes were able to penetrate the mucus barrier and reach the deeper epithelium. These findings showed great promise in enhancing the topical delivery of IFN α-2b as a local anti-viral medicament (Jøraholmen et al. 2017). In another exercise, clotrimazole-loaded coconut oil-core nanocapsules (Fig. 3.9) were developed for the vaginal delivery of clotrimazole and appeared as a promising alternative to treat vulvovaginal candidiasis. A prolonged release of clotrimazole was observed with no burst effect in the in vitro drug release experiment. Nanoencapsulation has not only improved the stability of clotrimazole against UV radiation but also its anti-candidal activity (Santos et al. 2014).

Endometriosis is a gynaecological disease condition prevalent in approximately 10% of women of childbearing age and is identified as one of the leading causes of infertility (Singh et al. 2015; Bedin et al.2019). It is characterized by the presence of endometrial-type tissues outside the uterus; particularly in ovaries and fallopian tubes. It is believed that oxidative stress, extracellular matrix degradation, as well as increased angiogenic activity have some contribution to the occurrence

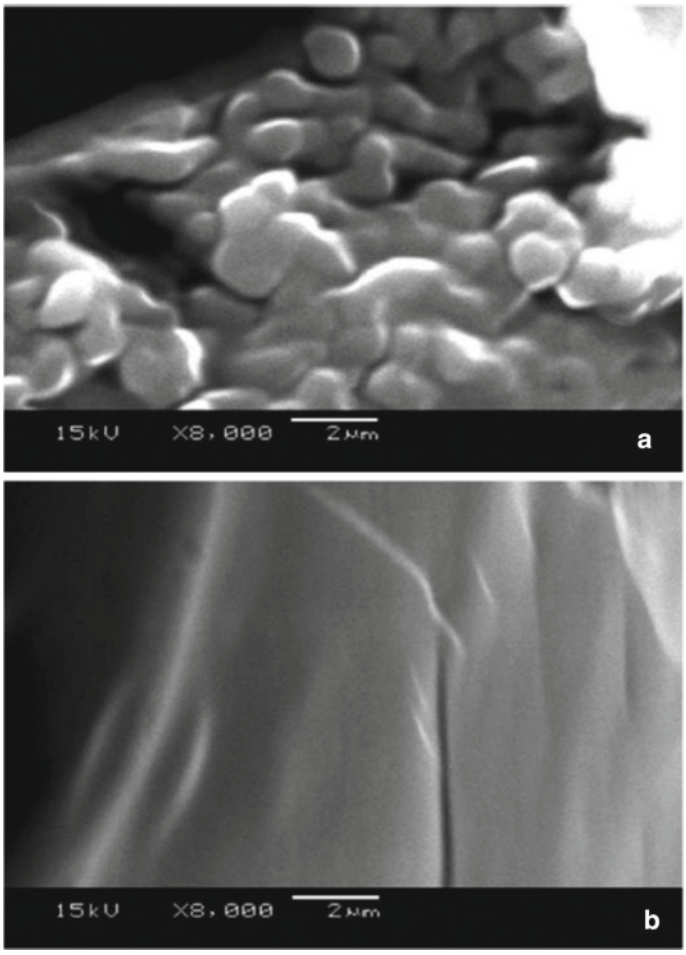

Fig. 3.9 SEM images of clotrimazole-loaded coconut oil-core nanocapsules NC-1 (**a**) and trehalose, the cryoprotectant (**b**) (Adapted with permission from Santos et al. 2014)

of endometriosis. Therefore, Singh et al. developed epigallocatechin gallate and doxycycline-loaded nanoparticles (Dox-EGCG NPs) and evaluated the therapeutic efficacy on endometriosis in mice. Interestingly Dox-EGCG NPs were found to be more effective than single drug-loaded NPs (Dox-NPs or EGCG-NPs) in terms of the capability to decrease oxidative stress, angiogenesis, and matrix metalloproteinases activity in mice with induced endometriosis (Singh et al. 2015).

Contraception provides both health and social benefits by preventing unintended pregnancies and abortions as well as facilitating family planning by the spacing of births. In the field of male contraception, the most widely used methods are the use of condoms and vasectomy. Out of these two methods, vasectomy is the most effective method, however, it suffers from limited reversibility. Therefore, a

reversible, medium-term (2–20 weeks) contraceptive method was introduced with the use of calcium alginate hydrogel, PEG-Au nanoparticles, and ethylene diamine tetraacetic acid (EDTA). This method was based on the physical blockage as well as chemical inhibition of sperm motility in the vas deferens (Bao et al. 2019; Yan et al.2022).

Copper possesses spermicidal activity and hence has been used as a female contraceptive in the form of intrauterine devices (IUDs). However, it is believed that a burst release of cupric ions from these Cu-IUDs occurs initially after insertion and it might be responsible for side effects such as irregular and heavy bleeding, spotting, and pelvic pain. Therefore, Hu et al. (2018a, b) introduced a nano-Cu/low-density polyethylene (nano-Cu/LDPE) intrauterine device (IUD) and evaluated its contraceptive effectiveness. In comparison to the existing Cu-IUDs, nano-Cu/LDPE-IUD displayed superior mechanical properties, corrosion behaviour, and prospective lifespan as well as it did not induce systemic toxicity in the tested animals (Hu et al. 2018b).

3.5 Conclusion

Different categories of nanomaterials like synthetic or natural polymers, lipids, and metals have emerged as novel delivery systems to target a therapeutic agent to a particular location or to accomplish a controlled release of a therapeutic agent at a target site. This strategy is useful to overcome issues like limited solubility, poor bioavailability, low stability, non-targeted/non-specific delivery and adverse side effects of the therapeutic agents and to enhance the therapeutic efficacy and effectiveness. Thus these nano-drug delivery platforms could be highly beneficial in the treatment of specific disease conditions and thereby to improve the quality of life of the people.

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Chapter 4
Nanotechnology in Virology

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Abstract Viruses are submicroscopic infectious agents causative of many diseases in humans including several deadliest infections that have emerged in the recent past. The rapid and accurate diagnosis of viral infections at the early stages is crucial in preventing the pathogen outburst. However, most of the currently available diagnosis methods are often laborious, time-consuming, and less sensitive. On the other hand, the limited availability of antiviral therapeutics and the adverse effects associated with traditional antiviral medicines necessitate the development of novel treatment strategies to combat viral infections. In recent years, different types of nanomaterials have been explored for the diagnosis and treatment of viral infections. This chapter provides an overview of some nanotechnology-based approaches developed for the rapid and high-quality diagnosis of viral infections. Further, antiviral treatment strategies involved with nanomaterials are also discussed with examples.

Keywords Antiviral · Diagnosis · Nanomaterials · Viral infections

4.1 Viruses: The Obligate Parasites

The discovery of the tobacco mosaic virus (TMV) is a historical landmark in science. Ivanowsky, who fathered the discovery of the virus, worked on tobacco mosaic disease that was already proven to have a microbial aetiology but known as a "*contagium vivum fluidum*-soluble living germ" by earlier scientists. Finally, it was reported that the causative agent was unable to grow independently and it required the presence of viable, dividing host cells to replicate (Artenstein 2012). The historical discovery of the TMV in 1892 was followed by the discovery of the foot-and-mouth disease virus (Coxsackievirus) in 1898. The first virus discovered in humans was the

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yellow fever virus in 1901 (Levine and Enquist 2007). New virus species infecting humans have been identified since then with a frequency of three or four per year (Woolhouse et al. 2012). Viruses are responsible for over two-thirds of all human infections (Woolhouse and Gaunt 2007).

The lack of treatments and immunization for infectious diseases has become a critical challenge due to the emergence of novel variants of human pathogenic viruses. Historically, certain viral infection outbreaks had affected the human population devastatingly. Amongst them, Spanish flu, Smallpox, Zika, Ebola, SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome), HIV-1, and the present pandemic; COVID-19 are significant. "Spanish flu" was an influenza pandemic that spread worldwide during 1918–1919 killing over 50 million people (Trilla et al. 2008). Genomic RNA fragments of the Spanish flu virus have been isolated from preserved tissues of the flu victims and three genes including the hemagglutinin (HA) gene were completely sequenced. These gene sequences confirmed that the causative agent of Spanish flu was an influenza A virus of the H1 N1 subtype. The virulence which is determined by the hemagglutinin gene was a recombinant one that originated from human-influenza lineage and swine-influenza lineage making the virus more virulent (Gibbs et al. 2001).

Smallpox (Variola) devastated mankind for centuries until it was eradicated in 1980 (Riedel 2005; Breman and Arita 1980). It affected all social classes. In the eighteenth century in Europe, around 400,000 people died of smallpox annually, and one-third of the survivors went blind (Riedel 2005). In England, the disease was called the "speckled monster" because of the symptoms (raised pustular lesions) that appeared suddenly. The fatality rate was 20–60% and the survivors were left with disfiguring scars. The fatality rate in infants was as higher as 80–98% (Riedel 2005). The respiratory tract is the usual portal of entry for the smallpox virus. Skin, conjunctival and trans-placental infections occur in rare instances (Moore et al. 2006).

SARS (Severe Acute Respiratory Syndrome) was the first global pandemic of the twenty-first century (Syed et al. 2003). It resulted in 8098 infected cases and a 9.6% death rate in 29 countries (Lau et al. 2008). The PCR test results obtained by blood and sputum samples indicated that SARS was caused by a novel coronavirus strain. The causative virus of this disease has been reported to possess an incubation period of 2–7 days, and the symptoms include prodromal pyrexia that could be associated with chills, malaise, rigours, myalgia, dyspnea, dry cough, and headache (Loon et al. 2004).

Middle East respiratory syndrome coronavirus (MERS-CoV) which was an emerging betacoronavirus of lineage C, is responsible to cause severe acute respiratory disease in humans. The MERS disease cases were reported in seven countries of the Arabian Peninsula in 2012 and the majority of the reported cases were from Saudi Arabia. The mortality rate of the infected persons was approximately 30–40% (Oboho et al. 2015). Zika virus (ZIKV) is an arbovirus (arthropod-borne virus) that is transmitted through mosquito vectors. It was first isolated from a primate in 1947 and later from mosquitoes in 1948 in Africa. ZIKV is transmitted to humans through the bite of infected mosquitoes. The clinical symptoms of Zika infection are non-specific and therefore can be misdiagnosed as other arboviruses like dengue and

chikungunya. Zika virus infection was associated with only mild symptoms before the huge French-Polynesian outbreak in 2013–2014. Apart from severe neurological complications, severe congenital malformations including microcephaly associated with ZIKV infection were also reported (Musso and Gubler 2016).

The Ebola virus outbreak was one of the deadliest infections which reported a fatality rate of 30–90% (Baize et al. 2014). The Ebola virus was first identified in 1976 after two unrelated outbreaks were reported in the Democratic Republic of the Congo (formerly known as the Republic of Zaire) and Southern Sudan. The name "Ebola" came after a small river closed to the epicentre of the Congo outbreak (Feldmann and Geisbert 2011). The clinical symptoms of the infection were fever, vomiting, severe diarrhoea, and haemorrhages (Baize et al. 2014). The most recent Ebola virus outbreak was originated in March 2014 and it was associated with a novel strain of Zaire species, the most virulent of the 5 Ebola species; EBOV (formerly Zaire Ebola virus), Sudan ebolavirus, Bundibugyo ebolavirus, Reston ebolavirus, and Tai Forest ebolavirus (Meyers et al. 2015). The Ebola pandemic was reported in five countries Guinea, Nigeria, Senegal, Liberia, and Sierra Leone (Baize et al. 2014). Ebola viral transmission occurs through direct contact via broken skin, mucous membranes, or by objects like needles. The body fluids of infected individuals including blood, saliva, vomit, and semen are infectious. Ebola had also been transmitted through direct contact with infected animals such as by handling infected animal carcasses and consuming bush meat (Muyembe-Tamfum et al. 2012).

Human Immunodeficiency Virus-1 (HIV-1) is another virus that has a devastating health impact on mankind. It is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS) (Reeves and Doms 2002). The AIDS pandemic continues spreading over many parts of the world. It is reported that over 34 million individuals have been currently infected with HIV whereas most infections are caused by HIV type-1(HIV-1) strains (Reeves and Doms 2002). AIDS, caused by HIV-1 infection, was first identified in 1981 after a common set of symptoms was reported amongst a group of homosexual males in the USA (Brennan and Durack 1981). The main route of transmission is through sexual contact and blood transfusion (Deacon et al. 1995). The symptoms of acute HIV-1 infection generally appear within days to weeks following the initial exposure and the symptoms like fever, fatigue, maculopapular rash, headache, pharyngitis, lymphadenopathy, myalgia, arthralgia, aseptic meningitis, retro-orbital pain, weight loss, gastrointestinal distress, night sweats, depression, and oral and genital ulcers are common amongst the patients (UNAIDS and WHO 1998).

The most recent threat to global health is the ongoing respiratory disease outbreak that was recently named "Coronavirus Disease 2019 (Covid-19)". This disease, identified in December 2019 is caused by a novel strain of coronavirus that is structurally similar to the severe acute respiratory syndrome (SARS) causing virus (Harapan et al. 2020). Respiratory arrest due to acute respiratory distress syndrome is the major cause of mortality caused by Covid-19 (Mehta et al. 2020).

4.1.1 Virus Structure and Classification

The development of gene sequencing technologies enables the rapid sequencing of viral genomes and thereby explains the fundamental aspects related to the evolution of viruses. It further allows the elucidation of antigenic determinants and viral molecular epidemiology (Cottam et al. 2009). Thus, genetic sequence characterization and determination of the viral origins have always been significant issues in virology (Holmes 2009). Meanwhile, the systematic discovery of new viruses has undergone a renaissance over recent years (Delwart 2012; Deeks et al. 2013). Initiating from improved strategies to clone and screen the cDNA libraries, the availability of new sequencing-based and amplification-based techniques has facilitated the characterization of not only isolated viruses but also uncultured viruses (Junglen and Drosten 2013).

The genetic diversity and dense population size of viruses ($>10^{31}$ virions in the biosphere) create a huge selective pressure on cellular organisms (Lwoff and Tournier 1966; Bellett 1967; Musso and Gubler 2016). Viruses are a determining factor of the revolution in molecular genetics of bacteria and phage biology towards the understanding of central biological processes such as replication, transcription, and translation (Lwoff and Tournier 1966, 1971; Pringle 1998; Junglen and Drosten 2013).

Viruses are small, obligate, and intracellular parasites that contain either an RNA or DNA genome covered by a virus-coded, protective protein coat (Gelderblom 1996). The reproduction of viruses depends on the complex metabolic and biosynthetic pathways of eukaryotic or prokaryotic-specific host cells. The main function of the virion (a virus particle) is to insert its genome (DNA or RNA) into the specific host cell and to make the host cell express the viral genome (transcription and translation). The genome of the virion, often with associated proteins, is packed inside a protein capsid that is symmetric. The nucleoproteins, together with the viral genome, form the nucleocapsid. Some viruses have envelopes whereas others remain as naked viruses (Horne and Wildy 1964).

Viruses are divided into groups based on their shape and size, chemical composition, mode of replication, and genomic structure. Helical nucleocapsids are abundant in many filamentous and pleomorphic viruses. They consist of protomers (a helical array of capsid proteins) arranged around a helical filament made out of nucleic acid. Icosahedral nucleocapsids are characteristic of many "spherical" viruses. The number of capsomeres and their arrangement is useful in virus identification and classification. The viral genome can be either DNA or RNA and the genomic arrangement can be circular or linear; single-stranded (ss) or double-stranded (ds) (Gelderblom 1996).

Apart from physical characteristics, genomic structure and mode of replication can also be used as the criteria for the classification of viruses, i.e. chemical composition and nucleic acid configuration. In addition, positive or negative sense of genomic ssRNA is considered in virus classification (Abrescia et al. 2012). Viruses are divided into groups at different hierarchical levels such as order, family, subfamily, genus, and

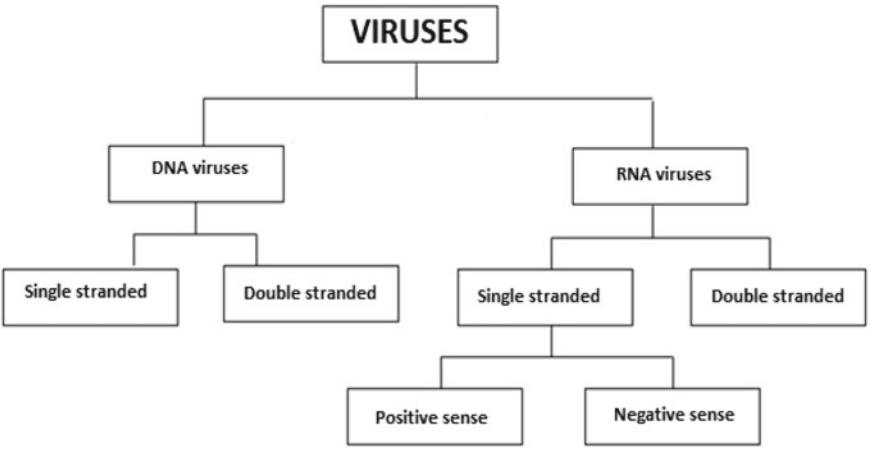

Fig. 4.1 Classification of viruses based on the genome

species. It is estimated that more than 30,000 virus isolates have been identified today and grouped into more than 3,600 species, 164 genera, and 71 families (Gelderblom 1996).

Two classification schemes have influenced the way of envisioning the virosphere; the Baltimore scheme (Baltimore 1971) classifies viruses into seven groups based upon the nature of the genome (type, number of strands, and for a single-strand genome, the sense). The groups (Fig. 4.1) are ds-DNA, ss-DNA, ds-RNA, positive (sense) ss-RNA, and negative (antisense) ss-RNA viruses. In addition, there are two more virus categories; single-stranded RNA viruses with reverse transcriptase, and double-stranded DNA viruses with reverse transcriptase (Abrescia et al. 2012).

The second classification by the International Committee on Taxonomy of Viruses (ICTV) targets to develop an internationally recognized criterion upon taxonomy for viruses, defining the names for virus taxa to communicate with the virology community. The agreed taxonomic levels consist of order, family, genus, and species. The organizational structure of ICTV classification indicates the host of a particular virus or virus group (Table 4.1). Therefore, ICTV classification is a modification of the Baltimore classification with the host type.

4.1.2 Techniques Used in the Diagnosis of Viral Diseases

In the past decades, the diagnosis of infectious diseases was achieved mainly by culturing biological specimens obtained from infected individuals. Although effective culturing techniques have facilitated the reliable detection of various bacterial, viral, and fungal pathogens, for certain infectious diseases, the conventional cultivation techniques have failed to detect the etiological agent. For instance, while it

Table 4.1 Categorization of virus families based on the host (grouped into seven Kingdoms)

Host						
Animalia	Plantae	Fungi	Protozoa	Archaea	Bacteria	Chromista
Adenoviridae	*Alphaflexiviridae*	*Alphaflexiviridae*	*Lavidaviridae*	*Ampullaviridae*	*Corticoviridae*	Endornaviridae
Alloherpesviridae	*Amalgaviridae*	*Barnaviridae*	*Marseilleviridae*	*Bicaudaviridae*	*Cystoviridae*	*Marnaviridae*
Alphatetraviridae	*Avsunviroidae*	*Betaflexiviridae*	*Mimiviridae*	*Clavaviridae*	*Inoviridae*	*Narnaviridae*
Anelloviridae	*Benyviridae*	*Bromoviridae*	*Narnaviridae*	*Fuselloviridae*	*Leviviridae*	*Phycodnaviridae*
Arenaviridae	*Betaflexiviridae*	*Chrysoviridae*	*Pseudoviridae*	*Globuloviridae*	*Microviridae*	Unassigned
Arteriviridae	*Bromoviridae*	*Endornaviridae*	*Totiviridae*	*Guttaviridae*	*Myoviridae*	*Rhizidiovirus*
Ascoviridae	*Caulimoviridae*	*Genomoviridae*		*Myoviridae*	*Plasmaviridae*	
Asfarviridae	*Closteroviridae*	*Megabirnaviridae*		*Plelipoviridae*	*Podoviridae*	
Astroviridae	*Endornaviridae*	*Metaviridae*		*Rudiviridae*	*Siphoviridae*	
Baculoviridae	*Fimoviridae*	*Narnaviridae*		*Siphoviridae*	*Sphaerolipoviridae*	
Bindaviridae	*Geminiviridae*	*Partitiviridae*		*Sphaerolipoviridae*	*Tectiviridae*	
Birnaviridae	*Genomoviridae*	*Pseudoviridae*		*Spiraviridae*		
Bornaviridae	*Metaviridae*	*Quadriviridae*		*Turriviridae*		
Caliciviridae	*Nanoviridae*	*Reoviridae*		Unassigned		
Carmotetraviridae	*Ophioviridae*	*Totiviridae*		*Salterprovirus*		
Caulimoviridae	*Partitiviridae*	Unassigned				
Circoviridae	*Phenuiviridae:*	*Rhizidovirus*				
Coronaviridae	*Tenuivirus*					
Dicistroviridae	*Phycodnaviridae*					
Filoviridae	*Pospivirioidae*					
Flaviviridae	*Potyviridae*					
Genomoviridae	*Pseudoviridae*					
Hantaviridae	*Reoviridae*					
Hepadnaviridae	*Rhabdoviridae*					
Hepeviridae	*Secoviridae*					
Herpesviridae	*Tolecusatellitidae*					
Iridoviridae	*Tombusviridae*					
Malacoherpesviridae	*Tospoviridae*					

(continued)

Table 4.1 (continued)

Host						
Animalia	Plantae	Fungi	Protozoa	Archaea	Bacteria	Chromista
	Tymoviridae Unassigned *Cilevirus* Unassigned *Idaeovirus* Unassigned *Ourmiavirus* Unassigned *Polemovirus* Unassigned *Sobemovirus* *Virgaviridae*					

(continued)

Table 4.1 (continued)

Host						
Animalia	Plantae	Fungi	Protozoa	Archaea	Bacteria	Chromista
Marseilleviridae						
Metaviridae						
Nairoviridae						
Nimaviridae						
Nodaviridae						
Nudiviridae						
Nyamiviridae						
Orthomyxoviridae						
Papillomaviridae						
Paramyxoviridae						
Parvoviridae						
Peribunyaviridae						
Permutotetraviridae						
Phenuiviridae:						
Phasivirus						
Phenuiviridae:						
Phlebovirus						
Phycodnaviridae						
Picobirnaviridae						
Picornaviridae						
Polydnaviridae						
Polyomaviridae						
Poxviridae						
Pseudoviridae						
Reoviridae						
Retroviridae						
Rhabdoviridae						
Roniviridae						
Togaviridae						
Tymoviridae						
Unassigned						
Deltavirus						

had been found out by epidemiological means that hepatitis is transmitted by various routes, culturing of specimens of infected individuals failed to figure out a causative agent (Prince 1968; Purcell et al. 1973; Feinstone et al. 1975). Similarly, the use of culture-based techniques for gastroenteritis caused by non-bacterial infectious agents in infants and young children was found to be fruitless (Connor and Barrett-Connor 1967; Kapikian 1975). Moreover, culturing techniques usually require a substantial period of incubation time before diagnosis. In the case of many viral agents, diagnosis relied on the cultivation of the causative agent, however, that cannot be made rapidly to be used in the treatment of an acute illness (Yolken 1980).

Because of these disadvantages, there is a necessity in developing methods of detecting infectious agents devoid of cultivation. In some circumstances, immune electron microscopy is a method of detecting viral agents (Almeida and Waterson 1969). Two etiological agents that cause hepatitis in humans namely hepatitis A and B (Almeida et al. 1971; Feinstone et al. 1975), and causative agents of diarrhoea, viz., Rotavirus and Norwalk virus (Kapikian et al. 1972) were first recognized using electron microscopy and it was used to describe the basic epidemiology of these causative agents. Although electron microscopy plays an important role in the understanding of viruses, the need for sophisticated technology has become a limiting factor for the general applicability of this technique. Therefore, there has been an urgent requirement for developing better ways of detecting non-cultivable antigens. Though numerous techniques have been developed, radioimmunoassay (RIA) is the one that has widespread usage for hepatitis A and B antigen detection. The advantages of RIA are high sensitivity and objectivity and it can be used to test a large number of specimens at a time. On the other hand, RIAs, which depend on the gamma-radiation emission by an isotope-bound immune-reactant, have numerous disadvantages such as radioactive isotopes possessing an inherent rate of decay which means the radiolabelled reagents lose activity over time. Therefore, frequent re-labelling, retesting, and re-standardization are essential. In addition, RIAs expose the user to a potential radiation hazard, and expensive equipment is required to measure the radiation restricting RIAs to central laboratories (Forghani et al. 1977).

Thus, there has been a great interest in developing assay systems that would retain the advantages of RIA and avoid its inherent problems. Enzyme immunoassay/enzyme-linked immunosorbent assay (ELISA) has become the closest to achieving this goal. In ELISA, an enzyme is used as the immunoglobulin marker which substitutes the radioactive isotope in RIA. A very small amount of enzyme-labelled immunoglobulin can react with a substrate to yield a visibly coloured reaction. Therefore, expensive detection equipment is not required. Enzyme-antibody conjugates can be pre-prepared and those conjugates have a negligible loss of activity with long storage time obviating the requirement of constant re-labelling and retesting of reagents (Kapikian et al. 1972; Voller et al. 1978; Wisdom 1976).

ELISA measures either antigen or antibody. To detect an infectious agent, the labelling of the antibody is usually more convenient than that of the antigen, because the latter may not be available in pure form. Sandwich ELISA is commonly used to detect the antigen in bodily fluids. In order to detect the targeted antigen, a specific antibody is used to capture it. The capture antibody is coated to a solid phase. After

an appropriate incubation period, the unbound antibodies are washed off and the test sample is added. After incubation, the excess sample is rinsed off and an aliquot of enzyme-labelled antibody is added. It binds to antigens that are bound to the capture antibody located in the solid phase. Followed by the washing step, the specific substrate to the enzyme is added. The enzyme will convert the substrate to a visible form or can be measured spectrophotometrically. This approach has the advantage of using only a single antibody (Wolters et al. 1976; Voller et al. 1978). The drawback of the ELISA system is that it requires longer incubation periods for increased sensitivity (Yolken 1980).

Conventional tests for virus detection like antigen-capture enzyme-linked immunosorbent assay (ELISA), immune-peroxidase monolayers assays (IPMA), and immune-fluorescent assays (IFA) are commonly used, but these methods are often laborious, time-consuming, and less sensitive (Liu et al. 2011; Xu et al. 2012a, b). Recently, PCR (Polymerase Chain Reaction) is identified as more sensitive, rapid, and specific for virus detection (Jiang et al. 2010; Podgórska and Stadejek 2010). When there are similar clinical symptoms, proper differentiation of the pathogens is essential for an accurate diagnosis of the disease. Separate amplification of each target is needed for monospecific PCR assays and thus is resource-intensive and expensive. Multiplex PCR assays employed in clinical diagnosis are significantly advantageous because several viruses can be amplified in a single reaction mixture simultaneously, making the diagnosis cost-effective (Liu et al. 2013). Usually, multiplex PCR assays distinguish probe hybridization after the completion of the PCR reaction. Utilization of real-time PCR assays for specific identification of the target sequence using fluorescent probes improves the assay's specificity and reduces hands-on time. Besides, the real-time PCR approach enables the performance of multiplex amplification and detection inside a single tube. In some selected real-time PCR systems, up to four amplification products are possible to be distinguished in a single tube (Wu et al. 2014). Despite the wide utility, Pourhosein et al. stated that conventional PCR and newly developed real-time PCR require an expensive device, the use of toxic and carcinogenic substances, fluorescence detectors, and skilled personnel for operation (Pourhosein et al. 2011).

In another study, Notomi et al. introduced a method for replicating nucleic acids known as Loop-Mediated Isothermal Amplification (LAMP). Four primers including two outer primers, two inner primers, and another two loop primers recognize about six to eight regions in the target DNA making the test highly sensitive (Notomi et al. 2000; Kimura et al. 2005). In the last two decades, LAMP has been popularized as an efficient, rapid, and cost-effective approach that can be used to detect various viral infections and diagnose causative agents (Pourhosein et al. 2011).

The findings of Ratcliff et al. revealed that unknown strain diversity remains undetected in PCR. For viruses that have high sequence diversity amongst strains, the primer and probe design requires to be constantly reviewed in newly published sequences. A very few mismatches as one or two in a primer reduces amplification efficiency, whereas a fluorescent probe reduces or eliminates the fluorescence signal strength. Some probes are highly vulnerable to mismatches in viral strain sequence diversity. Therefore, a false negative result ought to strain diversity is always possible.

In addition, poor reagent storage protocols where relevant reagents are subjected to repeated freeze–thaw would quickly decrease enzyme potency, reducing assay sensitivity and resulting in false negatives. Moreover, RNA is fragile and rapidly degraded due to the ubiquitous presence of RNases. As a consequence, clinically significant specimens should be analysed by alternative methods (Ratcliff et al. 2007).

The characteristics like small size, simple biology, and being obligate intracellular parasites emerge as challenges in virus detection. Due to the limited availability of treatment options, rapid and well-timed diagnosis of viral infections is essential, yet, most of the currently available methods are time-consuming, labour-intensive, and often expensive. In this respect, nano-scale materials could be employed to enhance the speed, detection limit, and accuracy, as well as to minimize the cost of the existing methods for virus detection (Mokhtarzadeh et al. 2017).

4.1.3 Antiviral Treatment Strategies

The development of antiviral vaccines marks two historical milestones. The biggest breakthrough was the discovery of the smallpox vaccine using the cowpox virus by Edward Jenner, and the other was the attenuation of the infectious virus by serial passage into a new host which led to the discovery of Louis Pasteur's rabies vaccine (Arnon 1980).

The success of vaccination or immunization against viral diseases depends on inducing an adequate and appropriate immune response towards protective viral antigens. Vaccine efficacy is evaluated in terms of the amount of induced antibodies and their potency, the time required for antibody production, the potential of recall, and the capacity of antibodies to influence the key steps in the virus pathogenesis (Arnon 1980).

After a pathogenic virus is recognized, different types of vaccines can be developed, which consist of virus, viral vector, nucleic acid, protein/peptide, or cell-based vaccines. Virus-based vaccines usually need patient-derived viruses, whereas other categories can utilize viral genomic sequences to accelerate antiviral vaccine development (Zhou et al. 2020).

Although immunization of individuals against viral pathogens with prophylactic vaccines is the most effective method, for most viruses such vaccines are not available. Therefore, antiviral drugs which can block the replication of the virus in infected hosts are important in combatting viral infections. These drugs have to induce strong and specific inhibitory action on virus replication without interfering with cellular processes (Haasnoot and Berkhout 2009). Zamecnik and Stephenson stated the sequence-specific inhibition of viral replication by antisense DNA oligonucleotides (Zamecnik and Stephenson 1978). However, antisense-based antiviral therapeutics are almost shuttered because of the problems associated with the toxicity of oligonucleotides, their instability in the serum, and the problematic delivery to the right target cells. Thus, a new generation of nucleic acid-based antiviral compounds such as ribozymes, DNAzymes, and decoy RNAs had been developed but those

were unable to overcome the previous problems. Those drawbacks could be overcome using chemically modified nucleic acids instead of using normal DNA/RNA oligonucleotides (Haasnoot and Berkhout 2009).

There are 37 licensed antiviral drugs according to the current armamentarium for chemotherapy of viral infections (De Clercq 2004). Nineteen compounds have been formally approved for the treatment of HIV infections; they are (i) nucleoside reverse transcriptase inhibitors (NRTIs), (ii) nucleotide reverse transcriptase inhibitors (NtRTI), (iii) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (iv) protease inhibitors, and (v) viral entry inhibitors. For the management of chronic hepatitis B infections, lamivudine, adefovir, and dipivoxil have been approved. Acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, trifluridine, and brivudine are used to treat herpes simplex virus and varicella-zoster virus infections. Ganciclovir, foscarnet, valganciclovir, fomivirsen, and cidofovir have proven to be effective in the treatment of cytomegalovirus infections in immune-compromised patients (AIDS patients with CMV retinitis). Amantadine and rimantadine, neuraminidase inhibitors like zanamivir and oseltamivir are available for the treatment and prophylaxis of influenza infections. Ribavirin has been used in the management of respiratory syncytial virus infections whereas the combination of ribavirin with pegylated interferon-α has been accepted as the therapy for hepatitis C virus infections (De Clercq 2004).

Highly active antiretroviral combination therapy (HAART) has been responsible for a gradual decrease in AIDS mortality cases since 1996, and most significantly changed the clinical profile of HIV from a sub-acute lethal disease to a chronic ambulatory disease. HAART includes a double nucleoside (NRTI) backbone and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or PI/r (ritonavir; pharmacologically enhanced protease inhibitor). Triple NRTI combinations are less effective than 2NRTIs/NNRTI and 2NRTIs/PI/r combinations (Yeni 2006).

The clinical utility of present-day treatment strategies is associated with several drawbacks. For example, the establishment of viral reservoirs (where continual viral replication takes place and is inaccessible to the present drug delivery methods) could be observed in the central nervous system (CNS) despite suppressive antiretroviral therapy (Hellmuth et al. 2015). Similarly, there are issues related to the use of RNA interference (RNAi) such as the inability of RNA to cross the cell membrane due to its size and anionic charge as well as toxicity (Adesina and Akala 2015). In view of these, novel carrier systems that are capable of traversing these membranes would be promising tools in antiviral therapy (Singh et al. 2017).

Lewis et al. stated that HAART (Highly active antiretroviral therapy) which is based on nucleoside reverse transcriptase inhibitors (NRTIs) has transfigured AIDS treatments in recent years. HAART can successfully suppress the replication of the virus in the long term, but it has significant toxicity, which can seriously affect treatment effectiveness. NRTI-related mitochondrial toxicity is the major toxicity that causes serious side effects such as lactic acidosis and hepatic failure. However, insufficient understanding of the mechanisms underlying mitochondrial toxicity has hindered efforts to discover novel antiviral drugs with better side-effect profiles (Lewis et al. 2003).

Herpes simplex virus (HSV) infection is treated using antiviral drugs such as acyclovir (ACV). Morfin and Thouvenot reported the resistance towards acyclovir (ACV) amongst immunocompromised patients and allogeneic bone marrow transplant patients. Based on these findings, certain mutations associated with the viral genome are responsible for the ACV resistance and therefore some strains of HSV are resistant to ACV. Consequently, this appears as a drawback of antiviral drugs in the management of viral infections (Morfin and Thouvenot 2003).

Considering the aforementioned drawbacks inherited from the current treatment strategies, there is a necessity for the discovery of novel treatment approaches to combat viral infections. Thus, the application of nanotechnology in antiviral therapy would be an innovative alternative to address the failures of conventional techniques.

4.2 Detection of Viruses Using Nanomaterials

Although several traditional methods with high sensitivity and high reproducibility are used in the identification of pathogens, some drawbacks such as the requirement of sophisticated, highly expensive instruments, trained people, high cost, and longer running time, necessitate the development of novel techniques in the detection of pathogens. As a result, sensitive, quick, and economically favourable modern detecting techniques are developed using nanomaterials. Nanomaterials are used in those techniques due to their unique features such as magnetic, electrical, and catalytic properties (Shinde et al. 2012). Usually diagnostic involves bioreceptors and transducers. Bioreceptor is a biological molecule such as an enzyme, protein, antibody, or a biological system like tissue, cell, or a whole organism that can specifically bind with the pathogen. It is normally immobilized on a suitable support medium and the specific interaction between the bioreceptor and the pathogen results in an alteration in physicochemical properties. This change is detected and measured by the transducer. Dendrimers, liposomes, carbon nanotubes, and nanoparticles are some examples of bioreceptors. Transduction can be obtained by modes like (i) optical (e.g. fluorescence-based detection, molecular beacons, refractive index detection, surface plasmon resonance-based biosensor), (ii) magnetic (e.g. μNMR technique, magnetic field sensors based on superconducting quantum interference device, magnetoresistive-based biosensors), (iii) electrochemical (e.g. anodic stripping voltammetry, amperometric sensors, potentiometric sensors, conductometric sensors, electrochemical impedance spectroscopy), or (iv) microgravimetric (bulk wave or quartz crystal microbalance, surface acoustic wave devices, magnetoelastic sensors). Due to the superior sensitivity, these nano-based diagnostics may play an important role, particularly when the infectious analyte concentration is at a low level (Shinde et al. 2012). Some instances where nanomaterials are used in the detection of viruses are summarized below.

4.2.1 Gold Nanoparticles (AuNPs)

Highly sensitive immunoglobulin M (IgM) capture ELISA kits are currently available for the rapid diagnosis of positive-sense single-stranded RNA genome containing the Japanese encephalitis virus (JEV). However, the low specificity in the differentiation between Japanese encephalitis and dengue is one of the major concerns with respect to the IgM-capture ELISA kit-based assays. As a result, techniques like conventional reverse-transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR-based assays have been employed to detect the JEV genome. Huang et al. evaluated the effect of AuNPs on RT-PCR and real-time quantitative RT-PCR assays in the detection of JEV. Interestingly, the amplification yield of the PCR product has increased in the presence of AuNP while shortening the PCR time. The expected product (306 bp amplified fragment) was produced after 35 amplification cycles in the absence of AuNP whereas this fragment was detected after 25 cycles in the presence of AuNPs at a concentration of 1.6 nM and 3.2 nM. However, the interaction of haemoglobin with AuNP and the presence of PCR inhibitors like lactoferrin and immunoglobin G (IgG) resulted in reduced performance of real-time RT-PCR in the blood-based assays in comparison to the culture medium-based detection assays. Therefore 1.6 nM AuNP was identified as the optimal concentration in a real-time RT-PCR blood-based detection assay (Huang et al. 2008). The increased efficiency of RT-PCR and real-time RT-PCR in the presence of AuNP can be related to the high thermal efficiency and thermal conductivity of AuNPs. Once suspended in water, AuNPs have the ability to cause thermal equilibrium with the liquid environment within a very short period like 10–200 ps. Furthermore, the selective interaction of AuNP with single-stranded DNA may reduce the mispairing between both templates and primers enhancing specificity and the amount of PCR product. Thus, Huang et al. concluded that the nanogold-based RT-PCR and real-time quantitative RT-PCR assays as simple, sensitive, and rapid early diagnostic tools for the detection and quantitation of JEV in tissue cultured and clinical samples (Huang et al. 2008).

Shawky et al. used unmodified AuNP for the detection of the hepatitis C virus (HCV) which is a positive-sense single-stranded RNA virus. Normally enzyme-linked immunoassays and recombinant immune blot assays are employed to detect anti-HCV antibodies while conventional RT-PCR is used in the qualitative detection of HCV-RNA. Real-time RT-PCR and/or branched DNA-based assays are used in quantitative detection. Although high sensitivity and specificity can be observed with these methods, features like time and labour intensiveness, high cost, and requirement of specialized equipment limit the above conventional detection methods. The method developed by Shawky et al. was an AuNPs-based colourimetric method that enabled the direct detection of unamplified HCV-RNA extracted from clinical samples. Usually, AuNPs display an intense red colour due to a specific phenomenon called plasmon resonance. Upon the aggregation of AuNPs, the blue colour appears. In this experiment, the RNA extracted from the serum samples of healthy volunteers and chronic HCV patients was mixed with the hybridization buffer and a specific primer targeting HCV-RNA. The reaction mixture was denatured, annealed, and

thereafter cooled to room temperature for 10 min before the addition of AuNPs. A colour change from red to blue was visualized in the HCV-positive specimens within one minute while the HCV-negative samples retained the original red colour (Fig. 4.2). In the absence of the HCV, the primers are free to stabilize AuNPs, hence, get adsorbed on AuNPs preventing aggregation. As a result, the original red colour remains. This assay was found to be highly sensitive with a short turnaround time and cost effective (Shawky et al. 2010).

Human immunodeficiency virus type 1 (HIV-1) is a single-stranded, positive-sense, enveloped RNA virus that is responsible for the development of acquired immunodeficiency syndrome (AIDS). The diagnosis of AIDS is achieved by the detection of HIV-1 RNA, capsid antigen (p24), and anti-HIV antibody. Tang and Hewlett investigated the feasibility of using AuNP-based biobarcode amplification (BCA) assay to enhance the detection sensitivity of HIV-1 p24 antigen. Interestingly, the HIV-1 p24 BCA assay has increased the detection limit of traditional colourimetric ELISA by 100–150-fold. Another advantage of this BCA assay was the relatively shorter period of time required to obtain results compared to conventional ELISA assay. Motivated by these observations, the researchers further modified the BCA assay with europium (Eu^+) NP-based immunoassay (ENIA) in which Eu^+ NPs had replaced AuNPs in the BCA assay. This has resulted in a further increase in the sensitivity while reducing the incubation time (Tang and Hewlett 2010).

Lee et al. used sialic acid stabilized AuNPs (SA-AuNP) for the detection of the influenza virus which is a negative-strand RNA virus. Viral hemagglutinin protein usually binds to sialic acid groups present on the surface of lung epithelial cells. In this method, sialic acid molecules on SA-AuNP surface interact with hemagglutinin leading to a colourimetric change in the SA-AuNP solution. The aggregation of SA-AuNPs on the virus surface resulted in an increased absorbance at 600–610 nm in the absorption spectra as shown in Fig. 4.3 (Lee et al. 2013).

Similarly, Liu et al. utilized functionalized AuNPs for the rapid and selective detection of the influenza A virus. In this approach influenza-specific monoclonal anti-hemagglutinin antibodies (mAb) were conjugated to AuNPs to produce mAb–AuNP probes. The virus possesses multiple recognition sites for the mAb–AuNP probes on its surface causing aggregation of the mAb–AuNP probes. This leads to a red shift in the absorption spectrum which is attributable to the plasmon coupling between adjacent AuNPs. The colour change from red to purple was visualized by the naked eye (Liu et al. 2015).

Basso et al. employed localized surface plasmon resonance (LSPR) shifting for rapid detection of the dengue virus which is a single positive-stranded RNA virus. Here, surface modification of the AuNPs was initially carried out to produce conjugates of AuNP-antibody. This allowed the detection of all four dengue serotypes (DENV-1, -2, -3, -4) with high specificity and selectivity (Fig. 4.4). Further, this new approach has significantly reduced the testing duration of around 1–3 days for conventional detection to less than 5 min (Basso et al. 2018).

Recently, Qiu et al. introduced a dual-functional plasmonic biosensor for the detection of the novel SARS-CoV-2 virus. The novel SARS-CoV-2 is a positive-sense, single-stranded RNA virus that is responsible for the outbreak of the novel

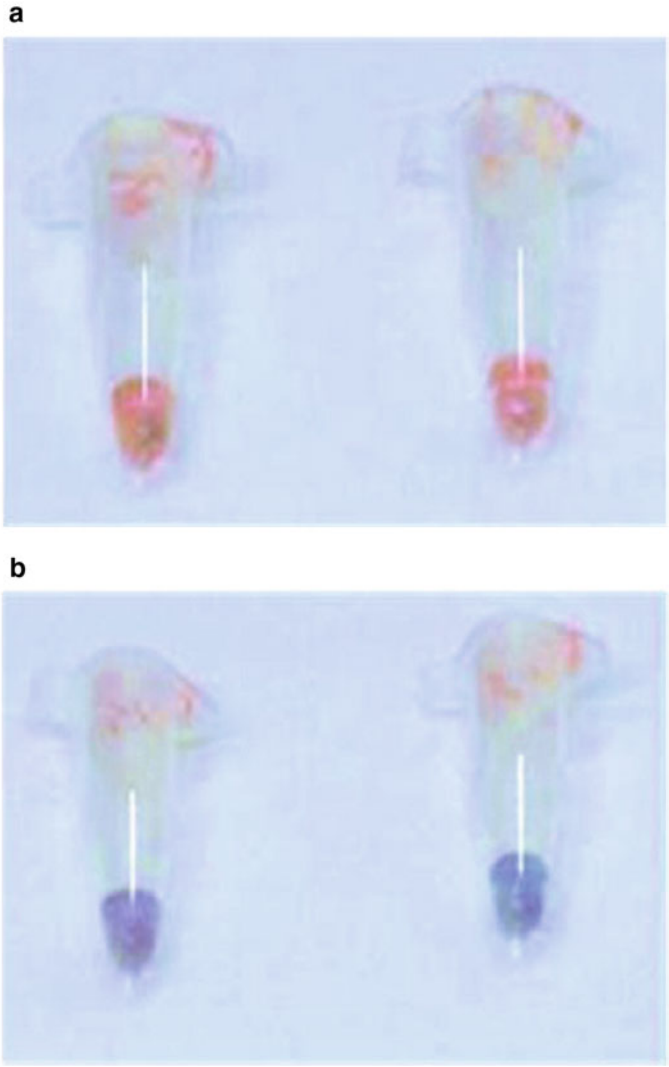

Fig. 4.2 Colorimetric HCV-RNA assay using unmodified AuNPs (The photographs were taken 1 min after the addition of AuNPs). **a** HCV-RNA negative samples, **b** HCV-RNA positive samples (Adapted with permission from Shawky et al. 2010)

coronavirus disease (COVID-19). The biosensor was developed by combining the plasmonic photothermal (PPT) effect and LSPR sensing transduction. The plasmonic chip with the two-dimensional distribution of gold nanoislands (AuNIs) was functionalized with complementary DNA receptors for SARS-CoV-2 sequence detection. The AuNIs chip was capable of generating the local PPT heat once illuminated at

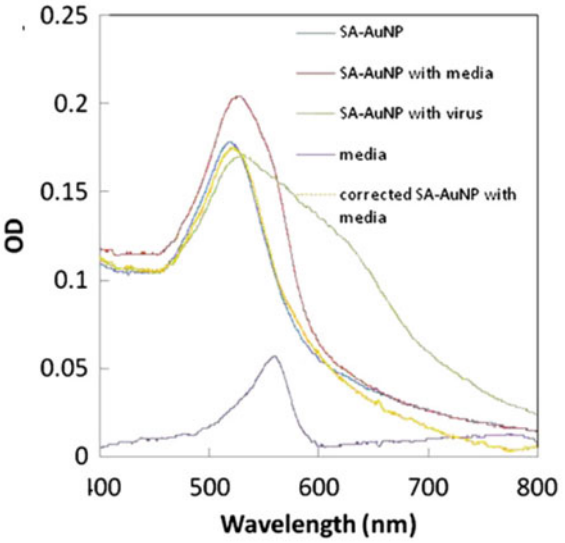

Fig. 4.3 Absorption spectra of AuNP solution under different conditions (Adapted with permission from Lee et al. 2013)

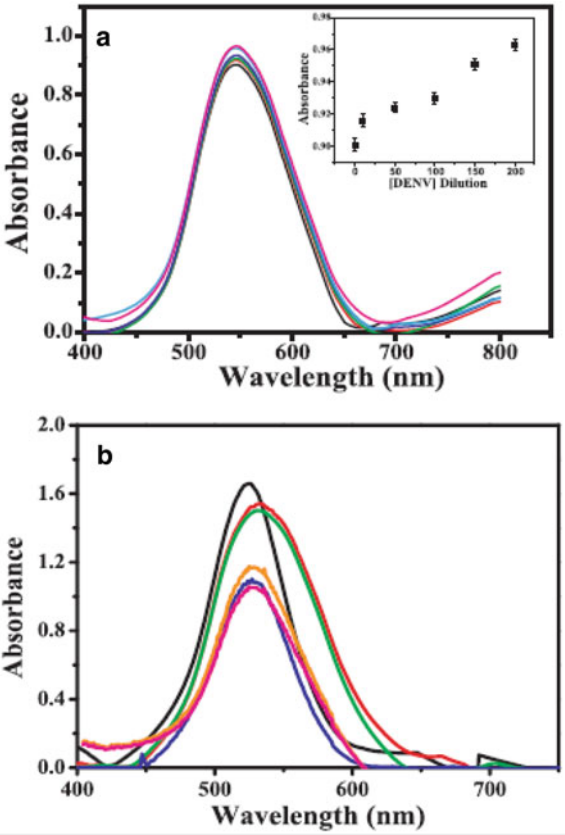

Fig. 4.4 **a** Variations of pool DENV concentrations. A sample pool DENV at titre 107 TCID50/mL diluted to 0.0 μL PBS (black line), 1/10 μL PBS (red line), 1/50 μL PBS (green line), 1/100 μL PBS (blue line), 1/150 μL PBS (pink line) and 1/200 μL PBS (violet line). Insert in figure calibration curve for pool DENV. **b** UV–Vis absorbance spectra of control negative. AuNPs (black line), MUA (red line), EDC-NHS (green line), antibody (blue line), ZIKV (orange line) and YFV (pink line) (Adapted with permission from Basso et al. 2018)

their plasmonic resonance frequency and transducing the in situ hybridization for highly sensitive and accurate detection of the virus (Qiu et al. 2020).

4.2.2 Silver Nanoparticles (AgNPs)

Silver nanoparticles are also used in virus detection. For example, AgNPs-thiol graphene quantum dots (GQD-SH) was exploited as a substratum to load antibody to detect hepatitis C virus core antigen (HCV). In this approach, AgNPs were immobilized on SH groups of GQDs through the formation of Ag–S bond. The anti-HCV (antibody) was loaded on the surface of the glassy carbon electrode through the interaction between $–NH_2$ functional group of the anti-HCV and AgNPs. Riboflavin was employed as a probe for the differential pulse voltammetry detection of HCV core antigen. The specific recognition between antibodies and antigens resulted in a decrease in the electrochemical signal. This method was found to be an effective tool for the detection of HCV core antigens with high efficiency and high sensitivity (Valipour and Roushani 2017).

A highly sensitive conductive nano label for sandwich-type electrochemical immunoassay was developed by Huang et al. using silver nanoparticle-graphene (AgNPs-G) for the detection of avian influenza virus H7 (AIV H7). The assay was performed with H7-polyclonal antibodies (PAbs) that were attached to the AgNPs-G surface (PAb-AgNPs-G). This electrochemical immunosensor was highly specific and sensitive towards the AIV H7 permitting rapid detection of the virus (Huang et al. 2016).

Teengam et al. employed AgNPs as a colourimetric reagent for DNA detection based on pyrrolidinyl peptide nucleic acid (acpcPNA)-induced nanoparticle aggregation. The positively charged lysine at the C-terminus of the acpcPNA probe induced aggregation of citrate anion-stabilized AgNPs in the absence of complementary DNA. However, in the presence of the complementary DNA, an anionic DNA-acpcPNA duplex has formed. In consequence, dispersion of the AgNPs has taken place giving rise to an observable colour change. The method was used for the detection of Middle East respiratory syndrome coronavirus (MERS-CoV) which is a positive-sense, single-stranded RNA virus as well as the human papillomavirus (HPV) which is a double-stranded circular DNA virus. The results of this study suggested its applicability as an alternative diagnostic tool for the simple, speedy, sensitive, and selective detection of infectious agents (Teengam et al. 2017).

4.2.3 Copper Nanoparticles (CuNPs)

Copper nanoparticles also play an important role in virus detection. Mao et al. introduced a colourimetric method using copper nanoclusters to detect the hepatitis B virus which is a double-stranded DNA virus (Mao et al. 2016).

Respiratory syncytial virus (RSV) is a negative-sense single-stranded RNA virus that causes cold-like symptoms in adults and children. Valdez et al. evaluated localized surface plasmon resonance (LSPR) shifting as an RSV detection method with the use of anti-RSV polyclonal antibody conjugated to metallic nanoparticles; Cu, Ag, and Au (Fig. 4.5). The results indicated that functionalized copper nanoparticles were more efficient and specific for RSV detection in comparison to silver and gold nanoparticles. Further, this study revealed that LSPR is a simple, inexpensive, and rapid alternative method for the development of new detection devices for RSV (Valdez et al. 2016).

4.2.4 Carbon Nanotubes (CNT)

The electrical properties of single-walled carbon nanotubes (SWCNTs) were utilized by Lee et al. to develop an electric immunoassay for the detection of swine-influenza virus (SIV) H1N1. The anti-SIV antibody was immobilized on the surface of SWCNTs using poly-L-lysine. The immunobinding of SIV on the sensor surface resulted in an alteration in the resistance of SWCNTs thin film. With increased SIV concentration, more SIV got adsorbed on the surface, increasing the resistance of SWCNT film. This technique was identified as an inexpensive, and ultrasensitive strategy for point-of-care detection (Lee et al. 2011).

Dias et al. demonstrated that carbon nanotube-screen printed electrodes (CNT-SPE) can be used for the early screening of patients infected with the dengue virus. An electrode for the selective detection of NS1 was developed in this study. First, the carbon nanotubes were carboxylated and dispersed in a carbon ink to develop a screen printed working electrode. A thin film of ethylenediamine was deposited on the electrode to facilitate the immobilization of anti-NS1 antibodies. Electrochemical responses were generated due to the action of H_2O_2 with the peroxidase-conjugated to anti-NS1. This method was identified to be cheaper and more practical than RT-PCR and ELISA, thus having a great promise for use in an epidemic situation (Dias et al. 2013).

In another study, an electrochemical sensor was constructed on the surface of multi-walled carbon nanotubes (MWCNTs) modified glassy carbon electrode for the detection of HIV. This method is a rapid, sensitive, and very simple method for the identification of p-24 antigen directly. The sensor was developed on multi-walled carbon nanotubes on the surface of a glassy carbon electrode (GCE). GCE was modified by using acrylamide, N,N′-methylenebisacrylamide, ammonium persulphate as the functional monomer, cross-linking agent, and initiator, respectively. Results have shown that biosensor specifically identifies HIV p-24 and displays a broader linear detection range, repeatability, reproducibility, and stability (Ma et al. 2017).

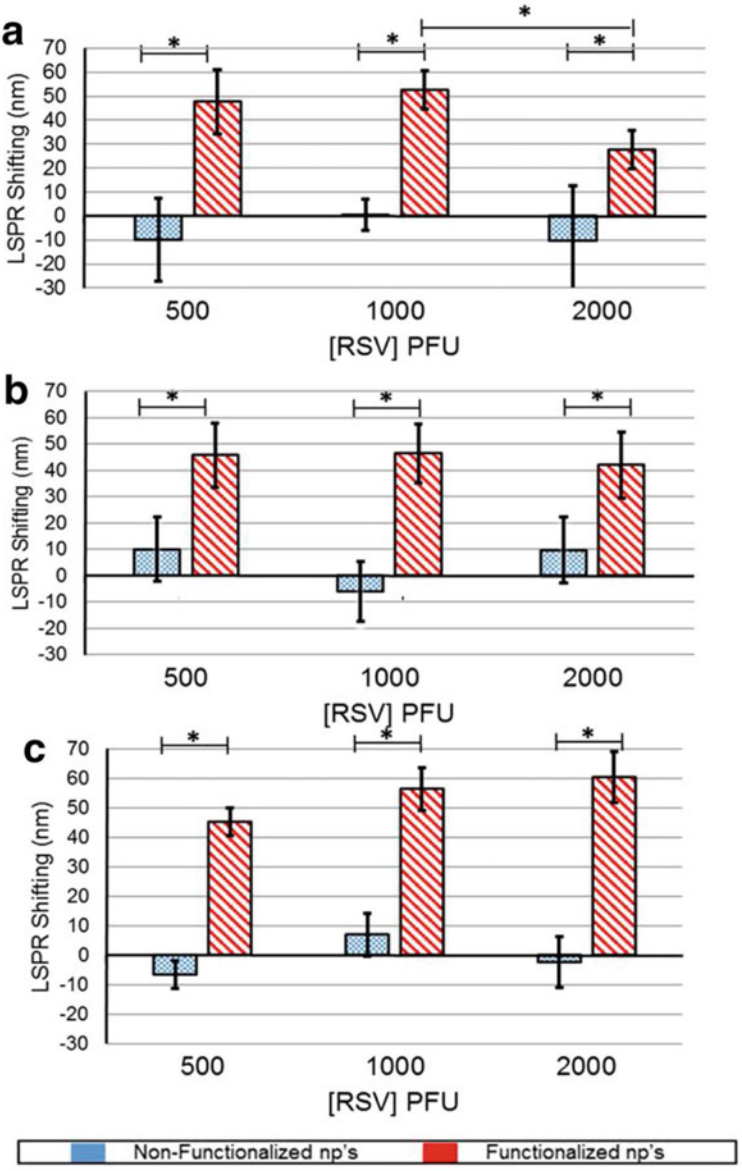

Fig. 4.5 Graph illustrates the LSPR shifting at different titres of RSV at 30 min (**a**), 60 min (**b**), and 120 min (**c**) for antibody-functionalized (red) and non-functionalized (blue) silver nanoparticles. The asterisk symbol represents the significance $p < 0.05$ (Adapted with permission from Valdez et al. 2016)

4.2.5 Quantum Dots (QD)

The size-tunable fluorescent ability and the simultaneous excitation of a single wavelength have made quantum dots a promising platform in optical encoding detection. Deng et al. prepared a QD-biosensor system (CdTe QD) by mixing both green QD biosensors and orange QD biosensors for the simultaneous and independent detection of H9 avian influenza virus and MHV 68 virus based on antibody−antigen interaction. The green and orange CdTe QDs have been identified as effective pH-sensitive fluorescent probes capable of monitoring the proton flux driven by ATP synthesis. Upon the loading of the H9 virus, a rapid increment of the fluorescent intensity at 535 nm was observed in green QD biosensors that have been labelled with an H9 virus antibody. This was attributed to the enhancement of the activity of the ATP synthetase by the antibody-antigen reaction. Similarly, the fluorescent intensity has markedly increased at 585 nm when MHV 68 was loaded. This has occurred due to the enhancement of the ATP synthetase activity of the orange QD biosensors. Moreover, with the loading of both those viruses together, a rapid change in both fluorescent intensities at 535 and 585 nm was observed. Interestingly, each part of the QD biosensors worked independently without interfering with each other in the fluorescence assays (Deng et al. 2007).

In a very recent study, an immunosensor was introduced using streptavidin-conjugated quantum dots (QDs/SA) for the detection of non-structural protein 1 (NS1) of the dengue virus. These NS proteins are accountable for the replication of new viruses in the host cell and the newly developed immunosensor was capable of detecting even a minute amount of the NS1 antigen. The QDs/SA was first conjugated to biotinylated NS1 antibody and thereafter utilized to detect the NS1 antigen. This appeared to be a highly sensitive, rapid, simple, and convenient method for the detection of the virus in the early stage of infection (Tran and Park 2021).

Roh et al. introduced a nanoparticle-supported aptamer probe for the detection of the hepatitis C virus. HCV NS3 was detected with the help of an imaging probe comprised of nanoparticle QDs with a carboxyl group and a 5′-end-amine-modified RNA oligonucleotide as a capturing probe. This QDs-based RNA aptamer displayed higher selectivity and specificity and appeared as a promising platform in diagnosis (Roh et al. 2010).

4.2.6 Other Nanomaterials

Yang et al. introduced a fluoroimmunoassay method for the detection of trace-level Hepatitis B Surface Antigen (HBsAg) with a detection limit of 0.1 ng/mL. This method used a fluorophore hybrid silica (FHS) nanoparticles-based labelling system and displayed excellent optical properties. The sensitivity of this novel FHS nanoparticles-based labelling system was significantly increased in comparison to

the corresponding immunoassay performed with direct fluorophore labelling (Yang et al. 2004).

A fluorescent aptasensor system was developed by Pang et al. for the detection of the hemagglutinin protein of the H5N1 virus. In this approach, anti-rHA aptamers were immobilized onto the surface of $Ag@SiO_2$ NPs and thiazole orange molecules were used as the fluorescent tag. Non-requirement of covalent labelling with fluorophores to the aptamer and the very low background noise were some advantages of this method (Pang et al. 2015). Likewise, an amplified electrochemical immunosensor based on 1-naphthol as an electroactive substance and Pt/CeO_2/graphene-oxide composites as a catalytic amplifier were constructed for sensitive detection of influenza (Yang et al. 2015). In another study, electrospun semi-conducting Mn_2O_3 nanofiber-based biosensor was developed to detect the dengue virus. This platform had combined the inherent advantages of metal oxide nanofibers and electrochemical transduction techniques enabling the detection of dengue consensus primer at zeptomolar range (Tripathy et al. 2017).

4.3 Antiviral Activity of Nanoparticles

The interaction between different nanomaterials and viruses is the subject of much research effort to find treatment strategies for infectious diseases caused by viruses. For example, Gaikwad et al. evaluated the antiviral activity of biogenic AgNPs against herpes simplex virus types 1 and 2 and human parainfluenza virus type 3. This study revealed that the interaction between silver nanoparticles and tested viruses was depended on the size and zeta potential of the AgNPs. Besides, the activity also depended on the source that was used to prepare AgNPs. The antiviral activity was conspicuous in the AgNP produced by *Fusarium oxysporum* and *Curvularia* species which were smaller in size in comparison to the AgNPs synthesized from other fungal sources. It was speculated that smaller-sized nanoparticles were capable of attaching the virus and thereby blocking the interaction of virus and host cell causing attenuation of viral replication (Gaikwad et al. 2013). In another study, AgNP displayed anti-HIV-1 activity at an early stage of viral replication by blocking viral entry, particularly the gp120-CD4 interaction. Usually, gp120 (glycoprotein exposed on the surface of the HIV envelope) binds to the primary cellular receptor CD4 on the host cell surface to initiate the infection. This study revealed that AgNP can bind to gp120 thus preventing CD4-dependent virion binding, fusion, and infectivity (Lara et al. 2010). Similarly, Xiang et al. evaluated the interaction of AgNPs with the H1N1 influenza A virus where MDCK cells were used as the infection model. Their results revealed that AgNPs can reduce H1N1 influenza A virus-induced apoptosis in MDCK cells (Xiang et al. 2011).

The antiviral activity of AgNP was determined against the hepatitis B virus (HBV) as well. Lu et al. employed the HepAD38 cell line as an infection model to study the in vitro anti-HBV activity of AgNP. A reduction in the extracellular HBV DNA formation of HepAD38 cells was observed in the presence of AgNP with mean

particle diameters of approximately 10 nm and 50 nm. Although these nanoparticles had a slight effect on the amount of HBV covalently closed circular DNA, the formation of intracellular HBV RNA was inhibited by AgNP (Lu et al. 2008). In another study, Baram-Pinto et al. found that AgNPs that were capped with mercaptoethane sulfonate can block the entry of herpes simplex virus 1 (HSV-1) into the cell by binding to the cell surface heparin sulphate (Baram-Pinto et al. 2009). Moreover, Hu et al. found that AgNP at a concentration of 100 μg/mL could completely inhibit the replication of HSV-2 virus. The formation of bonds between silver nanoparticles and the glycoprotein membrane of the HSV-2 virus was speculated to be the underlying mechanism for the antiviral activity. This resulted in an inhibition of the interaction between the virus glycoprotein and the receptor, thus preventing the internalization of the virus (Hu et al. 2014).

Recently Jeremiah et al. examined the antiviral effect of AgNPs against SARS-CoV-2. Amongst the AgNPs of different sizes, the nanoparticles of diameter around 10 nm at a concentration range of 1–10 ppm were found to be effective in inhibiting extracellular SARS-CoV-2. However, AgNPs at concentrations of 20 ppm and above exhibited cytotoxic effects. From the observations of the luciferase-based pseudovirus entry assay, it was hypothesized that AgNPs were capable of inhibiting the viral entry step by disrupting viral integrity (Jeremiah et al. 2020).

There are several research studies on the antiviral activity of AuNPs. Vijayakumar and Ganesan reported the antiviral activity of AuNPs stabilized with polyethylene glycol against HIV-1. These AuNPs were found to inhibit the viral entry by binding with gp120 and thereby preventing CD4 attachment (Vijayakumar and Ganesan 2012). Similarly, the antiviral activity of non-functionalized AuNPs against HSV-1 has also been reported. Paradowska et al. revealed that AuNPs were capable of reducing the cytopathic effect (the structural changes in host cells caused by viral invasion) of HSV-1 in Vero cells in a dose- and time-dependent manner (Paradowska et al. 2021).

Meléndez-Villanueva et al. reported the antiviral activity of biogenic AuNP prepared from garlic extract. These AuNPs inhibited the replication of the measles virus in Vero cells with an EC_{50} of 8.829 μg/mL. It was assumed that the positive surface charge of the AuNPs and the negative charge of the viral membranes facilitated the binding of AuNPs and the viral envelope, thus preventing cell adsorption and the onset of infection in the host cell (Meléndez-Villanueva et al. 2019).

Hemagglutinin is a highly conserved surface protein in different influenza virus (IV) strains that mediate the binding of the virus with the host cell receptors. As a result, it is considered an effective antiviral target. Kim et al. fabricated porous gold nanoparticles (PoGNPs) to cleave the disulfide bonds in hemagglutinin. The antiviral activity of PoGNPs was attributed to the blocking of viral attachment associated with membrane fusion, as a result of the cleavage of disulfide bonds in hemagglutinin. The potential utility of PoGNP against other enveloped viruses with considerable spike proteins on the surface (e.g. HIV, coronavirus) was also highlighted in this study (Kim et al. 2020).

Cuprous oxide nanoparticles (CO-NPs) also displayed antiviral activity. Hang et al. demonstrated the efficacy of CO-NPs against HCV in the HCVcc/Huh7.5.1

cell culture system. CO-NPs were capable of inhibiting the infectivity of cell culture HCV (HCVcc) as well as the entry of HCV pseudoparticle (HCVpp). Therefore it was concluded that the ability of CO-NPs to act as an anti-HCV agent was due to the interaction of CO-NPs with the virion surface. This interaction can block the receptor-binding sites on HCV envelope glycoprotein and inhibit both viral attachment and entry (Hang et al. 2015). Recently, Takeda et al. disclosed the effectiveness of copper iodide (CuI) nanoparticles in inactivating SARS-CoV-2 virus. CuI-doped film and fabric exhibited rapid inactivation of the virus. The virucidal activity was assigned to the destruction of viral proteins and the genome particularly due to the action of CuI-derived reactive oxygen species. These findings indicated the potential utility of CuI-doped film and fabric as anti-SARS-CoV-2 materials for surgical masks/protective clothes as well as to protect high-touch environmental surfaces (Takeda et al. 2021).

4.4 Treatment/Prevention of Viral Infections with the Use of Nanomaterial

Nanotechnological approaches can be considered a powerful tool to improve the activity of antiviral drugs. Encapsulation of antiviral drugs with nanoparticles is normally associated with features like controlled release kinetics, improved bioavailability, modified pharmacokinetics, and reduced adverse effects. Further, the physicochemical properties of nanocarriers may allow the targeting of specific sites and interaction with virus structures (Lembo et al. 2018). For example, the antiviral drug acyclovir (ACV) which is used to treat HSV infections, chickenpox, and shingles, has a short half-life and incomplete absorption. Therefore, Kamel et al. developed stealthy ACV nanoparticles with the use of polylactic acid, polylactic-co-glycolic (PLGA) 85/15 to increase the drug plasma half-life after intravenous administration. This approach increased the mean residence time of the drug 29 times (Kamel et al. 2009). Likewise, ACV-loaded galactose conjugated PLGA nanoparticles were also introduced (Gupta et al. 2013).

Gold nanoparticles (AuNPs) have been widely studied as a delivery carrier of different biopharmaceuticals. Lee et al. introduced a target-specific long-acting delivery system of interferon α (IFNα) using the hybrid materials of AuNP and hyaluronic acid (HA). This HA-AuNP/IFNα complex was employed in the systemic treatment of HCV infection (Lee et al. 2012). Similarly, Xu et al. introduced surface-engineered gold nanorods as DNA vaccine adjuvant for HIV treatment. These gold nanorods promoted cellular and humoral immunity along with T cell proliferation through activating antigen-presenting cells (Xu et al. 2012a, b).

Nanotechnology has also been employed in the vaccine development process to enhance antigen delivery and efficient induction of immunity. Various nanoparticle systems like polymeric nanoparticles, virus-like particles (VLPs), liposomes, and chitosan have been used for these purposes (Sulczewski et al. 2018). Nanoparticles are emerging as a highly promising vaccine platform for several infectious diseases

due to their ability to deliver antigens and many immunostimulatory molecules. The antigen can be encapsulated, adsorbed, and dispersed on the nanoparticle matrix. The slow-release vaccine antigens and recruitment of antigen-presenting cells are usually responsible for the immunostimulatory activity of nano-vaccines (Fig. 4.6) As a result, many research groups attempted to develop nano-vaccines against the Chikungunya virus, Dengue virus, Zika virus, Japanese encephalitis virus, and West Nile virus over recent years (Al-Halifa et al. 2019; de Souza et al. 2021).

Dhakal et al. used PLGA nanoparticles to encapsulate inactivated Swine influenza virus (SwIV) H1N2 antigens (KAg) and induced maturation of antigen-presenting cells in vitro. Pigs that were vaccinated twice with this preparation have developed significantly milder disease compared to non-vaccinated animals once challenged with a virulent heterologous influenza virus strain (Dhakal et al. 2017). In another study with the use of trimeric fusion protein from the respiratory syncytial virus (RSV) as a model immunogen, Francica et al. demonstrated that nanoparticle-forming thermoresponsive polymers (TRP) allow for co-delivery of Toll-like receptor agonists (TLRa) to enhance protective immunity (Francica et al. 2016).

Some of the vaccines developed against COVID-19 are nanotechnology-based. For example, mRNA-based Pfizer/BioNTech (BNT162b2) and Moderna (mRNA-1273) vaccines are delivered in lipid nanoparticle formulations (Dube et al. 2021). These nanoparticles help in protecting the encapsulated mRNA from ribonucleases and thereby improving the stability. Further, the nanoparticles facilitate the delivery of intact mRNA to the target site (Khurana et al. 2021). In addition, Geng et al. introduced a virus-like particle (VLP) vaccine that combines the effectiveness of virus-based vaccines and the safety of protein-based vaccines. Usually, VLPs are spherical supramolecular assemblies of viral capsid proteins but are free from genetic materials. Here, lumazine synthase nanoparticle protein was the structural scaffold while 120 copies of SARS-CoV-2 receptor-binding domain were the surface immunogen. Interestingly this VLP vaccine exhibited potent inhibition of SARS-CoV-2, SARS-CoV-1, and their variants (Geng et al. 2021).

Although nanomaterials show great promise in treating viral infections, many studies only investigated biocompatibility using in vitro experiments. Due to the complex interactions between nanomaterials and biological systems, particularly with small molecules, enzymes and other proteins, and cells, properly designed in vivo experiments are required to evaluate the behaviour of these materials under physiological conditions.

4.5 Conclusion

Viral infections have emerged as a major global health hazard, particularly in recent years. However, only a limited number of diagnostic tools and antiviral therapeutics are available to combat these viral infections. Nanotechnology-based novel approaches can provide a better platform to diagnose viral infections within a short time with high sensitivity in comparison to the existing viral detection methods. Also,

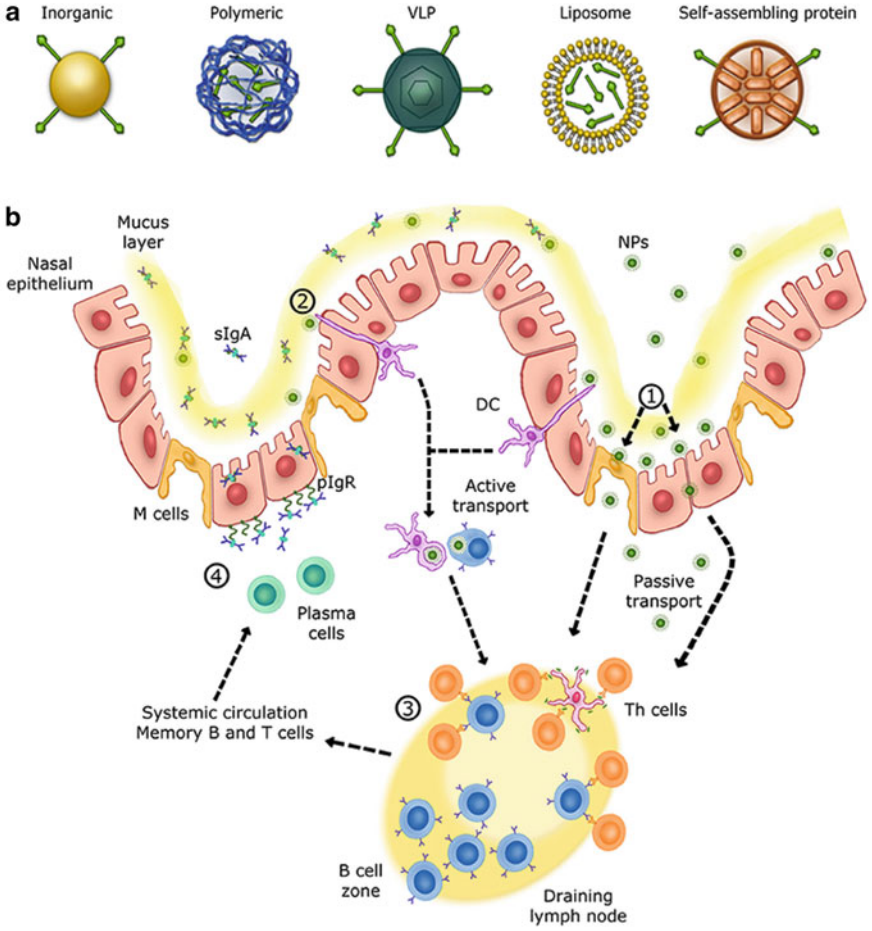

Fig. 4.6 Overview of the immune response in the upper respiratory tract **a** Schematic view of different nanoparticles used for intranasal vaccination. **b** Mechanisms of nasal-associated lymphoid tissues (NALTs) immune responses in the upper respiratory tract. (1) Nanoparticles are transcytosed from the mucus layer into the nasal epithelial tissues by micro-fold cells (M cells) or passively diffuse through epithelial cell junctions. (2) Other nanoparticles are captured and internalized by DCs (dendritic cells) from their extension through epithelial junctions and by other APCs, such as B cells. (3) Cells that have encountered nanoparticles migrate to the nearest lymph node in order to activate naive T helper cells. Once activated, T helper cells activate B cells that have encountered the same antigen presented by nanoparticles. Activated B cells proliferate in the lymph node (B cell zone) and, once mature, enter systemic circulation in order to reach the inflammation site. IgA + B cells locally differentiate into antibody-secreting plasma cells to produce IgA dimers. (4) IgA dimers are secreted via polymeric Ig receptor (pIgR) at the mucosal surface. NALT immune response induces long-lasting memory B and T cells able to trigger a rapid recall response (Adapted with permission from Al-Halifa et al. 2019)

nanomaterials may help to overcome issues like low solubility and bioavailability, burst release, development of resistance, and adverse effects which are often associated with conventional antiviral therapeutics. Therefore, nanotechnology appears as a novel and promising tool in the effective management of viral infections in future medicine.

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Chapter 5
Nanomaterials for Wound Healing and Tissue Regeneration

Mayuri Napagoda, Priyalatha Madhushanthi, and Sanjeeva Witharana

Abstract Being the largest organ and the protective shield of the human body, the skin is highly vulnerable to potential injuries. A cascade of biological events initiates after the injury to regenerate and repair the damaged tissue and this process is referred to as wound healing. Wound dressings have been introduced as a temporary protective physical barrier to prevent the invasion of pathogenic microorganisms and to keep the wound from dehydration while facilitating the healing process. In recent times, nanomaterials have emerged as a source for developing highly effective and innovative wound dressings. Particularly, several polymeric nanofibres have shown promising results as scaffolds for skin regeneration while some metal nanoparticles possess intrinsic antibacterial properties thus making them potential candidates for integration into wound dressings. Moreover, encapsulating drugs, biomolecules, and growth factors, within nanocarriers is also offering new treatment modalities, especially for chronic wounds. Therefore, this chapter provides an overview of the recent advances in nanotechnology-assisted wound healing and tissue regeneration and the applicability of nanomaterials in the treatment of chronic and acute wounds.

Keywords Nanocarriers · Nanomaterials · Tissue regeneration · Wound dressings · Wound healing

5.1 The Wound Healing Process

Skin is a complex organ and it is composed of the epidermis, dermis, and skin appendages like hair follicles, sebaceous glands, etc. It acts as a barrier and protects the internal organs from harmful ultraviolet rays, mechanical damage, and harmful pathogens. In addition, skin prevents the evaporation of water from the body and

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maintains the fluid imbalance and is involved in thermal dysregulation. Therefore, the integrity of healthy skin is of great importance in maintaining the physiological homeostasis of the human body. However, the skin is functioning as the protective shield of the body and becomes vulnerable to potential injury. Hence, wound healing is an essential process for the survival of organisms (Bentley 2004; Takeo et al. 2015; Sorg et al. 2017).

A wound is defined as a "disruption of normal anatomic structure and function of the skin". Generally, wounds can be categorized as acute wounds and chronic wounds. Acute wounds are caused by irradiation, exposure to heat, mechanical damages, etc. and are healable within 8–12 weeks. On the other hand, chronic wounds are caused due to diseases like diabetes, tumours, etc. and the healing would take more than 12 weeks (Zahedi et al. 2010).

Wound healing is a cascade of biological events which initiates after the injury to regenerate and repair the damaged tissue. The wound healing process can be described under four main phases; haemostasis, Inflammation, proliferation, and maturation (Dalisson and Barralet 2019). The haemostasis phase starts soon after wounding (within the first few minutes) and it involves vasoconstriction, platelet plug formation, activation of the coagulation cascade, and finally the formation of a fibrin plug (final clot). Vasoconstriction helps to reduce the blood flow while platelet adhesion, activation, and aggregation lead to platelet plug formation. Due to the activation of the extrinsic pathway (tissue factor pathway) and intrinsic pathway of blood coagulation, a cascade of enzymatic reactions takes place and ultimately causes the conversion of fibrinogen to fibrin. The fibrin monomers get polymerized to form a fibrin polymer mesh and this results in a cross-linked fibrin clot around the platelet plug (LaPelusa and Dave 2022). Within an hour after clotting, the second phase of the wound healing process, i.e. inflammation starts. Polymorphonuclear neutrophils (PMNs) are predominately found in the wound bed up to about two days after the injury. These PMNs perform several functions such as the release of free radicals to kill bacteria in the wound, phagocytosis of debris, and breakdown of damaged tissues through the action of proteases. After two days, these PMNs either undergo apoptosis (cell death) or get degraded by macrophages. Thereafter, monocytes enter the wound bed and get converted into macrophages. Matured macrophages secrete several growth factors and cytokines that promote angiogenesis, granulation tissue formation as well as re-epithelialization. Before the end of the inflammatory phase of wound healing, the factors which are released by the macrophages activate fibroblasts, endothelial cells, and keratinocytes from surrounding tissues and initiate the migration and proliferation processes. These will be followed by events like the laying down of a new extracellular matrix, the formation of granulation tissue to form a barrier between the wound and the environment, and the closing of the wound. Usually, the final phase of the wound healing process, i.e. maturation starts three days after an injury. In this phase, the collagen matrix which has been previously laid down is remodelled slowly (Dalisson and Barralet 2019).

5.2 Wound Dressings

According to the clay tablets of Mesopotamian origin that date back to 2500 BCE, Mesopotamians used milk or water to clean the wounds before dressing them with honey or resin (Daunton et al. 2012). In the early clay tablets, three healing gestures were described. Those were washing the wounds, making the "plasters", and bandaging the wound. These ancient "plasters" (the present-day equivalent of wound dressings) were mixtures of clay/mud, herbs, oils, etc. (Shah 2011). Similarly in ancient Greece (460–370 BCE), wine and vinegar were employed to clean the wounds while honey, wine, and oil were used as further treatments. Wool boiled in water or wine was used as bandages. In traditional Chinese medicine, green tea, liquorice, soaked mushrooms, and many herbal powders have been employed to promote granulation tissue, help in debridement and prevent infection (Daunton et al. 2012).

5.2.1 Traditional Wound Dressings

Over the years wound dressings have evolved from the crude application of herbal materials and animal fat to tissue-engineered scaffolds. Gauze, lint, plasters, cotton wool, as well as synthetic or natural bandages, can be considered traditional wound dressings. These are used as primary dressings (i.e. the dressing that makes physical contact with the wound surface) or secondary dressings (i.e. dressings that are used to cover the primary dressing) and help to prevent contaminations and thereby protect the wound.

Gauze is made using cotton fibres, rayon, and polyesters which can act against some bacterial infections. Mostly, sterile gauze pads are used to absorb fluids and exudates in open wounds. Although gauzes are widely used as wound dressings, there are several disadvantages associated. For example, gauzes should be changed from time to time and are not cost-effective. Moreover, excessive wound drainage causes it to become adherent to the wound and as a result, the patient would experience pain at the time it is being removed. Bandages are made using cotton wool, cellulose, or polyamide materials. Cotton bandages are used to keep light dressings in place, whereas high compression bandages and short-stretch compression bandages are used to provide sustained compression. Tulle dressings like Bactigras, Jelonet, and Paratulle (some commercially available tulle dressings) are impregnated with paraffin and these are appropriate for superficial clean wounds. Because of the inability to provide a moist atmosphere for wound healing, nowadays dry traditional wound dressings comprised of topical liquid and semi-solid formulations have been replaced by new dressings with more sophisticated formulations (Boateng et al. 2008; Dhivya et al. 2015).

5.2.2 Modern Wound Dressings

These kinds of dressings have been produced to keep the wound from dehydration and facilitate the healing process. Modern dressings are usually made up of synthetic polymers. Some examples are given below.

(a) Semi-permeable film dressings

These dressings are made of transparent and adherent polyurethane and are impermeable to bacteria but permit the transfer of water vapour, O_2, and CO_2 from the wound while providing autolytic debridement eschar. In the early days, the films were produced from nylon derivatives with adhesive polyethylene frames and the adhesive layer permits the dressing to adhere to intact skin. However, these nylon-derived film dressings were not used for highly exudating wounds because of their limited absorption capacity. Moreover, maceration of the wound and the surrounding healthy tissues were also observed with the use of these materials. These dressings are recommended as a primary dressing for dry and superficial wounds. Yet, these can also be used in more exuding wounds, as a secondary dressing on top of foam dressings or dressing pads. When applying to intact skin in vulnerable areas, these dressings can also help to reduce friction, though, the removal should be done with caution to prevent any damage to the epidermal layer of the skin by the adhesive (Abdelrahman and Newton 2011; Shi et al. 2020).

(b) Semi-permeable foam dressings

Semi-permeable foam dressings are made up of hydrophobic and hydrophilic foam, sometimes with adhesive borders (Morgan 2002). These dressings are usually produced from polyurethane and silicone and are available as adhesive or non-adhesive foam dressings (Abdelrahman and Newton 2011). The outer layer of the dressing protects the wound from a liquid due to its hydrophobic properties while allowing gaseous exchange and water vapour. Depending on the thickness of the wound, foam can absorb varying amounts of wound drainage. These dressings are designed mainly for lower leg ulcers and can be used for moderate to highly exudating wounds or granulating wounds (Ramos-e-Silva and Ribeiro de Castro 2002). Some foam dressings are demonstrated to be effective cavity fillers. The foam absorbs exudates preventing pooling and skin leakage while maintaining a low adherence to allow for easy, painless removal (Abdelrahman and Newton 2011).

(c) Alginate dressings

Alginate dressings are made out of calcium or sodium alginate derived from seaweeds. These dressings are usually recommended for moderately to heavily exuding wounds but are not suitable for dry wounds. Calcium component of the dressing functions as a haemostat thus useful in bleeding wounds (Abdelrahman and Newton 2011). Alginate dressings have achieved a good absorption capability through the formation of a strong hydrophilic gel which can limit the wound exudates and reduce bacterial contamination (Dhivya et al. 2015). Despite there were some concerns that alginate can inhibit keratinocyte migration, Thomas et al. revealed that

alginate speed up the healing process. This has occurred by activating macrophages to synthesize TNF-α that initiates inflammatory signals. After applying alginate dressings to the wound, the ions in the alginate exchange with the blood and form a protective film. Furthermore, these dressings require secondary dressings as they could dehydrate the wound causing a delay in healing (Thomas et al. 2000; Boateng et al. 2008).

(d) Hydrogel dressings

Hydrogels are a form of insoluble hydrophilic material made up of synthetic polymers like poly (methacrylates) and polyvinyl pyrrolidine. A high water content (70–90%) in hydrogels assists granulation of tissues and epithelium in a moist environment. In addition, the soft elastic properties of these dressings make them easy to apply and also to remove once the wound is healed without causing any damage. Furthermore, hydrogels reduce the temperature of cutaneous wounds, providing those with a cooling effect. These dressings are designed for chronic wounds, necrotic wounds, burn wounds, and also for pressure ulcers (Dhivya et al. 2015). These hydrogel dressings are produced as tubes or as flat sheets and by considering the depth and location of the wound the most suitable one should be selected (Abdelrahman and Newton 2011).

(e) Hydrocolloid dressings

Hydrocolloid dressings are amongst the most commonly used interactive dressings which have two layers named inner colloidal layer and the outer water-impermeable layer. These dressings are formed with the help of gel-forming agents such as carboxymethyl cellulose, gelatin, and pectin and are combined with other materials like elastomers and adhesives (Boateng et al. 2008; Dhivya et al. 2015; Vowden and Vowden 2017). Hydrocolloids have the properties of debridement and absorb wound exudates while being permeable to water vapour but impermeable to bacteria (Thomas 1992). These kinds of dressings can be used for pressure sores, superficial burns, and traumatic wounds. In addition to that, hydrocolloid dressings do not cause any pain during the removal. Therefore, such dressings can also be recommended for paediatric wound care management. Nevertheless, these are usually not recommended for neuropathic ulcers or wounds with a lot of exudates and are often used as a secondary dressing (Boateng et al. 2008; Dhivya et al. 2015).

(f) Antimicrobial dressings

Topical antimicrobial dressings are designed to minimize the growth of microorganisms in wounds and can be used in both chronic and acute wounds. These dressings are designed using silver and iodine. In addition, polyhexamethylene biguanide-based antimicrobial dressings that affect bacterial cell metabolism have recently been introduced. All these dressings are found to be effective against a broad range of microorganisms commonly associated with chronic wounds (Flores and Kingsely 2007; Abdelrahman and Newton 2011).

5.2.3 *Significance of Nanotechnological Approaches in Wound Healing and Tissue Regeneration*

Recent advances in nanotechnology have opened up new avenues for drug delivery applications, allowing the delivery of biomolecules or growth factors, which can be used in chronic wound healing. The small size and physicochemical properties of the nanomaterials facilitate the intracellular delivery of biomolecules or drugs by protecting them from degradation and improving the drug penetration into the wound. This enables the topical administration of drugs and increment of the half-life thus reducing the number of applications and costs. Furthermore, encapsulating drugs and biomolecules within nanocarriers would allow different drug release profiles that may match the requirements of the wound healing process (Blanco-Fernandez et al. 2021).

Therefore, this chapter summarizes the recent advances in nanotechnology-assisted wound healing and tissue regeneration and the applicability of nanomaterials like nanoparticles, nanofibers, self-assembled nanocarriers, etc. in the treatment of chronic and acute wounds.

5.3 Application of Nanomaterials in Wound Healing and Tissue Regeneration

Nanoparticles are very small particles typically with a diameter of 1–100 nm. They have been extensively used in biomedicine and tissue engineering applications. Nanoparticles play two roles in wound healing; i.e. with their intrinsic properties positive for wound healing, and as drug delivery systems (Blanco-Fernandez et al. 2021).

Nanoparticles are employed in wound dressing materials mainly due to their intrinsic antimicrobial properties. Moreover, nanoparticles are capable of penetrating cell membranes whereas conventional antimicrobial agents often have a limited ability to cross certain cell membranes. Nanoparticles exert antimicrobial properties by degrading cell membranes, blocking and altering enzymatic pathways, etc. (Yah and Simate 2015).

Metal nanoparticles such as silver, gold, copper, copper oxide, iron oxide, zinc oxide, and titanium dioxide possess antibacterial properties which are useful in wound healing. The antibacterial activity of the above-mentioned metal nanoparticles triggers by the formation of reactive oxygen species (ROS) as well as interaction with biomolecules such as DNA, proteins, or inhibition of enzymes (Chatterjee et al. 2014; Shaikh et al. 2019). For example, silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) exert antibacterial activity via several modes of action. These nanoparticles can disrupt the bacterial cell membrane, leach into the cytosol, and destabilize/disrupt membrane proteins, cytoplasmic proteins, and enzymes. This would ultimately lead to metabolic impairment and ultimately bacterial cell death.

Further, AuNPs and AgNPs can generate ROS creating oxidative stress. The presence of ROS can damage proteins and nucleic acids in bacterial cells and also inhibit the electron transport chain of bacterial cells leading to bacterial cell death (Joshi et al. 2020).

Silver has been widely used in the field of medicine for over centuries in the form of metallic silver, $AgNO_3$, and silver sulphadiazine, to treat open wounds, burns, and also to treat chronically infected wounds. With the introduction of antibiotics, the usage of silver compounds had drastically reduced. However, with the development of nanotechnology, silver metal in its nano form re-emerged as a prospective antimicrobial agent (Rai et al. 2009). The extremely small size and large surface area to volume ratios have made AgNPs an effective antimicrobial agent. The formation of "pits" was observed in the cell wall of *Escherichia coli* cells treated with AgNPs, while AgNPs got accumulated in the bacterial membrane increasing the permeability and ultimately resulting in cell death (Sondi and Salopek-Sondi 2004). A more recent study conducted using *Staphylococcus aureus* and *E. coli* revealed that AgNPs can completely inhibit the growth of bacterial cells by destroying the permeability of the cell membrane and decreasing the activity of some enzymes (Gomaa 2017). Xiu et al. suggested that the antimicrobial activity of AgNPs depends on the release of Ag^+, thus it can be modulated by manipulating oxygen availability, size and shape of the particles, and the type of coating (Xiu et al. 2012). Bhattacharya et al. studied the antibacterial activity of AgNPs coated with a functionalizing agent. Here, polyethylene glycol, tween 80, and sodium dodecyl sulphate were added separately to coat AgNPs. Amongst those, polyethylene glycol-coated AgNPs were found to be most effective against both normal and multi-drug resistant strains of bacteria. It was observed that intracellular ROS production in bacteria was high in polyethylene glycol-coated nanoparticles (Bhattacharya et al. 2012).

AgNPs can increase the rate of wound closure by promoting the proliferation and migration of keratinocytes. Further, AgNPs can facilitate the differentiation of fibroblasts into myofibroblasts and thereby promoting wound contraction (Liu et al. 2010). Tian et al. demonstrated that AgNPs can contribute to the wound healing process through their antimicrobial action, by reducing wound inflammation and modulating fibrogenic cytokines (Tian et al. 2007). Tannic acid (TA)-modified AgNPs were also found to have effective antibacterial activity against *Pseudomonas aeruginosa*, *S. aureus*, and *E. coli*. Furthermore, in a mouse splint wound model, TA-modified AgNPs enhanced wound closure, epithelialization, angiogenesis, and granulation tissue formation. TA-AgNPs also induced the expression of vascular endothelial growth factor-α (VEGF-α), platelet-derived growth factor-β (PDGF-β), and transforming growth factor-β1(TGF-β1) cytokines which are involved in the efficient wound healing process (Orlowski et al. 2018).

Gold nanoparticles (AuNPs) are widely used in tissue regeneration, wound healing, and also in drug delivery. On the surface of AuNPs, there is a possibility to conjugate different ligands like polypeptide sequences, antibodies, and proteins (Fathi-Achachelouei et al. 2019). For example, Gu et al. reported that AuNPs can be conjugated with existing antimicrobial drugs by preparing vancomycin-conjugated gold nanoparticles (Au@Van) (Fig. 5.1). These nanoparticles have

displayed increased activity against vancomycin-resistant *Enterococci* (VRE) and a notable activity against *E. coli* which is a Gram-negative bacterium normally unaffected by vancomycin (Gu et al. 2003).

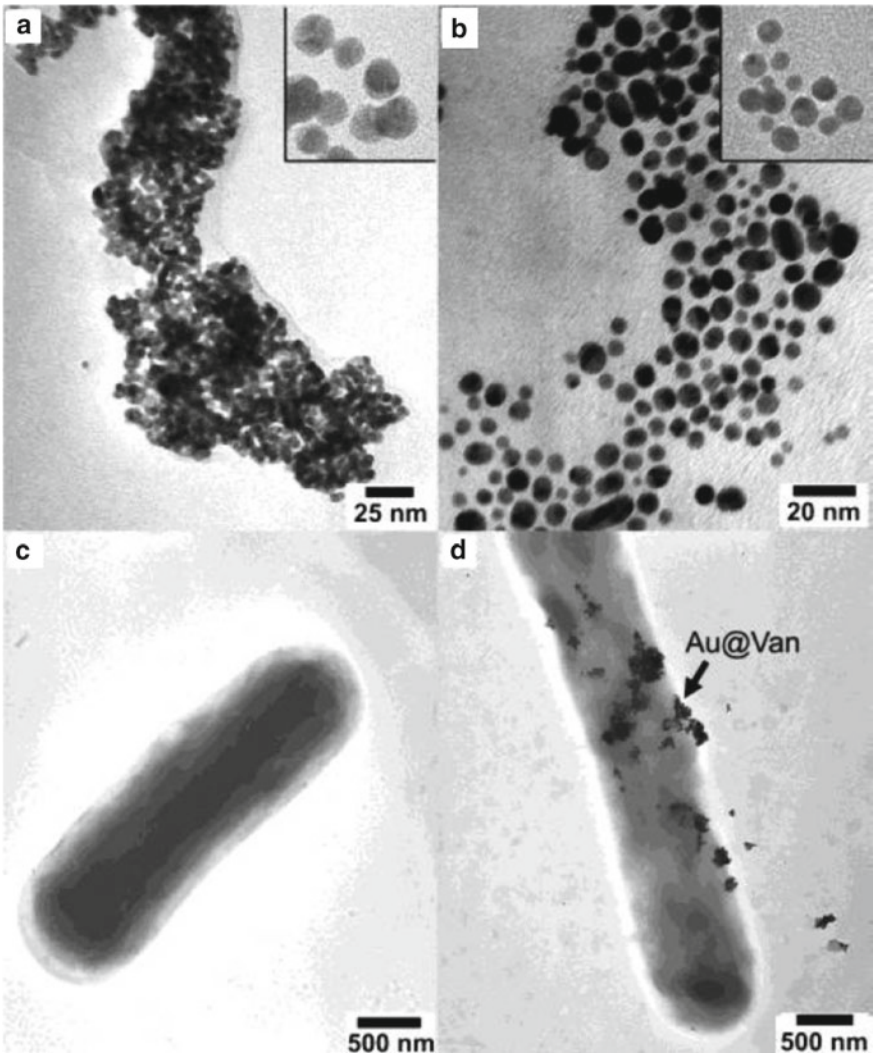

Fig. 5.1 Transmission electron micrographs of **a** Au@Cys and **b** Au@Van nanoparticles in the aggregated state after cryodrying at concentrations of 6.7 and 50 μg/mL (The insets show TEM images taken at the MIC concentration for Au@Cys and **b** Au@Van) and TEM images of *E. coli* after being treated by **c** Au@Cys and **d** Au@van nanoparticles at minimum inhibition concentrations (In this study Au nanoparticles conjugated with cysteine; Au@Cys were employed as the control) (Adapted with permission from Gu et al. 2003)

Recently, Korani et al. evaluated the antibacterial, antioxidant, cytotoxic, and cutaneous wound healing potential in AuNPs synthesized using *Abelmoschus esculentus* extract. Transmission electron microscopy (TEM) images indicated that the prepared nanoparticles were spherical and the size was around 75 nm. These nanoparticles exhibited significant antioxidant and antibacterial activities while being non-toxic. Interestingly, these AuNPs could accelerate wound closure, thus indicating the prospective application as a wound healing therapeutic (Korani et al. 2021).

AuNPs can be adjusted to strongly absorb near-infrared radiation and eventually transfer this energy in the form of heat into the surrounding environment. When nanoparticles are attached to bacterial cells, this localized heating during irradiation of near-infrared radiation can lead to irreversible cellular damage. On this basis, Norman et al. experimented with gold nanorods that have been covalently linked to pathogen-specific antibodies to selectively destroy one of the multi-drug resistance Gram-negative bacterium *P. aeruginosa* (Norman et al. 2008). Similarly, Gil-Tomas et al. attempted to covalently couple toluidine blue O–tiopronin to AuNPs and thereby enhance the antimicrobial activity against *S. aureus*. This conjugate entity showed activity under both 632.8 nm laser light and white light and appeared as a rapidly acting photosensitiser in the photodynamic therapy of wound and burn infections (Gil-Tomás et al. 2007). Moreover, Sherwani et al. demonstrated that photosensitizer conjugated-AuNPs can be used in the treatment of cutaneous *Candida albicans* infections. These nanoparticles have significantly reduced the fungal burden in topical skin wounds and tongues infected with *C. albicans* in mice (Sherwani et al. 2015).

Further, Naraginti et al. demonstrated that the application of green synthesized AgNPs and AuNPs formulations on open skin wounds in rats has accelerated the wound healing process. This has occurred via increased granulation tissue formation, collagen deposition, and re-epithelialization and thereby shortening the overall healing time. The topical application of AuNP formulation was found to be more effective than the application of AgNP formulation and the conspicuous wound healing activity observed with AuNPs was linked to its high anti-inflammatory and antioxidant activities (Naraginti et al. 2016).

Laser-tissue welding and laser-tissue soldering techniques have been introduced as an alternative approach to traditional suturing to repair skin. Although laser-tissue welding results in faster healing, it inherits several disadvantages like thermal damage and low penetration depths. In order to overcome these issues, exogenous chromophores have been employed to absorb the laser energy within the near-infrared window. In this respect, gold nanoshells have displayed great promise as exogenous near-infrared absorbers in laser-tissue welding. The in vivo experiments with rat skin wound healing model indicated that the aforementioned approach can result in a good wound healing response while minimizing the destruction of surrounding tissues and permitting the welding of thicker tissues (Gobin et al. 2005).

Zinc oxide nanoparticles (ZnONPs) possess antibacterial, anti-inflammatory, and skin regeneration properties and thus can be considered an ideal material for wound dressings and to speed up the healing of both acute and

chronic wounds. Hu et al. employed the electrospinning technique to fabricate zinc oxide/silver/polyvinylpyrrolidone/polycaprolactone (ZnO/Ag/PVP/PCL) nanofibres. In this approach, ZnONPs and AgNPs were mixed with PVP and PCL to obtain nanofibers. The ZnO/Ag/PVP/PCL bimetallic nanofibers displayed potent antibacterial activity and low cytotoxicity than the single metal nanomaterial-loaded nanofibres (Hu et al. 2018).

Copper plays many roles during the wound healing process. The antibacterial activity in copper is well-known while its involvement in skin re-modulation, stimulation of angiogenesis, and enhancement of anti-inflammatory power have also been documented. As copper in its nano form displays better bioavailability than the native bulk form, Thiwari et al. conducted experiments to synthesize copper nanoparticles (CuNPs) and to evaluate the wound healing activity. In this study, CuNPs were prepared using *P. aeruginosa.* Synthesized CuNPs have exhibited better antimicrobial efficacy and a higher margin of safety on HaCaT normal cell line in comparison to native copper. Further treatment with CuNP-gel showed an increased rate of wound healing in rats compared to native copper (Tiwari et al. 2014). Similarly, Xiao et al. hypothesized that copper metal–organic framework nanoparticles can be modified for the slow release of Cu^{2+}ions and thereby to enhance the rate of wound healing. Folic acid was added during the synthesis of nanoparticles as a stabilizer and the resulting folic acid-modified copper metal–organic framework nanoparticles slowly released copper ions and promoted cell migration in vitro. Further, the synthesized nanoparticles were capable of promoting angiogenesis, deposition of collagen, re-epithelialization, and accelerating wound closure rates (Xiao et al. 2018). Sankar et al. have synthesized copper oxide nanoparticles (CuONPs) using *Ficus religiosa* leaf extract and evaluated antibacterial, and wound healing activity. Interestingly, CuONPs have inhibited the growth of some pathogenic bacterial strains that can delay the wound healing process (Fig. 5.2). It increased the rate of wound healing in Wistar Albino rats (Sankar et al. 2015).

Similarly, CuNPs were synthesized from aqueous extract of the leaves of *Falcaria vulgaris* and the antioxidant, antimicrobial, and wound healing properties of the nanoparticles were evaluated. Notable antioxidant and antimicrobial activities were observed while the nanoparticles did not affect the viability of the human umbilical vein endothelial cell line. The in vivo experiments revealed that the treatment with CuNPs ointment remarkably increased the cutaneous wound healing process in rats induced with cutaneous wounds (Zangeneh et al. 2019).

Recently, Ahmed et al. reported an ecofriendly approach for the synthesis of CeO_2 nanoparticles and the wound healing potential of the synthesized nanoparticles was evaluated after incorporating them into a chitosan hydrogel membrane as a wound dressing. Here, the synthesis of CeO_2 nanoparticles was mediated by *Abelmoschus esculentus* extract which functioned as a reducing and stabilizing agent. The green synthesized nanoparticles exhibited bactericidal effects against both Gram-positive and Gram-negative bacterial species while the in vivo studies revealed that these nanoparticles were highly effective in treating wounds. CeO_2 nanoparticles induced collagen deposition and enhanced the tensile strength of skin and thereby promoted wound healing (Ahmed et al. 2021).

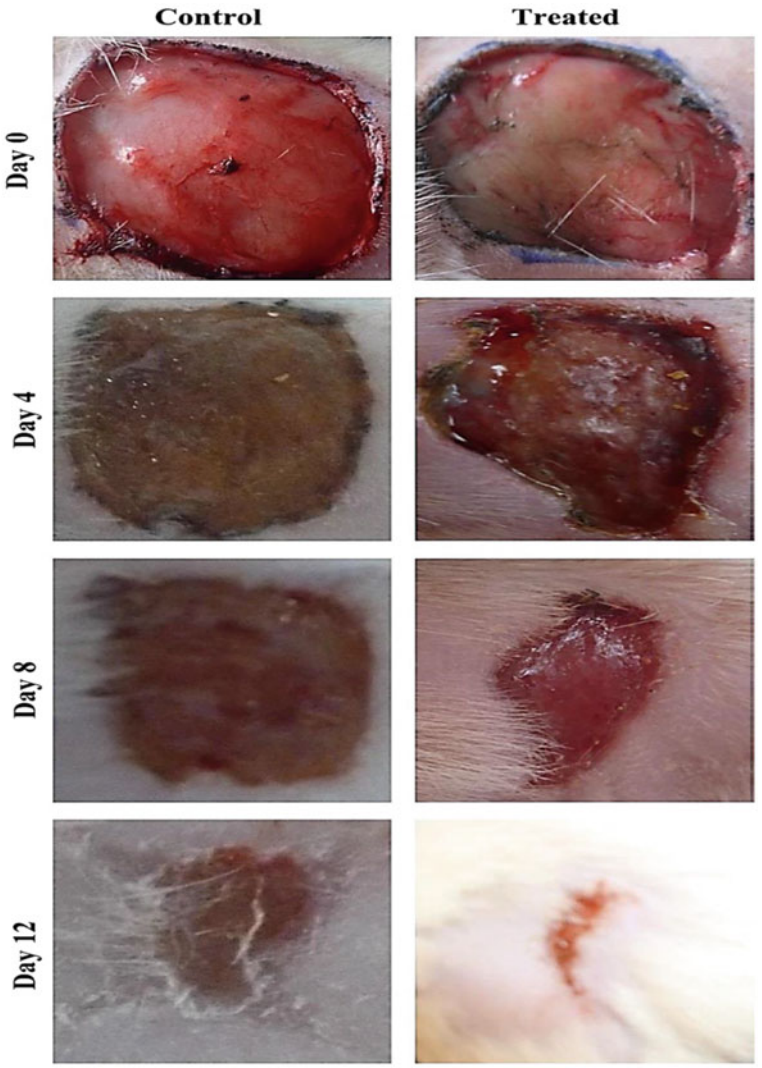

Fig. 5.2 Photographic illustration on different days of control and copper oxide nanoparticles treated animals (Adapted with permission from Sankar et al. 2015)

Different growth factors like epidermal growth factor (EGF), basic fibroblast growth factor, granulocyte–macrophage colony-stimulating factor, vascular endothelial growth factor (VEGF) are involved in the wound healing process. For example, EGF stimulates epithelial cell proliferation and the synthesis of the extracellular matrix while VEGF stimulates new vessel growth and increases vascular permeability. Similarly, basic fibroblast growth factors (bFGFs) promote fibroblast proliferation and neovascularization. Ribeiro et al. attempted to encapsulate VEGF and

EGF in chitosan microparticles which were then embedded inside a dextran-based hydrogel. The in vitro and in vivo assays revealed the applicability of the above system as a biocompatible approach to improve mechanical, chemical, and biological protection of the damaged skin and thereby to re-establish the skin architecture (Ribeiro et al. 2013). In an early study, Elçin et al. used calcium alginate microspheres as VEGF carriers and examined the in vivo wound healing potency on the Wistar rat model. The results indicated that VEGF-encapsulated alginate microspheres were capable of promoting vigorous angiogenesis, thus showing great promise in tissue engineering (Elçin et al. 2001).

AuNPs have recently emerged as gene delivery vectors. Wang et al. attempted to functionalize ultra-small gold nanoparticles (AuNPs@LL37) with the antimicrobial peptide LL37 which exerts various immunomodulatory functions. Thereafter, the suitability of this system for the topical treatment of diabetic wounds was investigated. Here AuNPs@LL37 system was combined with pro-angiogenic (VEGF) plasmids (AuNPs@LL37/pDNAs) and this has significantly improved the gene transfection efficiency in keratinocytes. It promoted angiogenesis and inhibited bacterial infection in diabetic wounds ultimately resulting in accelerated wound closure rates (Wang et al. 2018).

Lipid nanoparticles have been widely investigated over recent years as a safe and cost-effective drug delivery system. Moreover, these lipid nanoparticles also help to protect the drug from biological degradation. Solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid drug conjugates are some examples of lipid nanoparticles widely employed in pharmaceutical research (Attama et al. 2012).

Impaired wound healing and the development of non-healing diabetic foot ulcers are common amongst patients suffering from diabetes mellitus. The overproduction of tumour necrosis factor α (TNFα) causes further damage to the wound due to the up-regulation of cellular apoptosis, formation of reactive oxygen species, and degradation of the matrix. Therefore, Kasiewicz and Whitehead developed lipid nanoparticles (LNPs) loaded with siRNA-specific for TNFα. The topical application of the nanopreparation decreased TNFα mRNA expression in the wound in both diabetic and non-diabetic mice. Further, TNF α knockdown has increased the wound healing process in diabetic mice compared to untreated controls (Fig. 5.3). These findings demonstrated that LNP-based RNA interference therapy can decrease the severity and duration of chronic diabetic wounds (Kasiewicz and Whitehead 2018).

The topical administration of nanoparticle-based delivery systems is often facilitated by incorporating into either semi-solid hydrogels or solid scaffolds like fibrin-based biomaterials. Human recombinant EGF-loaded solid lipid nanoparticles (rhEGF-SLN) and human recombinant EGF-loaded nanostructured lipid carriers (rhEGF-NLC) were embedded in either semi-solid hydrogels or fibrin-based solid scaffolds and the performance of each wound dressing-the delivery system was investigated. The results indicated that both rhEGF-SLN and rhEGF-NLC nanocarrier systems were equally effective with respect to their in vitro performance. However, the long-term stability of fibrin-based scaffolds suggested that fibrin-based scaffolds

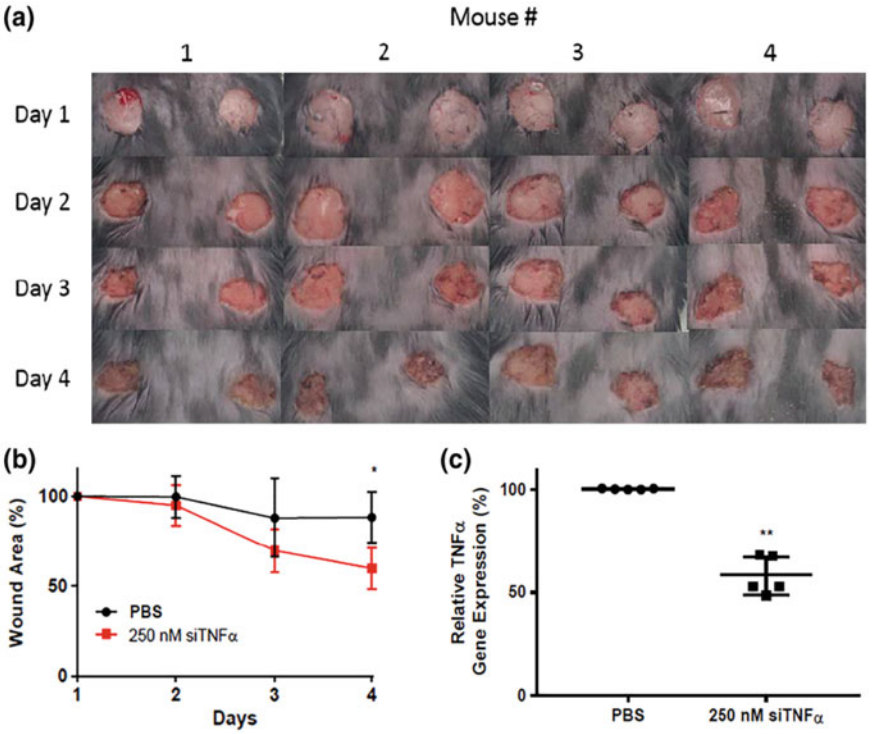

Fig. 5.3 LNP-mediated TNFα gene silencing accelerated wound healing in diabetic mice. **a** Mice were double-wounded on day 1 and treated on days 2 and 3 with either PBS (control, left wounds) or LNPs containing siTNFα (250 nM, right wounds). Mice were sacrificed on day 4. **b** The wound area is represented as a percent of its original size. By day 4, nanoparticle-treated wounds are statistically significantly smaller than PBS-treated wounds. **c** Wounds treated with lipid nanoparticles containing siRNA specific against TNFα (siTNFα) experienced a 43% reduction in TNFα expression compared to PBS-treated control wounds. In each panel, error bars represent s. **d** (n = 4–5, $*p < 0.05$, $**p < 0.01$) (Adapted with permission from Kasiewicz and Whitehead 2018)

might be the ideal approach to formulate rhEGF-loaded lipid nanoparticles (Gainza et al. 2015).

Saporito et al. attempted to load eucalyptus and rosemary essential oils into solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) prepared from natural lipids. Thereafter the antimicrobial activity of these lipid nanoparticles was determined against *S. aureus* and *Streptococcus pyogenes*. The in vitro proliferation enhancement, as well as wound healing potential on normal human dermal fibroblasts, was also tested while the rat burn model was used to determine the wound healing activity in vivo. The nanostructured lipid carriers made up of olive oil and loaded with eucalyptus oil demonstrated promising activity in both in vitro and in vivo assays. The synergic effect of eucalyptus oil with olive oil which is rich in oleic acid was proved to be responsible for antimicrobial activity and wound repair promotion

(Saporito et al. 2017). Similarly, Rosseto et al. developed solid lipid nanoparticles and nanostructured lipid carriers as topical propolis delivery systems for wound healing purposes. Good cell viability was observed in human immortalized keratinocytes treated with propolis and by-product extracts entrapped systems. Moreover, in vivo wound healing process was accelerated by SLN modified with propolis (SLN-2 and SLN-4) as depicted in Fig. 5.4 (Rosseto et al. 2017).

Various electrospinning nanofiber scaffolds have been evaluated as wound dressings by many researchers. The electrospinning method is a quick, cost-effective, and repeatable method that can be used to obtain nanomaterials formed by the deposition of polymer nanofibers. Natural polymers (e.g. collagen, gelatin, chitosan, silk fibroin), as well as synthetic biodegradable polymers like poly(vinyl alcohol) (PVA), polyacrylonitrile (PAN), poly(vinyl acetate) (PVAc), polyurethane (PU), polylactic acid (PLA), poly(ε-caprolactone) (PCL), poly(lactide-co-glycolide) (PLGA), are employed in the development of these nanofibrous mats. Electrospinning results in an interconnected three-dimensional network with features like the large surface area to volume ratio and high porosity that can facilitate wound homeostasis while also allowing gas and nutrient exchange. Further, loading drugs or other biomolecules into these fibres is also possible (Abrigo et al. 2014). Particularly, in the treatment of chronic non-healing wounds, much attention is given to dressings that are capable of preventing microbial infiltration while maintaining a balanced moisture and gas exchange environment. In this respect, electrospun wound dressings with the ability to simultaneously impair or treat infection and encourage cell proliferation/wound healing are receiving much attention (Abrigo et al. 2014; Coelho et al. 2018).

Gonis et al. also conducted experiments to develop bioactive wound dressing materials capable of promoting wound healing as well as being involved in the regeneration of healthy tissue. These experiments led to the development of poly (1,8 octanediol-co-citrate) and poly (acrylic acid) nanofibrous scaffolds. Antibacterial potential, hydrogel-like water uptake capability, and the capacity to deliver growth factor at physiologically relevant concentrations are some important features of this

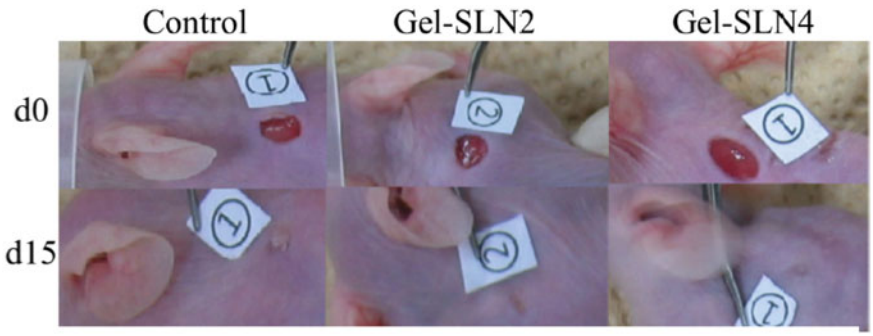

Fig. 5.4 Results of wound closure test—representative photographs showing the wounds and the different treatments in SKH1-mice after treatment (d0) and 15 days after (d15) (Adapted with permission from Rosseto et al. 2017)

nanofibrous scaffold. Further, it demonstrated low toxicity and cellular adhesion while enhancing the proliferation of skin fibroblasts (Gonis et al. 2017).

Fibrin is a coagulation protein that plays an important role in the wound healing process. It is derived from its precursor fibrinogen, a soluble protein synthesized by the liver and found in blood plasma. Fibrin is often utilized as a scaffold for wound healing and also as a drug carrier. For example, Alphonsa et al. developed ciprofloxacin-loaded and fluconazole-loaded fibrin nanoparticles in order to achieve sustained delivery of encapsulated antimicrobial drugs to treat microbial infected wounds. A good antimicrobial activity was observed in the above drug-loaded systems while maintaining adequate cell viability of human dermal fibroblast cell lines (Alphonsa et al. 2014). Similarly, Losi et al. developed a fibrin-coated poly(ether)urethane-polydimethylsiloxane scaffold containing poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with recombinant human VEGF and bFGF (Losi et al. 2013).

Nanofibrous scaffolds fabricated with natural polymers such as collagen in the presence of bioactive metabolites have great promise in tissue engineering. Kandhasamy et al. conducted experiments to develop collagen-coated ostholamide (OSA) electrospun nanofiber scaffold and thereafter to evaluate the wound healing potential. Ostholamide (OSA) was derivatised from osthole, a natural coumarin with antimicrobial, anti-inflammatory, and antioxidant activities. Here, OSA was added to polyhydroxybutyrate (PHB) and gelatin (GEL), which were used as templates for electrospun nanofibers. Thereafter, the electrospun PHB-GEL-OSA scaffolds were coated with collagen and this resulted in PHB-GEL-OSA-COL nanofibrous scaffold that mimics the extracellular matrix (Fig. 5.5).

The PHB-GEL-OSA-COL nanofibers have displayed exceptional mechanical stability, which is important in wound healing. Moreover, the presence of OSA provided antibacterial properties to this nanofiber scaffold. Further, the nanofibrous scaffold was able to release OSA in a controlled manner and it was found to be stable against enzymatic degradation, a property that is important for the repair and remodelling of tissues during wound healing. The in vivo experiments conducted using Wistar rats revealed enhanced wound healing in rats treated with PHB-GEL-OSA-COL nanofibrous scaffold in comparison to untreated rats (Fig. 5.6) (Kandhasamy et al. 2017).

Hyaluronic acid (HA) is a component of the extracellular matrix involved in the wound healing process. It is widely used as a hydrogel scaffold for wound healing applications. Pereira et al. developed polymeric films containing hyaluronate, sodium alginate, and polyvinyl alcohol (PVA) and loaded with *Aloe vera* extract and vitamin E. Although this formulation was found to be more effective than the conventional vitamin E cream, it showed a burst release of the vitamin and was not occlusive (Pereira et al. 2014). Therefore, a new form of hyaluronic acid-coated lipid nanoparticles was formulated for topical administration on burns. Here, vitamin E-loaded nanoparticles based on hyaluronate and lecithin were developed with the use of the cationic lipid dioctadecyldimethylammonium bromide (DODMA). The prepared nanoparticles were subsequently embedded in a polymeric film containing *Aloe vera* extracts. Interestingly, a slow release of the vitamin was observed along with more

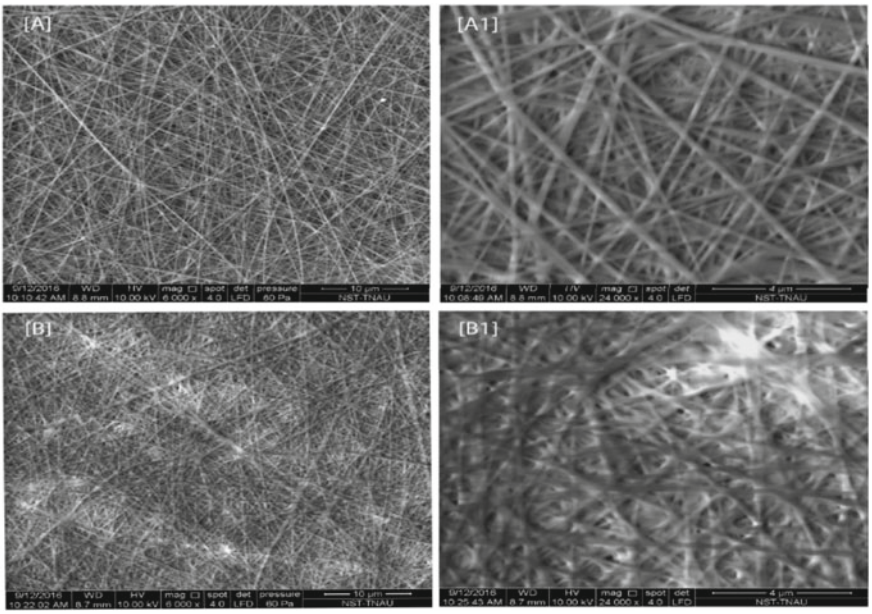

Fig. 5.5 SEM micrographs of nanofibers; **a** and **a1** PHB-GEL-OSA (overlay view at 6000X and 24000X) and **b** and **b1** PHB-GEL-OSA-COL (Collagen coated PHB-GEL-OSA fibres at 6000X and 24000X magnification) scaffold (Adapted with permission from Kandhasamy et al. 2017)

efficient occlusion properties in comparison to the formulation containing the vitamin but in the absence of nanoparticles. Further, this nanoparticle-loaded polymer film reduced the water loss through damaged skin while protecting the vitamin against degradation from light and oxygen (Pereira et al. 2016).

Recently Buntum et al. formulated semi-solid poly(vinyl alcohol) (PVA) hydrogels containing essential oil-loaded chitosan nanoparticles for wound dressing applications. Essential oils of clove and turmeric were used in this study. The essential oil-loaded chitosan nanoparticles in semi-solid PVA hydrogels helped to sustain and prolong the release rate of essential oil from the hydrogels. Moreover, the investigated semi-solid PVA hydrogels were found to be non-toxic to the cells as those were able to retain the viability of NCTC clone 929 and NHDF cells above 70% (Buntum et al. 2021).

Oxidative stress adversely affects tissue repair and regeneration, thus the removal of excessive ROS may result in an accelerated healing process. In this respect, antioxidant biomolecules play a significant role with their ability to neutralize free radicals. Therefore, Kandhasamy et al. designed a quinone-based chromenopyrazole (QCP) antioxidant-laden silk fibroin nanofiber scaffold (QCP-SF) for skin tissue engineering applications. For this, 3-methyl-4-(4-oxo4H-chromen-3-yl)-1-phenylbenzo[6,7]chromeno[2,3-c]pyrazole-5,10 (1H,4H)-dione (QCP) derivatives inherited with potent antioxidant activity were synthesized and incorporated into

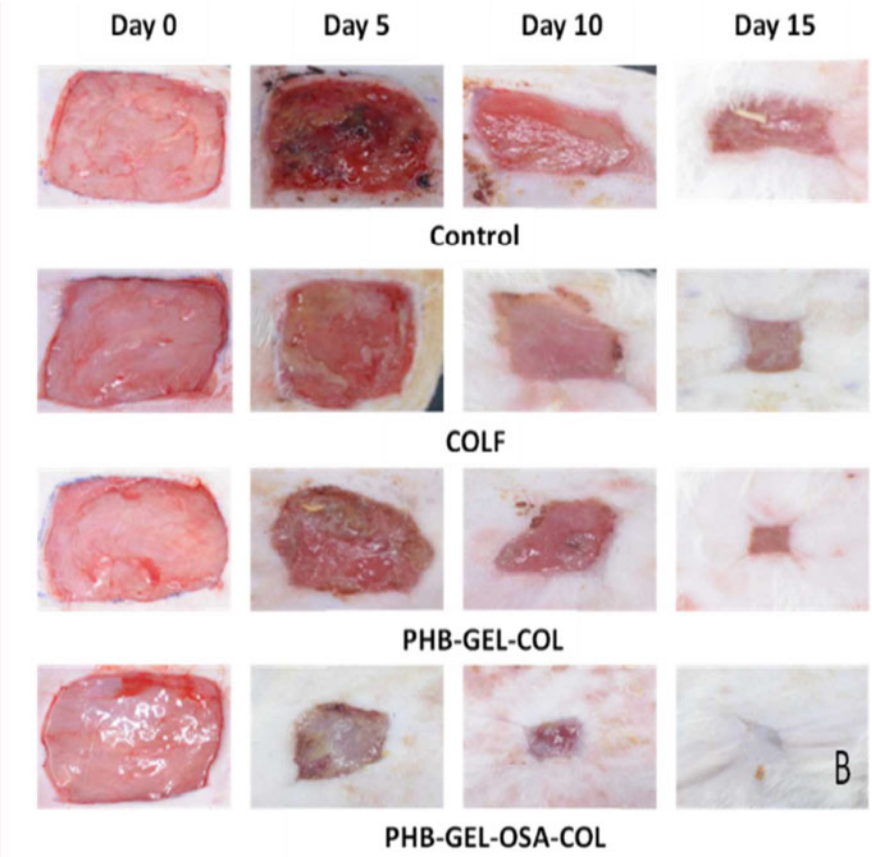

Fig. 5.6 Photographs showing wound closure efficiency in the control (untreated wound), collagen scaffold (COLF), PHB-GEL-COL fibres, and PHB-GEL-OSA-COL electrospun nanofibrous scaffold (Adapted with permission from Kandhasamy et al. 2017)

silk fibroin nanofiber scaffold. Silk fibroin (SF) is a natural protein derived from cocoons of silkworm *Bombyx mori* and it has various tissue engineering applications mainly due to its ability to enhance re-epithelization, adhesion, and massive proliferation of keratinocytes, fibroblasts, and endothelial cells. Upon the incorporation of QCP, the nanofiber scaffold displayed an increased cell attachment and proliferation in NIH 3T3 fibroblasts and rat bone marrow stem cells (rBMSCs). Moreover, QCP-SF scaffold displayed a rapid cell migration in the scratch assay and hence an excellent wound healing activity (Kandhasamy et al. 2019).

Vitamin E-loaded silk fibroin nanofibrous scaffolds have also been developed for wound healing applications. Vitamin E (α-tocopherol) functions as an antioxidant as well as a stabilizing agent in biological membranes. The water-soluble derivative of vitamin E, *RRR*-α-tocopherol polyethylene glycol 1000 succinate (VE TPGS) was

loaded into silk fibroin nanofibrous mat and the wound healing activity was evaluated by Sheng et al. Sustained release behaviour of VE TPGS from the nanofibrous mat was observed in the in vitro study. Moreover, this novel scaffold encouraged the proliferation of skin fibroblasts and enhanced the survival of the cells against oxidative stress (Sheng et al. 2013). In another study, vitamin E-loaded starch nanoparticles were incorporated into silk fibroin/poly(vinyl alcohol)/*Aloe vera* nanofibers system prepared by the electrospinning method. The incorporation of *Aloe vera* and vitamin E silk fibroin/poly(vinyl alcohol) nanomatrix enhanced the fibroblast attachment, proliferation, and collagen secretion while the presence of vitamin E has exerted an antioxidant effect facilitating the wound healing process (Kheradvar et al. 2018).

In another study, soy protein isolate/silk fibroin (SPI/SF) nanofibrous scaffolds were developed and the wound healing potential was evaluated. These scaffolds were found to be non-toxic to normal mammalian cells and were capable of healing full-thickness wounds in rats within 14 days (Varshney et al. 2020). Similarly, nanofibrous scaffolds comprised of gum tragacanth/poly (ε-caprolactone) have also been developed for tissue engineering/wound dressing applications. Gum tragacanth is a mixture of water-soluble polysaccharides of plant origin reported with wound healing potency. Poly (ε-caprolactone) is an aliphatic polyester with good mechanical properties which is widely employed in biomedical applications due to its biocompatibility, slow biodegradability, and non-toxicity. The fabricated scaffold was capable of enhancing fibroblast adhesion and proliferation and also displayed antibacterial activity against Gram-positive and Gram-negative bacterial species (Ranjbar-Mohammadi and Bahrami 2015).

Rathinavel et al. fabricated a nanoscaffold comprised of functionalized SBA-15 (Santa Barbara Amorphous) polycaprolactone (PCL) and curcumin for wound healing applications. The high biocompatibility and cell adhesion property of amine-functionalized SBA-15 as well as antimicrobial potential in curcumin have made this nanoscaffold an effective wound healing therapeutic. The in vivo experiments with Wister rats indicated that the treatment with this nanoscaffold has led to re-epithelization, collagen deposition, and formation of granulation tissue resulting in 99% scar-less wound healing within 21 days (Rathinavel et al. 2021).

The combination of different nanotechnological approaches can result in the development of more sophisticated and improved wound healing materials. For example, Chen et al. synthesized a novel wound dressing with the use of AgNP-loaded Konjac Glucomannan composite sponges (KGM/AgNP). In this approach, AgNPs were prepared with egg whites and then KGM powder was added while vigorously stirring. Thereafter this mixture was freeze-dried to obtain a nanocomposite sponge. This sponge possessed properties like antibacterial activity against *E. coli* and *S. aureus*, good cytocompatibility, higher capacity of water absorption and retention as well as significant mechanical properties. In addition, animal experiments demonstrated that the fabricated nanocomposite was capable of enhancing wound healing and promoting fibroblast growth and epithelialization (Chen et al. 2018).

Furthermore, Ding et al. prepared a chemically cross-linked spongy bilayer composite as a wound dressing. The upper layer was formed from chitosan-Ag

nanoparticles cross-linked with genipin. Genipin is a compound isolated from the fruit of *Gardenia jasminoides* and it is being widely used to cross-link the free amino groups on chitosan. The lower layer of the bilayer composite was made of chitosan cross-linked with genipin and partially oxidized *Bletilla striata* polysaccharide. *B. striata* is a plant employed in traditional Chinese medicine and several in vitro assays have reported that polysaccharides obtained from this plant can enhance the proliferation of human vascular endothelial cells and the expression of vascular endothelial growth factor. The newly developed wound dressing material exhibited good water retention, mechanical strength, L929 cell proliferation, and antimicrobial activity. The in vivo studies revealed that the new wound dressing material has accelerated the healing rate of cutaneous wounds in mice (Ding et al. 2017).

Similarly, Yang et al. employed some of the structural components of β-lactam antibiotics; i.e. 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA), and 7-amino-desacetoxy-cephalosporanic acid (7-ADCA) to modify the surfaces of AuNPs. Thereafter these modified AuNPs were incorporated into electrospun poly(ε-caprolactone) (PCL)/gelatin fibres to fabricate a wound dressing material. The antibacterial activity studies indicated that APA-coated AuNPS were more effective than AuNPs coated with 7-ACA and 7-ADCA to inhibit the growth of *P. aeruginosa* and *Klebsiella pneumonia* as well as clinically isolated MDR (multidrug resistant) strains. Furthermore, the in vivo bacteria-infected wound healing study showed that it has a remarkable ability to treat MDR bacteria-infected wounds (Yang et al. 2017).

Thanusha et al. developed a hydrogel composed of the triterpenoid asiatic acid, zinc oxide nanoparticles, and copper oxide nanoparticles which were incorporated into the gelatin and glycosaminoglycans (hyaluronic acid and chondroitin sulphate). Thereafter the efficacy of this hydrogel composite in healing second-degree burn wounds was determined using Wistar rats. The presence of either ZnONPs or CuONPs in the hydrogel composite resulted in a higher antibacterial activity compared to the hydrogel composite scaffold. Moreover, a significant wound healing activity was seen in Wistar rats treated with hydrogel composite in comparison to the control (NeuSkin™ and cotton gauze). The histopathology study demonstrated that re-epithelialization, arrangement of collagen fibres, and angiogenesis were facilitated by the above treatment (Fig. 5.7). Further, a decrease in TNF-α and an increase in MMP-2 expression were observed with the treatment and supposed to be contributed to the healing process (Thanusha et al. 2018).

The wound healing potential of phytochemical-decorated AuNPs was evaluated by Lee et al. The skin regeneration study revealed that the treatment of gallic acid-isoflavone-covered AuNPs/protocatechuic acid-isoflavone-covered AuNPs on the dorsal skin of rats has resulted in a thicker epidermis, a decrease in the metalloproteinase-1 level and an increased activity in superoxide dismutase. Moreover, phytochemical-decorated AuNPs treatment on surgical and burn wounds in Sprague–Dawley (SD) rats has improved the expression of VEGF and angiopoietin-2 and induced the proliferation of granulation tissue and keratinocytes. Further, the study revealed that nanoparticles exert an anti-inflammatory function during the wounding healing process while gallic acid-isoflavone-covered AuNPs can activate

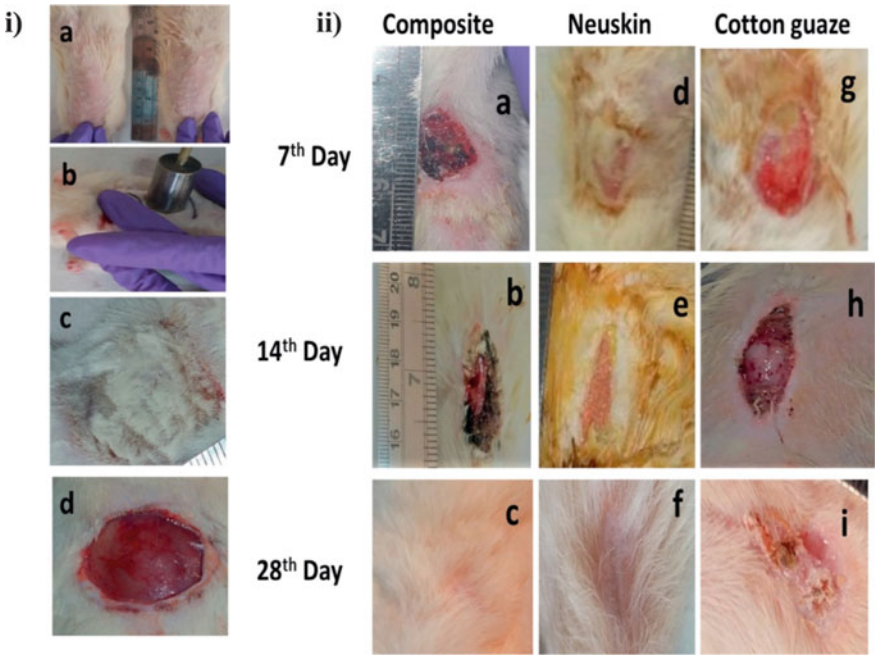

Fig. 5.7 Photographic images of (i) procedure followed for applying second-degree burn wound: **a** removal of hairs, **b** applying burn punch (2 cm^2) of 95 °C for 20 s, **c** formation of burn, **d** after 24 h, removed epidermal burnt position-0th day. (ii) **a–c** hydrogel composite applied wound on 7d, 14d and 28d, **d–f** NeuSkinTM applied wound on 7d, 14d, and 28d, **g–i** Cotton gauze (Control) applied wound on 7d, 14d, and 28 day (Adapted with permission from Thanusha et al. 2018)

the transforming growth factor β (TGF-β) function for skin regeneration (Lee et al. 2015).

Tissue-engineered skin substitutes are emerging as an effective therapeutic option to treat patients with skin damage caused by congenital defects, burns, etc. Although collagen-based dermal substitutes (e.g. Dermagraft, Apligraf) are used as skin substitutes, the issues like antigenicity could lead to unsatisfactory outcomes. As a result, several synthetic or natural polymers have been investigated and silk fibroin has shown great promise as an alternative material for the construction of dermal substitutes (Park et al. 2016). Although nanofibers fabricated by electrospinning silk fibroin possess desirable biocompatibility, the small pore size makes it difficult for cells to infiltrate these electrospun silk fibroin. Therefore, Lee et al. developed a method using NaCl crystals to control the pore size of the electrospun silk fibroin and thereby allowing cells to easily infiltrate into the nanofibers and proliferate internally. The newly developed three-dimensional electrospun silk fibroin nanofiber did not show any cytotoxicity while displaying increased cell infiltration in NIH 3T3 fibroblast cells. The in vivo experiments revealed that it could accelerate re-epithelialization and wound closure and was equally effective as Matriderm. However, it almost

completely degraded without inducing wound contracture like with Matriderm. Therefore, this electrospun silk fibroin nanofiber displayed a high potential to be used in human patients with full-thickness skin defects (Lee et al. 2014). In the follow-up study, a skin substitute was developed by using the 3-D electrospun SF nanofiber scaffold. The high interconnectivity between pores and the porosity as well as the water uptake abilities have facilitated cell infiltration and proliferation leading to increased infiltration of fibroblasts and the stratification of keratinocytes. These observations further confirmed the suitability of the 3-D electrospun silk fibroin nanofiber scaffold for skin tissue engineering (Park et al. 2016).

An important concern during tissue engineering is to mimic the fibrous structure of the extracellular matrix. The development of scaffold materials with good biodegradability, surface properties, mechanical strength as well as controlled drug release potential is important in tissue engineering. Li et al. fabricated core-sheath nanofibers comprised of poly (ε-caprolactone) and silk fibroin blends. This scaffold resulted in an improved cell attachment and proliferation and sustained drug release (Li et al. 2011). In another study, the natural antioxidant fenugreek was incorporated into silk fibroin nanofibers and the wound healing potential of this nanofiber scaffold was evaluated using full-thickness excisional wounds in the rat model. The developed material has displayed excellent mechanical, thermal, and antioxidant properties and resulted in complete re-epithelialization and enhanced collagen deposition accelerating the wound healing process (Selvaraj and Fathima 2017).

Bioactive glasses (BGs) are normally employed for hard tissue regeneration. However, these can also be used to prepare wound dressings because of their ability to stimulate rapid haemostasis, fibroblasts proliferation, and angiogenesis. Particularly the nano-scale bioactive glasses were found to be more effective than conventional bioactive glasses due to the larger specific surface area. Wang et al. developed an easy-to-use hydrogel by mixing bioactive glasses with gelatin. This hydrogel caused a rapid cutaneous-tissue regeneration and tissue-structure formation in rats (Wang et al. 2017).

Based on the aforementioned examples, it is clear that nanomaterials have a great potential to be employed as wound healing therapeutics and tissue engineering scaffolds in near future.

5.4 Conclusion

Skin is the largest external organ in the human body and it can be damaged due to various reasons. Therefore, wounds may occur on the skin very often. Wound healing is a complex process and it may get delayed due to several factors. Nowadays traditional wound dressings have been replaced by more sophisticated modern wound dressings. Nanomaterials are playing an important role in the development of highly effective wound dressings. Tissue engineering scaffolds nanoparticles, nanofibers, as well as nanocomposites, appear as excellent wound healing agents and tissue engineering materials due to the intrinsic antimicrobial activity, cytocompatibility,

low toxicity, etc. Hence, nanomaterials have an inevitable future as wound dresses and skin substitutes.

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Chapter 6
Applications of Nanotechnology in Dermatology

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Abstract Nanodermatology is an exponentially growing area and this chapter provides a general overview of the potential applications of nanotechnology in dermatology. In order to contextualize this topic, different examples of nanosystems are presented along with a brief discussion on the anatomy and physiology of the skin. Then, the significance of nanomaterials as a diagnostic tool is highlighted with several examples while the role of nanotechnology in effective, targeted, and efficient drug delivery is presented in detail. In addition, the recent and anticipated advances in nanotechnology for cosmeceuticals are also discussed. Thus the chapter covers a vast array of applications of nanomaterials in skin care. Although nanodermatology has an inevitable future, the possible toxic effects and the related safety concerns could not be neglected. Thus special emphasis is given to discuss current controversial views on cytotoxicity and carcinogenicity associated with nanomaterials.

Keywords Nanocarriers · Nanocosmeceuticals · Nanodermatology · Skincare · Toxicity

6.1 An Introduction to Nanodermatology

Nanotechnology is a multifaceted field that involves the design, synthesis/production, characterization, and application of structures, devices, and systems where the shape and size are controlled at the nanometre scale. These nanometre-sized objects possess remarkable self-ordering and assembly behaviours which are rather different from the

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macro objects and also with an increased surface area to volume ratio (Emerich and Thanos 2003). In consequence, nanoparticles exhibit many novel physiochemical, electronic, optical, mechanical, catalytic, and thermal properties that are not present in the bulk form (Misra et al. 2008; DeLouise 2012). These unique properties of the nanomaterials have been harnessed by the scientific community over the past few decades and it has led to rapid development in many scientific areas from engineering to biomedical sciences (Hulla et al. 2015).

Nanotechnology has proven an enormous promise due to its broad spectrum of applications in diagnostic, therapeutic, and preventive medicine. Over recent years, different types of nanomaterials (Table 6.1) such as liposomes, nanocapsules, solid lipid nanoparticles, dendrimers, cubosomes, fullerenes, nanocrystals, and niosomes have been widely employed in the field of medicine. Typically, nanoparticles have been categorized on the basis of shape, size, and structure as well as their physical and chemical properties.

Amongst the different branches of nanomedicine, one of the exponentially growing areas is nanodermatology, which has revolutionized the diagnosis and treatment of several skin diseases. Moreover, nanomaterials have influenced the field of cosmeceuticals with the unique and enhanced properties conquered by the particles at the nano level, compared to the bulk materials. Different innovations in nanodermatology are outlined in Table 6.2 and will be discussed in detail in this chapter.

To get a better understanding of the impact of nanomaterials in dermatology and cosmetics, a brief overview of the structure of the human skin is given below.

6.2 Skin, the Outer Covering of the Body

The skin is the largest organ in the human body and functions as a barrier against pathogenic microorganisms, parasites, viruses, heat, and UV radiation. From the outside to the inside, the skin comprises three major structural layers, namely, epidermis, dermis, and hypodermis. The epidermis is the outermost layer of the skin which encompasses five sub-layers or strata; stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. The major cell type found in the epidermis is known as keratinocytes while melanocytes (pigment cells) and Langerhans cells (immune cells) are also distributed within the epidermis. The dermis lies beneath the epidermis and consists of dense irregular connective tissues. It also contains mechanoreceptors, thermoreceptors, hair follicles, sebaceous glands, sweat glands, lymphatic and blood vessels. The most underneath layer is the hypodermis (subcutis) and is consisted of a network of collagen fibres and adipocytes (fat cells).

The skin is susceptible to a variety of diseases, disorders, and injuries ranging from relatively benign microbial infections to more fatal conditions like skin cancers. Skin diseases appeared as one of the most common human illnesses and pervade all cultures, occur at all ages, and affect between 30 and 70% of individuals, with even

Table 6.1 Different types of nanomaterials

Type of nanomaterial	Characteristic feature
Liposome	Concentric bilayered vesicles in which the aqueous volume is entirely enclosed by a lipid bilayer composed of natural or synthetic phospholipids (Raj et al. 2012)
Solid lipid nanoparticles	Prepared from solid lipids dispersed in water or in an aqueous surfactant solution (Ekambaram et al. 2012)
Nanocapsules	Consist of a liquid/solid core in which the drug is placed into a cavity, which is surrounded by a natural or synthetic polymer membrane (Kothamasu et al. 2012)
Cubosomes	Discrete, sub-micron, nanostructured particles of bi-continuous cubic liquid crystalline phase. It is formed by the self-assembly of liquid crystalline particles of certain surfactants when mixed with water and a microstructure at a certain ratio (Spicer et al. 2003)
Niosomes	Non-ionic surfactant-based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulting from the organization of surfactant macromolecules as a bilayer (Rajera et al. 2011)
Buckminsterfullerenes/buckyballs	60 carbon atoms formed in the shape of a hollow ball (Nagda et al. 2010)
Dendrimer	Unimolecular, monodisperse, micellar nanostructures, with a well-defined, regularly branched symmetrical structure and a high density of functional end groups at their periphery (Raj et al. 2012)
Carbon nanotubes	Allotropes of carbon, made of graphite, have been constructed in cylindrical tubes with nanometre scale in diameter and several millimetres in length (He et al. 2013)
Metal and metal oxide nanoparticles	Formed from inorganic materials, such as gold, silver, zinc, platinum, silica, etc.
Nanocrystals	Aggregates comprising several hundred to tens of thousands of atoms that combine into a cluster (Raj et al. 2012)
Quantum dots	Nanocrystalline semiconductor material made from cadmium selenide with exclusive spectroscopic and optical properties (Hegazy and Abd El-Hameed 2014)

higher rates in at-risk subpopulations (Hay et al. 2014). As the outermost barrier of the body, the skin is constantly being challenged by microbial invaders, including the commensal microorganisms that comprise the normal flora of the skin as well as the pathogenic microorganisms responsible for infections (Brodell and Rosenthal 2008). Similarly, the skin is constantly exposed to solar ultraviolet (UV) radiation as being the largest organ and the external boundary of the body. The exposure of the skin to ultraviolet radiation induces the formation of reactive oxygen species, which in turn

Table 6.2 Potential applications of nanodermatology

Application	Examples
Diagnostic application	Quantum dots and gold nanoparticles in the diagnosis of melanoma, carbon nanotubes for diagnosis of skin infections (Kim et al. 2010; Zheng et al. 2010; Zelada-Guillén et al. 2012)
Transdermal drug delivery	Corticosteroids in nanolipid carrier system to treat psoriasis and atopic dermatitis (Doktorovova et al. 2010)
Wound healing	Curcumin-ampicillin solid lipid nanoparticles, topically applied NO-releasing silane hydrogel-based nanoparticles (Martinez et al. 2009; Alihosseini et al. 2016)
Treatment of skin cancers	Doxorubicin-filled cationic solid lipid nanoparticles, camptothecin-loaded polyvinyl alcohol-functionalized multi-walled nanotubes, and gold nanoparticles (Sahoo et al. 2011; Choi et al. 2015; Huber et al. 2015)
Inflammatory skin diseases	Topical semi-solid formulations of poly(lactic acid) (PLA) nanoparticles as anti-inflammatory drug vehicles for local treatment of inflammatory skin diseases (Boisgard et al. 2017)
Treatment of acne	Azelaic acid-loaded poly-dl-lactide/glycolide copolymer nanoparticles (Reis et al. 2013)
Treatment of diseases in hair	Minoxidil encapsulated in neutral liposomes and finasteride carried in liposomes to treat alopecia (Kumar et al. 2007; Jain et al. 2010)
Sunscreens	TiO_2 and ZnO nanoparticles in sunscreens (Lu et al. 2015)
Moisturizers, anti-ageing creams and other cosmeceuticals	Solid lipid nanoparticle-based topical formulations of N-6-furfuryl adenine against ultraviolet radiation-induced skin photodamage, solid lipid nanoparticle containing retinyl palmitate with anti-wrinkle effects (Jeon et al. 2013; Goindi et al. 2015)
Antiseptic agents	Silver and other metal nanoparticles against microbial infections (Besinis et al. 2013)

causes an imbalance between pro-oxidants and antioxidants, generating oxidative stress. This is usually associated with inflammation, immunosuppression, impaired wound healing, DNA damage, and activation of signalling pathways that affect gene transcription, cell cycle, proliferation, and apoptosis. These alterations ultimately promote carcinogenesis (Amaro-Ortiz et al. 2014; Dunaway et al. 2018). Moreover, the depletion or disturbance of lipids in the stratum corneum leads to a perturbation of the barrier function of the skin resulting in psoriasis and atopic dermatitis (Sahle et al. 2015). The findings of the Global Burden of Disease 2010 study revealed the importance of dermatology on a regional and global level and indicated that the burden of skin conditions is high in both high- and low-income countries (Hollestein and Nijsten 2014). The Global Burden of Disease Study 2013 further confirmed

that skin diseases remain a major cause of disability worldwide (Karimkhani et al. 2017). Acne vulgaris, urticaria, eczema, and pruritus have been identified as some of the common skin conditions that cause a huge burden in the global context of health. Therefore, over recent years increased attention has been paid to different strategies in the diagnosis and treatment as well as the prevention of skin diseases. In this respect, nano-designed materials and devices have proved to be more efficient, effective, and versatile than their traditional counterparts. Thus nanodermatology is an inevitable tool to reduce the burden of skin diseases and thereby to improve the quality of life of people.

6.3 Nanoparticles as Diagnostic Tools

6.3.1 Quantum Dots in the Detection of Skin Cancers

Skin cancer is considered the most common malignant disease particularly seen amongst Caucasians and each year more than a million new cases are being reported worldwide. Skin cancer can be classified based on the cells it originates from and its clinical behaviour. The most common types are basal cell carcinoma, squamous cell carcinoma (both are nonmelanocytic skin cancers), and malignant melanoma (D'Orazio et al. 2013; Simões et al. 2015; Orthaber et al. 2017).

Out of these, nonmelanocytic skin cancers are the most common malignancy found in humans whereas melanoma accounts for about 75% of skin cancer deaths worldwide (Goldstein and Tucker 1993; Rigel and Carucci 2000). Melanoma usually originates in melanocytes, however, it can also begin in other pigmented tissues like in the eye or the intestine. Malignant melanoma can be cured by surgical resection at its early stages, but once it has progressed to the metastatic stage, the treatment would be extremely difficult and usually, it is unresponsive to current therapies (Gray-Schopfer et al. 2007). The dermatological examination, dermoscopy, and surgical biopsy with pathohistological biopsy are conventional approaches involved in the diagnosis of skin cancers, however, extensive research has been conducted to develop more efficient and high-throughput methods for early prognosis and effective therapy of melanoma and other skin cancers.

One approach for the early detection of melanoma is based on quantum dots. Quantum dots (QDs) are engineered fluorescent nanoparticles with unique optical and chemical properties (Fang et al. 2017). Upon excitation by a suitable light source, QDs emit fluorescence from visible to infrared wavelengths, and in comparison to a conventional fluorophore, optical properties such as high brightness, resistance to photo-bleaching, and tunable wavelength are superior in QDs. Cadmium/selenide (Cd/Se) QDs are amongst the most widely investigated QDs. These are typically prepared with a Cd/Se core, a ZnS shell and, an outer surfactant layer of either trioctylphosphine oxide (TOPO) or octyldecylamine (ODA) molecules (Zheng et al.

2010). Owing to the high specificity and sensitivity, the quantum dots immunofluorescent label has been widely employed as a promising approach for biomedical imaging in cancer cells. Since human melanoma cell adhesion molecule CD146 is overexpressed on the surface of melanoma cells, it has been utilized as an important target for melanoma diagnostics by Zheng and co-workers. CD146 is a transmembrane glycoprotein that is comprised of five extracellular immunoglobulin (Ig) superfamily-related domains, a transmembrane region, and a short cytoplasmic region. CD146 is expressed on advanced and metastatic melanoma cells, however, it is not expressed on normal melanocytes. In that approach, PEG–COOH capped highly fluorescent CdSe/ZnS QDs were conjugated with streptavidin to prepare QD–SA label. Then, this QD–SA was used to link with biotinylated goat antimouse IgG and mouse anti-human CD146 to label CD146 overexpressed on live and fixed cells by fluorescence-activated cell sorting (FACS) and confocal microscopy. Labelling of target cells was shown to have high brightness, photostability, and specificity and the results demonstrated the suitability of the construction based on QD–SA label, biotinylated IgG and CD146 antibody for detection of melanoma cells (Zheng et al. 2010).

Kim et al. experimented with antibody-conjugated quantum dot nanoprobe in a co-culture system that could mimic an in vivo-like tumour microenvironment in which cancer cells grow with normal cells. A melanoma–melanocyte co-culture model was employed in this study to assess the specific binding of QDs conjugated with melanoma antibodies. The images were analysed with automated confocal microscopy and it revealed that conjugation of antibodies to the QD has significantly improved the melanoma specificity, while unconjugated antibody alone suffered from non-specific binding to melanocytes. Thus this study has demonstrated a rapid and sensitive cancer cell detection system that could be employed in the development of high-throughput screening platforms for early cancer diagnosis and anti-cancer therapeutics (Kim et al. 2012).

Although QDs are appearing as an advanced version of classical fluorescence-based imaging approaches, the major disadvantage of using QDs in biological systems is their toxicity associated with the formation of reactive oxygen species (ROS) and the release of heavy metal ions such as Cd^{+2} from the core of QDs (Diaz-Garcia et al. 2018). In order to address this issue a new generation of cadmium-free QDs has been developed, without affecting the spectroscopic advantages characteristic of QDs, but lacking detrimental effects linked to the presence of cadmium (Xu et al. 2016). With these advancements, it could be hypothesized that QDs would replace the conventional diagnosis methods for skin cancer, in years to come.

6.3.2 Gold Nanoparticles for the Diagnosis of Melanoma

To improve the treatment outcomes and thereby the survival of patients, factors like early diagnosis, accurate staging, and image-guided resection of melanomas play a crucial contribution. The low sensitivity and specificity as well as the poor spatial resolution, shallow penetration, and/or ionizing radiation inherited to the

conventional techniques limit the achievability of those main objectives. As a solution, the combination of high-resolution photoacoustic tomography (PAT) with gold nanocages (AuNCs) was introduced (Kim et al. 2010). The high electron density of gold nanoparticles (AuNP) makes them ideal candidates for bioimaging and these have been applied to assess different bio-specific molecular interactions (Bagheri et al. 2018). Kim et al. proved that AuNCs can serve as a novel contrast agent for in vivo molecular PAT of melanomas with exquisite sensitivity and high specificity, once those are bioconjugated with [Nle^4,D-Phe^7]-α-melanocyte-stimulating hormone (Fig. 6.1). Interestingly, these bioconjugated AuNCs enhanced contrast ~300% more than PEGylated AuNC which was employed as the control (Kim et al. 2010).

Similarly, tagging melanoma cells with AuNP demonstrated a 34% increase in photoacoustic signal in comparison to the unmodified melanoma cells and this offers greater sensitivity in stationary metastasized melanoma detection systems using photoacoustics (McCormack et al. 2011).

6.3.3 Carbon Nanotubes for the Diagnosis of Skin Infections

The current diagnosis of infectious diseases caused by microorganisms, viruses, and parasites is based on microscopic analysis, culturing, immunoassays, and polymerase chain reaction (PCR). However, these diagnostic protocols are with some shortcomings, for example, microscopy lacks sensitivity in many clinical scenarios and culturing is highly time-consuming. Although highly sensitive, immunoassays such as ELISA are labour intensive and challenging to implement multiplex detection. PCR offers molecular specificity but it involves complex sample preparation and the potential for false-positive is high (Sin et al. 2014). Thus there are continuous efforts on the development of more sensitive and rapid diagnostic techniques for infectious diseases and as a result, biosensors came into the picture.

A biosensor is an analytical device that converts molecular recognition of a target analyte into a measurable signal via a transducer (Sin et al. 2014). The physical, chemical, electrical, and optical characteristics of carbon nanotubes (CNTs) enable them to serve as one of the best-suited materials for the transduction of signals associated with the recognition of analytes, metabolites, or disease biomarkers (Tîlmaciu et al. 2015). For example, a network of single-walled carbon nanotubes (SWCNTs) has been reported as the first biosensor to be used to detect *Staphylococcus aureus* in real-time (Zelada-Guillén et al. 2012). *S. aureus* is considered a leading opportunistic pathogen that is responsible for a variety of different infections ranging from superficial skin lesions, through deep-seated abscesses to life-threatening sepsis (Pollitt et al. 2018). In the study of Zelada-Guillén et al. SWCNTs functioned as an ion-to-electron potentiometric transducer while anti-*S. aureus* aptamers were the recognition element. The CNTs were functionalized with aptamers using non-covalent (i.e. adsorption of drop-casted pyrenil-modified aptamers onto the external walls of the SWCNTs) or covalent bond formation (between amine modified aptamers and

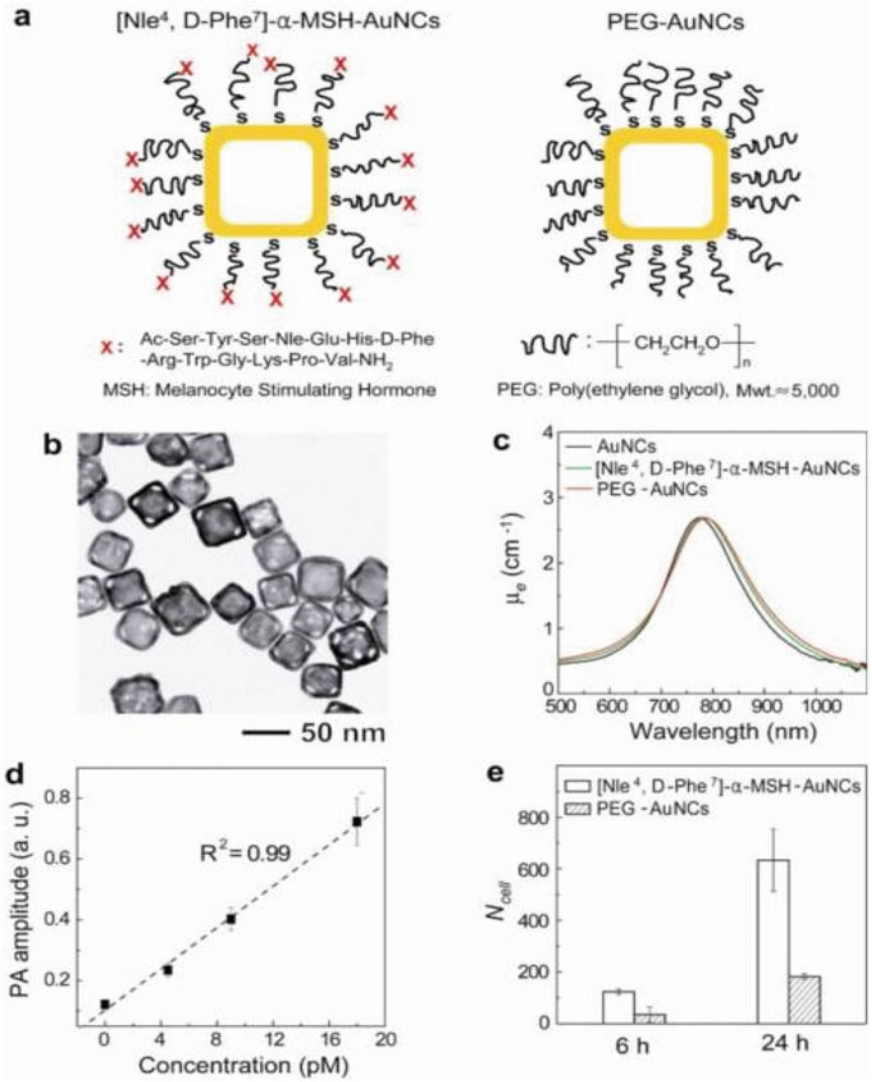

Fig. 6.1 Schematic, characterization, photoacoustic properties, and cellular uptake of the AuNCs. **a** [Nle^4, D-Phe^7]-α-MSH-AuNCs (left) and PEG-AuNCs (right). **b** A typical transmission electron microscopy image of the AuNCs. **c** UV–vis-NIR spectra of the AuNCs (18 pM) conjugated with different chemical agents. μ_e is the extinction coefficient. **d** Plot of the PA amplitude as a function of AuNCs concentration. **e** In vitro uptake of [Nle^4, D-Phe^7]-α-MSH-AuNCs and PEG-AuNCs by B16 melanoma cells as determined by ICP-MS. Here N_{cell} denotes the number of AuNCs per B16 melanoma cell ($p = 0.0005$ at 6-h and $p = 0.007$ at 24-h) (adapted with permission from Kim et al. 2010)

carboxylic groups introduced in the SWCNTs) and were tested with real samples from freshly excised pig skin which was contaminated with *S. aureus*. Although a higher sensitivity was observed in the non-covalent approach, a better value was observed for the minimum concentration of detection with covalent functionalization (Zelada-Guillén et al. 2012). Thus, these innovative approaches could pave the way towards microbial analysis systems that may offer extremely low detection limits and close to real-time responses.

6.4 Therapeutic Applications of Nanotechnology

A new era has dawned in modern medicine with the introduction of various nano-based drug delivery systems that are capable of improving the pharmacological and therapeutic properties of drugs. In general, the routes of drug administration are broadly classified into two categories; namely systemic route (that includes oral, sublingual, rectal, vaginal, urethral, intravascular, intramuscular, subcutaneous, and inhalation routes) and local/topical route of drug administration (Verma et al. 2010). The compounds that are applied to the skin either remain on the skin surface (topical) or penetrate through the skin layer (transdermal) to exert their effects in the target sites located within the skin or in deeper areas. Penetration of topically applied compounds can occur through three possible routes, i.e. through appendages (hair follicles, sebaceous glands, sweat glands), through the corneocytes (transcellular), or through the matrix in the stratum corneum (intercellular).

Although transdermal delivery is considered an efficient mode of drug delivery, the structure of the epidermis especially the arrangement of the *stratum corneum* limits the penetration of drugs (Schoellhammer et al. 2014). As a result, various methods have been explored to enhance the transport of drugs across the *stratum corneum.* Nanotechnology is one of the approaches used today to modify the drug permeation/penetration with the controlled release of the active ingredients. Especially the polymeric nanoparticles are capable of modifying the activity of drugs and protecting their stability, delaying and controlling the release while increasing the adhesivity or the time of permanence in the skin (Guterres et al. 2007; Gupta et al. 2013).

6.4.1 Nanocarriers to Treat Psoriasis and Atopic Dermatitis

Psoriasis is a non-infectious, autoimmune disease characterized by dry, itchy, and scaly skin patches. In this condition, keratinocytes possess a shortened life cycle resulting in some alterations in the desquamation process. The topical delivery of drugs is recommended for psoriasis for local action and to avoid the first-pass metabolism. Nevertheless, conventional anti-psoriatic drug formulations such as

creams, ointments, and gels are becoming outdated due to poor percutaneous absorption and patient incompliance due to greasiness and stickiness (Vincent et al. 2014; Abdelgawad et al. 2015). Some drugs such as methotrexate, cyclosporin, clobetasol propionate, calcipotriol, betamethasone, tazarotene, temoporfin, and tretinoin are used to treat psoriasis, however, the application of a high dose of these drugs using conventional formulations would create toxicity (Vincent et al. 2014). To minimize the toxic effects and to improve the targeting effects, novel drug delivery systems have been introduced especially for mild and moderate psoriasis.

Solid in oil (S/O) nanocarriers have been introduced as vehicles for the transdermal delivery of methotrexate for the treatment of severe and moderate psoriasis. It is believed that the side effects like hepatic toxicity, loss of vision, headache, hair loss, etc. often associated with the systemic use of methotrexate could be reduced by effective topical delivery of methotrexate targeting the diseased cells (Vincent et al. 2014). Yang et al. *developed a solid in oil nanosuspension* as a reverse micellar system into which a methotrexate-coated non-ionic surfactant complex was incorporated. The stability of methotrexate-surfactant complexes was enhanced by the addition of the basic amino acids L-arginine and L-lysine. A transdermal delivery experiment was conducted with the S/O nanocarrier and a permeation behaviour of methotrexate through Yucatan micropig skin was evaluated with a Franz diffusion cell. The oil-based nanocarriers were found to be effective in the penetration through the stratum corneum. Further, the incorporation of urea has dramatically improved the release property of methotrexate from the S/O nanocarrier (Yang et al. 2012). In another study, Singka et al. synthesized a nanogel based on co-polymerised N-isopropylacrylamide and butylacrylate and loaded it with methotrexate. Finite doses were then applied to excised porcine epidermal membranes mounted in Franz diffusion cells. The results of this study demonstrated that the nanogel could undergo de-swelling and the expulsion of methotrexate was influenced by a change in temperature during penetration through the skin. Further, it revealed that the incorporation of saturated Na_2CO_3 could enhance methotrexate flux from the nanogel (Singka et al. 2010).

The main catechin found in green tea (-)-epigallocatechin-3-gallate (EGCG), has proven to mitigate inflammation and induce the expression of keratinocyte differentiation markers caspase-14 and p57/KIP2 promoting epidermal differentiation and cornification. However, the development of EGCG for the treatment of psoriasis has been restricted due to its limited bioavailability. Thus a chitosan-based polymeric nanoparticle formulation of EGCG (nanoEGCG) was developed and the efficacy of nanoEGCG versus native or free EGCG was evaluated in vitro and in vivo imiquimod (IMQ)-induced murine psoriasis-like dermatitis model. This study revealed that nanoEGCG formulation represents a promising drug delivery strategy with enhanced drug delivery and effectiveness to treat psoriasis and other inflammatory skin diseases (Chamcheu et al. 2018).

Clobetasol propionate is a highly lipophilic corticosteroid widely used for the treatment of psoriasis as well as atopic dermatitis, which is an inflammatory skin disease most frequently found in children and characterized by dry, itchy, scaly, and red skin. A topical oil in water (O/W) nanoemulsion was prepared by Alam et al.

and clobetasol propionate (CP) was incorporated in the disperse phase of the oil. The efficacy of this nanoemulsion was assessed by different types of in vivo experiments which revealed that the CP-loaded nanoemulsion has significantly increased nucleoside triphosphate diphosphohydrolase activity in lymphocytes. The membrane protein nucleoside triphosphate diphosphohydrolase is responsible for the hydrolysis of extracellular ATP which is responsible for cell proliferation, differentiation, and inflammatory processes. Any signs of irritation were not observed during the in vivo irritation studies, thus indicating that the developed nanoemulsion with potent anti-inflammatory action was safe for human use (Alam et al. 2013).

Fluticasone propionate is a glucocorticoid applied topically in the treatment of atopic dermatitis and psoriasis. To improve the safety profile and decrease the adverse side effects commonly reported in topical corticotherapy, nanostructured lipid carriers (NLC) consisting of glyceryl palmito-stearate, PEG-containing medium-chain triglycerides mixture, polysorbate 80, and soybean phosphatidylcholine were prepared for topical delivery of fluticasone propionate (Doktorovova et al. 2010). In a recent study, the topical glucocorticoid hydrocortisone (HC) along with hydroxytyrosol (HT)-loaded chitosan nanoparticles (CSNPs) were prepared (Fig. 6.2) and analysed for their adverse effects on healthy human skin. Various parameters including transepidermal water loss, erythema intensity, and irritation score as well as blood haematology, blood biochemistry, and adrenal cortico-thyroid hormone levels of the tested individuals were evaluated while skin biopsy was obtained to assess histopathological changes in the skin. Transepidermal water loss and erythema intensity did not indicate any signs of local irritation, redness, and toxicity, which were further confirmed by a normal Draize skin irritation scoring system and skin hematoxylin and eosin staining results. Similarly, no significant change was observed in blood haematology, blood biochemistry, and adrenal cortico-thyroid hormone level which indicated the non-systemic toxicity of the nano-formulation in treating atopic dermatitis (Siddique et al. 2017).

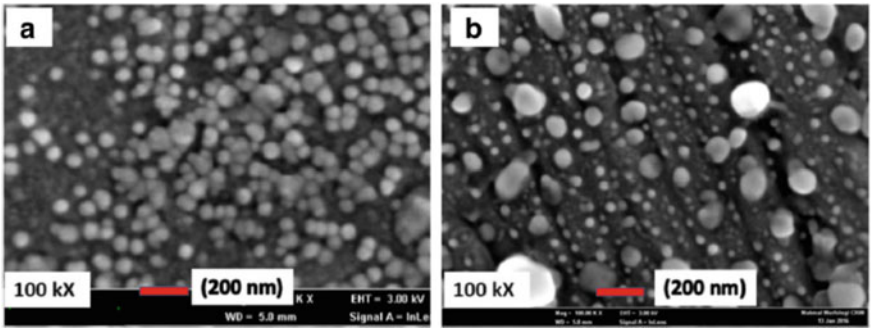

Fig. 6.2 Comparable SEM micrographs of both small- (**a**) and large–scale (**b**) HC-HT CSNP (adapted with permission from Siddique et al. 2017)

6.4.2 The Significance of Nanoparticles in the Treatment of Other Inflammatory Skin Diseases

Rosacea is a common chronic relapsing inflammatory skin condition with persistent erythema followed by telangiectasia, papules, pustules, lymphoedema, and fibrosis. Dysregulation of the immune system, changes in the nervous and the vascular system, and macro- and micro-organisms such as *Demodex* mites and *Staphylococcus epidermidis* are believed to be triggers of this condition (Rivero and Whitfeld 2018). Investigations at the molecular level propose that the overexpression and increased activity of the serine protease Kallikrein 5 (KLK5) has a role in rosacea and other inflammatory skin diseases. Thus the use of inhibitors of the enzyme, for example, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF•HCl), as well as anti-human recombinant Kallikrein 5 (antiKLK5) antibody, would be useful in the treatment of this disease (Limón et al. 2018). However, the low bioavailability limits the above applications. As a solution, gold nanoparticles (GNP) coated with a mixture of hydroxyl- and carboxyl-terminated thiolates (GNP.OH/COOH) were synthesized. These carboxyl groups were used to further functionalize the nanoparticles with the serine protease inhibitor AEBSF•HCl either electrostatically (GNP.COOH AEBSF) or covalently (GNP.AEBSF) or with the antiKLK5 antibody (GNP.antiKLK5). Cell viability assays revealed a significant reduction in the cytotoxicity of free AEBSF once it gets incorporated into the nanoparticles. The functionalized nanoparticles GNP.AEBSF and GNP.antiKLK5 inhibited intracellular KLK5 activity in HaCaT cells and diminished secretion of IL-8 under inflammatory conditions triggered by TLR-2 ligands (Limón et al. 2018). Thus the above study demonstrated the potential of GNP as a new intracellular delivery approach for small drugs as well as antibodies in the treatment of rosacea.

Seborrheic dermatitis is an inflammatory condition that causes scales on the scalp, face, and other parts of the body. The inflammation starts to gradually increase and is usually characterized by scaly, itchy, flaky skin (Paralikar 2015; Dave et al. 2017). It is assumed that an inflammatory immune reaction to the fungus *Malassezia furfur* has an involvement in the aetiology of this disease, hence, antifungal agents are widely employed in the treatment of seborrheic dermatitis. Ketoconazole is one of the antifungal drugs used for this purpose. To achieve maximum therapeutic efficacy, a novel vesicular system of ketoconazole with the herbal plant extract of *Azadirachta indica* was developed by Dave et al. A thin film hydration method was employed to design a liposomal preparation. This herbal formulation of liposome-loaded ketoconazole displayed a synergistic antifungal effect while controlled particle size and zeta potential indicated that the drug in this formulation was stable at a prolonged duration of storage and had optimum percentage cumulative drug release (Dave et al. 2017). These findings would inspire the development of highly effective novel formulations for the treatment of seborrheic dermatitis.

As inflammation is instinctive to almost all skin disorders, there are many attempts to develop more efficacious and safer alternatives to the currently available steroids, antibiotics, and calcineurin inhibitors to treat chronic conditions like dermatitis. In

this respect, lipid nanoparticles of tetrahydrocurcumin (THC)/"white curcumin" had shown great promise. The in vitro and in vivo studies on lipid nanoparticles of THC-based hydrogel revealed that the prepared formulation to be non-irritating, and stable with enhanced anti-inflammatory activity than the free THC in the gel. The above study indicated the efficacy of the above nano-formulation against skin inflammation and opened up new therapeutic avenues for several skin diseases (Kakkar et al. 2018).

6.4.3 Nanoformulations for the Treatment of Acne

Acne vulgaris is a common dermatological disorder and is usually associated with inflammation of pilosebaceous units by *Propionibacterium acne*, a Gram-positive bacterial species. Development of seborrhoea, comedones, erythematous papules, pustules, nodules, or pseudocysts is amongst the clinical presentations of this condition (Rathi 2011). Topical and oral antibiotics and retinoids are regularly prescribed to these patients. Topical treatment is employed as the first choice in mild and moderate acne while systemic therapy is applied in severe and moderate cases (Jain et al. 2014). To improve the physicochemical properties or to improve the pre-clinical efficacy of these drugs, several colloidal carriers like liposomes and mixed vesicles, lipid nanoparticles, and microemulsions have been introduced by various research groups over the past few years. Some of these developments are given below.

Tretinoin (all-trans retinoic acid) is employed in the topical treatment of psoriasis and acne. However, a high dose and prolonged action of tretinoin are responsible for skin irritation. Thus encapsulation of tretinoin in vehicles would increase the stability of the drug as well as it would make a convenient way to deliver the drug. Thus solid lipid nanoparticles (SLN) containing tretinoin were prepared by Ridolfi et al. with and without the addition of the biopolymer, chitosan, and were subjected to in vitro cytotoxicity studies in keratinocytes and the antibacterial activity studies against *P. acne* and *S. aureus*. The SLN-chitosan-tretinoin exhibited high encapsulation efficiency as well as high physical stability in the tested period of one year. Further, those were not found to be cytotoxic to keratinocytes and a significant antibacterial activity was observed against *P. acne* and *S. aureus*. Therefore these findings supported the utility of chitosan-SLN for encapsulating tretinoin and thereby to increase its therapeutic efficacy in the topical treatment of acne and psoriasis (Ridolfi et al. 2012).

Adapalene is a second-generation retinoid effective in treating acne vulgaris and attempts were made to develop adapalene-loaded SLNs for effective topical delivery. Jain et al. employed a hot homogenization method to formulate adapalene-loaded SLNs which have been characterized for particle size, polydispersity index, entrapment efficiency, and drug release properties. Adapalene SLNs were incorporated into carbopol hydrogel for topical delivery and in vitro drug release, skin permeation, biodistribution, rheological behaviour, and texture profile analysis were conducted. The SLNs-based adapalene gel has shown its potential in targeting the skin epidermal layer and reducing systemic penetration. The optimal therapeutic response and improved therapeutic efficacy as well as minimal penetration across

the epidermis with an interception of minimal side effects demonstrated the suitability of SLNs as a novel carrier for the topical delivery of adapalene (Jain et al. 2014).

Azelaic acid is a naturally occurring dicarboxylic acid that is used as a topical application in the treatment of comedonal and inflammatory acne (papulopustular, nodular, and nodulocystic) as well as several other dermatological conditions (Fitton and Goa 1991). However, side effects and low compliance have restricted several topical treatments with azelaic acid. Thus, a topical acne treatment was developed by Reis et al. with azelaic acid-loaded poly-DL-lactide/glycolide copolymer nanoparticles. The encapsulation efficiency of this formulation was found around 80% while differential scanning calorimetric analysis confirmed a strong interaction between the polymer and the drug. Controlled and pulsatile release profile was observed in the in vitro drug release studies while cytotoxicity assays as well as occluded patch test confirmed that the formulation excipients were safe, hence, the suitability of azelaic acid-loaded nanoparticles to be an efficient and safe treatment for acne (Reis et al. 2013).

6.4.4 Nanotechnology for Hair Care

Hair is composed of keratin and has two structures, namely, the hair follicle (the sac-like structure located within the dermis, from which the hair develops and sprouts) and hair shaft (the part of the *hair* that protrudes from the skin). The hair follicle functions as an entry point for topically applied compounds and contributes to the transport of drugs and cosmetics into the skin. Therefore, it plays a significant role in the penetration and permeation of topically applied nanosized particles. Different types of nanosystems, for example, polymeric nanoparticles, metallic nanocrystals, liposomes, lipid nanoparticles, etc. have been employed for the management of follicular permeation thus, for treating several skin diseases (Fang et al. 2014).

Androgenic alopecia is the most common form of human hair loss and it is reported that around 50% of adult men by the age of 50 and almost all Caucasian men by age 80 would get affected (El-Domyati et al. 2009; Gomes et al. 2014; Rosen et al. 2015). Minoxidil and finasteride are the only two treatments approved by the US Food and Drug Administration for hair loss. To address the corrosive adverse effects such as dryness, irritation, burning, etc. associated with the use of minoxidil, the drug was encapsulated into solid lipid nanoparticles. The study revealed that these novel formulations were as efficient as commercial solutions for skin penetration without the corrosive potential and suggested that the solid lipid nanoparticles suspensions could constitute a promising formulation for hair loss treatment (Padois et al. 2011).

Finasteride, a 5-α reductase inhibitor that prevents the peripheral conversion of testosterone to dihydrotestosterone, is another drug used in the treatment of androgenic alopecia. Since the oral administration of this drug is associated with a variety of side effects, attempts were made to encapsulate finasteride into topical liquid crystalline nanoparticles (LCN) as a viable alternative to oral administration. In this study

finasteride-loaded LCN exhibited a controlled release profile with less than 20% of the drug released in the first 24 h (Madheswaran et al. 2013). In a recent study, Roque et al. prepared poly(lactic-co-glycolic acid) nanoparticles with finasteride by using a modified method of emulsification/solvent diffusion. High encapsulation efficiency was achieved for finasteride and in vitro release assays in physiological conditions indicated a prolonged release of the drug where 100% of finasteride was released after a period of three hours. Further, in vitro permeation study demonstrated that nanoparticles had low levels of penetration of finasteride, improving its time residence on the skin. The excipients and vehicles used in nanoparticle preparation did not cause any erythema or other adverse reaction in any of the volunteers and were compatible with the skin. These results suggested the suitability of the new formulation for dermal delivery of finasteride for alopecia treatment (Roque et al. 2017).

Similarly, PLGA nanoparticles loaded with a variety of hair-growing ingredients such as hinokitiol (Fig. 6.3), gylcyrrhetinic acid, and 6-benzylaminopurine exerted a 2–2.5 fold greater scalp-pore permeability compared to controls and the in vivo assays demonstrated enhanced hair growth in tested mice (Tsujimoto et al. 2007). In another study, nanocapsules containing hinokitiol were infused into a hair cleansing shampoo and hair tonic, and the hair growth-promoting effect was evaluated in vivo. The study demonstrated a significantly higher degree of hair growth compared to saline and minoxidil solution controls (Hwang and Kim 2008).

On the other hand, unwanted or excess hair could also have adverse psychological effects and impair quality of life. As an alternative to the widely used laser hair removal method, nanoparticles capable of removing unwanted hair have been developed and patented recently. According to the study of Harris and Kim, topical application of plasmonic nanoparticles localized to target structures within the hair follicle can be activated by light to localize thermal damage. Thus it suggested that topically applied nanomaterials are successful carriers for hair-altering ingredients, and unveils a novel therapeutic option for hair loss and hair removal (Harris and Kim 2014).

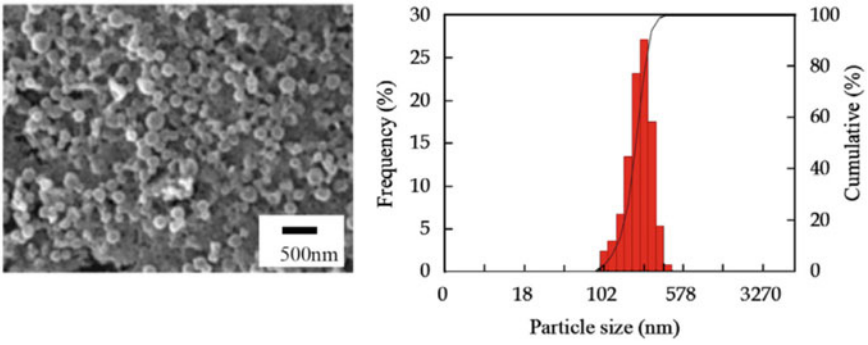

Fig. 6.3 Photo of hinokitiol-loaded PLGA nanospheres and their particle size distribution. **a** SEM photo of PLGA nanospheres **b** particle size distribution of PLGA nanospheres (adapted with permission from Tsujimoto et al. 2007)

6.4.5 Nanomaterials for Wound Healing and Infection Control

Wound healing in the skin is a highly coordinated and regulated process that occurs through several phases of haemostasis, inflammation, proliferation, and remodelling and involves multiple cellular and molecular events tightly controlled by numerous growth factors, chemokines, and cytokines. The dynamic relationship between skin and microbiome is also a crucial contributor to the outcome of this process (Hamdan et al. 2017). As a naturally occurring biological process, wound healing may require a longer duration, hence novel nanotechnological approaches have been developed over the years to shorten this duration. The nano-scale particles have a high probability of interaction with the biological target and are also capable of displaying enhanced penetration into the wound site. In consequence, nanomaterials would be able to deliver a sustained and controlled release of therapeutics resulting in an accelerated healing process (Parani et al. 2016). Nanoparticles, nanofibers, nanocomposites, nanoengineered hydrogels, and self-assembled nanostructures are a few examples of commonly investigated nanostructures and some of the developments in this arena are discussed below.

Chitosan is derived from the partial deacetylation of chitin and consists of D-glucosamine and N- acetyl-D-glucosamine. It has been extensively evaluated for a range of biomedical applications for example; tissue engineering, infection control, wound healing, therapeutic delivery, haemostatic application, etc. (Parani et al. 2016). Recently, Vedakumari et al. synthesized chitosan-fibrin (CS-FN) nanoparticles and those were investigated for antibacterial and wound healing potential. These nanoparticles completely inhibited the growth of *Escherichia coli* and *Staphylococcus* and did not exert any toxic effects on fibroblast cells even at a higher concentration of 100 μg/mL. The open excision wounds made in male albino rats were treated with saline as the control or CS-FN nanoparticles once in two days for up to 10 days. Wounds treated with CS-FN nanoparticles were completely healed on day 14, whereas it took significantly more time, i.e. 22 days in the control. Histological as well as biochemical analyses proved increased synthesis of collagen with active migration of fibroblasts and epithelial cells in CS-FN treated wounds (Vedakumari et al. 2015). In another study, a hydrophilic and porous nanofibrous network was produced with deacetylated (CS-DD)/arginine modified (CS-A) chitosan and used as a wound dressing (Antunes et al. 2015). Male Wistar rats were wounded and treated with CS-DD or CS-A while in the control group, the wounds were washed with PBS (pH = 7.4). The in vitro assays revealed that the membrane was non-cytotoxic, and provided a three-dimensional matrix with a significant contact area to which human fibroblasts adhere and proliferate. Treatment with CS-DD or CS-A showed faster-wound closure than that of the control group, however, the effect was significant with the application of CS-A nanofibers in comparison to non-modified membranes. Therefore, these results clearly indicated the utility of nanofibrous chitosan-based scaffolds for accelerated wound healing (Antunes et al. 2015).

During each phase of wound healing, cytokines and growth factors are involved in the modulation of various cellular processes. For example, vascular endothelial growth factor (VEGF) is a key mediator for angiogenesis and granulation tissue formation in the early stage of healing while platelet-derived growth factor (PDGF) is crucial for inflammation, granulation, re-epithelialization, and remodelling processes (Xie et al. 2013). Therefore, experiments were conducted to develop a dual growth factor-releasing nanoparticle-in-nanofiber system in the hope of accelerating the wound healing process. Here VEGF was electrospun into chitosan and poly(ethylene oxide) (PEO) nanofibrous scaffolds to promote angiogenesis in the short term. Additionally, poly lactic-co-glycolic acid (PLGA) nanoparticles loaded with platelet-derived growth factor-BB (PDGF-BB) were dispersed in the nanofibers. These nanofibrous composites were capable of delivering VEGF quickly and PDGF-BB in a relayed manner, supporting fibroblast growth while exhibiting antibacterial activities. In vivo studies revealed significantly accelerated wound healing potential in these nanofiber/nanoparticle scaffolds in comparison to the control suggesting a promising treatment approach for normal and chronic wound healing (Xie et al. 2013).

Curcumin, a plant secondary metabolite is a well-known topical wound healing agent for both acute and chronic wounds. However, the limited bioavailability, stability, and photosensitivity of this compound have hindered its therapeutic applicability. To overcome these issues, curcumin was encapsulated in poly (lactic-co-glycolic acid) (PLGA-CC) nanoparticles (Chereddy et al. 2013). Although exogenous lactate released from PLGA polymer is capable of accelerating angiogenesis and wound healing, the study was focused on enhancing the activity in the presence of curcumin. In vivo studies based on a full-thickness excisional wound healing mouse model revealed a higher wound healing activity in PLGA–curcumin nanoparticles in comparison to PLGA or curcumin alone. A significant re-epithelialization, granulation tissue formation, and anti-inflammatory potential were observed with PLGA–curcumin nanoparticles. In addition to the sustained release of curcumin, these nanoparticles protected curcumin from light degradation while enhancing its water solubility (Chereddy et al. 2013). Krausz et al. also proved that curcumin nanoparticles (curc-np) were capable of inhibiting the in vitro growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* in a dose-dependent manner while enhancing wound healing in an in vivo murine wound model (Krausz et al. 2015).

In another study, biocompatible quercetin and curcumin nanovesicles were developed and their effectiveness in counteracting phorbol ester 12-O-tetradecanoylphorbol-13-acetate induced lesions and inflammation was investigated in vitro and in vivo (Castangia et al. 2014). The polyphenolic compounds, quercetin, and curcumin were incorporated into liposomes or into innovative penetration enhancer containing vesicles (PEV) and used as drug delivery systems. These bionanovesicles showed a significant anti-inflammatory activity resulting in an inhibition of the onset of skin wounds. The protective effect was more prominent in the curcumin-PEV formulation and it suggested that the topical application of

curcumin-loaded nanovesicles could provide efficient protection against skin wounds (Castangia et al. 2014).

Nitric oxide (NO) has a significant role in wound healing, as it is involved in angiogenesis, migration of epithelial cells, and proliferation of keratinocytes. Therefore the topical application of NO is identified as a preventive and therapeutic strategy against superficial skin infections, including MRSA infections (Ghaffari et al. 2006). NO is capable of modulating immune responses and is a significant regulator of wound healing (Soneja et al. 2005). Friedman et al. developed a NO-releasing platform using silane hydrogel-based nanotechnology (Friedman et al. 2008) where the hydrogel/glass composite was synthesized using a mixture of tetramethylorthosilicate, polyethylene glycol, chitosan, glucose, and sodium nitrite in sodium phosphate buffer. The sustained release rate and the total concentration of NO were modulated by altering the methods for nanoparticle production, such as changing the molecular weight of polyethylene glycol and the concentration of nitrite encapsulated. The nitrite was reduced to NO within the matrix and a fine powder comprising nanoparticles containing NO was obtained after lipolyzation. Once exposed to an aqueous environment, the hydrogel properties of the composite allowed for the opening of water channels inside the particles, facilitating the release of trapped NO over extended periods (Martinez et al. 2009). These nanoparticles displayed antimicrobial activity against MRSA in a murine wound model and an acceleration of infected wound closure was observed in NO-treated groups (Figs. 6.4 and 6.5). However, NO-nanoparticles exerted minimal toxicity on cultured fibroblasts suggesting their potential to develop into topically applied antimicrobials for the treatment of cutaneous infections and wounds (Martinez et al. 2009). In a more recent study, NO-releasing poly(lactic-co-glycolic acid)-polyethylenimine (PEI) nanoparticles (NO/PPNPs) were developed to achieve prolonged NO release, antibacterial efficacy, and wound healing activity. These nanoparticles exhibited potent bactericidal efficacy against MRSA and *Pseudomonas aeruginosa* and were capable of binding on the surface of the bacteria. Furthermore, NO/PPNPs accelerated wound healing and epithelialization in a mouse model of a MRSA-infected wound while not exhibiting any toxicity to healthy fibroblast cells (Nurhasni et al. 2015).

Similarly, the nitric oxide-releasing nanoparticle (NO-np) platform was investigated as a treatment approach for burned infections caused by *Candida albicans* using a murine burn model. An effective antifungal activity, as well as an accelerated rate of wound healing in cutaneous burn infections, were observed with NO-np suggesting its potential to serve as a novel topical antifungal agent to be used for the treatment of cutaneous burn infections and wounds (Macherla et al. 2012).

Silver has a long empirical history as an antimicrobial agent and was widely used until the discovery of antibiotics in the early 1900s for the prevention of infections during wound healing. Silver was applied in different forms and it gradually changed over the years, for example, from silver nitrate solution at the beginning of the nineteenth century to silver sulfadiazine cream formulations in the 1960s and nowadays in the form of metallic silver nanoparticles (Parani et al. 2016). Not only as a wound and burn dressing, nanosilver is also employed as a water disinfectant and a room spray. Several commercial products such as ACTISORB Silver 220® dressings are

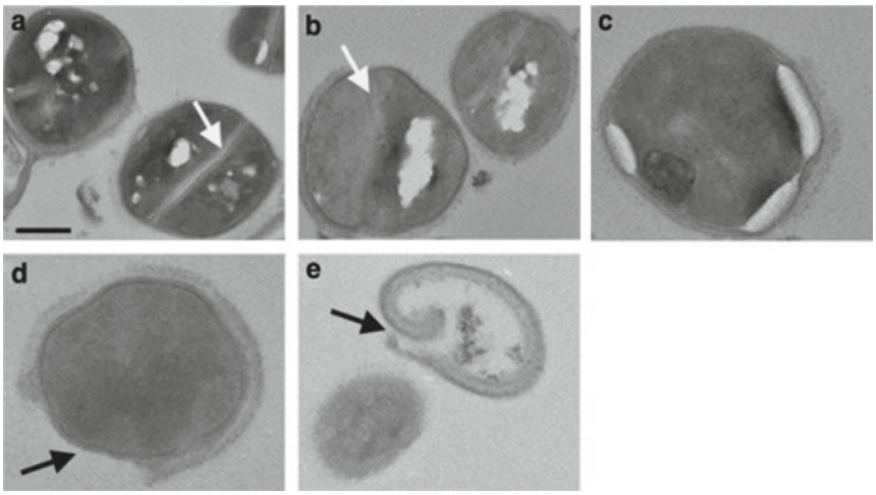

Fig. 6.4 NO-releasing nanoparticles have antimicrobial activity against *Staphylococcus aureus* (*SA*) Transmission electron microscopy (TEM) of *SA* exposed to sustained release of NO by nanoparticles revealed cell wall damage and lysis. **a** Growth of *SA* in the absence of NO-np showed intact cell wall architecture (control). Growth of *SA* in the presence NO-np after 1 (**b**), 4 (**c**), 7 (**d**), and 24 hours (**e**) showed increasing destruction of cell wall architecture, oedema, and cell lysis. White and black arrows denote bacterial cross-wall and cell wall damage after NO treatment, respectively. Bar = 0.2 μm (adapted with permission from Martinez et al. 2009)

currently available in the market (Chen and Schluesener 2008: Papakostas et al. 2011).

Silver nanoparticles have been employed in numerous clinical trials in the therapy of wounds, especially burns and chronic wounds like diabetic ulcers (Boroumand et al. 2018). In an animal trial study, Lu et al. observed that inorganic particles such as silica could firmly adhere to open wounds. As a result, AgNPs were integrated into mesoporous silica nanoparticles through the aid of disulfide bonds (Ag-MSNs) and resulted in prominent antibacterial activities with low cellular toxicity (Lu et al. 2018). Similarly, biocellulose functionalized with AgNPs has demonstrated significant activity against Gram-negative bacterial species while promoting wound healing (Pal et al. 2017).

Silver nanoparticles have proven potent antimicrobial activities against bacteria and fungi and in combination with antibiotics, with synergistic, additive, or antagonistic effects (Jain et al. 2009). An antimicrobial gel formulation containing silver nanoparticles was developed by Jain et al. by a proprietary biostabilization process and the acute dermal toxicity studies in Sprague–Dawley rats showed that the gel formulation was completely safe for topical application (Jain et al. 2009). Similarly, vancomycin-capped gold nanoparticles effectively inhibited the growth of vancomycin-resistant enterococci (VRE) (Gu et al. 2003) while chitosan nanocomposites prepared from chitosan and gold nanoparticles enhanced wound epithelialization (Hsu et al. 2011).

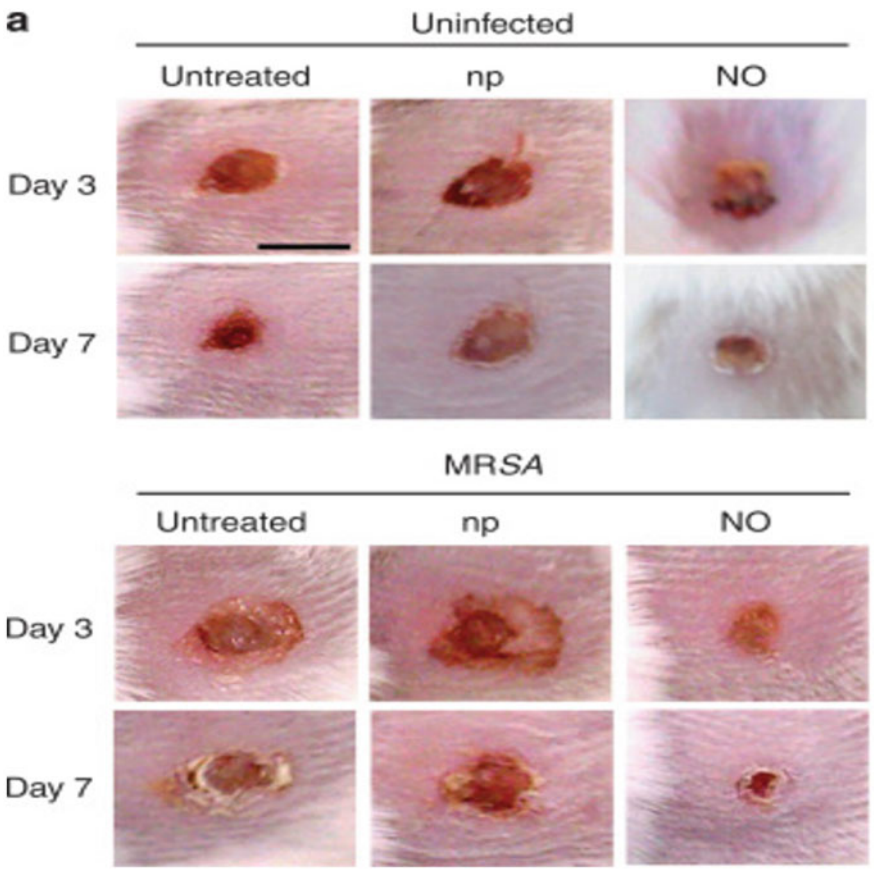

Fig. 6.5 NO-np increased the wound healing rate in mice. **a** Wounds of Balb/c mice uninfected and untreated, uninfected treated with nanoparticles without nitric oxide (NO) (np), uninfected treated with nitric oxide through nanoparticle (NO-np), untreated methicillin-resistant *Staphylococcus aureus* (MR*SA*)-infected, np-treated MR*SA*-infected, and MR*SA*-infected treated with NO-np, days 3 and 7. Bar = 5 mm (adapted with permission from Martinez et al. 2009)

Besides, there are numerous reports on nanomaterials that have been investigated for wound healing potential. For example, silica-gold core–shell materials were developed for the treatment of cutaneous wounds (Li et al. 2015) while zinc oxide nanoflowers were capable of promoting angiogenesis and chemotaxis of cells (Barui et al. 2012). Moreover, the sparingly soluble antimicrobial drug ciprofloxacin was intercalated in layered silicate montmorillonite nanoparticles and gelled in biodegradable gelatin to composite three-dimensional hydrogel with controlled release property for wound dressings (Kevadiya et al. 2014).

There were many attempts to create core–shell nanocomposites by combining two metals (Mihai et al. 2019), for example, AuZnO core–shell nanocomposites were formulated using biogenic gold nanoparticles (AuNPs) extracted from

Hibiscus sabdariffa plant with a thin layer of ZnO. These nanocomposites displayed antibacterial and anti-biofilm effects against *S. aureus* and methicillin-resistant *S. haemolyticus*, through the release of reactive oxygen species (Khan et al. 2018).

MicroRNAs that are involved in the control of transcription of certain genes, thus regulating pro-inflammatory cytokine synthesis have been utilized by Zgheib et al. in their study. Here microRNA-146a (miR-146a) were conjugated with cerium oxide nanoparticles to enhance the diabetic wound healing process (Zgheib et al. 2019).

Based on the aforementioned shreds of evidence, it is clear that nanomaterials could improve current therapeutic approaches for infection control and accelerate the wound healing process significantly.

6.4.6 Nano Approaches in the Treatment of Skin Cancer

A variety of nanoparticles have been extensively investigated in order to develop an efficient and effective treatment approach for skin cancers. This includes liposomes, dendrimers, carbon-based nanoparticles, inorganic nanoparticles, and protein-based nanoparticles. Nanoparticles could allow anti-cancer drugs to reach the cancer site specifically and, thereby enhance the treatment efficacy, especially in metastasized skin cancers (Dianzani et al. 2014). Some examples of the use of nanomaterials to treat different forms of skin cancers are illustrated below.

Doxorubicin is one of the widely used chemotherapeutic agents and the latest findings revealed that conjugation of doxorubicin to gold nanoparticles would be an effective delivery method of this drug against a melanoma cell line (Zhang et al. 2012). Similarly, the anodic iontophoresis of doxorubicin-loaded cationic solid lipid nanoparticles (DOX-SLN) was developed to increase the distribution and tumour penetration of doxorubicin. In this study, the iontophoresis of cationic DOX-SLN resulted in increased penetration of doxorubicin by approximately 50-fold. The in vivo experiments revealed that the DOX-SLN iontophoretic treatment was effective in inhibiting the growth and survival of tumour cells which was accompanied by an increase in keratinization and consequent cell death (Huber et al. 2015). In another study, cationic solid lipid nanoparticles containing doxorubicin were developed and the cytotoxicity and uptake in B16F10 murine melanoma cells were investigated. The stearic acid composition in the lipid phase was altered in these formulations which resulted in a high entrapment efficiency (97%) in formulations with higher amounts of stearic acid. This suggested the possible interactions between the cationic charges on doxorubicin molecules with the negative charges in stearic acid. The encapsulation of doxorubicin has led to a significant increase in cytotoxicity to melanoma cells, thus indicating the potential of these nanoparticles for the treatment of skin cancer (Taveira et al. 2012).

5-fluorouracil (5-Fu) is a chemotherapeutic agent that is applied topically, particularly for precancerous conditions like Bowen's disease, actinic keratosis, or superficial basal cell carcinoma (Goon et al. 2015). However, the topical application of 5-Fu

has several drawbacks which include inadequate frequency and/or length of treatment, insufficient drug concentration, as well as poor penetration into the epithelium, which contribute to tumour recurrence (Goette 1981; Dianzani et al. 2014). To address these problems and to improve the penetration of 5-Fu, albumin/drug-loaded magnetic nanocomposite spheres carrying 5-Fu were fabricated by Misak et al. The in vivo assays revealed significantly superior therapeutic effects in these nanocomposite spheres with increased efficacy to inhibit tumour growth (Misak et al. 2013). Similarly, to target this drug into tumour cells and thereby to avoid unwanted systemic absorption, shell-enriched solid lipid nanoparticles that were loaded with 5-Fu were developed by Khallaf et al. and tested in vivo. Reduced inflammatory reactions with reduced degrees of keratosis and reduced symptoms of angiogenesis were observed in mice treated with these SLN in comparison to the mice treated with 5-Fu alone (Khallaf et al. 2016). In an early attempt, polybutylcyanoacrylate nanoparticles loaded with 5-Fu were locally applied once a day for 35–40 days in patients with basal cell carcinoma. A complete tumour resolution was observed in 31 of 32 patients as confirmed by histological investigations and this demonstrated an alternative treatment approach for patients who are not surgical candidates (Hadjikirova et al. 2005).

Camptothecin (CPT) was initially isolated from the medicinal plant *Camptotheca acuminate* and was identified as a topoisomerase inhibitor, but with poor water solubility. Multiwalled carbon nanotubes (MWCNTs) and graphene oxide (GO) were functionalized with highly hydrophilic and biocompatible poly(vinyl alcohol) (PVA) to increase their aqueous solubility and CPT was loaded onto these drug carriers. MWCNT–PVA and GO–PVA formed stable complexes with the CPT via non-covalent interactions while a higher cytotoxic activity was observed with CPT-loaded nanosystems (MWCNT–PVA–CPT and GO–PVA–CPT) compared to the free CPT alone. The cytotoxic activity was found to be more prominent in MWCNT–PVA–CPT (Sahoo et al. 2011).

Paclitaxel was also isolated from a plant extract and is presently of common use in the treatment of several important malignancies. In order to reduce the toxicity and the limited efficacy due to water-insolubility, nanoparticles albumin-bound paclitaxel (Nab-PTX) have been developed. Hersh et al. conducted a phase II clinical trial to evaluate their efficacy and safety in previously treated and chemotherapy-naive patients with metastatic melanoma (Hersh et al. 2010). This study demonstrated that nab-paclitaxel was well tolerated and active in both groups of patients and was further supported by the study of Kottschade et al. which was a phase II clinical trial using nab-PTX and carboplatin in melanoma patients (Kottschade et al. 2013).

Non-thermal atmospheric pressure plasma has emerged as a promising tool in the treatment of melanoma skin cancer. Although it could efficiently destroy cancer cells, its selectivity is low. Choi et al. demonstrated that treatment with a combination of non-thermal atmospheric pressure plasma and anti-NEU (human epidermal growth factor receptor 2) antibody-labelled gold nanoparticles could effectively and selectively kill melanoma cells. NEU protein is frequently overexpressed in the cell membrane of melanoma cells, and this was targeted by anti-NEU antibody-labelled

gold nanoparticles. The study indicated that labelled nanoparticles have preferentially targeted melanoma cells rather than normal keratinocytes (Choi et al. 2015). A similar study was conducted by conjugation of an antibody against phosphorylated-FAK (focal adhesion kinase) to gold nanoparticles (Choi et al. 2017). FAK is also a protein that is overexpressed in melanoma cells and in the above study, treatment with phosphorylated-FAK-gold nanoparticles (p-FAK-GNP) decreased the viability of G361 cells in a time-dependent manner by inducing apoptosis. A much higher lethality was observed in the combined treatment with plasma and p-FAK-GNP suggesting that these new approaches could be very effective for treating melanoma (Choi et al. 2017).

Nanoparticles are being employed in gene silencing therapy, a technique that emerged recently to treat different types of cancers. Gene silencing involves either interruption or suppression of the expression of a gene at transcriptional or translational levels and small interfering RNA (siRNA) are frequently used for this purpose. siRNAS are double-stranded RNA molecules that are capable of interfering with the translation of specific mRNAs based on their sequence. Although this approach appears to be very promising, the most significant problem associated with it is the inability to deliver sufficient siRNA into the cytoplasm of target cells (Thakor and Gambhir 2013). In this respect, nanolipids and gold nanoparticles have been developed to specifically deliver siRNA to their target tissues (Miele et al. 2012). For example, anisamide-targeted liposome-polycation-DNA nanoparticles containing *N*,*N*-distearyl-*N*-methyl-*N*-2-(*N′*-arginyl) aminoethyl ammonium chloride (DSAA) as a carrier lipid were developed to specifically deliver siRNA to cultured B16F10 melanoma cells, which express the sigma receptor (Chen et al. 2010). Further, Alshamsan et al. revealed that nanoparticles based on polyethylenimine (PEI) modified with stearic acid could be a promising carrier for siRNA delivery for the efficient STAT3 (signal transducer and activator of transcription 3) downregulation in B16 melanoma cells (Alshamsan et al. 2010).

With all these novel developments, it is possible to predict that nanotechnology would become an inevitable tool to treat skin cancers efficiently.

6.5 Nanocosmeceuticals

Cosmeceuticals are responsible for maintaining a good appearance, changing the appearance, as well as keeping the skin and its surroundings in good condition and are widely employed to treat conditions like photoaging, hyperpigmentation, wrinkles, and hair damage. Nowadays, nanotechnology has opened up new avenues in the cosmeceutical industry with the introduction of nanoemulsions, nanocapsules, nanopigments, liposome formulations, niosomes, nanocrystals, solid lipid nanoparticles, etc. These nanomaterials are capable of improving the stability of the cosmetic ingredients, efficiently protecting the skin from harmful ultraviolet rays, increasing the aesthetic value of the products, targeting active ingredients to the desired site,

and controlling the release of active ingredients for prolonged effect (Lohani et al. 2014).

Sunscreen agents are widely used as protectants against the harmful ultraviolet (UV) radiation that falls within the UV-B (290–320 nm) and UV-A (320–400 nm) regions. Titanium dioxide (TiO_2) and zinc oxide (ZnO) are amongst the inorganic physical sun blockers frequently incorporated into sunscreens products to obtain broad-spectrum UV protection. However, the formation of an opaque film after the application is one of the major drawbacks associated with the incorporation of bulk TiO_2 and ZnO into these formulations. Thus, nanosized TiO_2 and ZnO came into the picture as those are capable of eliminating the undesired opaqueness without reducing the UV-blocking efficacy of the sunscreen product (Smijs and Pavel. 2011).

Skin ageing is accelerated by many external factors such as exposure to chemicals, pollutants, and UV radiation and is manifested as drying out, loss of elasticity and texture, thinning, damaged barrier function, the appearance of spots, and, finally, wrinkles (Lohani et al. 2014). Thus, a variety of anti-ageing products are available in the market and many cosmeceutical companies have employed nanotechnology to enhance the efficacy of these products. For example, L'Oreal has developed an anti-wrinkle cream containing nanosomes of Pro-Retinol A while Lancôme introduced a cream that contains nanoencapsulated triceramide (Lohani et al. 2014). At a laboratory scale, Suter et al. incorporated heptapetide P7 (a peptide that could stimulate the skin's own self-defence mechanisms against oxidative stress) into solid lipid nanoparticles. The in vivo studies resulted in active peptide delivery into skin cells and demonstrated a statistically significant decrease in DNA damage in comparison to the placebo (Suter et al. 2016).

N-6-furfuryl adenine (N6FA) which is also known as "kinetin" is a bioactive natural phytochemical compound. Solid lipid nanoparticle-based topical formulation of N6FA was developed by Goindi et al. in order to evaluate its efficacy against UV-radiation-induced skin photodamage. The optimized formulation was characterized in terms of particle size, drug entrapment efficiency, zeta potential, and pH while its stability, spreadability, ex-vivo skin permeation, and photoprotective effects against UV-induced skin damage were also investigated. The investigations demonstrated that N6FA-loaded SLNs were capable of preventing photodamage due to chronic UV exposure and the effects were comparable with the widely used commercial formulation, "Garnier wrinkle lift anti-ageing cream" (Goindi et al. 2015).

Administration of antioxidants to the skin is one of the strategies to reduce ageing and also to achieve protective effects on the skin. These antioxidants are capable of quenching free radicals and other reactive species that are potent enough to cause severe damage to cells and cell components of the skin. However, the stability of these antioxidant compounds may not always be sufficient to guarantee their effects on the skin while the delivery of these compounds into deeper layers of the skin may not always feasible. Thus different types of nanomaterials have been developed for the encapsulation of antioxidant substances (Vinardell and Mitjans 2015). For example, niosomes have been prepared with different proportions of polyglyceryl-3 dioleate or glycerol monooleate and cholesterol enabling the accumulation of resveratrol in the stratum corneum (Pando et al. 2013). Moreover, the co-encapsulation of

resveratrol and curcumin in niosomal systems resulted in a promoted ability to reduce free radicals due to a synergic antioxidant action (Tavano et al. 2014). On the other hand, tocopherol has been incorporated into nanocarriers to produce a non-irritant, stable, as well as cosmetically appealing aqueous formulation that could induce a high release of tocopherol (Mahamongkol et al. 2005; Ben-Shabat et al. 2013). Recent investigations revealed that a nanoemulsion system formulated with *Eysenhardtia platycarpa* leaf flavanones was capable of enhancing anti-ageing activity (Domínguez-Villegas et al. 2014) while nanosized phytobioactive compounds, such as curcumin and vegetable oils, were capable of enhancing the skin appearance by various antioxidative mechanisms (Naz and Ahmad 2015; Badea et al. 2015).

In addition, various other cosmeceutical products have been formulated in the recent past via nanotechnological approaches. Liposomes, nanoemulsions, and solid lipid nanoparticles are widely used in moisturizing formulations to achieve prolonged effects. A cleanser soap with a blend of nanosilver and natural ingredients was developed by Nano Cyclic Inc. to remove pathogenic microorganisms and to diminish age spots and sun-damaged skin (Lohani et al. 2014). Similarly, niosomes containing lauric acid and curcumin have displayed an enhanced antimicrobial activity against acne-caused skin infections (Liu and Huang 2013). In terms of hair care products, the development of curcumin-based liposomes resulted in 70% enhanced penetration of the curcumin to the hair growth, irrespective of liposome type in the size range of 213–320 nm (Jung et al. 2006; Ganesan and Choi 2016). The use of sericin nanoparticles in hair cosmeceuticals bestows gloss and softness and promotes the maintenance of colouration in dyed hair (Pereda et al. 2012). Further, silica nanoparticles have been used in lipsticks to improve the homogenous distribution of pigments (Viladot et al. 2013) while nail paints having nanosized particles displayed improved toughness, mar resistance, and impact resistance of the mammalian nails (Amato et al. 2007). In addition, the incorporation of silver and metal oxide nanoparticles with antifungal activity in nail polish is speculated to be useful in the treatment of fungal toenail infections (Lohani et al. 2014).

Thus it is obvious that nanotechnology-based cosmeceuticals could offer an advantage of diversity in products, enhanced bioavailability of active ingredients, and increase the aesthetic appeal of these products with prolonged effects. However various concerns have been raised about the possible penetration of nanoparticles through the skin and the potential hazards of these nanocosmeceuticals to human health (Lohani et al. 2014) with controversial views on the pros and cons of these products.

6.6 Concerns on Nanotoxicity

On par with the tremendous increment in the usage of nanomaterials in dermatology, there have been considerable efforts to investigate and understand the interaction of nanomaterials with the skin. Since there could be various limitations, for instance, toxicity, tissue deposition, and long-term oncological potential, it is essential to have

a better understanding of their chemistry in order to minimize or avoid potential negative effects on human health and the environment (Antonio et al. 2014).

For example, there were many concerns about the development of reactive oxygen species from metal oxide nanoparticles due to the photocatalysis process occurring upon exposure to UV radiation. Moreover, some people question whether nanoparticles are small enough to penetrate the epidermis and be absorbed into the human bloodstream, causing toxicity with long-term use (McSweeney 2016). However previous studies suggested that this photocatalytic process might not be significant, as the nanoparticles do not penetrate below the level of the stratum corneum. Yet some studies suggested that nanoparticles might breach that barrier under certain circumstances although the majority of those studies have used animal skin models rather than human skin (Tran and Salmon 2011). More recently, it was reported that the antioxidant systems in the skin are capable of neutralizing reactive oxygen species generated by these metal oxides. In addition, numerous in vitro and in vivo studies conducted in both animal and human skin, demonstrated that nanoparticles were confined to the level of the stratum corneum after topical application, even in the skin where the barrier function had been altered. The shedding and turnover of the stratum corneum could further prevent the accumulation of nanoparticles and based on this evidence the EU's Scientific Committee on Emerging and Newly Identified Health Risks concluded that the topical use of TiO_2 and ZnO in cosmetic products does not pose a risk to humans. However, it is recommended that until more data are available, the use of these nanoformulations at sites with severely impaired barrier function should be minimized (Jansen et al. 2013).

The commercialization of products containing silver nanoparticles with antibacterial properties is increasing over the years at an accelerated pace while the number of toxicological concerns related to nanosilver also continues to grow as well. However, current in vitro toxicological investigations based on cell cultures, lower-order lifeforms, or embryonic organisms are insufficient for an in-depth understanding of the toxicity of these nanoparticles. Further, this lag far behind due to the limited availability of appropriate tools for in vivo characterization and assessment on higher-order organisms. Nevertheless, it is believed that the recently developed imaging modalities could be useful for monitoring the in vivo transport and the fate of silver nanoparticles and thereby could contribute towards new insights into the toxicological mechanisms of these nanomaterials (Stensberg et al. 2011).

As signified above, reliable and reproducible screening approaches are required to investigate specifically the vast number of bio-physicochemical interactions at the nano/bio interface and thereby to assess the hazardous effects of these nanomaterials. Nel et al. developed a predictive toxicological approach with the use of mechanisms-based high-throughput screening in vitro. This enabled making predictions about the physicochemical properties of nanomaterials to correlate pathology or disease outcomes in vivo. The in vivo results were used to validate and improve the in vitro high-throughput screening and to establish structure–activity relationships (Nel et al. 2012). However, this approach was also not perfect as there were several drawbacks associated, including the inability to predict the real immediate

toxic effects in humans, or the consequence of chronic exposure, such as oncogenic potential. Hence the development of new techniques to evaluate the accurate correlations between in vitro and in vivo investigations is imperative while a better understanding of the physiochemical, molecular, and physiological processes of nanoparticles is important to turn nanodermatology into a reliable and sustainable diagnostic, treatment, and preventive modality (Hasanzadeh et al. 2015).

6.7 Conclusion

Nanomaterials are employed in dermatology as diagnostic tools and also to achieve effective, targeted, and efficient delivery of drugs. In addition, the recent and anticipated advances in nanotechnology contribute to the development of novel cosmeceuticals suggesting that nanodermatology has an inevitable future.

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