Engineering Materials

Anand Krishnan
Anil Chuturgoon *Editors*

Integrative Nanomedicine for New Therapies

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Preface

Nanotechnology is gaining popularity in modern medicine and helping to improve our quality of life. The rapidly changing pace of life necessitates rapid progress in scientific and medical research to ensure the individuals' good health and well-being. The nanotechnology "tsunami" offers novel insights into diseases and several life-threatening illnesses. These insights include rapid diagnosis and therapy of diseases such as cancer and infectious diseases. This book integrates the science of nanotechnology and medicine and its applications in nanomedicine. The information is intended for a wide audience researching in science and medicine.

The work covers basic techniques in the synthesis of nanomaterials, their biohazards, applications in diseases, drug delivery systems and their novel aspects as nutraceuticals. Taken together, the information provides the state-of-the-art solutions previously difficult to treat diseases, with limited adverse health outcomes due to therapy.

This book appeals to researchers in chemistry, physics, biochemistry, drug discovery, nutrition and medicine interested in cutting-edge research with relevance to progress in contemporary biomedicine. Some specific disease applications are included and covered extensively by including the latest innovative and relevant information of nanomedicine.

All the chapters of this book were prepared by expert researchers who have made substantial contributions to the fields of nanotechnology, drug design and delivery and nanomedicine. Each chapter is presented well and contains illustrations that will captivate the reader's interest.

We believe that that this book will substantially increase our knowledge on and applications of nanotechnology in medicine to help curb the exponential increase in human diseases.

Bloemfontein, South Africa — Anand Krishnan
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The original version of this book was revised: Book Editor affiliation updated and co-author's name has been corrected in the Chapter "Nanomedicines in Tuberculosis: Diagnosis, Therapy and Nanodrug Delivery". The correction to this book can be found at https://doi.org/10.1007/978-3-030-36260-7_15

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About the Editors

Dr. Anand Krishnan, D.Tech., PrchemSA has expertise in organic chemistry/medical biochemistry/integrative medicine/nano(bio)technology/drug discovery. He received his doctoral degree in organic chemistry in the Department of Chemistry, Durban University of Technology, in collaboration with the Department of Medical Biochemistry, University of KwaZulu-Natal, in 2014. He completed his master's degree in organic chemistry from Bharathiar University, India, and bachelor's degree in chemistry from Madurai Kamaraj University, India. He was Postdoctoral Researcher at Durban University of Technology, South Africa, from November 2014 to November 2016. He has published many scientific articles in international peer-reviewed journals and has authored many chapters as well as review articles. He was recognised for his contributions and received awards from national and international organisations. He has been awarded Best Postdoctoral Researcher Award for 2016 and 2017 from Durban University of Technology and Young Scientist Researcher Award 2016 from Pearl Foundation. He is Member of various editorial boards of the journals of the international reputation. His research interests include organic synthesis, catalysis, heterocyclic chemistry, cannabis chemistry, drug discovery, phytonanotherapy, extracellular vesicles, nanotoxicology and chemical pathology.

Currently, he received prestigious Innovation Post-doctoral Research Fellowship from National Research Foundation (NRF), South Africa and conducting research at the Department of Chemical Pathology, School of Pathology, Faculty of Health Sciences and National Health Laboratory Service (NHLS), University of the Free State, Bloemfontein, South Africa.

Prof. Anil Chuturgoon, Ph.D. is Head of Discipline of Medical Biochemistry in the School of Laboratory Medicine and Medical Sciences at the University of KwaZulu-Natal (UKZN), Durban, South Africa. He completed his B.Sc., B.Sc. (Hons) and M.Sc. degrees at the University of Natal. He thereafter obtained his doctoral degree in toxicology and epigenetics from UKZN. He is passionate about research and shaping young minds to think critically. He is Member of several scientific associations and editorial boards. He received the Distinguished Teacher Award for excellence in teaching. He is NRF-rated Scientist and was placed in the top 30 UKZN researchers for 2016, 2017 and 2018. He has successfully supervised over 50 masters' and 27 doctoral students. He has over 110 refereed publications, 12 chapters in books and over 200 conference proceedings.

Basic Techniques to Investigate the Nanostructured Materials

Navaneethan Duraisamy, Kavitha Kandiah and Balagurunathan Ramasamy

Abstract This chapter is to deliver the basic methods for the characterizations of nanostructured materials. There are different shapes of materials existing in the nanomaterials such as particles, sheets, roads, dots, balls and films. The crystalline structures and surface morphology of nanomaterials are clearly calibrated using advanced techniques such as X-ray diffraction, Field emission scanning electron microscopy and transmission electron microscopy. The chemical compositions and purity of materials are examined by Energy dispersive X-ray analysis, Fourier transform infrared analysis and X-ray photoelectron spectroscopy. The biological studies of nanomaterials are examined using bioactivity, anti-microbial activity and bio degradability. This review gives a comprehensive understanding of the physico-chemical and biological nature of the nanomaterials.

1 Introduction

NANO is the heart core research field in the emerging technology; utilize nanomaterials in the range of 1–100 nm sizes with different shapes. The size of the materials and shapes are having a potential for various applications in biomedical, energy harvesting, energy storage, fertilizers and agriculture. The tuning of sizes and shapes are more important to adopting the materials for targeting application. Designing the specific structure of nanomaterials would be investigated using various advanced technical tool in the field of material science and technology (Wu et al. 2018; Murty et al. 2012; Duraisamy et al. 2013, 2018). But, it is not applicable to use same technical tools for all types of samples (conducting polymers, metal oxides, metal

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sulphides, carbon materials, biomolecules and etc.) due to the influence of materials handling and sensitivity. The selection of tools is mainly depend on the materials nature, for example, Kirk et al. reported that the surface structure of biological sample can be examined using environmental scanning electron microscopy (ESEM) but not in normal scanning electron microscopy (SEM) because it is more important to maintain the nature (alive) of biological sample. However, other than biological samples, normal SEM technique is more preferable to determine the shapes, sizes and surface of materials (ref-my paper). In order to analysis the biological sample at high resolution (in depth view) under freezing condition, Cryo-SEM is more feasible than ESEM but expensive (Kirk et al. 2009).

In this chapter, we have focused on the basic technical tools for the investigation of nanomaterials such as X-ray diffraction (XRD), Fourier transform infrared spectrometer (FT-IR), Raman spectroscopy, Dynamic Light Scattering, Brunauer–Emmett–Teller surface area analysis and mechanical characterization, Scanning electron microscopy, Transmission electron microscopy (TEM) and nano indentation. The above tools are significantly play an vital role to analysis the structural crystallinity, crystalline size, phase nature, presence of functional groups, surface purity, surface area, shapes, sizes, presence of defects and mechanical strength of the materials. This chapter is providing strong background to understand the basic tools for the investigation of nanomaterials, where strongly support to bring out the anticipated materials in the field of science and technology.

2 X-Ray Diffraction Analysis

The crystallographic nature of prepared samples was evaluated by X-ray diffraction (XRD) analysis. The XRD patterns were used for structural determination of the prepared samples. The structural crystallinity of TiO_2 nanoparticle (pure) and its composites were examined by XRD spectrometer (X'Pert PRO; PANalytical, the Netherlands) with a radiation source of CuK_α ($\lambda = 1.5406$ Å) at 30 mA current with an accelerating voltage of 40 kV. The synthesized samples were scanned at 2θ (angle) and a range of 10–80° with an increment of 0.05°. The diffraction patterns of the synthesized samples were confirmed with help of standard reference data, that is, Joint Committee on Powder Diffraction Standards (JCPDS). The average crystallite size was determined using Scherrer formula (Choi et al. 2004; Kavitha et al. 2013a):

$$D = \frac{k\,\lambda}{\beta \cos\theta} \tag{2.1}$$

where D is the crystal size, k—Scherrer constant, λ—wavelength (1.5406 Å), β—full width at half maximum and θ—diffraction angle.

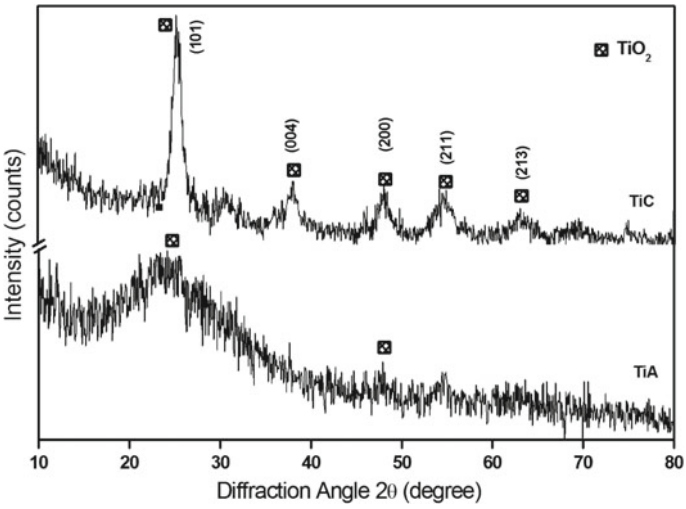


Fig. 1 X-ray diffraction patterns of prepared TiO_2 nanosamples

2.1 Metal and Metal Oxide

The crystalline nature of the TiO_2 nanoparticles at 393 K (TiA) and 673 K (TiC) is analysed by X-ray diffraction (XRD) analysis, as shown in Fig. 1. It is ascertained that TiA is amorphous in nature, which is in line with the results of previous studies (Yin et al. 2001). The high-intensity peak seen in TiC at 25.328° (2θ) is in accordance with the characteristic diffraction peak of TiO_2 anatase phase (JCPDS file no. 21-1272). The peak intensity and sharpness of TiC show that sintered samples do not have any impurities. The crystallite size of the TiO_2 nanosample is determined by Scherrer equation (Choi et al. 2004; Kavitha et al. 2013a). Owing to the amorphous nature of TiA, no peak is observed to calculate the crystallite size, whereas TiC shows 8 nm crystallite sizes.

2.2 Carbon and Polymer Materials

The XRD pattern of graphite, graphene oxide (GO), graphene (rGO) and polymer is shown in Fig. 2. The high-intensity peak appearing at 10–12.4 (2θ) is in accordance with the characteristic diffraction peak of graphene oxide and peak at 24.7–26.6° is for reduced graphene oxide and graphite, (JCPDS no. 89-7213). In addition, the peak intensity and peak sharpness shows that the samples do not have significant impurities. According to the Scherrer formula and 2θ angle, the average crystallite size of the extracellular reduced graphene oxide is calculated as 5 nm and GO is 7 nm (Kavitha et al. 2013b).

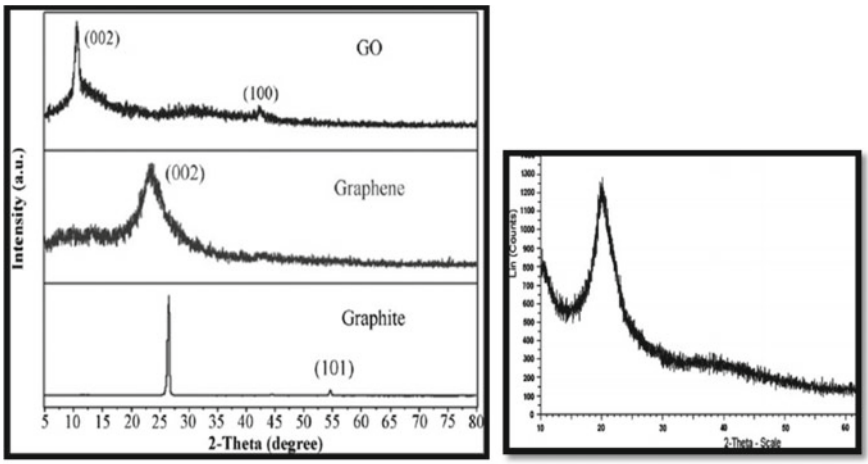


Fig. 2 X-ray diffraction patterns carbon and polymer nanosamples

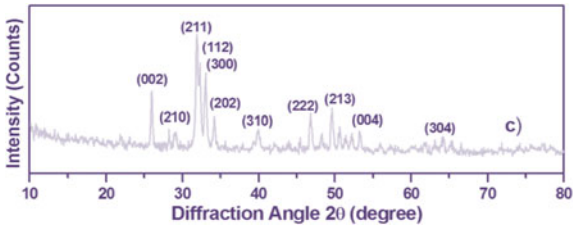


Fig. 3 XRD analysis of pure HAp obtained at 150 °C

2.3 Ceramic Materials

Figures 3 shows the XRD pattern of HAp treated at 150 °C for time period of 15 h at 150 °C.

All the peaks of d spacing are identified and matched well with a standard JCPDS file (09-0432). The XRD patterns confirmed the high crystalline nature of HAp, where identified on the basis of intensity and peak width. No other crystalline phase is present besides HAp. The crystallite size is calculated from Scherrer's formula. The crystallite size was 26.9232 nm. The crystallinity of HAp increased with an increasing the reaction temperature and time (Rajkumar et al. 2011).

2.4 Polymers and Composites

Figure 4 illustrated the XRD pattern of temperature-variant composite samples. The obtained broad diffraction patterns (25.32° and 26.6°) were confirmed the formation

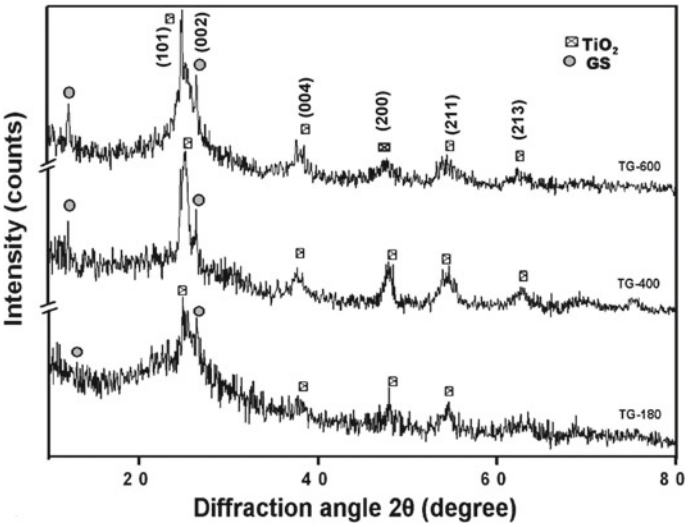


Fig. 4 XRD analysis of titania–graphene composite

of crystalline titania-graphene oxide composite (JCPDS file no. 21-1276) (Zhang et al. 2010; Krishnamoorthy et al. 2013).

However, a small hump at 12.7° was clearly identified with increasing the sintering temperature of titania-graphene oxide nanocomposite, which was due to trace amount of graphite oxide (Krishnamoorthy et al. 2013). The average crystallite size was 3.01, 3.13, 3.23 nm respectively for 180, 400 and 600 °C. As similar with previous studies, this study also conforms the addition of carbon into the titania reduces the crystallite size (Manivasakan et al. 2010; Krishnamoorthy et al. 2013). Moreover, this study disclosed that the crystallite size increased with increasing sintering temperature of composite materials, which was due to effect of temperature during the nucleation process (Manivasakan et al. 2010).

3 Dynamic Light Scattering

The particle size distribution was calculated via particle size analyser (Nanophox; Sympatec, Clausthal-Zellerfeld, Germany). This was simply working on the principle of dynamic light scattering with three-dimensional photon cross-correlation method. Here, light source was He–Ne laser with a maximum intensity of 10 mW (λ = 632.8 nm).

A stable colloidal solution was obtained by dispersing the nanoparticles in an aqueous solution using a sonochemical reactor (Vibra-Cell; Sonics, USA). The solution of dispersed nanoparticles was taken into the liquid cell and measured from 1 to1000 nm at 90° (scattering angle).

The particle size distribution (PSD) measured for both samples TiA and TiC was illustrated in Fig. 5a, b, confirming that the PSD of synthesised samples is below 50 nm. However, the PSD of TiA (25–50 nm) is wider than that of the TiC (16–28 nm) (Kavitha et al. 2013a, c). This reduction is due to the uniform arrangement of

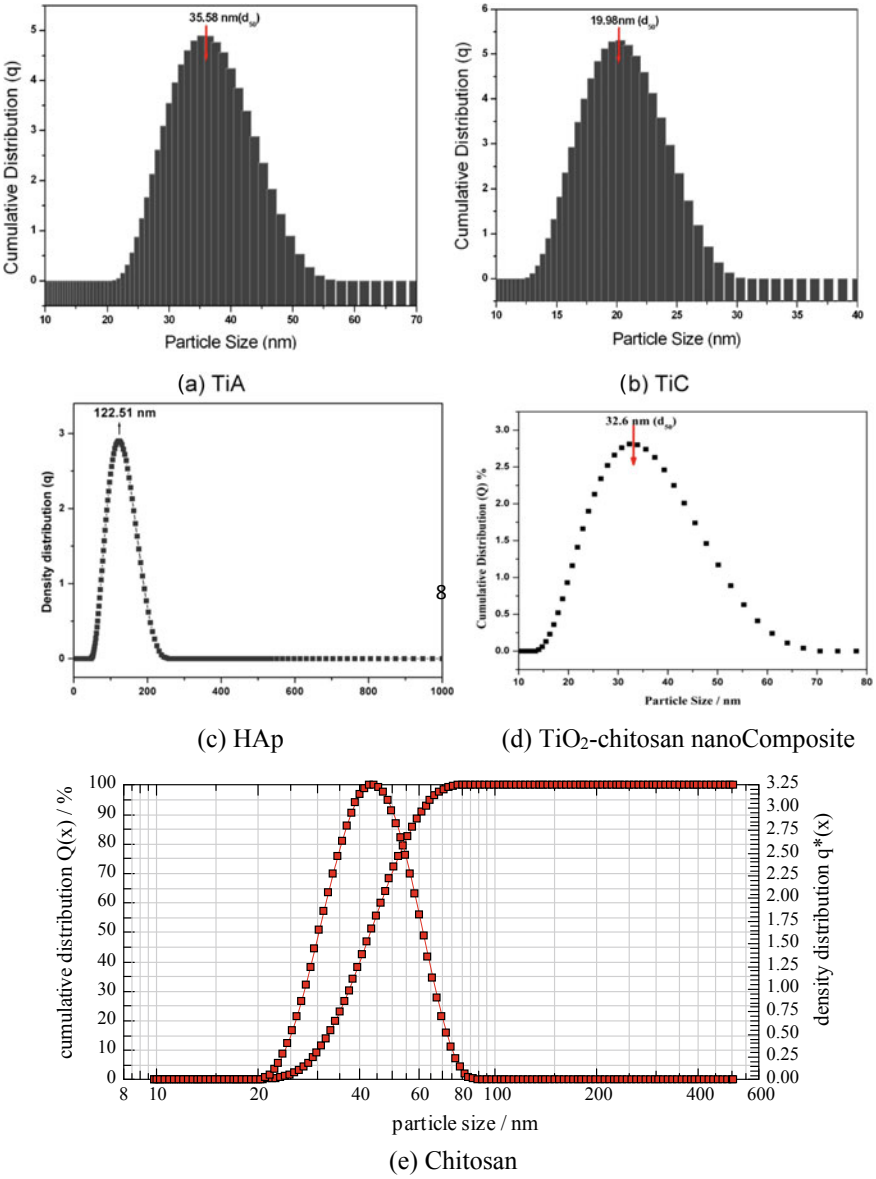


Fig. 5 Particle size distribution of nano-TiO_2, HAp, nanocomposite and polymer

lattice and complete removal of unnecessary components present in the sample during sintering (Manivasakan et al. 2010). Figure 5c illustrates the particle size distribution of the HAp obtained at 423 K for 15 h using a laser particle size analyzer. The mean particle size distribution of HAp is 122.51 nm (Rajkumar et al. 2011). Figure 5d shows the 15–65 nm of particle size distribution of TiO_2-chitosan nanocomposite material (Kavitha et al. 2013a, c). Figure 5e shows the PSD of chitosan is obtained from 20 to 80 nm.

4 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectrometer (FTIR; Spectrum 100; PerkinElmer, USA) was used to identify the functional group of prepared samples based on the atomic vibrations of a molecule. The peaks appearing in the absorption spectrum correspond to the frequency of the samples. The functional groups and chemical bonds in pure TiO_2 nanoparticles and their composites were analysed using FTIR spectroscopic studies. For FT-IR study, a small pellet was prepared via simple mixing of examine sample with known materials of potassium bromide in the ratio of 2:200 (w/w), which was pressed under hydraulic pressure pellet maker (pressing weight was 125 kg cm^{-2}). The absorption spectra analyses were carried out on the pellets at a λ range of 4000–400 cm^{-1} with 1 cm^{-1} resolution.

4.1 Metal Oxide and Ceramics

The functional groups of the prepared TiO_2 nanoparticles are listed in Fig. 6a shows that the broad peaks obtained at 400–900 cm^{-1}correspond to the characteristic peak of Ti–O–Ti stretching mode (Kavitha et al. 2013a, b). While sintering the TiO_2, the

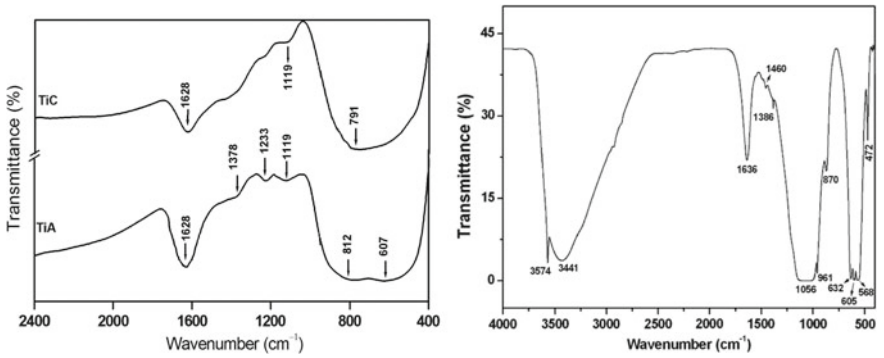


Fig. 6 FTIR analysis of TiO_2 nanosamples

broad Ti–O–Ti covalent band is narrowed down to 550–800 cm^{-1} due to transformation of Ti (OH) into TiO_2. The vibration bands obtained at 1119 and 1233 cm^{-1} were assigned, respectively, to the stretching and bending modes of Ti–O–C, and Ti–OH surface groups (Manivasakan et al. 2010, Al-Sagheer and Merchant 2011, Zawadzki and Kaczmarek 2010). The Ti–OH group accelerates the formation of apatite nucleation due to the release of Ca^{2+}, Na^{+} or K^{+} ions (Nakayama and Hayashi 2007). The CH_3 stretching is observed near 1380 cm^{-1}. The presence of carbon band in TiO_2 was the influence of inadequate evaporation of solvents during the synthesis. In addition, the peak observed at 1628 cm^{-1} was responsible for hydroxyl group, which is gradually reduced while sintering the sample TiA into TiC.

FTIR has been employed to analyse the characteristic bands of the prepared samples. The FTIR spectra of the HAp are prepared by hydrothermal treatment at 423 K for 15 h as shown in Fig. 6b. The characteristic bands of the PO_4^{3-} groups are observed at 472, 568, 605, 961 and 1056 cm^{-1} (Rajkumar et al. 2011, 2013). The peaks observed at 632 cm^{-1} are due to O–H bending deformation mode. The stretching vibration of lattice OH^{-} ions is attributed at 3574 cm^{-1}. The peaks at 1460, 1386 and 870 cm^{-1} are due to atmospheric carbon dioxide (Chen et al. 2007; Zhao et al. 2009). The bands registered at 3441 and 1636 cm^{-1} were ascribed to adsorb H_2O (Kavitha et al. 2013c, Vallet-Regi 2001). Based on the FTIR result, it can be concluded that there is no obvious peak for any other impurity.

4.2 Carbon and Composite Materials

FTIR analysis of GO and rGO (Fig. 7a) shows, the presence of C–OH, C–O and C–O–C peaks in GO nanosheets but, not prepared rGO. Instead of oxygen and hydroxyl groups, rGO has C–C band, which confirms the occurrence of reduction process. FTIR is clearly shows and validate that, the reduction of multiple thick GO layers to thin rGO layer in nano dimension (Kavitha et al. 2013b, d; Krishnamorrthy et al.

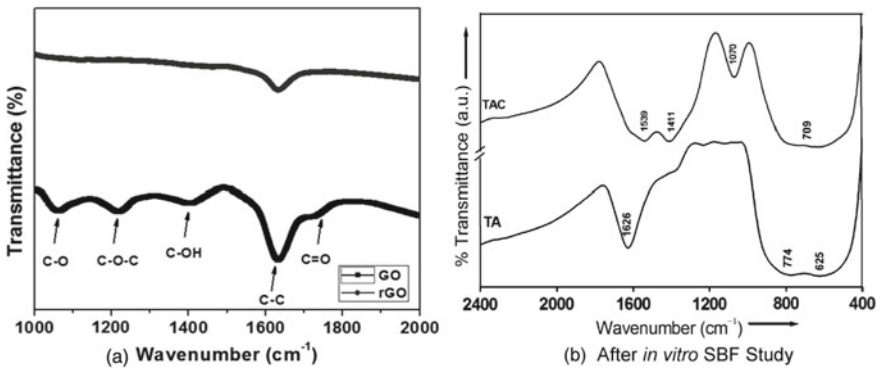


Fig. 7 Fourier transform infrared spectroscopy of graphene sample

2013). Similarly, Fig. 7b shows the HAp formation on nano-TiO_2 and nano-TiO_2–chitosan coated Ti-6Al-4 V. In addition, the presence of functional groups such as Ti–O–Ti (400–800 cm^{-1}), Ti–OH (1106), Ti–O–C (1233 cm^{-1}), and C–O (1392 cm^{-1}) and CO_3^{2-} (1415 cm^{-1}) peak after bioactivity were also confirm the formation of HAp. This result supported the TAC coating on Ti-6Al-4 V, helps in the enhancement of cell growth for tissue engineering applications.

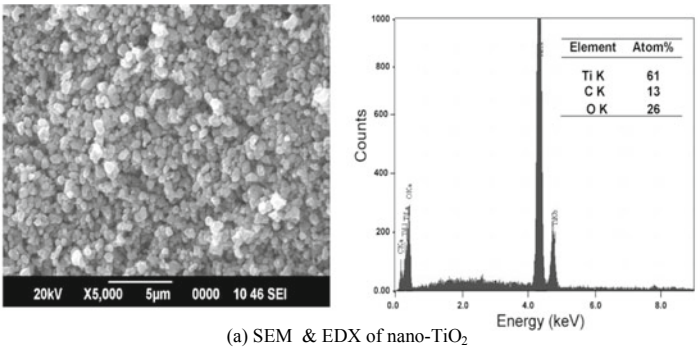
5 Microscopic and Spectroscopic Analyses

Transmission electron microscopy (TEM) working at 120 kV a beam of electron is transmitted through the specimen. An image was generated by the interaction of the electrons transmitted via sample. Further, the image was enlarged and followed by clear focus on a fluorescent display or a layer of photographic film. The morphology and particle size of the pure TiO_2 nanoparticle and its composites were examined by analysing the image obtained from TEM (CM 200; Philips, USA). Well-dispersed material was loaded on TEM grid and followed by drying with a help of IR lamp for ~30 min. The images of the samples were then captured from dried grids. The image of TEM exhibited a zooming up to 1,000,000 × with a good resolution than 10 Å. A particle size was measured from the randomly selected areas of each sample, and then, the average size was calculated. In addition, selected area electron diffraction (SAED) pattern of the samples was obtained. The obtained images showed the crystalline nature and lattice arrangements of the prepared samples.

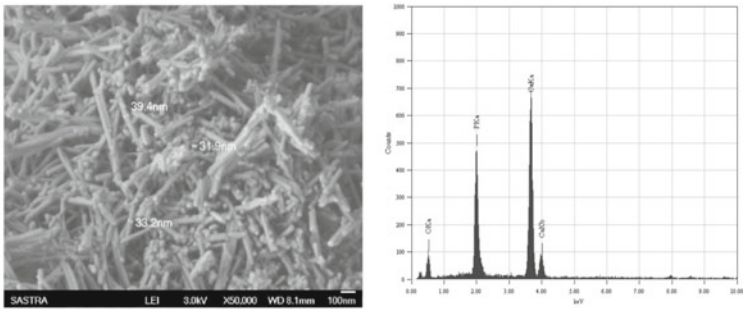
In SEM, a high-energy electron beam with 25 keV (accelerating voltage) was used to investigate the materials surface (morphology, shape and size). In addition, an elemental analysis of the material was also evaluated using energy-dispersive X-ray coupled with SEM (SEM–EDX; JSM 6390; JEOL, Japan). For the SEM–EDX analysis, preparation of samples was carried out by smearing 1 μg sample on the carbon tape (conductive and adhesive nature) mounted on a brass stub and then, the samples were gold or platinum coated (thickness around 5–10 nm achieved within ~60 s) via applying a current of 20 mA using a magnetron sputtering. The deposition of calcium (Ca) and phosphate (P) on the surface of the 1.5 SBF-incubated samples was quantified before and after the bioactivity study using X-ray fluorescence spectrometry (XRF; EDX-720; Shimadzu, Japan).

A FE-SEM image, the particles reveals spherical like TiO_2 (Fig. 8a), nanorod like HAp (Fig. 8b), leaf like GO (Fig. 8c) and leaf and spherical mixed morphology of Nanocomposites (Fig. 8d) (Kavitha et al. 2013d; Rajkumar et al. 2011). In addition, the EDX of all the prepared nano-samples shows the purity and exact material preparation. There is no contamination was found in EDX of all the nano-samples (Fig. 8).

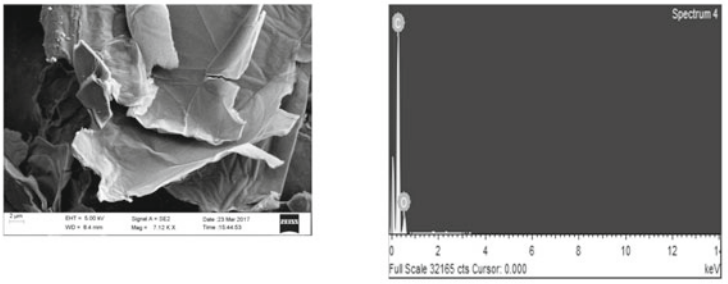
The primary particle sizes of the prepared nanosample are shown in Fig. 9. TEM images confers the exact size of each defined crystallite existed in the particles. In addition, all the prepared particles are diffracted at 50 nm range and the average



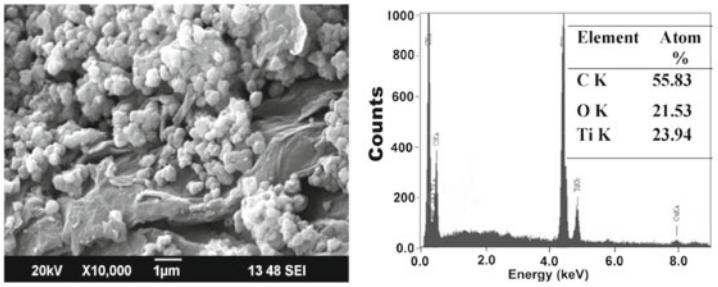
(a) SEM & EDX of nano-TiO_2



(b) SEM & EDX of nano-HAp

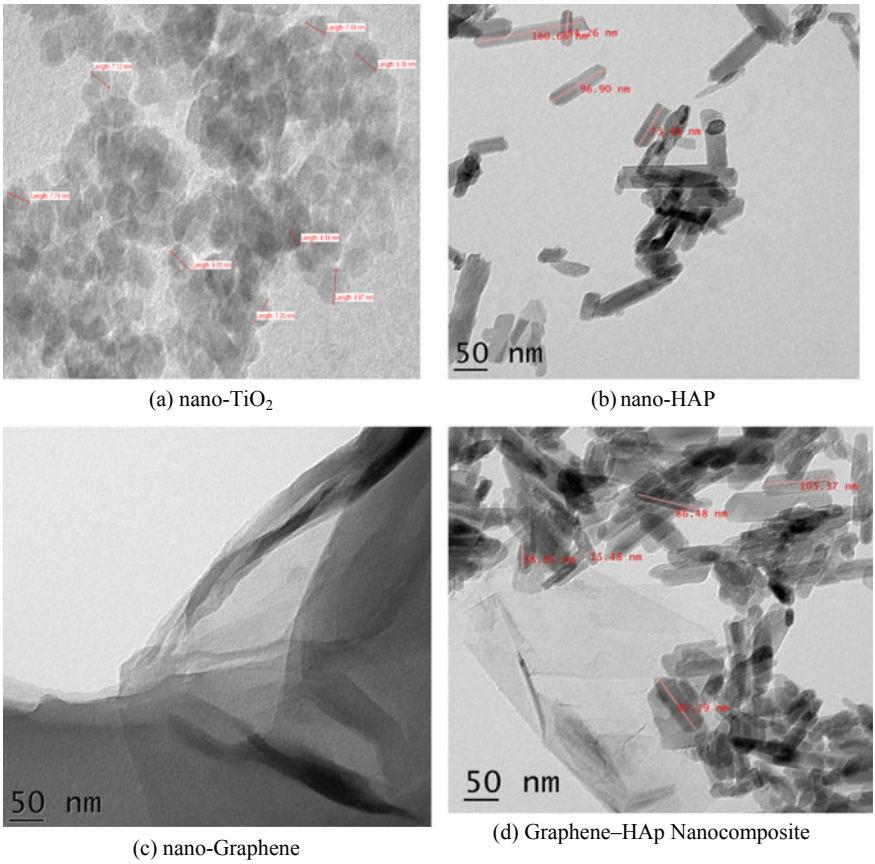


(c) SEM & EDX of nano-Graphene oxide (GO)



(d) SEM & EDX of TiO_2-Graphene nanocomposite

Fig. 8 SEM and EDX of nanosamples



(a) nano-TiO_2 (b) nano-HAP

(c) nano-Graphene (d) Graphene–HAp Nanocomposite

Fig. 9 TEM images of prepared nanosamples

mean particle size is noted. The particle size of TiO_2, HAp and composite is respectively as 3.5 nm, 8.19 nm and 8.86 nm. Moreover, the sintering temperature, rotation speed, pH, precursor material and synthesis procedure were a vital role to determine the particle size and agglomeration of the finer particles (Manivasakan et al. 2010, Kavitha et al. 2013d, Rajkumar et al. 2011; Xu et al. 2006).

6 Brunauer–Emmett–Teller Surface Area Analysis

The specific surface area (SSA) of the samples was calculated with respect to the Brunauer–Emmett–Teller (BET) method (Barrett et al. 1951) using BET Surface Area Analyser (Autosorb AS-1MP; Quantachrome, USA). A well-known isotherm equation for multilayer adsorption was proposed by Brunauer, Emmett and Teller

in 1938. Gas sorption (both adsorption and desorption) at the surface of dry solid powder was commonly used to determine the surface area of the nanomaterials. In this method, the nanoparticles were first heated and degassed under vacuum for 3 h to eliminate the physisorbed moisture. The degassing temperature of the samples depends on the melting and decomposition nature of its own. The physisorption analysis was carried out with N_2 adsorption–desorption measurements under liquid-nitrogen temperature (77 K). A very low working temperature was used to avoid unnecessary thermal damage on the materials surface. Different quantities of gas molecules were adsorbed and desorbed at various amounts of gas. The pore structure (distribution of pore size and its micro/mesopore volume) of the prepared samples was performed by Barrett–Joyner–Halanda method using BET Surface Area Analyser (Manivasakan and Rajendran 2011).

The obtained SSA of the prepared nanocomposites shows TiO_2-graphene at different temperature. In addition, the increase of SSA with respect to higher sintering temperature is shown in Fig. 10 (Kavitha et al. 2013d). The full isotherms of the prepared Nanocomposites at different ratio of TiO_2-graphene: TG.25, TG.5, TG1, TG2, and TG4 samples were found to be 167.98, 186.52, 212.85, 216.04, and 234.56 (Fig. 11) Kavitha et al. 2013b). SSA, total pore volume, and pore diameters were increased with increasing graphene content in the composites. However, the surface area and total pore volume of TGS nanocomposites were initially reduced up to 0.5 g (wt%) of graphene (TG.5), which was due to the total occupation of TiO_2 with low graphene content (Kavitha et al. 2013b). In addition, higher content of TiO_2 tend to collapse the basal spacing of graphene sheet, where confirmed by SEM (Fig. 4) and TEM (Fig. 5). This result was clearly confirmed that TG1, TG2, and TG4 samples exhibited optimal ratio for the improved interactions with the bone-inducing cells. The large surface area and pore size of TG1–TG4 possess three dimensional way of cell growth with healthier cells and nutrients attachment from the body as defined as earlier (Kavitha et al. 2013b, Zhang et al. 2010, 2012, Akhavan et al. 2010).

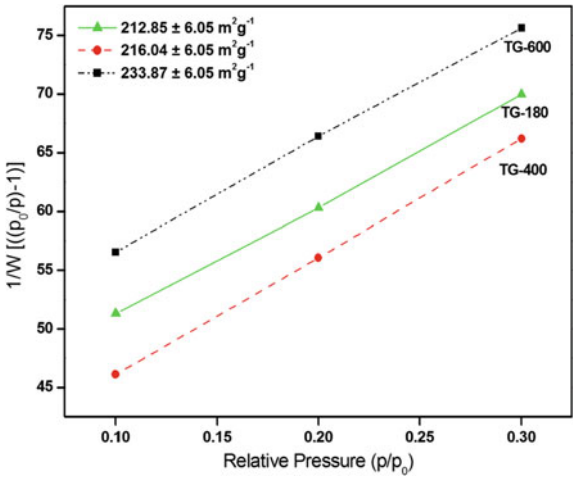


Fig. 10 BET surface area analysis of titania–graphene composites

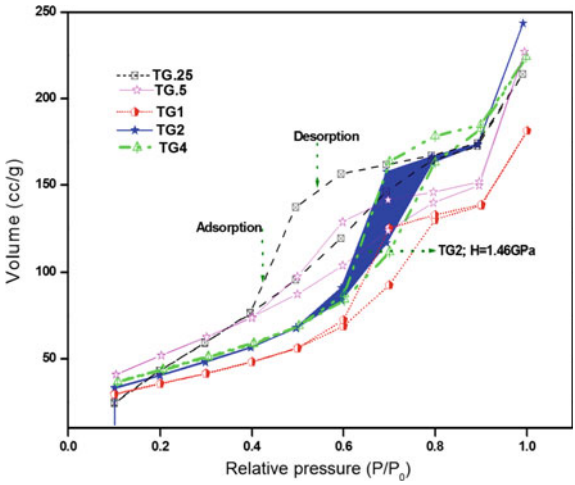


Fig. 11 BET full isotherm of the cure of titania–graphene nanocomposites

7 Mechanical Characterisation

The mechanical properties such as nano-hardness (H) and Young's modulus (E), of the nanocomposites were evaluated by TriboIndenter (Quasistatic nanoindentation, TI-700; Hysitron, USA). The measured force versus displacement curve obtained from the indentation shows the mechanical properties of the materials. Indentation was carried out with a Berkovich pyramidal indenter at a constant load of 1 μN. Both loading and unloading times were keeping consent as 5 s at rate of 200 nm s^{-1} at maximum force of 1000 μN. A small volume of materials with a scan size of 10 × 10 μm was covered during the measurements. The mechanical properties of specimen were obtained using a standard relationship between the applied indentation load (P) and the measured penetration depth (h), as given below (VanLandingham et al. 2001; Yuan et al. 2008):

$$H = P_{\max}/\mathrm{A}(h_c) \tag{2.2}$$

$$E = \sqrt{\pi} \,.\, S/2\sqrt{A(h_c)} \tag{2.3}$$

where, P—applied maximum force, A(h_c)—the area of function, A—area and h_c—contact depth and S—contact stiffness from slope of the loading and unloading curves.

Mechanical testing (H and E values) of the nanocomposite is evaluated by comparison of displacement vs. force plot, using Tibo Scan V8.2 software. The measured nanohardness of pure TiO_2 (1.80 GPa) and TiO_2–chitosan (0.29 GPa) nanocomposites is shown in Fig. 12 illustrated the result of reduction in hardness and Young's

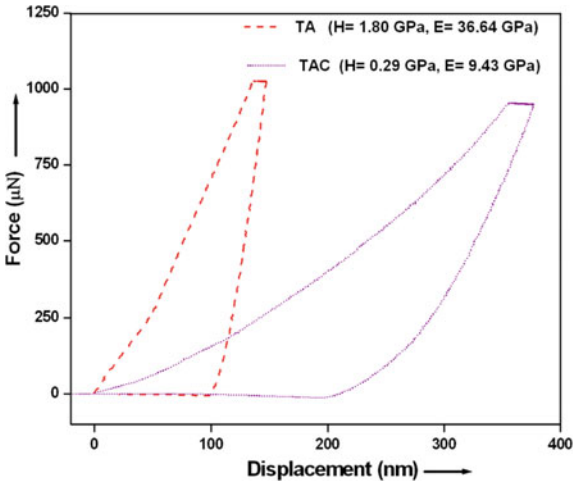


Fig. 12 Mechanical indentation results of titania–chitosan composites

moduli, while adding chitosan to TiO_2. Previous studies were support the fact of prepared composite (Kavitha et al. 2013c; Zhang et al. 2010, 2012; Akhavan et al. 2010) is hard enough to be considered a biomaterial for bone regeneration application.

8 Raman Spectra

Figure 13 illustrated the Raman analysis of TiO_2-graphene composites. The bands at 148, 396, 519 and 639 cm^{-1} were confirmed the presence of TiO_2 (Kavitha et al. 2013d). A non-destructive analysis disclosed the G and D band of graphene, where the bands observed at ~1588 and 1355 cm^{-1} along with the 2D band at 2679 cm^{-1} (Zhang et al. 2010, 2012; Akhavan and Ghaderi 2010; Selvam et al. 2013). The G and D bands intensity were increased with increasing the graphene concentration in the composites. But TiO_2 bands were reduced in composite (influence of the graphene concentration)

The slight bands shift (TiO_2 and graphene bands) towards lower wave number was detected due to the composite formation. Raman spectrum confirmed the presence graphene and the formation of TiO_2-graphene composites. Similarly, Fig. 14 shows the Raman spectra of HAp-graphene Nanocomposites. In Fig. 14, the graphene peaks such as G and D bands and HAp peak at 960–990 cm^{-1} is shown. However, in composite, the HAp peaks are suppressed by domination of graphene peaks.

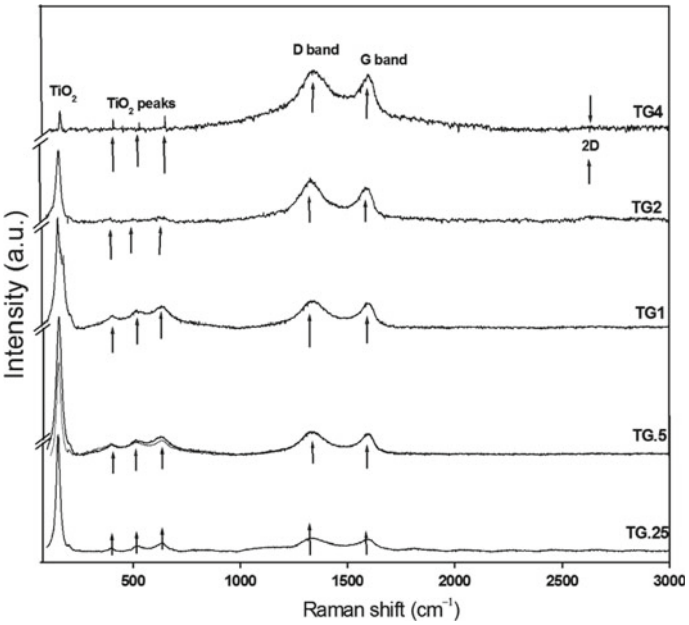


Fig. 13 Raman spectra of the TiO_2-graphene nanocomposites

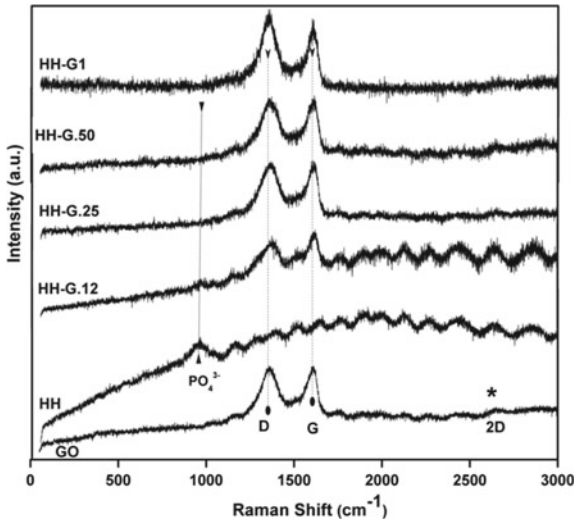


Fig. 14 Raman spectra of the HAp-graphene nanocomposites

9 Conclusion

The characterization techniques are played a significant role to investigate the physico-chemical properties of nanostructured materials. The detailed structural analysis and chemical bonding between the different molecules and surface purity were clearly explained by XRD, Raman, FT-IR analysis. The presence of elements in the synthesized materials was elucidated using EDX, XRF and XPS analysis. Here, confirmation of rGO from GO and the interaction between metal oxides and rGO were investigated. There different types of surface morphologies such as sheet structure of rGO, particles shapes of TiO_2, road like shape of Hap were clearly explained by SEM and TEM analysis. Surface to value ratio of nanomaterials (metal oxides, polymers, carbon and composites) was measured by BET. Nano-Indentation technique was used to determine the mechanical strength of materials (pure TiO_2: 1.80 GPa) and TiO_2–chitosan: 0.29 GPa) nanocomposites) for biomedical application.

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The Importance of Nano-materials Characterization Techniques

Yazan Al Thaher, Balakumar Chandrasekaran and Sarojini Jeeva Panchu

Abstract The potential application of nanotechnology in the medical field ranges from nanomaterials and biological devices, to nanoelectronics biosensors, can be extended to molecular nanotechnology like biological machines. Nanomaterial characterization is a keystone for the development and adoption of nanomaterials for certain applications. The unique and novel physico-chemical properties of nanomaterial gave rise to a number of characterization techniques. Therefore, nanoparticles are characterized to study various physical and chemical features such as composition, structure size, morphology, surface area, optical properties, surface composition, oxidation state, and electrochemistry. The characterization of nanomaterials should not be limited to a single technique, because usually multiple measurements are needed to capture all pertinent nanomaterial characteristics. Hence, in this chapter, details of different characterization techniques such as transmission electron microscopy (TEM), atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray diffraction (XRD), dynamic light scattering (DLS), infrared spectroscopy (IR), and zeta potential (ZP) are discussed.

Keywords Nanomaterials · X-ray diffraction · Transmission electron microscopy · Dynamic light scattering · Zeta potential · Scanning electron microscopy

1 Introduction

Nanomedicine utilizes nanomaterials for the diagnosis, prevention and therapy of various diseases, which has witnessed increasing research interest in recent years. Nanomaterial is defined as the particles having a size between 1 and 100 nm, or at least having one dimension in the range of sub-nanometer to 10 nm (Petros and DeSimone 2010; Duncan and Gaspar 2011). Research on nanomaterials has been conducted in

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many fields such as science, technology and medicine (Webster 2006). Nanomaterials have distinct physicochemical properties in terms of shape, size, composition, surface properties, molecular weight, solubility, and stability. These properties are critical factors determining their physiological behaviour; hence the characterization of nanomaterials is indispensable to confirm the quality and safety during the nanomedicine development process. Some examples of nanomaterials include dendrimers, liposomes, silver nanoparticles, gold nanoparticles, silica nanospheres, quantum dots, fullerenes, carbon nanotubes, carbon nanorods, micelles and solid lipid nanoparticles (Duncan and Gaspar 2011; Mahajan et al. 2013).

It is very imperative to recognize the characteristic features of nanomaterials, because they have unique physicochemical properties that affect the in vivo distribution and behaviour, respectively. The physiological interactions for nanomaterials through tissues in the body may differ from conventional medicines e.g. biodistribution, passage, phagocytosis, endocytosis, renal body clearance. Therefore, it is essential to develop robust and reliable techniques for characterizing nanomaterials, to provide guidance for assessing the safety, quality control and toxicity of nanomaterials (Powers et al. 2006). The characterization of nanomaterials focuses on special parameters not encountered in conventional larger non-nanomaterials. These special parameters include size, porosity (pore size), size distribution, shape, surface area, zeta potential, wettability, aggregation, adsorption isotherm (adsorption potential), impurities, and distribution of conjugated moieties.

There are sophisticated and diverse methods have been employed for the characterization of the nanomaterials. For example, techniques in optical and electron microscopy, light scattering, surface scanning, circular dichroism, zeta-potential, X-ray scattering, magnetic resonance spectroscopy, and mass spectrometry (Sapsford et al. 2011). In this chapter, we presented an overview about the unique physicochemical properties of nanomaterials. In addition, we discussed the principle, applications, advantages and limitations of different modalities employed to explore the physicochemical characteristics of nanomaterials (Table 1).

2 Overview of Physicochemical Properties

2.1 Size

The EU Commission (2011) defined a nanomaterial as one that consists of 50% (by number) or more of particles having a size between 1 and 100 nm, which is primarily based on particle size (EU Commission 2011). Nanomaterial size for a non-spherical particle is generally defined as an equivalent diameter of a spherical particle with equivalent selected physical properties in the same environment (Powers et al. 2006; Shekunov et al. 2007). For example, measuring the hydrodynamic diameter of a particle using Stokes-Einstein equation to calculate the effective size from the diffusion coefficient.

Table 1 Some of the techniques used for the assessment of the physicochemical properties of nanomaterials

Technique	Physicochemical properties analysed	Strengths	Limitations	References
Scanning electron microscopy	Size and size distribution Shape Aggregation and Dispersion	Direct measurement of the size/size distribution and shape of nanomaterials High resolution (down to sub-nanometer)	Dry samples required Conducting sample or coating conductive materials required Biased statistics of size distribution in heterogeneous samples Expensive equipment	Reimer (1998), Aznar et al. (2009)
Transmission electron microscopy	Size and size distribution Shape Aggregation Dispersion	Direct measurement of the size/size distribution and shape of nanomaterials with higher spatial resolution than SEM Several analytical methods coupled with TEM for investigation of electronic structure and chemical composition of nanomaterials	Ultrathin samples in required Samples in non-physiological condition Sample damage/alternation Poor sampling Expensive equipment	Williams and Carter (2009), Chen and Wen (2012)
Atomic force microscopy	Size and size distribution Shape Structure Sorption Dispersion Aggregation Surface properties	3D sample surface mapping Sub-nanoscale topographic resolution Direct measurement of samples in dry, aqueous or ambient environment	Over estimation of lateral dimensions Poor sampling and time consuming Analysis in general is limited to the exterior of nanomaterials	Parot et al. (2007), Tiede et al. (2008)
Dynamic light scattering	Hydrodynamic size distribution	Non-destructive/invasive manner Rapid and more reproducible measurement Measures in any liquid media, solvent of interest Hydrodynamic sizes accurately determined for monodisperse samples Modest cost of apparatus	Insensitive correlation of size fractions with a specific composition Influence of small numbers of large particles Limit in polydisperse sample measures Limited size resolution Assumption of spherical shape samples	Sapsford et al. (2011), Lin et al. (2014)

(continued)

Table 1 (continued)

Technique	Physicochemical properties analysed	Strengths	Limitations	References
Infrared microscopy	Structure and conformation of bioconjugate Surface properties	Fast and inexpensive measurement Minimal or no sample preparation requirement	Complicated sample preparation Interference and strong absorbance of H_2O Relatively low sensitivity in nanoscale analysis	Cantor and Schimmel (1980)
Zeta potential	Stability Surface charge	Simultaneous measurement of many particles	Electro-osmotic effect Lack of precise and repeatable measurement	McNeil (2011), Brar and Verma (2011)
X-ray diffraction	Size, shape and structure for crystalline materials	Well-established technique High spatial resolution at atomic scale	Limited applications in crystalline materials Only single conformation/binding state of sample accessible	Cantor and Schimmel (1980), Wu (2009)

The size of the nanomaterial plays a crucial role in determining its fate in vivo, most importantly determining the enhanced permeability and retention effect and modulating the interactions with biological entities (Tenzer et al. 2011). Also, the size regulates the distribution and navigation of nanomaterials in blood stream and between different tissues, penetration across drug physiological barriers, and even induction of cellular localization and cell specific interactions (Feng 2004; Ferrari 2008; Jiang et al. 2008).

Recently, there has been a rising concern about the safety of nanomaterials and their related toxicity and adverse health effects (Oberdörster et al. 2005; Karlsson et al. 2009). One example on size dependent toxicity, gold nanoparticles (NPs) ranging from 8 to 37 nm caused cytotoxic effects in mice with physiological changes detected in lung, liver and spleen tissue samples, which have not been observed with mice treated with smaller or larger nanoparticles (3, 5, 50, 100 nm) (Chen et al. 2009). Another example, the apoptotic effect of smaller size silver NPs against certain cell lines, and 20 nm silica nanoparticles exhibiting more toxicity than 100 nm silica (Sosenkova and Egorova 2011; Kim et al. 2012; Park et al. 2013). However, the relationship in other types of nanomaterials, with certain chemical composition, between size and its toxicity may not be obvious e.g. titanium oxide and iron oxides (Park et al. 2007; Buzea et al. 2007; Karlsson et al. 2009). The effect of size and shape for nanomaterials on the toxicity profile should be investigated case to case basis, since no consensus on the increased toxicity related to size because of the wide differences in the behaviour of nanomaterials.

Determination of size distribution for a population of particles is done using various methods available, but each has its own optimal size range for measurement (Babick et al. 2016). These methods include, primarily, counting a small number of particles from a population e.g. transmission electron microscope (TEM) and scanning electron microscope (SEM). Secondarily, methods that count the average behaviour of a large number of particles from a population e.g. Dynamic light scattering and X-ray scattering. There are methods that separate the particles based on the size or density prior to determining the size distribution using suitable methods e.g. centrifugation, field-flow fractionation, size-exclusion chromatography (Hodoroaba and Mielke 2015; Zhou et al. 2017). The polydispersity of the sample can necessitate the use of a fractionation method, but the effect of the particle separation process on the nanoparticle dissolution and aggregation need to be investigated, especially for reactive nanoparticles.

2.2 Surface Properties

The interactions of nanomaterial with surrounding species is determined by the characteristics of the surface that is governed by the atomic or physical surface structure (Powers et al. 2006). These characteristics include surface composition, surface energy, wettability, surface charge (Vertegel et al. 2004; Powers et al. 2006). The surface composition and energy are relevant to the dissolution, aggregation and accumulation of nanomaterials. Surface charge is often measured by zeta potential and have potential effect on receptor binding and physiological distribution between barriers. These parameters are intrinsically relevant to the superficial layers but not to the bulk materials. However, characterization of all these parameters together is impractical and determination of these parameters requires independent validation for each nanomaterial system.

Zeta potential is an extensively used surface property for nano-sized particles as the magnitude and sign of charge have a significant effect on the fate, toxicity and interaction of nanoparticles in physiological systems. The passive uptake of charged nanoparticles makes them as useful agents for tumour targeted drug delivery. On the other hand, poly(D,L-lactide-co-glycolide)-formulated nanoparticles containing chitosan are employed for localization and sustaining gene delivery (Baoum et al. 2010). An enhanced cellular uptake was observed for positively-charged nanomaterials than neutral or negatively-charged counterparts (Baoum et al. 2010; Asati et al. 2010; Liu et al. 2011). Polystyrene conjugated nanoparticles with a positive surface charge due to amino-functionality were identified to be cytotoxic through DNA damage mechanism (Liu et al. 2011). In addition, positively-charged silica nanoparticles showed cytotoxicity towards macrophage NR8383 cells (Bhattacharjee et al. 2010). Conversely, silica with higher negativity decreased the cytotoxicity while comparing with silica NPs of the same size exhibiting weaker negative charge (Park et al. 2013).

Determination of zeta potential can be done using various methods in both aqueous and non-aqueous media including electrophoresis, electro-osmosis, electro-acoustic

methods (Delgado et al. 2007; Lowry et al. 2016). The zeta potential can be calculated through measuring the electrophoretic or electroacoustic mobility after suspending the nanoparticles in a suspension medium. However, the zeta potential obtained is a model value rather than a direct measurement, thereby leading to variability and difficulty of comparison between different laboratory values. Moreover, the presence of polymers or polyelectrolytes on the surface of nanomaterial can even complicate zeta potential measurement, and the reported zeta potential should be considered as an "apparent" zeta potential (Lowry et al. 2016).

The dissolution rate of the nanomaterial is an important parameter that can greatly influence its fate and toxicity potential in environmental or physiological fluids (Unrine et al. 2012; Dale et al. 2015). The release of free ions in solution is determined by the nanomaterial solubility which is measured at equilibrium. However, equilibrium is hard to achieve in open biological systems or slowly dissolving materials. The dissolution rate is more relevant for comparing the different processes that led to the build-up of ions in biological systems (Misra et al. 2012; Vencalek et al. 2016). The dissolution rate is dependent on different factors such as pH, ligands, flow conditions, etc., that are particular for each specific system (Vencalek et al. 2016).

Another system-dependent property is agglomeration which is an inherent thermodynamic instability of nanoparticle colloidal suspensions. Agglomeration plays an important role in determining the toxicity, fate of nanoparticles, affects the reactivity, dissolution rate and the hydrodynamic diameter of nanoparticles. Agglomeration relies on particle properties e.g. size and charge and coating type. Also, medium properties affect agglomeration such as pH, ionic strength, composition, particle concentration, etc. Aggregation could happen between particles with the same material (homoaggregation), or to a different material (heteroaggregation), or to stationary surface (deposition) (Hendren et al. 2015).

2.3 Shape

The nanomaterial can play an important role in the transport, delivery and elimination, targeting and internalization (Euliss et al. 2006; Decuzzi et al. 2009). Phagocytosis of drug delivery carriers was highly dependent on carrier shape (Champion and Mitragotri 2009), also the efficiency of drug delivery carriers was highly influenced by controlling the shape of the carrier (Champion et al. 2007). In addition, the in vivo circulation time and adhesion of nanomedicine throughout the circulatory system can be modified by controlling the shape of drug loaded nanomaterials (Geng et al. 2007; Doshi et al. 2010).

The shape of nanomaterial can affect its biological fate, cellular uptake, biocompatibility and retention in tissues and organs (George et al. 2012; Park et al. 2007). For example, the lower toxicity of spherical shaped nickel nanoparticles compared to the dendrimer shaped ones towards zebrafish embryos. Also, the higher toxicity of silver nanoparticles when tested against *Escherichia coli* and Zebrafish embryos compared to the spherical or rod-shaped nanoparticles (Ispas et al. 2009; George

et al. 2012). Therefore, studying the particle shape is frequently performed using electron microscopy. Image analysis softwares (e.g. Image J) are available to count the particles and to determine their shape, as well.

2.4 Composition and Purity

The composition of nanomaterials has a wide variety of constituents because of different structural types approved for production or potential nanomedicines. Structural types of nanomaterials and their derivatives can range from simple nanoparticles, liposomes, micelles, dendrimers, emulsion, quantum dot, fullerenes, carbon nanotubes (CNTs), metals and metal oxides, lipids, proteins, DNA, etc. Composition of nanomaterials defines its properties and affects their toxicity, delivery, transport and biodistribution. Therefore, chemical analysis of nanomaterials is complex but important for nanomedicine medical applications (Etheridge et al. 2013). Many studies addressed the toxicological concerns about nanomaterials of different composition. For instance, the general cytotoxicity observed in quantum dots with metallic complexes consisting of used metals such as selenium and cadmium (Hardman 2006). Another example, is the induced inflammatory neutrophil response of Titanium dioxide when intratracheally instilled in rat and mouse lungs (Sohaebuddin et al. 2010).

2.5 Interaction Between Nanomaterials and Biological Environments

Nanomaterials in biological environment have few undesirable effects such as coagulation, aggregation and non-specific absorption due to various intermolecular interactions occurring between the interface of nanoparticles and biological fluids. (Nel et al. 2009). Intrinsic physicochemical properties of the nanomaterials e.g. shape, crystallinity, composition, heterogeneity, porosity and surface geometry are essential factors to be differentiated before their application to biological systems. However, extrinsic properties (size distribution, dissolution, aggregation and agglomeration) cannot be measured individually in the system because they are governed by other factors related to their presence in different biological environments (French et al. 2009; Nel et al. 2009). For instance, the particle size distribution may vary as particles undergo dissolution over a period of time (Bian et al. 2011). However, the initial size distribution of the nanoparticles can be determined independently. Hence, nanomaterials should be characterized under different physical states, in vivo and in vitro environments.

3 Techniques for Physicochemical Characterization

3.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) uses beams of electrons for imaging, unlike optical microscopy which uses light sources and glass lenses to illuminate specimens to magnify images. SEM employs beam of the accelerated electrons and electrostatic or electromagnetic lenses to give greater depth of field and higher magnification (100,000×). The high energy electron generated by SEM are focused at the surface of solid samples to create a variety of signals, after they interact with sample reflecting the atomic composition and topographical details of the surface. The incident electron beam scanned across the surface of the sample, and an electron detector detects the emitted electrons for different positions in the scanned area. The morphology to the sample surface is recorded as a digital image after displaying the brightness recorded from the intensity of the emitted electrons (Reimer 1998).

Adjusting the SEM measurements and optimizing the analysis depend on the type of electrons emitted. The sample electron emission consists of elastic and inelastic scattering events. Elastic collision of incident electrons with sample atom nuclei causes the ejection of high-electrons, while lower energy electrons are emitted because of inelastic scattering as secondary electrons. These electrons emitted after collisions with the sample nuclei because of the considerable energy difference lost from loosely bound electrons to sample atoms (Aznar et al. 2009).

SEM analysis requires simple sample preparation that include drying and contrasting samples to determine the size, size distribution and shape of the nanomaterials. The sample preparation may cause shrinkage of the specimen and change the characteristics of the nanomaterials. This can only be used for certain biological samples which also can have problems during scanning through an electron beam to acquire charge and deflect the electron beam leading to imaging faults. This can be resolved by coating the biomolecules with ultrathin layer of electrically conducting material (Bogner et al. 2005; Hall et al. 2007). However, the use of new microscopy techniques allows the determination of the topography of nanomaterials without the need for drying of the sample such as environmental SEM, or cryo-SEM. The environmental SEM technique involves the analyses to be performed without coating, drying or freezing for hydrated material samples (Bogner et al. 2005). The environmental SEM allows the samples to be imaged in their natural state without the need for modification, because the sample chamber is operated in high humidity and a low-pressure gaseous environment of 10–50 Torr. Therefore, the charging artefacts can be eliminated and coating samples with a conductive material is no longer necessary (Tiede et al. 2008) While, the cryo-SEM involves careful freezing of the samples and has been used for the characterization of microspheres and nano-emulsions. Cryo-SEM method is often required in electron microscopy to image surface groups attached to NPs, the size of nanomaterial cannot be studied in physiological conditions (Hall et al. 2007). SEM application is only restricted to samples 200 nm in size because

of the low resolution of SEM. Also, SEM has the disadvantage of a destructive sample preparation, preventing its analysis by other modalities. In addition, the small number of sample particles in the scanning region can lead to biased statistics of size-distribution of heterogeneous samples (Bootz et al. 2004).

3.2 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is the most effective tool for the characterization of nanomaterials at spatial resolution from the atomic level (1–100 nm) up to micrometre level, by providing direct images and chemical information about nanomaterials. TEM provide higher resolution than SEM because it uses powerful electron beams, therefore TEM gives greater detail and provides information about crystallinity and granularity of the nanoparticles. TEM is essential in many fields of nanotechnology research including the drug nanocarriers and their morphology before and after drug incorporations at different concentrations (Williams and Carter 2009). TEM images can be generated from incident electrons transmission through the sample. The magnification of TEM is primarily determined by several factors like the ratio of the distance between image plane and objective lens, as well as the distance between specimen and objective lens, respectively. Consequently, the incident electrons interact with the specimen and transform the unscattered electrons to scattered electrons (Williams and Carter 2009).

TEM often can be coupled to a wide variety of analytical techniques for different applications e.g. energy dispersive X-ray diffraction which can be used for investigating the chemical composition of nanomaterials (Tiede et al. 2008). TEM has a high spatial resolution to study the morphology and structure of nanomaterials when compared to SEM, which allows better determination for the size, shape and degree of aggregation and dispersion (Hall et al. 2007). In addition, TEM can be used for the determination of dynamic displacement of nanomaterials in an aqueous environment (Chen and Wen 2012). Wet scanning transmission electron microscopy (STEM) imaging system allows transmission observation of particles totally submerged in a liquid phase. Therefore, the STEM wet mode doesn't require the addition of contrast agents or dyes and allows observation in nanoscale resolution (Bogner et al. 2005).

The use of TEM is accompanied with certain limitations. First, the small sample size can lead to biased statistical size analysis as in any other high-resolution imaging technique to view a small sample section. Another drawback is the 2D images of 3D specimens losing depth sensitivity for the samples. Also, measurement by TEM is a destructive technique preventing further analysis of the same sample by other techniques, where only thin sample section is required for beam penetration. In addition, sample preparation for TEM is a time consuming and may alter the sample structure, where the specimen thickness should be of less than 50 nm in particular case. Finally, TEM samples can be destroyed or even damaged by the exposure to high-voltage electron beams (Williams and Carter 2009). Figure 1 shows TEM images for amino functionalized silica nanoparticles layered with different polyelectrolytes

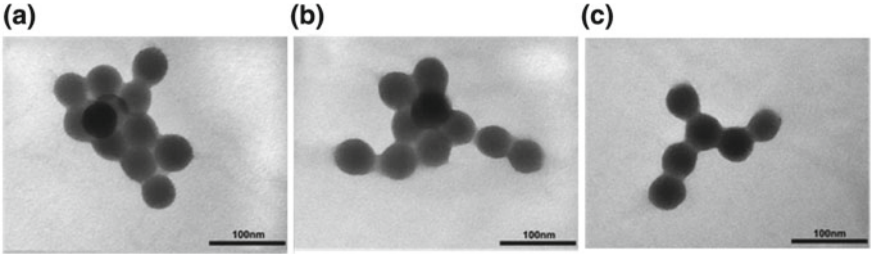

Fig. 1 TEM images for amino functionalized silica nanoparticles layered with different types of polymers adapted from Al Thaher et al. (2018)

and gentamicin as antimicrobial agent (Al Thaher et al. 2018). These nanoparticles have application in the preparation of antimicrobial bone cement used for providing prophylaxis from postsurgical infections (Al Thaher et al. 2017; Thaher et al. 2018), which are layered with different types of hydrolzable and non-hydrolyzable polymers using Layer-by-Layer coating technique (Alotaibi et al. 2018).

3.3 Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) is a surface probe microscopy technique imaging method which includes a micro-machined cantilever usually made of silicon or silicon nitride. The cantilever has a sharp tip from one end for the detection of deflection of cantilevered tip that are caused by van der Waals (vdW) and electrostatic repulsion, along with an attraction between the tip and the atom at the measured surface. The oscillating cantilever scans over the surface of the specimen with demonstrated resolution on the order of fractions of a nanometer (Tiede et al. 2008). AFM can be used for studying nanomaterials shape, size, structure, dispersion and aggregation, like other microscopy techniques e.g. SEM and TEM. There are three different scanning modes in AFM studies including static mode (noncontact mode), dynamic mode (contact mode) and tapping mode (intermittent contact mode) (Sapsford et al. 2011). AFM can be used for the characterization of dynamics between nanomaterials in biological situations and for studying the size and shape of nanomaterials under physiological conditions (Parot et al. 2007).

AFM is capable of imaging a variety of biomaterials at nanometer scale in aqueous fluid, without causing significant damage to many types of native surfaces (Parot et al. 2007). However, one disadvantage of the AFM is that the size of the cantilever tip is generally larger than the dimensions of the nano-surface examined, causing overestimation of the dimensions of the sample (Tiede et al. 2008). The single-molecule force microscopy (SMFM) was developed to overcome the lack of AFM capability to locate specific molecules. In SMFM, the cantilever tip carries a ligand,

a chemical group, or a cell adhesion molecule, which can probe functional molecules on cells surface (Francius et al. 2008).

3.4 Dynamic Light Scattering (DLS)

The size of particles is a crucial factor for determining their properties and safety, especially for polymers and colloids in biological systems. Dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering, is one of the light scattering techniques most widely used to measure hydrodynamic size, shape, structure, aggregation state, and biomolecular conformation (Sapsford et al. 2011). DLS can measure small particle size from one nanometer up to submicron scale in solution or suspension by a monochromatic light source. DLS monitors the fluctuations caused by the Brownian diffusion of spherical particles, where the movement of the particles is linked to an equivalent hydrodynamic diameter. A beam of laser light is focused into a sample solution and the intensity of the Doppler shift of the incident radiation is measured by a photon detector, which is time-dependent on the fluctuations. The Stokes–Einstein equation is used to calculate the particle size which relates the particle diffusion timescale to the equivalent sphere hydrodynamic diameter. This equation accounts on both the viscosity of the solution and the temperature in the sample medium (Lin et al. 2014).

The main advantages of DLS are the short measuring time (few minutes), accuracy in determination of the size for monodisperse particles, measuring samples in different range of concentrations even in diluted samples of high molecular weight species can be measured. Also, the DLS apparatus and running cost is low and more reproducible compared to other methods (Lim et al. 2013). DLS is typically used to study the stability of formulations at different time and temperature variations. Also, for rapid determination of the presence of aggregates in formulations prepared by different procedures in monodisperse samples. DLS allows measurements under physiological conditions, mimicking the in vivo conditions. Therefore, DLS is a useful method for the determination of hydrodynamic size of nanomaterials in biological fluids (Brar and Verma 2011).

On the other hand, there are several disadvantages encountered with the DLS. First, the difficulty to measure size in samples with particular composition, or the presence of aggregates in the sample. Also, the presence of dust particles or small particles in the range of (1 nm–3 μm) which can interfere with the measurement. In addition, measuring heterogenous samples with DLS is limited, because of the difficulty in the analysis for mixed particles population and resolving the data various size distributions. Finally, the DLS assumes a spherical shape for the particles measured which decreases the measurement accuracy for samples with non-spherical nature. Therefore, it is often essential to use complementary size characterization methods (Brar and Verma 2011). Figure 2 shows DLS of nano chitosan peptide that has potential application for treating age-related macular degeneration, the measurements were performed with a Malvern Zeta sizer (Malvern Instrumentation Co, Westborough,

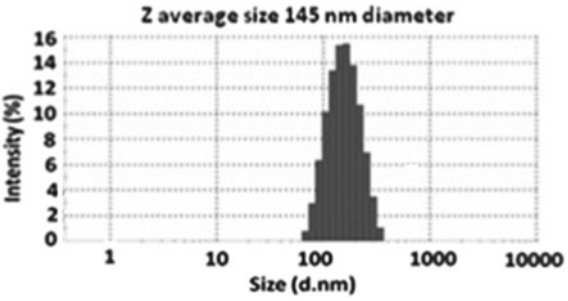

Fig. 2 DLS for chitosan nanoparticles adapted from Jayaraman et al. (2012)

MA). It shows a hydrodynamic size of 145 nm after dispersing the nanoparticles in aqueous medium at 25 °C (Jayaraman et al. 2012).

3.5 Infrared Spectroscopy (IR)

Infrared (IR) radiation absorption occurs when a molecule possesses a time variant dipole moment the frequency of the incident IR light is the same as the molecule oscillating frequency. The energy gained by the absorption of IR radiation induces molecule covalent bond bending, stretching or twisting. The stationary state of molecular vibration is considered the normal mode for the molecule; the vibrations consist of different coupled pairs of covalent bonds or atoms which make a combination of the normal modes. Thus, the IR spectrum revealing absorption (or transmission) versus incident frequency offer a fingerprint for the structure of the studied molecule (Cantor and Schimmel 1980).

Fourier transform infrared (FTIR) spectroscopy is commonly employed for the characterization of nanomaterials, where the certain spectral bands used to study the conjugation of nanomaterials to biomolecules and to reveal the conformational states of bound biomolecules (Tom et al. 2007). FTIR can also be used to study nano-scaled materials, e.g. the confirmation of molecules covalently grafted onto carbon nanotubes. Nanoparticles can be directly studied owing to inherent infrared absorptions or can be characterized by the functional groups on their surface (e.g., hydroxyl and carboxyl groups present at graphene quantum dots). In addition, IR spectroscopy allows the identification of different ligands attached to the surface of nanoparticles according to their vibrational signatures in a non-destructive, rapid and precise manner.

Most commonly, IR spectroscopy has been applied for characterizing metallic nanoparticles produced via a large variety of approaches. IR spectra reveal the vibrational characteristic peaks of the components present in the surrounding media of the nanoparticles. Biological extracts of IR can reveal proteins and metabolites through different functional groups (alcohols, amines, ketones, carboxylic acids and aldehydes). Also, IR spectroscopy has the potential of revealing the surface structure and the

oxidation state of metal-oxide nanoparticles has been confirmed using CeO_2 nanoparticle by exploring the presence or absence of strongly IR absorbing O-H stretching modes associated with O-H-OH complexes present at fully oxidized nanoparticles (Huang and Beck 2015; Yallappa et al. 2015).

3.6 Zeta Potential (ZP)

Zeta potential is an essential particle property that can be rapidly measured using light-scattering techniques. In an ionic solution, oppositely charged ions are firmly bound to the surface of a charged particle, forming a thin liquid layer called the Stern layer. The Stern layer is covered by an outer diffuse layer made of loosely associated ions. The Stern layer and diffuse layer together called the electrical double layer (Brar and Verma 2011). The particle surface is strongly bound to oppositely charged ions (Stern layer) where non-hydrated co-ions and counter ions are adsorbed at the surface. The last layer (the diffuse layer) consists of mobile co-ions and counterions. This layer is associated with the slip plane, which is an imaginary plane that separates immobile ions at the surface from mobile ions in solution. Brownian motion or any external force cause a tangential motion for the charged particle, where the ions in the diffuse layer migrate with the charged particle causing shearing with the ions staying in the bulk outside the layer (McNeil 2011). Zeta potential is defined as the electric potential on the shear surface, which can be measured by the velocity of the charged particles towards the electrode while the sample solution is exposed to an external electric field.

The zeta potential is used to analyse the electronic state of the particle surface, which help in predicting the stability of formulations that include these particles. The interaction between poorly charged or uncharged nanoparticles results in the formation of aggregates, which lead to instabilities (Brar and Verma 2011). The value of ± 30 mV is normally used to assume the stability of nanoparticles; zeta-potential values greater than $+30$ mV and lower than -30 mV are indicative of stable conditions, while values between -30 and $+30$ mV indicate unstable conditions that favour aggregation, coagulation, and flocculation (Sapsford et al. 2011).

Most commonly used method for determining the zeta potential is the electrophoretic light scattering technique that can measure the velocities of many charged particles in liquids. However, this method has problems in precision and reproducibility of the measurement, and sensitive to environmental changes such as alteration of pH or ionic strength. Therefore, measuring zeta potential in diluted solutions may not reflect the real value of concentrated solutions or suspensions (Xu 2008).

3.7 X-Ray Diffraction (XRD)

X-ray diffraction (XRD) is used to analyse the tertiary structures of crystalline or polycrystalline materials at the atomic scale. In XRD, a collimated X-ray beam is directed at the sample, and the scattering intensity and type is detected at specific angles. The detected scattered X-rays reflect the arrangement of the crystalline material, using Bragg's Law: $2d \sin\theta = n\lambda$, where n is an integer, λ is the wavelength, θ is the scattering angle, and d is the interplanar distance. The scattering angle is inversely proportional to the interplanar distance at a determined wavelength (Cantor and Schimmel 1980).

XRD is commonly employed analytical technique for the determination of structure at the atomic scale, orientation, phase, size of the crystals. It is also used for amorphous materials which displays broad diffraction peaks. XRD is an important technique used in pharma-industries for the evaluation of crystalline behaviour, the crystallinity (drugs, excipients and metabolites), drug carriers and the crystallinity phases of contaminants (Wu 2009). Though XRD is a well-established method, it suffers with drawbacks like difficulty in growing crystals and the ability of getting results from single binding state or conformation of the samples (Sapsford et al. 2011). Further, low intensity of diffracted X-rays affects mainly low atomic numbered materials when compared with electron diffractions.

Figure 3 shows the XRD spectrum for iron magnetic nanoparticles performed with a SPECS system (Berlin, Germany) equipped with a Phoibos 150 1D-DLD analyser. It shows the signals for different samples that correspond to different binding energies C1s (284.6 eV), O1s (530.1 eV) and Fe 2p (710.9 eV). The atomic concentrations can be calculated from the XPS results (Kurlyandskaya et al. 2017) (Fig. 3).

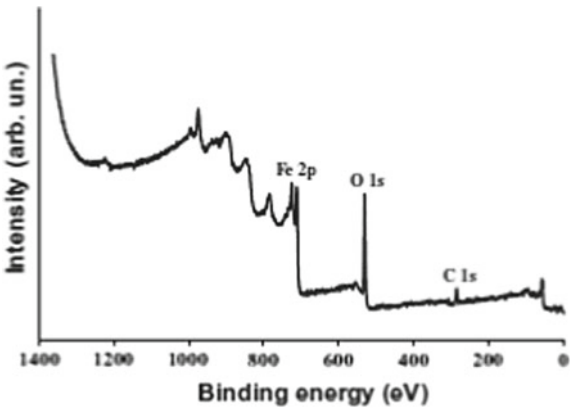

Fig. 3 XRD for Iron magnetic nanoparticles adapted from Kurlyandskaya et al. (2017)

4 Conclusion

Physico-chemical characteristics of nanomaterials have a considerable effect on their potential utility in biomedical field. Thus, it is crucial to determine the characteristics of these nano-biomaterials for their safety and toxicity. Nanomaterials have potential influence on physiological interactions ranging from molecular to systemic level, thereby making the development of nanomedicine for effective delivery of drugs, a promising field of research. The growing production and development of nanomaterials as nanomedicines requires the wisdom and the demand of standard guidelines from regulatory bodies. Rapid, sensitive, precise, valid and robust characterization techniques of nanomaterials are vital to different regulatory guidelines warranting safety of nanomaterials and nano-formulations. Finally, an understanding of individual technique including their strengths and limitations, provides us a clear picture in selecting the appropriate characterization techniques for potential nanomedicines.

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Biohazards of Nanomaterials

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Abstract Nanoparticles (NPs) refer to materials that have a range of 1–100 nm in one or more dimension. These can be sourced from a variety of engineered, targeted materials and in naturally occurring forms. Many of which, have not been systematically evaluated for their hazardous nature. Extensive use of nanotechnology in daily products, as well as drug delivery systems, has lead to their accumulation and proved to be a biohazard. Some studies in the recent past have reported potential toxic effects of these NPs (Silver NPs, Cerium NPs which have proven ecotoxicological impact in the freshwater environment).

1 Introduction

Nanomaterials (NMs) are units or entities that have a range of 1–100 nm in at least one or more dimension. These materials possess unique electronic, optical and mechanical properties. Nano formulation applies to the tools of nanotechnology and its understanding for prevention and treatment of disease, mainly to improve the action of the medicinal agent. It utilizes nanomaterials such as nanoparticles and nanorobots in this regard. Nano-technological products *now a days* have become a routine. Apart from drug delivery, nanomaterials (NMs) have found their way into our lives through different means i.e., skin, inhalation and digestive route and are now being utilized in managing (e.g., antibacterial NPs, metal oxide NPs and carbon based NPs) and in detecting environmental pollution, nano-biosensors uses nanoparticles, nanotubes, and nanowires with bio-recognition materials (Kuswandi 2018). NPs are utilized in treating air pollutants such as volatile organic compounds (VOCs), sulphur dioxide, nitrogen oxide, carbon disulfide, carbon monoxide, atmospheric lead etc. and effectively treat soil pollutants such as mercury, cadmium, lead, copper, chromium

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and trichloroethylene. Water pollutants such as organic pollutants, pathogens and industrial discharge containing heavy metals and its salts, these pollutants cannot be degraded naturally and tend to change the quality of the water (Kühnel et al. 2018; Ibrahim et al. 2016; Ajdary et al. 2018).

Engineered and generated metallic nanoparticles (NPs, like NiO, CuO, Mn_3O_4 nanoparticles), which are generated as a result off spin-off from metallurgical and welding technologies, are one of the most dangerous source of environmental and occupational hazard. Katsnelson et al. (2015), reported the use of bio-protectors to control the adverse effect of nanoparticles in organism using natural methods. These bio-protectors include biochemical, amino acid, different poly unsaturated fatty acid and metal ion like sodium glutamate, apple pectin, "Complivit-Se" (the source of vitamins, Se and Cu); "Complivit–Ca" (the source of vitamins and Ca); Glycine, Acetyl-cysteine and "Eicosavitol" (the source of omega 3 PUFA). High toxicity and genotoxicity of these NPs at low levels has been effectively reduced with the help of these bio-protectors. These bio-protectors aid in relieving the toxic effects by enhancing the natural process of biotransformation and elimination of these toxins. This as a result reduces the dose of toxic substance that are retained in the target organs of organism (which is termed as "toxicokinetic effects") and enhancing the functional reserves at all levels, and increasing and imparting its "toxicodynamic effects" (Katsnelson et al. 2015).

Engineered nanomaterials (ENMs) have tremendous ability to distribute from the point of contact to the secondary organs (like cardiovascular, central nervous, hepatic and renal systems, etc.). The toxicological effects observed in these organs include cytotoxicity, dysfunction of cellular and physiological processes, oxidative stress and inflammation. Insoluble NMs show low translocation as compared to NMs with either slow or fast solubility when assessed for pulmonary exposure. Dermal uptake is relatively less, whereas gut follows the pulmonary pattern of translocation (Kermanizadeh et al. 2015).

Nanomaterials in the cosmetic products are usually in the form of the preservatives, colorants and sunscreen. Nam et al. (2013) studied the genotoxic effect of nanoparticles like the silver, gold, titanium and zinc using SOS chromotest. From their studies, it was hard to assess the toxicity of TiO_2 due to its low solubility at 100 mg L^{-1}, for silver, gold and zinc the maximum NPs exposure range was 3.23, 32.3 and 100 mgm L^{-1}. At this range as evident from the maximum IF values (IF_{max}) which were below 1.5 for all the chemical, the NPs and the ion of the silver, gold and zinc exerted no genotoxic effect in the SOS chromotest. Based on their (Nam et al. 2013) studies theses metals were therefore classified as non-genotoxic.

In 2013, Kumar and Dhawan outlined the involvement of cellular processes in ENMs induced genotoxicity and its role in carcinogenicity. DNA damage leads to mutation through inactivation of tumor suppressor and genome stability of genes which further lead to growth of malignant tumors. They gave a four step approach to test nanomaterial toxicity, which include characterization (NM property in suspension and in powder form), in silico testing (QSAR studies and interaction with genetic materials and protein), in vitro (Effect of NM on target cells, dose response, cytotoxicity, genotoxicity, carcinogenicity and mechanistic studies) and in vivo tests

(validation through bio-kinetics, translocation and long-term studies) (Kumar and Dhawan 2013).

Study on the toxicity of nano sized TiO_2, ZrO_2, Fe°, Fe_2O_3, and Mn_2O_3 on *Saccharomyces cerevisiae* towards the oxygen consumption and the membrane integrity was carried out by Otero–Gonzâlez and his coworkers (2013). The dispersion of the nanoparticles in the bioassay medium was also undertaken. When compared to all the NPs, Mn_2O_3 showed highest inhibition of oxygen consumption (50% O_2 consumption at 170 mg L^{-1}) and damage of the plasma membrane (approximately 30% of cells with compromised membrane at 1000 mg L^{-1}) whereas Fe° caused low toxicity and TiO_2, ZrO_2 and Fe_2O_3 had no toxic effect on the yeast. Use of dispersant, decreases the inhibition caused by Mn_2O_3 at low concentration suggesting that the association of the dispersant with NPs may have influence on the interaction of the NPs with the cell. All the NPs showed high tendency for aggregation in the bioassay medium, the dispersant decreases the aggregation and increases the stability of the NPs like TiO_2, ZrO_2, and Mn_2O_3.

Gebel et al. 2014 carried out a study on *Drosophila melanogaster* an invertebrate to evaluate the effect of metal NPs on its fitness. Copper NPs and micro particles slowed the development process, the sperm competition and adult longevity was reduced. However, NPs were found to be less toxic than the large intake of copper. Silver NP on the other hand were found to be more toxic when assessed for pupal, larval survival rates and its climbing ability. It was found that silver did not affect the adult longevity and the reproductive processes (Gebel et al. 2014).

Juganson et al. developed a database NanoE-Tox based on existing literature on ecotoxicology of eight ENMs with different chemical composition: carbon nanotubes (CNTs), fullerenes, silver (Ag), titanium dioxide (TiO_2), zinc oxide (ZnO), cerium dioxide (CeO_2), copper oxide (CuO), and iron oxide (Fe_2O_3). The database consolidates figures from 224 articles and listed 1,518 toxicity values (EC_{50}, LC_{50}, NOEC). 35% of the data is concerned with eco-toxicity of silver NPs, followed by titanium dioxide, cerium oxide and zinc oxide. The data was originating from studies carried out on crustaceans (26%), bacteria (17%), fish (13%), and algae (11%). Based on the median toxicity values of the most sensitive organism the silver showed the highest toxicity while the iron oxide showed least toxicity. The order of toxicity in descending order is Ag > ZnO > CuO > CeO_2 > CNTs > TiO_2 > Fe_2O_3 (Juganson et al. 2015).

NPs also affect the efficiency of the renal system. The in vitro and in vivo studies proved that the ENMs from metal, carbon and silica exert cytotoxic effects and nephrotoxic effects at tubular and glomerular level. Cytotoxic effects of ENMs include induced oxidative stress, decreased cell viability, mitochondrial or cytoskeleton dysfunction, cell membrane and DNA damage. Tubular nephrotoxic effects include cellular fragments and proteinaceous liquid in the tubule, degeneration of tubular epithelial cell and renal interstitial fibrosis. Glomerular nephrotoxicity include proliferation of mesangial cells, changes in bowman's space and swollen glomeruli (Iavicoli et al. 2016).

EU Project under Nano safety cluster "NANOVALID" evaluated the ecotoxicity of seven well-characterized ENMs (Ag, CuO, MWCNTs, TiO_2, ZnO, SiO_2 and Au).

In four different laboratories the studies were undertaken using 15 different bioassay methods at 100 mg/L concentration.

High dissolution rates of silver, zinc and copper proved them toxic in majority of the assays, which later proved toxic when tested with soluble metal salts. The most sensitive species tested was *Daphnia magna* (towards Ag NMs, 24-h EC_{50} 0.003 mg L^{-1} of Ag), algae *Raphidocelis subcapitata* (ZnO and CuO, 72-h EC_{50}, 0.14 mg L^{-1} of Zn and 0.7 mg L^{-1} of Cu respectively) and murine fibroblasts BALB/3T3 (CuO, 48-h EC_{50} = 0.7 mg L^{-1} of Cu). MWCNTs showed toxicity only towards rat alveolar macrophages (EC_{50} = 15.3 mg L^{-1}) assumingly due to high aspect ratio and TiO_2 towards *R. subcapitata* (EC_{50} 6.8 mg L^{-1} of Ti) due to agglomeration of TiO_2 and entrapment of algal cells.

Four in vitro (eco) toxicity assays recommended are

- 48-h *D. magna* immobilization (OECD202),
- 72-h *R. subcapitata* growth inhibition (OECD201),
- 30-min *Vibrio fischeri* bioluminescence inhibition (ISO2010) and
- 48-h murine fibroblast BALB/3T3 neutral red uptake in vitro (OECD129) representing crustaceans, algae, bacteria and mammalian cells, respectively.

The results proved efficient for toxicity evaluation and shortlisting of hazardous NMs (Bondarenko et al. 2016)

Kim et al. (2015) studied the role of secondary pollutants. The workforce employed in production of engineered nanomaterials (ENMs) should be skilled in storage and handling of these chemicals, as the chemical rooms located near ENMs proved to be unintentional nano-aerosol generators. Mobile or stationary state measurements and on-line chemical analysis was used to identify the source of these nanoaerosols. The nanoaerosols present in storage rooms were much higher than the ENMs workplace. Particle formation occurred via gas-to-particle conversion of oxygenated VOCs and accumulated precursors. They emphasized the importance of secondary pollutants as particulates generated from liquid and gaseous phase of these hazardous chemicals. The rate of generation of these spherical nanoaerosols was estimated to be 63.8 particles $cm^{-3}s^{-1}$.

2 Nanomaterials

2.1 Titanium Dioxide

Titanium dioxide (TiO_2) NPs are among the most or are one that is used maximum in the consumer products (such as paints) and in the pharmaceutical products. Widespread use of such products has led to the presence and release of this into the environment. In humans, the major route of exposure to TiO_2 NPs is through skin and inhalation. Increase in the presence of TiO_2 NPs in the environment may pose serious threat to nonspecific organisms, like the bacteria. It may have some harmful

effect on the aquatic organisms (Gupta et al. 2016; Nam et al. 2013; Shukla et al. 2014; Elgeti et al. 2018; Demir et al. 2015; Tavares et al. 2014; Valdiglesias et al. 2013; Shukla et al. 2011; Sario et al. 2018; Zou et al. 2014; Shukla et al. 2013; Otero-González et al. 2013).

Shukla et al. (2011) reported the cytotoxic effect of TiO_2 NPs on human epidermal cells (A431). Cytotoxicity was assessed by MTT and NR uptake assays after 48 h of exposure. It was evident that genotoxic potential amplified ROS levels and oxidative stress leading to oxidative DNA damage and micronucleus formation.

- Fpg-modified Comet assay carried out with 0.8 $\mu g\ mL^{-1}$ titanium dioxide NPs and higher concentrations for 6 h showing significant induction in DNA damage (2.20 ± 0.26 vs. control 1.24 ± 0.04).
- Micronucleus formation increased significantly (14.67 ± 1.20 vs. control 9.33 ± 1.00).
- Reduction in glutathione levels (15.76%) with a related increase in lipid hydroperoxide (60.51%; $p < 0.05$) and reactive oxygen species (ROS) generation (49.2%; $p < 0.05$) after 6 h of exposure (Shukla et al. 2011).
- Immunoblot analysis revealed an increased expression of p53, BAX, Cyto-c, Apaf-1, caspase-9 and caspase-3 and decreased the level of Bcl-2 thereby indicating that apoptosis induced by TiO_2 NPs occurs via the caspase-dependent pathway.
- TiO_2 NPs induce DNA damage and cause apoptosis in HepG2 cells even at very low concentrations (1 $\mu g\ mL^{-1}$). Hence the use of TiO_2 NPs should be carefully monitored (Shukla et al. 2013).

Kumar et al. also studied the effect of TiO_2 NPs in *Salmonella typhimurium* exhibit normal and uniform distribution without aggregation. The treatment groups contained TiO_2 NPs in the concentration range of 8–8000 ng mL^{-1}. Results indicate that the uptake of NPs at concentration range of 8–80 ng L^{-1} increased after 1 h. Formation of micelles and protein coat facilitate the entry of NPs. TiO_2 NPs showed weak mutagenicity in TA1537 and TA98 strains and *E. coli* (WP2uvrA) in Ames test reducing the carcinogenic potential of these NPs (Kumar et al. 2011a). A significant decrease in *E. coli* viability was observed by flow cytometry live-dead discrimination assay and plate count method, which was concentration dependent. Electron microscopy and flow cytometry also emphasized that the internalization was without agglomeration. The TiO_2 NPs showed significant DNA damage after ENM treatment. Oxidative stress leading to genotoxicity was demonstrated by increasing levels of malondialdehyde, ROS, lactate dehydrogenase, hydroperoxide ions (Kumar et al. 2011b).

Valdiglesias and his coworkers (2013) investigated the effect of TiO_2 NPs on human neuronal cells (SHSY5Y) at a conc. of 80–150 $\mu g\ mL^{-1}$. The NPs effectively internalized in a dose–dependent manner but did not reduce the viability of neuronal cells. The effects did not led to any oxidative damage (Valdiglesias et al. 2013).

TiO_2 NPs at a dose of 100 mg/kg body weight administered to mice orally for 14 days to assess their toxic effects. Liver cells showed significant oxidative stress. Additionally, an intrinsic pathway of apoptosis activation suggested increased expression of BAX, p53, Caspase 9 and 3 and decreased expression of anti-apoptotic Bcl-2

protein. High accumulation in liver will lead to DNA damage and apoptosis through intrinsic pathway (Shukla et al. 2014).

A study was conducted on the coexistence of TiO_2 NPs and Silver NPS in aquatic environment on ciliated protozoans (*Tetrahymena pyreformis*) as an animal model. The release of silver ions as well as their effect on the oxidative biomarkers studied.

- 12000 lx continuous illumination lead to a 20% decrease in silver ion release when compared to dark conditions.
- Continuous illuminations in presence of TiO_2 NPs lead to a 64.3% decrease in silver ion concentration in comparison to plain AgNPs suspension.
- Presence of TiO_2 NPs at concentration of 1.5 mg L^{-1} (18.8 μM) reduced the toxicity of 1.5 mg L^{-1} (13.9 μM) AgNPs by 28.7% via adsorption of silver ions on TiO_2 NP surface in natural light and increased by 6.93% at 12,000 lx of continuous light due to formation of activated TiO_2-Ag NP complex.
- SOD activity increased in light controlled samples by 1.96 times as compared to dark controls.
- TiO_2 NPs resulted in decrease in CAT activity by 36.1% in light.
- TiO_2 NPs reduce the risk of Ag NP in natural light, but in continuous light it enhances the environmental risk of Ag NPs (Zou et al. 2014).

Demir et al. (2015) evaluated the in vitro genotoxic effect of TiO_2 (21 and 50 nm) and TiO_2 MPs on Mouse embryonic fibroblast (NIH/3T3) and human embryonic kidney (HEK293) cells for soft-agar anchorage independent cell transformation ability.

- Comet assay when complemented with FPG enzyme showed no induction of oxidative DNA damage.
- Cell-transformation promoting cell-anchorage independent growth in soft-agar was induced on long term exposure to TiO_2 NPs.
- TiO_2 MPs showed negative results.
- Higher doses (1000 μg mL^{-1}) resulted in genotoxic/transforming effects, but high dose reduces the risk of environmental toxicity (Demir et al. 2015).

Sario et al. studied the effect of TiO_2 NPs on *Drosophila melanogaster's* development and DNA damage. 72 h survival assay reported no adverse effect but genotoxic effects were visible at 8 μg mL^{-1} as assessed through eye-spot SMART assay (Sario et al. 2018).

2.2 Zinc Oxide

The consumer products that includes cosmetics, food packaging, imaging, biosensor and pharmaceutical products are flooded with Zinc oxide NPs. In this, way human and plants are more prone to the exposure of ZnO NPs. These ZnO NPs administered through different routes (inhalation, dermal or oral) (Patel et al. 2016; Senapati et al.

2015b; Kumar et al. 2015; Kwon et al. 2014b; Demir et al. 2014; Nam et al. 2013; Sharma et al. 2012b; Du et al. 2018).

Liver acts as the primary metabolizing organ and acts as a target after the NPs enter the body.

- Evaluation on Human liver cells HepG2 for its genotoxic and apoptotic potential, and molecular mechanism of cellular toxicity assessed.
- Decrease in cell viability and apoptosis observed on incubation of HepG2 (12 h) with ZnO NPs (14–20 $\mu g\ mL^{-1}$).
- Oxidative stress mediated DNA damage occurred as revealed by increase in Fpg sensitive sites.
- ROS triggers a decrease in MMP and increase in Bax/Bcl2 leading to apoptosis.
- ZnO NPs induced p53 phosphorylation, activated JNK and p38 pathway (Sharma et al. 2012b).

Oral toxicity of ZnO NPs was studied by Sharma et al. and reported

- Accumulation of NPs occurred in liver after sub-acute oral exposure (300 mg/kg for 14 days) led to cellular injury.
- An increase in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) serum levels, and observed pathological lesions in liver.
- Lipid peroxidation induces oxidative stress.
- Increase in the Fpg-specific DNA lesions in liver due to the DNA damage as evident from Comet assay.
- apoptosis in the liver of mice that was revealed by the TUNEL assay (Sharma et al. 2012a).

Human lymphoblast cell line TK6 utilized to assess the genotoxic potential of ZnO NPs ($\leq$35 nm and 50–80 nm).

- Comet assay revealed genotoxic reactions at a concentration of 100 $\mu g\ mL^{-1}$ in the selected samples.
- Percent DNA in tail was higher when exposed to 35 nm particles.
- ZnO NPs (50–80 nm) induced significant net oxidative DNA damage as shown by treatment with endonuclease III and Fpg.
- Inhibition of DNA damage at early stage of exposure.
- MP induced DNA damage was repaired faster than NP induced damage (Demir et al. 2014).

Observations on the effect of NPs on healthy individuals and patients suffering from lung cancer, asthma and chronic obstructive pulmonary disease (COPD) studied to evaluate the cellular pathways in the lymphocytes.

- A significant dose dependent increase in expression of tumor suppressor protein p53 (40, 60 and 110%), Ras p21 (30, 52 and 80%), c-Jun N-terminal kinases (JNKs, 28, 47 and 78%) in lung cancer patient samples.
- In COPD patients a similar trend was observed, an increase p53 (26, 45 and 84%), Ras p21 (21, 40 and 77%), JNKs (17, 32 and 69%) was observed after 6 h of ZnO ENPs (Kumar et al. 2015).

To understand the principle and molecular mechanism involved in immunotoxic response, Senapati et al. (2015b) used Human monocytic cell line (THP-1) as model. They used Comet and micronucleus assays to assess the NP induced DNA damage.

- Increase in ROS and pro-inflammatory cytokines (TNF-α and IL-1β) with a concentration dependent (0.5–20 μg mL^{-1}) decrease in glutathione levels.
- The expression levels of mitogen activated protein kinase (MAPK) cascade proteins such as p-ERK1/2, p-p38 and p-JNK were also significantly induced.
- ZnO NPs induce oxidative and nitrosative stress in human monocytes, leads to increased inflammatory response via activation of redox sensitive NF-κB and MAPK signaling pathways. (Senapati et al. 2015b).

2.3 Gold Silver

NPs of gold and silver are increasingly being used now-a-days in cosmetic products such as sunscreens, colorants and preservative due to their antimicrobial properties. Pollutants affecting species at the population level generate ecological instability in natural systems. The success of early life stages, such as those of aquatic invertebrates, is highly affected by adverse environmental conditions. Gold nanoparticles exert toxicity with DNA damage, accompanied by ROS production, but they do not inhibit yeast growth and viability. Diverse exposure patterns of gold NP causes multigenerational nano-toxicity. Silver released into the environment from emerging nanotechnology represents such a threat. Sediments are pools for numerous pollutants, which aggregate and/or associate with depositing suspended particles (Nam et al. 2013; Ávalos et al. 2018; Moon et al. 2017; Moretti et al. 2013; de Alteriis et al. 2018; Elgeti et al. 2018; Bastos et al. 2017; Yang et al. 2014; Oliver et al. 2014; Masrahi et al. 2014; Martirosyan et al. 2014; Ivask et al. 2014; García-Alonso et al. 2014; Nymark et al. 2013; Schlich et al. 2017).

Gold, silver and zinc NPs classified as non-genotoxic from the studies carried out on *E. coli* by Nam et al. (2013) using SOS chromotest. In vivo at concentration of 500 μM Gold and silver NPs had no harmful effects (Moretti et al. 2013).

Genotoxic evaluation of PVP-coated AgNPs showed no chromosomal damage at the early stage that may be due to the reduction in the ion leaching or aggressive agglomeration, which reduces the cellular uptake of AgNPs (Nymark et al. 2013)..

The antibacterial properties of the silver NPs make it an ideal candidate for most of the consumer products. The antibacterial property is due to the release of silver ion.

In this study, they used a complete genome library of *E. coli* consisting of approximately 4000 single gene deletion mutant to explain which one of the physiological pathways are involved in *E. coli* in its response to different silver nanoparticles.

- 9-43.5 nm NPs and a potential range of −10 mV to +33 mV was studied. In their study they used 9-43.5 nm NPs with −10 mV to +33 mV potential range

- Silver NPs with size of 9 nm exhibited a response like silver ion.
- Silver NPs with functionalized polyethyleneimine.
- Overall, the bacterial response pathway depends upon physicochemical properties of the NPs, especially the membrane potential (Ivask et al. 2014).

There are large number of literature present on the development of marine annelids like *Platynereis dumerilii* and is considered as an ideal model to study the exposure in coastal environments. García-Alonso and his coworkers exposed the eggs, larva, juvenile and adults of *P. dumerilii* to different concentrations of citrate silver NPs, humic acid silver NPs, capped silver NPs and silver nitrate as dissolved silver.

- Larvaes and eggs showed high mortality and abnormal development, fertilized eggs were sensitive to all formulations which indicates that early stages of marine coastal organisms are mostly affected by silver NPs.
- The adult and the juvenile stages were the most tolerant stages.
- Humic acid silver NPs showed high toxicity response and the uptake of humic acid Ag NPs was also higher in *P. dumerilii* than any other forms. Silver NPs were more toxic than Silver nitrate (García-Alonso et al. 2014).

Natural organic matter (NOM) play an important role in the aquatic environment by altering its quality and it also affect the behavior of NPs. Nematodes like *Caenorhabditis elegans* on exposure to Ag NP suspensions with or without NOM of two kind Suwannee River and Pony Lake fulvic acids (SRFA and PLFA, respectively) showed that PLFA had a better response towards toxicity then SRFA. Moreover, the total silver content in the tissue indicated that PLFA reduced the uptake of silver ions by the organism but not the uptake of citrate coated silver NPs. PLFA reduced total uptake of silver ions but not of citrate coated silver NPs. NOM–Ag NP composites were formed in medium and in nematodes on co-exposure to PLFA and prevented the $AgNO_3$- and CIT-Ag NP induced cellular damage (Yang et al. 2014).

In vitro cytotoxic studies on human spermatozoa using gold and silver NPs undertaken by Moretti et al. 2013. In their experiment, they incubated semen samples with 30–500 μM Gold/Silver NPs. Eosin Y test was used to determine the sperm viability and WHO guidelines was fallowed to assess the sperm motility

- The toxic effect of the silver NPs on the human spermatozoa motility and viability was found to be dose dependent and the silver NPs showed higher toxicity which was not significant
- It was found that the silver NPS was not detectable while the gold was confined to the spermatozoa only
- As cited by (Moretti et al. 2013), even at higher concentration of (250–500 μM) the silver and the gold NPs had no harmful effect the human spermatozoa.

Oliver et al. in 2014 studied the bioaccumulation and toxicity of silver in fresh water snail after the dietary exposure to the AgNPs coated with polyvinyl pyrrolidone (PVP) and evaluated the effect of the water hardness and presence of humic acid on the uptake of Ag by the soft tissue, which would affect the physiology. The results indicated that water hardness and the presence of humic acid did not affect the

bioaccumulation and the toxicity of Ag after dietary exposure. However, it effected significantly on membrane interaction and on the transformation of NPs. Absorption of the silver NPs in the freshwater snails was more efficient when mixed with diatoms. The experimental results indicated that the quality of water or controlling the quality was of least importance, as it had no effect on the bioaccumulation. Whereas the dietary exposure played an important role in the bioaccumulation.

The use of silver NPs in the consumers products is increasing sue to its antibacterial properties. Much of these AgNPs make their way into the wastewater streams, out of which 10% of them ended up in the aquatic ecosystem with unknown hazardous effect on the ecological system. Colman et al. in 2014 reported the effect of AgNPs on the aquatic ecosystem, he compared the effect of two AGNPs size (12 and 49 nm) to ionic form of silver

- They used 19 wetland mesocosms and added Ag to 360 L compartments to reach the Ag to 2.5 mg L^{-1}. Silver treatments with two coated controls in triplicates and compared to four replicate controls.
- Silver treatments were toxic to aquatic plants releasing significant quantities of dissolved chloride and organic carbon.
- Dissolved methane concentrations rose to 40-folds relative to control in all three silver treatments (Colman et al. 2014).

The physico chemical properties of the test media, affects the dissolution and aggregation of the NPs like silver, copper, Zinc and its toxicity to *Daphna magna*. The acute toxicity to Daphna *magna* was highest in the moderately hard water EC50 values of 1950, 4.94, 980 μg/L. for Ag, CuO, and ZnO, respectively. From their studies, they concluded that the toxicity of Ag NPs may be accounted to both the particulate and dissolved fraction whereas the toxicity of copper and zinc NPs was accounted to the dissolved fraction (Seo et al. 2014).

Martirosyan et al. (2014) investigated the effect of acute toxicity of Ag NPs with less than <20 nm alone and on co-administration with food matrix component phenolic compounds (PCs) on the cell based models of the gastrointestinal tract to assess the potential human health complications. In their study, they used co-culture model of Caco-2 and RajiB cells for accurate simulation of the gastrointestinal tract.

- Silver NPs at 40 μg mL^{-1} (EC_{50}) were cytotoxic to Caco-2 cells, and silver NPs led to oxidative stress starting from ca 45 μg mL^{-1}.
- Silver NPs lead to opening of tight junctions as demonstrated by immunofloresecence staining.
- Quercetin and Kaempferol protected the caco-2 cells from NPs induced toxicity and maintains the epithelial barrier integrity, disrupted by NPs. Resveratrol showed no protective effect (Martirosyan et al. 2014).

In a study carried out by Masrahi et al. (2014), the impact of silver (silver ions, silver NPS, and PVP coated silver NPs) on nitrification of soil process using soil slurry nitrification method with dissolution and sorption isotherms reported that the Ag (+)/Ag – NPs strongly sorbs in the soils and suppressed the nitrification process. At the same dose, the PVP coated silver NPs suppressed the nitrification processes

more significantly when compared to Silver ions. Whereas the toxicity of coated NPs could not be assessed (Masrahi et al. 2014).

Use of sewage sludge as fertilizers on agricultural lands has led to the significant increase of NPs in the soil over decades Schlich et al., investigated for over a period of 25 months the ecotoxicity and fate of silver nanomaterials with desired environmental condition in outdoor lysimeters. In their study, they applied two silver nanomaterial with 1.7 and 8 mg/kg dry matter through sewage sludge into the soil. Comparison between the laboratory samples and the outdoor samples every 180 days revealed that in long term the sludge applied AgNM remained immobile between the soil and leacheates. Uptake of the Ag NM by the root suggest that the chemical condition in the rhizosphere caused the remobilization of Ag NM applied in sludge. At higher concentration silver nanomaterials inhibited the microflora in the soil. Whereas at lower concentration it did not produce any effect.

Silver NPs also lead to reduction in development as evidenced by larval, pupal survival rates and larval climbing ability but Silver NPs had no effect on longevity and reproduction rates on *Drosophila melanogaster*. Larva treated with silver NPs had reduced gut microbiota diversity with rise in *Lactobacillus brevis* and decrease in acetobacter when compared to control or silver MPs treated groups. Therefore change in microflora of gut in a metazoan model may contribute to NP mediated toxicity, and proves that these metal NPs prove to be an emerging contaminant (Han et al. 2014).

A study was carried out by Raj et al. to elucidate the effect of Silver NPs on behavior and metabolism of *Drosophila melanogaster* reared on food with and without silver NPs, and from their study they suggested that

- When the larva was feed with silver NPs it showed abnormal behavior—such as poor crawling and inability to climb (in both adult and larva).
- Ingestion of large amount of Ag NPs at larval stage affected the metabolic activity significantly. There was decrease in lipid droplets, lipid droplets are the organelle in drosophila that store lipid in it.
- It also caused the increase in the ROS in larval tissue.

The above results are of concern as the AgNPs are more commonly used in consumer products (Raj et al. 2017).

2.4 Silica NPs

Silica dioxide (SiO_2) and amorphous silica has been used in various industrial products, including paints and coatings, plastics, glass, synthetic rubbers and adhesives. Synthetic amorphous silica (SAS) is used in pharmaceutical tablets and as additives in processed foods. Intake of SAS in food is estimated as 9.4 mg/kg/day of which 1.8 mg kg^{-1} day^{-1} is in nano size range (5–200 nm) (Ajdary et al. 2018; Holden et al. 2014; Kühnel et al. 2018; Kuswandi 2018; Demir and Castranova 2016; Hashimoto and Imazato 2015).

Genotoxicity assessment of 20 and 100 nm negatively charged SiO_2 NPs was carried out by Kwon, Kim et al., as per OECD guidelines. 4 tests were carried out for genotoxicity evaluation i.e., bacterial mutation assay, in vitro chromosomal aberration, comet assay and micronucleus test. The results showed that the selected NPs possess no genotoxicity in vitro as well as in vivo conditions. No statistical differences were observed in any of the tests. After evaluation of test results the authors claim that different exposure routes can induce genotoxicity in various organs. (Kwon et al. 2014a).

Rats exposed to oral dose of synthetic amorphous silica (SAS) and NM-202 (nanostructured silica for OECD testing) at 100, 1000 and 2500 mg/kg/day for 28 days or highest dose of SAS for 84 days.

- Intestinal content of mid- high dose groups possessed gel like properties when compared to low dose groups.
- Exposure to both the forms for 28 days showed no elevated tissue silica levels, neither the biochemical markers in blood nor the isolated cells showed toxicity.
- After 84 days of SAS exposure, silica was found to be accumulated in spleen, and in liver fibrosis was observed in NM202 treated group (Van der Zande et al. 2014).

RAW264 cells were evaluated for biological response against aluminum (13 nm) and silica NPs (12 nm). The cultured cells exposed to NPs. Hoechst/PI apoptosis assay and WST-8 cell viability for comet assay using confocal microscopy and genotoxicity for micronucleus analysis showed.

- Nuclei and DNA damage as observed through immunostaining genotoxicity testing.
- Cytotoxicity and genotoxicity are interrelated in the study.
- NPs can be observed as aggregates in vesicles, but nonexistent NP internalization can be seen in nucleus or cytoplasm.
- The morphological changes can be attributed to ionization of aluminum and silica NPs i.e., chemical change due to low pH of the vesicles.
- Concentrations over 200 $\mu g\ mL^{-1}$ are enough to induce genotoxic and cytotoxic effects (Hashimoto and Imazato 2015).

Gene mutation approaches (in mammalian cells) used for genotoxicity assessment. SAS NPs (7.172 and 7.652 nm) in fine colloidal form were taken for study on mouse lymphoma assay (L5178Y/Tk $\pm$) to evaluate the mutagenic effects of different concentrations of NPs (0.01–150 $\mu g\ mL^{-1}$). SAS NPs show concentration dependent mutagenicity. Induced mutant frequency (IMF) of SAS NPs (7.172 nm), SAS NPs (7.652 nm) and colloidal SiO_2 were 705.5×10^{-6}, 575.5×10^{-6} and 57.5×10^{-6} respectively. These results prove genotoxic potential of NPs (Demir and Castranova 2016).

Rats treated with Silica NPs at 50 mg/kg body weight (b.w.) caused DNA damage in liver that was small and statistically insignificant which was measured by standard comet assay, whereas an significant increase in the damage was observed at 4 h by the hOGG1 comet assay, which was constant with the mode of action of involving ROS. Histopathological studies on the liver indicated damaged and the involvement of the

white blood cells like the neutrophils. Further the genomic analysis and response pattern of the key genes involved in the inflammation and oxidative stress also supported the damage of the tissue mediated through the inflammatory response along with phagocytizing the damaged cells. At low dose of 5 mg kg^{-1} b.w. of silica NPs no changes were observed either histopathological or in gene array. The result indicated that the DNA damaged observed was due to secondary inflammatory response (Pfuhler et al. 2017).

2.5 Copper NPs

Copper NPs find their way into the ecosystem through waste waters containing ENM from consumer goods as they are present in various articles and proper disposal is necessary as it affects the water treatment processes. The toxicity in the aquatic environment is also affected by the nature and composition of the water body. Aquatic invertebrates are mostly affected by these pollutants which affect their development and production and their average life span. Mostly studies are carried out in simulated media which rule out presence of other natural constituents which affect the testing and toxicity results as reported in various studies (Stevenson et al. 2017; Katsnelson et al. 2015; Elgeti et al. 2018; Handral et al. 2016; Ajdary et al. 2018; Heinlaan et al. 2016; Kwon et al. 2014b; Du et al. 2018; Naasz et al. 2018; Kuswandi 2018).

Drosophila melanogaster an invertebrate was assessed for copper NPs and MP toxicity by Han et al. (2014). The results reveal that Copper NPs and MPs significantly slowed the development, and reduced longevity as well as sperm competition. Copper NPs had relatively lesser toxicity than its bulk counterpart (Han et al. 2014).

Presence of copper NPs, MPs and copper hydroxide-based fungicide affected the proper functioning of the septic tank which was regained after three weeks post exposure. The study proved that presence of copper in the septic tank lead to improper removal of the total organic carbon, and changes in pH which are not optimal. The septic system analysis included changes in phenotype and genotype of the microbial community in the septic tank and water quality evaluations.

- Copper NPs lead to 63% reduction in biological oxygen demand (BOD) which is normal.
- Reduced pH below optimum anaerobic fermentation range, suggesting incomplete degradation of organic waste.
- Copper fungicide increased the total organic carbon (TOC) to 57%, above typical range and leads to increased BOD.

Changes in TOC and BOD signify improper functioning of the septic system in presence of copper NPs (Taylor and Walker 2016).

A NanoValid program was carried out by EU FP7 to evaluate the effect of NPs in natural freshwaters silver and copper were selected as model NPs due to their high toxicity in freshwater. Freshwater was collected from Switzerland (Lake Greifen and

Lake Lucerne), OECD 202 and 236 *Daphne magna* and *Danio rerio* embryo assays were use to evaluate toxicity.

- Dissolution was evaluated through ultra-filtration, ultracentrifugation and metal specific sensor bacteria.
- Copper NPs aggregated and settled fast as compared to silver NPs which remained stable.
- Silver NPs were found to be more toxic to *D. magna* and *D. rerio* embryos (48 h EC_{50}1–5.5 μg/L and 96 h EC_{50}8.8–61 μg/L)in standard media as well as natural waters.
- Copper NPs differed in toxicity to *D. magna* in standard media (48 h EC_{50}0.9–11 mg L^{-1}) to fresh water from Lake Lucerne (5.5–26 mg L^{-1}) and Lake Greifen (5.7–75 mg L^{-1}).
- Zebrafish embryos showed no toxicity up to 100 mg L^{-1} independent of the media used.
- The results showed that natural water is easily affected by these NPs as compared to standard media due to composition difference and lays a point that evaluations related to toxicity should be carried out in realistic models (Heinlaan et al. 2016).

2.6 Chromium Oxide

Chromium oxide NPs are mostly used as catalysts for aromatic compound manufacture, pigment and as abrading agent. It becomes necessary to study its effect on human health due to its increasing applications (Senapati et al. 2015a; Singh et al. 2016; Naasz et al. 2018). Senapati et al. designed a study to find out the genotoxic and apoptotic effects of chromium oxide NPs on lungs (A549 cell lines). Comet assay and cytokinesis block micronucleus assay found out that NPs lead to DNA damage and also chalked out the molecular mechanism of its toxicity i.e., increased levels of reactive oxygen species (ROS). Increased ROS levels decreased the mitochondrial membrane potential (MMP) and increase in levels of BAX/Bcl-2, which lead to mitochondria mediated apoptosis leading to cell death (Senapati et al. 2015a).

NPs (34.89 ± 2.65 nm) and microparticles (3.76 ± 3.41 μm) (MPs) of chromium oxide were evaluated for a 28-day repeated toxicity study. 30, 300 and 1000 mg/kg/day oral treatments were designed in Wistar rats. Comet assays, micronucleus and chromosomal aberration assays were carried out to assess genotoxicity. DNA damage occurred mostly in liver and peripheral blood leucocytes at a dose of 1000 mg/kg body weight per day of NPs and MPs. Biodistribution occurred mostly in kidneys and least in brain signifying a dose-dependent distribution. Chromium was excreted mainly through feces than urine. NPs displayed significant tissue accumulation and higher absorption than MPs (Singh et al. 2016).

2.7 Carbon Nanoparticles

Morphology and size of carbon nanotubes (CNTs) may influence the toxicity and its impact on human health. Fullerenes affect *Chironomus riparius* by delaying female emergence time leading to impaired adult breeding. Tavares et al., evaluated the genotoxicity of nanosized TDO, synthetic amorphous silica and multiwalled CNTs in human lymphocytes. A cytokinesis block micronucleus assay was carried out and it inferred that the TDO NPs and two multiwalled CNTs showed increase in frequency of micronucleated binucleated cells, but a dose response relationship was not observed. This increases the tendency of these TDO NPs and multiwalled CNTs to become genotoxic. Amorphous silica on the other hand, was unable to induce micronuclei formation (Tavares et al. 2014; Waissi et al. 2017)

Another study proved that the effect of length and diameter of CNT affects its lung deposition, quantification of total and regional deposition of CNT was done by a stochastic bronchial network model. The model shows that transport of particles occurs along random paths, various forces and torque act along the translocation path of inhaled air.

- Small CNTs less than 1 nm are filtered along the extra thoracic airways.
- Intermediate CNTs up to 10 nm deposit in alveoli, (potentially hazardous).
- Large CNTs approx. 100 nm have minimal deposition.
- Thick CNTs have low deposition than small and intermediate CNTs, thick and long graphite CNTs have problems in clearance from the alveoli and present barriers for macrophages and epithelial cells (Sturm 2016).

2.8 Cerium

Drosophila melanogaster acts as a suitable model for in vivo testing of cerium oxide NPs and cerium sulphate exposure. Egg to adult viability, gene expression, uptake through intestines and intracellular ROS production by hemocytes, antigenotoxicity and genotoxicity was evaluated.

- Hsp genes were detected and TEM images confirmed the internalization of cerium oxide NPs and hemocytes.
- Neither toxicity nor genotoxicity was observed for any form of cerium.
- Cerium oxide NPs reduce the intracellular ROS production and genotoxic effects of potassium dichromate.
- *D. melanogaster* serve as an important animal model for NP material, and the role of intestinal barrier in studying the transposition of nanomaterial via ingestion (Alaraby et al. 2015).

2.9 Manganese

Saccharomyces cerevisiae was used as a model organism to test for toxicity of various metals (titanium, zirconium, iron, manganese) based on cell membrane integrity and oxygen consumption. Polyacrylate was taken as a dispersant to check the influence of aggregation on their toxicity. O_2 consumption was inhibited maximally by manganese oxide NPs (50% at 170 mg L^{-1}) and cell membrane damage (30% damage at 1000 mg L^{-1}). Iron showed low toxicity and titanium, zirconium caused no toxicity to the yeast. Supplementation with a dispersant decreased the inhibition by manganese NPs at low concentrations which infers that dispersant has an effect on interaction between the NPs and cells (Otero-González et al. 2013).

2.10 Palladium

Palladium (Pd) belongs to platinum group and is a rare metal. It is mainly used as catalyst in synthetic procedures, in jewelry making, biomedical, effluent treatment, electronic and engineering sectors. The results demonstrate that palladium NPs exert pro-inflammatory and cytotoxic effects in vitro and exerts alterations in different organs in vivo (Leso and Iavicoli 2018).

Renal toxicity of the palladium NPs was studied in female Wistar rats that was exposed intravenously to different concentration (0.012, 0.12, 1.2 and 12 μg/kg) of Palladium NPs. Renal toxicity was assessed by investigating the urinary excretion of retinol binding protein, β(2)-microglobulin and albumin. Results indicated that at concentration of 12 μg/kg there was significant increase in the level of urinary retinol binding protein and β(2)-microglobulin when compared to the controls. In addition the ultrastructural studies on the kidneys indicated or revealed significant changes in the epithelial tissue of proximal and distal tubules. Fontana et al. (2015) suggested from their experiment that the palladium NPs were able to exert significant changes in the renal dysfunction, at same time without affecting glomerular filtration (Fontana et al. 2015).

Effect of Palladium NPs on reproductive system of Wistar rats at different intravenous doses was studied by Leso et al. (2018). The quantitative changes in the serum concentrations of different sex hormones were also assessed. The results demonstrate that the highest exposure dose significantly abridged the estradiol and testosterone concentrations, while increasing the luteinizing hormone levels in treated animals indicating abnormal reproductive function (Leso et al. 2018).

2.11 Rare Earth Oxides

NPs prepared from rare earth oxides possess particle- specific cytotoxicity against gram negative *E. coli* (pBR322) as evidenced from a study carried out by He et al. (2015). Four rare earth oxides were selected for study lanthanum oxide, cerium oxide, gadolinium oxide, ytterbium oxide. Dissolution studies were carried out in presence of test organism to evaluate the ion related toxicity which was quantified with the help of X-ray absorption fine structure spectroscopy. The results indicated that presence of *E. coli* in media facilitated dissolution and the additional ion release results in ion related toxicities towards the organism. If the dissolution conditions are overlooked *i.e.*, presence of organism leading to particle–cell contact results in incorrect interpretation of particle-specific toxicity (He et al. 2015) (Table 1).

3 Genotoxicity Studies

Assessment of a chemical agent to test whether it is leading to changes in the genetic information within a cell causing mutations. The following tests are frequently used to assess the genotoxicity of a nanomaterial (Naasz et al. 2018; Juganson et al. 2015; Elgeti et al. 2018; Handral et al. 2016; Ajdary et al. 2018; Demir et al. 2015; Demir and Castranova 2016; Kumar et al. 2011a; Concu et al. 2017; Iavicoli et al. 2016; Moretti et al. 2013; Bermejo-Nogales et al. 2017; Laux et al. 2018; Ibrahim et al. 2016; Kumar et al. 2015; Das et al. 2017; Gebel et al. 2014).

3.1 Comet Assay

To asses nanogenotoxicity comet assay is one of the most common assays used. It is the simple method used to measure the DNA damage such as break in the single strand of the DNA or the double strand. There are many variations or modification done but the basic principle remains same. In this procedure the cells are exposed to formulation for 24 h. in fetal bovine serum (FBS) free culture medium with or without formamidopyrimidine DNA glycosylase (FPG) incubation. The treated cell were trypsinized and washed with phosphate buffer saline (PBS) twice and then subjected to centrifuge, the glass slides to be used were first washed dried and later coated with normal melting agarose then the cells were dispersed and finally embedded in the low melting point agarose. Then the prepared slides were immersed in in lysis solution [2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris-base, pH adjusted 10 with NaOH; 1% (v/v) Triton X-100 added immediately before use] for 60 min at 4 °C. the slides are removed and treated with 0.3 M NaOH and 0.001 M EDTA aqueous solution and the incubated in dark for 40 min ate 4 °C. Then the prepared gels were subject to the electrophoresis (0.8 Vcm^{-1}) in dark at 4 °C for 30 min. The gels were then washed

Table 1 Toxicity profile of various nanomaterials

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Aluminum oxide and silicon oxide	Cultured macrophages (RAW264)	WST-8 cell viability, Hoechst/PI apoptosis assay, micronucleus analysis, comet assay	Nuclei and DNA damage, NP concentrations above 200 $\mu g\ mL^{-1}$ induce cytotoxic and genotoxic effects to cells	Hashimoto and Imazato (2015)
Carbon nanotubes	Stochastic bronchial network	Aerodynamic/thermodynamic diameter concept and related empirical deposition formulae	CNT approx. 10 nm are potentially hazardous	Sturm (2016)
Cerium	*Drosophila melanogaster*	Egg-to-adult viability, gene expression, ROS	NPs significantly reduced the genotoxic effect of potassium dichromate and the intracellular ROS production	Alaraby et al. (2015)
Chromium oxide	Human lung epithelial cells (A549)	Comet assay, cytokinesis block micronucleus assay	Mitochondria-mediated apoptosis, cell death	Senapati et al. (2015a)
Chromium oxide	Wistar rats	Comet, micronucleus, chromosomal aberration (CA) assays	Size dependent toxicity	Singh et al. (2016)
Coexistent silver and titanium NPs	*Tetrahymena pyriformis*	Oxidative stress biomarkers studied	TDO NPs reduce the environmental risks of silver NPs in natural light, In continuous light TDO NPs enhance the environmental risks of silver NPs	Zou et al. (2014)
Copper	Model septic tank	Water quality evaluation, Microbial characterization	Improper functioning of septic tank during Copper NPs exposure	Taylor and Walker (2016)
Copper and nickel	*Drosophila melanogaster*	Wing spot assay	Nickel NPs found to be genotoxic than copper	Carmona et al. (2018)

(continued)

Table 1 (continued)

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Copper and silver NPs	*Drosophila melanogaster*	Adult longevity and reproduction rates	Copper was found toxic to adult insects Silver showed toxicity in development stages Silver reduced the microbial diversity of the larval gut	Han et al. (2014)
ENMs (Silver, copper and zinc oxide)	*Daphnia magna*	Acute toxicity test	Copper and Zinc showed toxicity in dissolved state Silver showed toxicity in dissolved as well as particulate form	Seo et al. (2014)
ENMs (Titanium dioxide, MWCNTs, and silicon NPs)		Cytokinesis-block micronucleus assay	No micronuclei formation due to silica Genotoxic effect of Titanium and MWCNTs	Tavares et al. (2014)
ENMs (ZnO, MWCNTs, cerium and silicon NPs)	Topminnow (*Poeciliopsis lucida*) liver cell line (PLHC-1), rainbow trout *(Oncorhynchus mykiss)* fibroblast-like gonadal cell line (RTG-2)	Cytotoxicity	ZnO NPs alter lysosome function and metabolic activity, multi-walled carbon nanotubes (MWCNTs) caused plasma membrane disruption at higher concentrations	Bermejo-Nogales et al. (2017)
Fullerene	*Chironomus riparius*	Multi-generation chronic ecotoxicology test	Impaired breeding, revealed by delayed female emergence time	Waissi et al. (2017)
Gold	*C. elegans*	Multi-generational effect, lipofuscin accumulation	Decreased reproduction, increased abnormalities in the reproductive organs	Moon et al. (2017)

(continued)

Table 1 (continued)

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Gold	*Drosophila melanogaster*	Comet assay, SMART	30 nm gold NPs indicate lack of mutagenic and recombinogenic activity	Ávalos et al. (2018)
Gold	*Saccharomyces cerevisiae*	Comet assay	DNA damage	de Alteriis et al. (2018)
Iron oxide, citrate-coated (maghemite) nanoparticles (IONPs)	Female guppy *Poecilia reticulata*	Comet assay, micronucleus (MN) test and erythrocyte nuclear abnormalities (ENA) frequency	P. reticulata erythrocytes are target of ecotoxicity of IONPs	Qualhato et al. (2017)
Polystyrene PS NPs	Green microalga *Dunaliella tertiolecta* (prey), Brine shrimp *Artemia franciscana* (predator)	Growth inhibition test, Long term toxicity test	Inhibition of algal growth, mortality in brine shrimps, increase in molting, triggering apoptotic pathway by cathepsin L-like protease	Bergami et al. (2017)
Polyvinyl alcohol (PVA), Cellulose nanofiber (CNF)	Cress and Spinach	Controlled compost test	No negative effects on germination or development	Salehpour et al. (2018)
Quantum dots	*Mytilus galloprovincialis*	qPCR	Particle specific effects after observed, dual role of MTs in the QD metabolism	Rocha et al. (2018)
Rare earth oxides (lanthanum oxide, cerium oxide, gadolinium oxide, ytterbium oxide)	*E. coli* (pBR322)	X-ray absorption fine structure (XAFS) spectroscopy	Cytotoxicity observed	He et al. (2015)
Silica	Mouse lymphoma cells L5178Y/Tk±	Point mutation, chromosome alterations	Concentration dependent mutagenicity, genotoxicity	Demir and Castranova (2016)

(continued)

Table 1 (continued)

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Silica	Rats	standard comet assay and hOGG1 glycosylase-modified comet assay	Weak DNA damage observed	Pfuhler et al. (2017)
Silica	Sprague–Dawley rats	Hydro- dynamic chromatography (HDC) inductively coupled plasma mass spectroscopy (ICP-MS) HDC ICP-MS	Silica is accumulated in spleen, liver fibrosis is seen after 84 day study	Van der Zande et al. (2014)
Silicon dioxide	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2uvrA	Genotoxicity	No genotoxicity of Si as per OECD guidelines	Kwon et al. (2014a)
Silver	Co-culture model of Caco-2 and RajiB cells	Cytotoxicity, ROS, Immunoflorescence	Quercetin and kaempferol, protect Caco-2 cells from silver NPs induced toxicity	Martirosyan et al. (2014)
Silver	*E. coli*	High throughput screening method	Positively charged particles are responsible for ROS formation at the cell membrane	Ivask et al. (2014)
Silver	Human hepatoma cell line (HepG2)	Cell viability, apoptosis, cell cycle and cyclins gene expression	Decreased cell proliferation and viability	Bastos et al. (2017)
Silver	*Lymnaea stagnalis*	Toxicity and bioaccumulation	Water chemistry has no effect on uptake of NPs	Oliver et al. (2014)
Silver	*Platynereis dumerilii*	Waterborne exposure, Toxicity testing	Concentration dependent toxicity was observed	García-Alonso et al. (2014)

(continued)

Table 1 (continued)

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Silver	Wetland mesocosms	Silver concentration, dissolved organic carbon	Silver treatments were toxic to aquatic plants, leading to a significant increase of dissolved organic carbon and chloride. Dissolved methane concentrations increased 40-fold relative to controls	Colman et al. (2014)
Silver nanomaterial	Wheat and Canola	Lysimeter	Inhibition of soil microflora	Schlich et al. (2017)
Silver nanoparticles	*Daphnia pulicaria*	Standardized chronic toxicity tests	Increased toxicity at low environmentally relevant food ration	Stevenson et al. (2017)
Silver nanoparticles	*Drosophila melanogaster*	Behavioral assays	Behavioral abnormalities, reduction in lipid droplets in drosophilia	Raj et al. (2017)
Silver NPs	*Caenorhabditis elegans*	Natural organic mater	High amplitude swelling of intestinal epithelium and gonadal tissue	Yang et al. (2014)
Silver NPs	High throughput screening (HTS) platform	Cytokinesis-block micronucleus (CBMN) assay	Genotoxicity is lymphocyte sub-type dependent and is particularly pronounced in CD2+ and CD4+ cells	Vecchio et al. (2014)
Titanium dioxide	*Drosophila melanogaster*	Somatic Mutation and Recombination Test (SMART), comet assay (neuroblasts)	Increased fly prolificacy. Increase genotoxicity, 8 $\mu g\ mL^{-1}$ dose	Sario et al. (2018)

(continued)

Table 1 (continued)

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Titanium dioxide	HepG2 cells	Fpg-Comet assay	DNA damage and apoptosis via caspase dependent pathway	Shukla et al. (2013)
Titanium dioxide	Human embryonic kidney (HEK293), Mouse embryonic fibroblast (NIH/3T3) cells	Micronucleus analysis, comet assay	Genotoxic/transforming effects detected at the higher dose tested (1000 $\mu g\ mL^{-1}$)	Demir et al. (2015)
Titanium dioxide	Human epidermal cells (A431)	Cellular uptake, cytotoxicity, cell viability, genotoxicity	Mild cytotoxicity and genotoxicity	Shukla et al. (2011)
Titanium dioxide	Human SHSY5Y neuronal cells	Viability, cytotoxicity, genotoxicity and oxidative damage	Dose-dependent cell cycle alterations, apoptosis by intrinsic pathway, and genotoxicity (not related with double strand break production)	Valdiglesias et al. (2013)
Titanium dioxide	Mice	Oxidative stress, DNA damage, tumor suppressor and proapoptotic protein expression in liver cells	DNA damage in liver cells and apoptosis	Shukla et al. (2014)
Titanium dioxide	*Paramecium caudatum* (predator), *Escherichia coli* (prey)	Surface interaction	Increased internalization, growth rate affected, bacterial ingestion	Gupta et al. (2016)
Zinc oxide	HepG2 cells	Genotoxicity assessment, cytotoxicity, apoptosis, Oxidative stress markers	cell viability decrease, cell death by apoptosis	Sharma et al. (2012b)
Zinc oxide	Human epidermal cells A431	Cytotoxicity	Cell death, ROS generation and induce cell cycle arrest in S and G2 M phase	Patel et al. (2016)

(continued)

Table 1 (continued)

Nano material	Model/cell line/crop	Toxicity assay method	Response	Reference
Zinc oxide	Human lymphoblastoid cell line TK6	Comet assay	DNA damage is induced by ZnO NP (50–80 nm), with induction of oxidative damage	Demir et al. (2014)
Zinc oxide	Human monocytic cell line (THP-1)	Comet assay, micronucleus assay	DNA damage, Oxidative and nitrosative stress in human monocytes, increased inflammatory response	Senapati et al. (2015b)
Zinc oxide	Lymphocytes (healthy volunteers, lung cancer, asthma, COPD patients)	Cell viability, immunoblot analysis	Overexpression of p53, p21, genotoxicity, carcinogenicity	Kumar et al. (2015)
Zinc oxide	Male Swiss albino mice	Fpg-modified Comet assay, TUNEL assay	DNA damage and apoptosis	Sharma et al. (2012a)
Zinc oxide	*Typha angustifolia* leaves	Effect on fungal sporulation	Visible light enhanced inhibition of fungal sporulation rate	Du et al. (2018)

with cold distilled water twice and stained, it was then viewed under microscope. (Sario et al. 2018; Doktorovova et al. 2014; Pfuhler et al. 2017; Senapati et al. 2015b; Shukla et al. 2011; Kwon et al. 2014a; Nymark et al. 2013; Demir et al. 2015; de Alteriis et al. 2018; Magdolenova et al. 2014; Cowie et al. 2015; Iglesias et al. 2017; Møller & Jacobsen 2017; Kwon et al. 2014b; Nam et al. 2013).

3.2 Micronucleus Assay

Cultured cells exposed to nanoparticle formulation. Cytochalasin B was added to the cell cultures after exposure of 6 h., to induce binucleation in dividing cells. The cells were later trypsinized for 20 min in PBS containing 10% FBS and centrifuged. PBS is added to pellet and centrifuged, further the collected pellet incubated with 5 ml of hypotonic solution for less than 2 min. The cells were again centrifuged and fixed with methanol-acetic acid and then in 97:3 methanol-acetic acid. The cells spread on a slide and dried overnight. The cells were further stained rinsed and analysed (Vecchio et al. 2014; Tavares et al. 2014; Qualhato et al. 2017; Nymark et al. 2013; Demir et al. 2015; Magdolenova et al. 2014; Senapati et al. 2015a; Iglesias et al. 2017; Laux et al. 2018; Nam et al. 2013; Sario et al. 2018; Demir and Castranova 2016; Kumar and Dhawan 2013; Pfuhler et al. 2017; Kwon et al. 2014a).

3.3 Chromosome Aberrations Test

Cell cultures were exposed to NPs for 24 and 48 h. Colcemid was added during last 4 h and the cells are collected by trypsinization and grown by hypotonic treatment followed by triplicate treatment with methanol-glacial acetic acid (3:1). Microscopic slides was prepared through air drying technique and stained with Geimsa 4% for 5 min in Sorensen's buffer and analyzed.

The mitotic index, i.e., the proportion of total cells that underwent mitosis at the time of growth, was calculated from 1000 cells per replicate (Møller and Jacobsen 2017; Nymark et al. 2013; Magdolenova et al. 2014).

Ames test or Bacterial reverse mutation test

This is the most common test used to test whether a chemical causes DNA mutation in the test organism, this test uses bacteria as the organism. Any chemical that causes mutation is mutagenic and may be carcinogen as cancer is most commonly related to the mutation. The positive result indicates that the chemical is mutagenic and may be cancerous. In this test Salmonella bacteria is used. In this procedure Freshly cultured cells were taken and mixed with cofactor mix. 30% liver fraction was prepared in 3.5 ml of cofactor mix. 0.6% agar and 0.5% NaCl was added in 100 ml distilled water. Agar solution was sterilized by autoclaving. A mixed aqueous solution of L-histidine (0.5 mM L^{-1}) and D-biotin (0.5 mM L^{-1}) for tester strain and tryptophan

(0.5 mM L^{-1}) for *E. coli* (WP2 uvrA), was added to agar medium immediately before use. Hundred microlitre of overnight grown tester strain culture (109 CFU mL^{-1}) incubated with metabolic activation mix (liver fraction) or sodium phosphate buffer (0.1 mM, pH 7.4 for liver fraction) at 37 °C for 30 min at 180 rpm along with the desired concentrations of the nanoparticles. After incubation, 2 mL of agar at 45 °C was supplemented to the mixture and then poured on a plate of minimal glucose agar media. The agar plates were left to solidify and were incubated for 48 h and finally the revertant colonies were counted (Kumar et al. 2011a; Kumar and Dhawan 2013; Kwon et al. 2014b).

4 Cell Line Suitability

NanoTEST project was launched to study the genotoxic potential of NPs of titanium dioxide NPs (20 nm), iron oxide (8 nm) both oleic acid coated and uncoated, rhodamine coated silica NPs (25 and 50 nm), polylactic glycolic acid polyethylene oxide NPs. Detection of strand break was done by negative control Endorem O and oxidized DNA lesions through alkaline comet assay. Statistical evaluation proves that titanium dioxide NPs and oleic acid coated iron oxide NPs were genotoxic in the experimental conditions used. The TK6 cells, BeWo b30, human lymphocytes and kidney cells seem to be the most reliable for detecting a dose-response (Cowie et al. 2015). Bermejo et al., revealed the suitability of fish cell lines for establishing hazard rankings of ENMs for testing and assessment (Bermejo-Nogales et al. 2017). An exhaustive Table 1 is provided as reference to check the suitability of a model for genotoxicity and other studies.

To detect a suitable model for genotoxic and non-genotoxic response following cell lines can be considered.

Cultured macrophages (RAW264)
Human lung epithelial cells (A549)
Mouse lymphoma cells L5178Y/Tk+/−
Co-culture model of Caco-2 and RajiB cells
Human hepatoma cell line (HepG2)
Human embryonic kidney (HEK293)
Mouse embryonic fibroblast (NIH/3T3) cells
Human epidermal cells (A431)
Human SHSY5Y neuronal cells
Human lymphoblastoid cell line TK6
Human monocytic cell line (THP-1)
Fibroblast-like gonadal cell line (RTG-2)
Kidney (monkey Cos-1 and human HEK293 cells)
Liver (rat hepatocytes and Kupffer cells)
Liver cell line (PLHC-1)
Lung (human bronchial 16HBE14o cells)

Placenta (human bewo b30)
Primary cells and cell lines derived from blood (human lymphocytes and lymphoblastoid TK6 cells)
Rainbow trout (*Oncorhynchus mykiss*)
Topminnow (*Poeciliopsis lucida*)
Vascular/central nervous system (human endothelial human cerebral endothelial cells)

(Bastos et al. 2017; Bermejo-Nogales et al. 2017; Aravind and Dhanya 2016; Bhattacharya et al. 2017; Aravind and Dhanya 2016; Senapati et al. 2015b; Demir et al. 2014; Ávalos et al. 2018; Kleandrova et al. 2014; Gebel et al. 2014; Dhawan and Sharma 2010; Tavares et al. 2014; Iglesias et al. 2017; Demir and Castranova 2016; Bondarenko et al. 2016; Shukla et al. 2013; Moon et al. 2017; Ajdary et al. 2018; Shukla et al. 2011; Iavicoli et al. 2016; Nymark et al. 2013; Hashimoto and Imazato 2015; Doktorovova et al. 2014; Laux et al. 2018; Du et al. 2018; Kwon et al. 2014b; Naasz et al. 2018).

5 Conclusion

Nanoparticles are more commonly and increasingly being used in various consumer product such as cosmetics, pharmaceuticals, drugs, preservatives and targeted drug delivery system. This as a result makes it a easy source in environment which may cause hazard to the non-targeted organs including humans. Many of the nanoparticles at higher concentrations are known to have deleterious effects on development and abnormal behavioral characteristics in the experimental organisms. The Nano genotoxicity studies through various assays and tests reveals the toxicity of these materials which may be hazardous in long term. However, at lower concentration these NPs have more desirable results than larger dose. In these conditions such formulations should be used wisely to overcome the unintentional undesirable effects to the living organism including humans.

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Biosynthesized Nanoparticles and Their Biological Applications

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Abstract The application of nanoparticles in the medicinal field offers some extraordinary usages like diagnosis, imaging, controlled and targeted delivery drug carrier, implantations, device making etc. In this chapter dealt with the recent development of technology for the nanoparticles synthesis through natural way such as plant extract, micro-organism and their biological applications. The building up of reliable, eco-friendly, and nontoxic approaches for synthesis of nanoparticles are most important to develop their medicinal applications like synthesis of metals, polymer, ceramic materials etc. These biosynthesized nanoparticles are relatively new, safe and eco-friendly with a number of the application without any toxic effects. The skill for nanoparticle synthesis includes maximum production, reducing time and cost to get distinct shape and size, to increase the stability of the nanoparticles and optimization of specific natural materials for different applications.

Keywords Biosynthesis · Medicinal applications · Microorganisms · Nanoparticles · Template · Reduction

1 Introduction

Recently, nanotechnology is considered to be the most assuring technology, widely used in the field of medicine, energy, electronics, environment, etc. (Disanto et al. 2015). Nanotechnology involves the synthesis of nanoparticles (NPs) of size varying between 1 and 100 nm (Sharma et al. 2009). Up until now, NPs have been set up from metal and non-metal, polymeric materials and bioceramics. The most of NPs having medicinal applications are metal NPs, liposomes, polymers, and ceramic materials etc. (Dreher 2004). In the field of medicine, NPs are being used as a novel movement structure for drugs, proteins, DNA, and monoclonal antibodies (Amarnath Praphakar et al. 2019; Nowrouzi et al. 2010; De Jong and Borm 2008; Lewinski et al. 2008).

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Chemically produced NPs are extra harmful to human when compared to huge particles of a similar compound substance. Normally it is suggested that toxicity corresponds to the size of the NPs (Yang and Watts 2005; Mostafalou et al. 2013). Also, we require an effective innovation in the synthesis of NPs, not only the physical and chemical methods also it is now possible to take account of the use of biological materials such as plants and microorganisms (Mohanpuria et al. 2008). Hence there is another stream of nanotechnology prevailing, which is bio-nanotechnology that coordinates the principles of natural components and biology with chemical and physical strategies to produce nano-sized particles having a particular capacities (Kathiresan et al. 2009). Bio-Nanotechnology represents an commercial alternative for physical and chemical methods. The chemical methods of NPs synthesis such as electrochemical method, hydrothermal, sol-gel, sonochemical, flame spray pyrolysis, co-precipitation, microwave and etc., using various reducing and stabilizing agents which is responsible for several biological and ecological hazards (Vinothini et al. 2019a; Ali et al. 2016).

This book chapter deals with the details of recent and works on biosynthesized NPs via plant sources and micro-organisms with their biological applications. Newly, NPs synthesis by using the source of natural plants products and microorganisms has been broadly studied. Biosynthesized nanotechnology refers to the use of nanotechnology to enhance the natural maintainability of ways for delivering negative exteriority. The development of reliable, clean, non-toxic, easily scaled-up technology, eco-friendly and green chemistry approaches for the synthesis of NPs are of most useful to increase their biological applications. Biosynthesized nanotechnology is a green approach to reduce potential environmental and human health risks and its products with new nano; metals, metal oxides, polymer, ceramic materials that are more eco-friendly for the duration of their life cycle (Fig. 1). In this chapter, initially discussed the potential of natural plant extracts and microorganisms assisted NP synthesis and followed the NPs applications in the medicinal field.

2 Biosynthesis of Nanoparticles by Natural Plants and Microorganisms

Since the beginning of 1900s, known the plant isolated have the capability to reduce the metal ions, while, the mechanism of the reducing agents included was seldom understood (Kumar and Yadav 2009). In recent times, phyto and microbial nanotechnology has given new ways to the synthesis of NPs is an eco-friendly, simple, fast, stable, and economical technique. This technology has benefits; as well as biocompatibility, adaptability, and the medical suitable of synthesizing NPs using the general dissolvable water as a decreasing medium (Noruzi 2015). The routes for generation of NPs mostly include either an either a “top-down” (consecutive slicing of a bulk material to inducenano-sized particle) method or a “bottom-up” (build-up

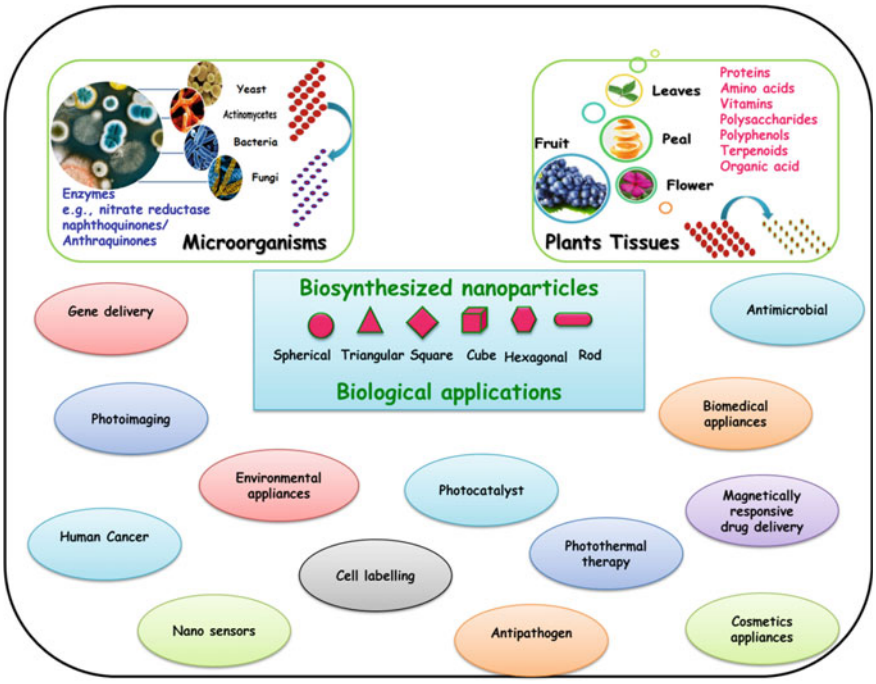

Fig. 1 Biosynthesized nanoparticles and their biological applications

of a material from the bottom: atom by atom, molecule by molecule or cluster by cluster) method (Fig. 2) (Isaacoff and Brown 2017).

Biomolecules existing in plant concentrates and microorganisms could be used to diminish metal particles to NPs in a coordinated biosynthesis process. This biogenic decrease of metal particle to base metal is quick, promptly drove at room temperature, weight, and effectively scaled up. Union utilizing plant concentrates and microorganisms are eco-accommodating. The decreasing operators included diverse water-dissolvable plant metabolites (for example terpenoid, alkaloids, polyketides, and phenolic mixes), co-compounds and microorganisms (Fungus, Yeast, Bacteria and so forth) gold (Au) and Silver (Ag) NPs have been the particular point of convergence of plant-interceded amalgamations, in perspective on their applications (Mittal et al. 2013). The idea of the plant extricates, the grouping of the plant concentrate and metal salt, the temperature contact, pH and development time is known to impact the pace of age of the NPs, their amounts and other features displayed in Fig. 3 (Dwivedi and Gopal 2010). The majority of the microorganisms have been creating inorganic NPs over either intracellular (relocating particles into the microbial cell to frame NPs) or extracellular (catching the metal particles on to the outside of the cells and diminishing particles) technique within the sight of enzymes (Zhang et al. 2011).

Regularly, a plant extricate based bio-reduction incorporates blending the fluid concentrate with a watery arrangement of the important metal salt. The response

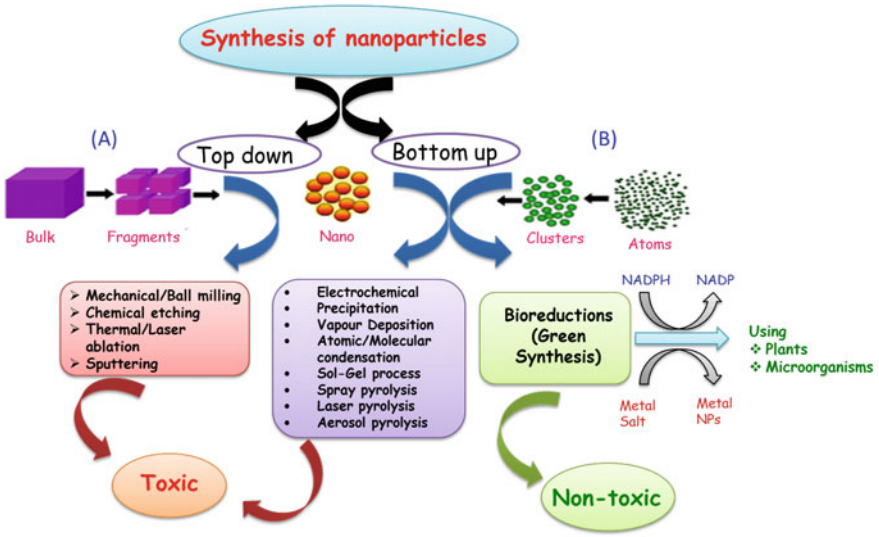

Fig. 2 Different methods for the synthesis of nanoparticles. (**A**) Top to bottom method and (**B**) bottom to top method

emerges at room temperature and it is generally finished inside a couple of minutes. Noteworthy, the varieties of plants species are collected from various parts of the world. The source of the plant extract is recognised to impact the characteristics of the NPs. Plant extract prepared from one species in different labs may give individual results. This is the primary disadvantage of utilizing crude natural products for the biosynthesis of NPs. Biological synthesis of metallic NPs, oxide NPs, alloy NPs, sulphide NPs, magnetic and nonmagnetic NPs and other types of miscellaneous NPs by using the plant extracts have been discussed in this chapter (Gowri et al. 2019).

2.1 Metallic Nanoparticles (MNPs)

In recent times, MNPs of Gold, Silver, Nickel, Cobalt, Zinc, Copper, Iron, platinum and Palladium are effectively biosynthesized by both plants and microbes. NPs utilizing the different plant parts, as well as leaves, fruits, stems, roots, and their extracts, from the medicinal herbals, are utilized for the synthesis of MNPs. In-plant separates based NP blend, the concentrate is different alongside an answer of the metal forerunner at room temperature (Mittal et al. 2013).

The pH, temperature, contact time, concentration, etc., of phytochemical and metal salts are known to influence the rate of NP formation, formation quantity, quality, and stability (Dwivedi and Gopal 2010). The microorganisms need longer cultivation duration to reduce into metal ions, however, water-solvable phytochemicals can diminish metal ions in a significantly a short span (Jha et al. 2009b). The

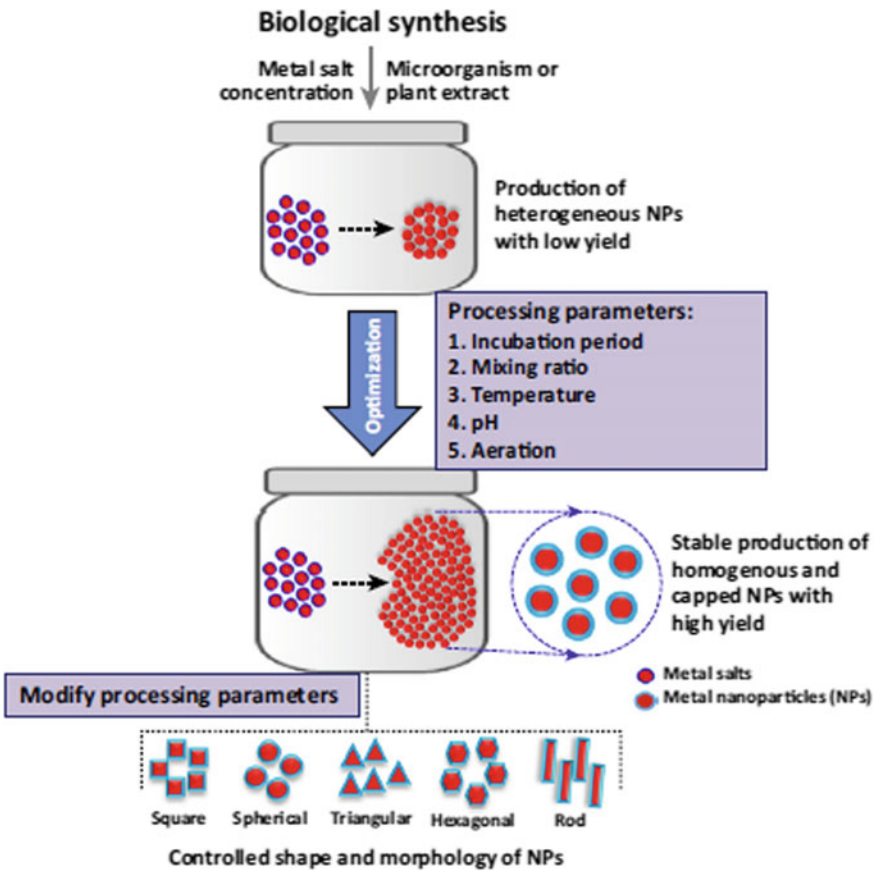

Fig. 3 Various types of nanoparticles by biosynthetic process

contents of the leaf extract additionally influences the NP synthesis as various leaf extracts include variable groupings of biochemical reducing agents (Mukunthan and Balaji 2012). The principle phytochemicals in charge of the synthesis of NPs are recognized as terpenoids, carboxylic acids, flavones, aldehydes, amides and ketones (Jeevanandam et al. 2016). Various characteristic metal NPs formed by plants and microorganisms are given in Tables 1 and 2 (Fig. 4).

2.1.1 Au and AgNPs Synthesis

Among the biological synthesis of metallic NPs, gold and silver NPs (Au and AgNPs) have attracted in considerably more attention as a outcome of their biocompatibility, high chemical, thermal stability and high potential for utilization of promising applications in science and medicine field because of its eco-friendly method and also

Table 1 Biosynthesis of MNPs and alloy NPs by natural plants

S. No.	Plants	MNPs	Particle size (nm)	Shape	References
1.	*Azadirachta indica*	Ag, Au and Ag core–Au shell	5–35	flat, plate-like spherical, peculiar core-shell structure	Shankar et al. (2004)
2.	*Tamarindus indica*	Au	–	Triangular	Ankamwar et al. (2005a)
3.	*Pelargonium Graveolens*	Au	21–70	Triangular, Spherical rods, flat and sheets	Shankar et al. (2003)
4.	*Cinnamomum camphora*	Ag, Au	55–80	Spherical, Triangular	Huang et al. (2007)
5.	*Aloe vera*	Ag	5–50	Octahedron	Chandran et al. (2006)
6.	*Cymbopogon flexuosus*	Au		Hexagonal, Triangular	Shankar et al. (2005)
7.	*Emblica officinalis*	Ag, Au	10–20, 15–25	–	Ankamwar et al. (2005b)
8.	*Medicago sativa*	Au	4–10	fcc twinned, crystal and icosahedral	Gardea-Torresdey et al. (2002)
9.	*Chilopsis linearis*	Au	1.1		Rodriguez et al. (2007)
10.	*Sesbania*	Au	6–20	Spherical	Sharma et al. (2007)
11.	*Medicago sativa*	Ag	35–40 at pH 10	Spherical, disk and irregular	Mohapatra et al. (2015)
12.	*Euphorbia prostrate*	Ag, TiO_2	12.82 ± 2.50 and 83.22 ± 1.50	Spherical	Abduz Zahir et al. (2014)
13.	*Mangifera indica*	Ag	14	Spherical and hexagonal	Sreekanth et al. (2016)
14.	*Piper nigrum*	Ag	10–60	Rod-shaped	Mohapatra et al. (2015)
15.	*Nigella sativa*	Ag	15	Spherical	Amooaghaie et al. (2015)
16.	*Piper betle*	Ag	48–83	Spherical	Ramachandran et al. (2016)

(continued)

Table 1 (continued)

S. No.	Plants	MNPs	Particle size (nm)	Shape	References
17.	*Eucalyptus globulus*	Ag	1.9–4.3 and 5–25 nm with and without a microwave treatment respectively	–	Ali et al. (2015)
18.	*Saraca indica*	Ag	23 ± 2	Spherical	Perugu et al. (2016)
19.	*Rosa 'Andeli'*	Ag	0.5–1.4	Spherical	Suárez-Cerda et al. (2015)
20.	*Orange* and *pineapple*	Ag	10–300	Spherical	Hyllested et al. (2015)
21.	*Pinus densiflora*	Ag	30–80	Oval, triangular	Velmurugan et al. (2015)
22.	*Cymbopogon citratus*	Au	20–50	Spherical, triangular, hexagonal and rod	Murugan et al. (2015)
23.	*Mushroom*	Au	20–150	Triangular, prism, hexagonal, spherical	Philip (2009)
24.	*Diospyros kaki*	Pt	2–12	Spheres and plates	Song et al. (2010)
25.	*F. decipiens*	Pd	2–22	Spherical	Sharmila et al. (2017)
26.	*Cinnamomum camphora*	Pd	3.6–9.9	Quasi-spherical	Yang et al. (2010)
27.	*Calotropis gigantean*	Ni	<60	–	Din et al. (2018)
28.	*Butea monosperma*	Ni	56–60	Flowers petal-like	Tayade et al. (2018)
29.	*Artocarpus heterophyllus*	Cu	132	–	Sharon et al. (2018)
30.	*Punica granatum*	Cu	–	Spherical	Padma et al. (2018b)
31.	*Ginkgo biloba*	Cu	15–20	Spherical	Nasrollahzadeh and Sajadi (2015)
32.	*Catharanthus roseus*	Pd	40	Spherical	Kalaiselvi et al. (2015)
33.	*Artocarpus gomezianus*	Zn	>20	Spherical	Suresh et al. (2015)

(continued)

Table 1 (continued)

S. No.	Plants	MNPs	Particle size (nm)	Shape	References
34.	*Lawsonia inermis*	Fe	21	Hexagonal	Naseem and Farrukh (2015)
35.	*Gardenia jasminoides*	Fe	32	Rock like appearance	Elango and Roopan (2015)
36.	*Cocos nucifera*	Pb	47	Spherical	Elango and Roopan (2015)
37.	*Coleus aromaticus*	Au–Ag alloy	28	Nanospheroids	Vilas et al. (2016)
38.	*Azadirachta indica*	Au–Ag alloy	50–100	Core/shell-type	Shankar et al. (2004)
39.	*Coriandrum sativum*	(Au Ag, Sr) tri-metallic alloy	70	Spherical	Binod et al. (2018)

cost effective techniques (Amarnath Praphakar et al. 2018; Mehnath et al. 2018; Jain et al. 2006). The properties of Au NPs are the easy surface functionalization, facile synthesis methods (Ghosh et al. 2008), tuneable surface plasmon resonance (SPR) (Huang and El-Sayed 2010; Narayanan and Sakthivel 2011), and low harmfulness (Boisselier and Astruc 2009) and AgNPs are used to prevent the expansion of a multiplicity of microorganism in the human system. They are used in catheters, cuts, consumes and wounds to shield them against contamination (Catauro et al. 2004; Szeto and Li 2005). In addition to their antimicrobial activities and anti cancerous to biological NPs have been proved to be a highly active fibre-based optical sensor for the sensing of H_2O_2 and could be used in several industrial applications (Tagad et al. 2013).

Nanoparticle Synthesis Using Plants

Several plants and microorganisms are successfully utilized for the proficient and fast synthesis of Au and AgNPs. However, manage in excess of the size and shape of Au and AgNPs has been prepared using the Leaf extracts of *Azadirachta indica* (Shankar et al. 2004), *Tamarindus indica* (Ankamwar et al. 2005a), *geranium Pelargonium graveolens* (Shankar et al. 2003), *Cinnamomum camphora* (Huang et al. 2007), *Aloe vera* (Chandran et al. 2006), *lemongrass Cymbopogon flexuosus* (Shankar et al. 2005) and fruit extract of *Emblica officinalis* (Ankamwar et al. 2005b). Also *Medicago sativa* (Gardea-Torresdey et al. 2002), *Chilopsis linearis* (Rodriguez et al. 2007) and *Sesbania* seedlings (Sharma et al. 2007) as reducing agent have been indicated, the potential in reducing Au(III) ions to form Au(0) gold NPs. Although, in AgNPs synthesized by using Ag salts as a precursor to form silver NPs Ag(0) with *Medicago sativa* (Mohapatra et al. 2015), *Euphorbia prostrate* (Abduz Zahir et al. 2014), *Mangifera indica* (Sreekanth et al. 2016), *Piper nigrum* (Mohapatra et al. 2015), *Nigella sativa* (Amooaghaie et al. 2015), *Piper betle* (Ramachandran et al.

Table 2 Biosynthesis of MNPs and alloy NPs by microorganisms

S. No.	Microorganisms	MNPs	Particle size (nm)	Shape	References
1.	*Pseudomonas deceptionensis*	Ag	10–30	Spherical	Jo et al. (2015)
2.	*Brevibacterium frigoritolerans*	Ag	10–30	Spherical	Singh et al. (2015a)
3.	*Bacillus methylotrophicus*	Ag	10–30	Spherical	Wang et al. (2015)
4.	*Weissella oryzae*	Ag	10–30	Spherical	Singh et al. (2015c)
5.	*Bhargavaea indica*	Ag, Au	30–100	Ag-anisotropic, Au-flower	Singh et al. (2015b)
6.	*Bacillus pumilus*, *Bacillus persicus*, and *Bacillus licheniformis*	Ag	77–92	Triangular, hexagonal, and spherical	Elbeshehy et al. (2015)
7.	*Streptomyces* sp. LK3	Ag	5	Spherical	Karthik et al. (2014)
8.	*Yarrowia lipolytica* NCYC 798	Ag	15	Spherical	Apte et al. (2013)
9.	*Extremophilic yeast*	Ag, Au	Ag 20; Au 30–100	Irregular	Mourato et al. (2011)
10.	*Candida utilis* NCIM 3469	Ag	20–80	Spherical	Waghmare et al. (2014)
11.	*Sargassum wightii*	Au	8–12	Planar	Singaravelu et al. (2007)
12.	*Brevibacterium casei*	Au, Ag	10–50	Spherical	Kalishwaralal et al. (2010)
13.	*Trichoderma viride*	Ag	5–40	Spherical	Fayaz et al. (2010)
14.	*Phaenerochaete chrysosporium*	Ag	50–200	Pyramidal	Vigneshwaran et al. (2006)
15.	*Aspergillus flavus*	Ag	8.92 ± 1.61	Spherical	Vigneshwaran et al. (2007)
16.	*Aspergillus fumigatus*	Ag	5–25	Spherical	Bhainsa and D'Souza (2006)
17.	*Verticillium* sp.	Ag	25 ± 8	Spherical	Bakshi et al. (2017)
18.	*Fusarium oxysporum*	Ag	5–50	Spherical	Bakshi et al. (2017)

(continued)

Table 2 (continued)

S. No.	Microorganisms	MNPs	Particle size (nm)	Shape	References
19.	*Shewanella algae*	Pt	5	–	Sinha and Khare (2011)
20.	*Enterobacter* sp.	Hg	2–5	Spherical	Liu et al. (2011)
21.	*Shewanella* sp.	Se	181 ± 40	Spherical	Lee et al. (2007)
22.	*Sargassum algae*	Pd	5–10	Octahedral	Momeni and Nabipour (2015)
23.	*Pyrobaculum islandicum*	U(VI), Tc(VII), Cr(VI), Co(III), Mn(IV)		Spherical	Kashefi and Lovley (2000)
24.	*Desulfovibrio desulfuricans*	Pd	50	Spherical	Yong et al. (2002)
25.	*Rhodosporidium diobovatum*	Pb	2–5	–	Seshadri et al. (2011)
26.	*Fusarium oxysporum*	Au–Ag alloy	8–14	Core/shell-type	Senapati et al. (2005)
27.	*Yeast*	Au–Ag alloy	9-25	Core/shell-type	Zheng et al. (2010a)
28.	*Fusarium semitectum*	Au–Ag alloy	10-35	Spherical	Dasaratrao Sawle et al. (2008)
29.	*Neurospora crassa*	Au–Ag alloy	100	Quasi-spherical	Castro-Longoria et al. (2011)

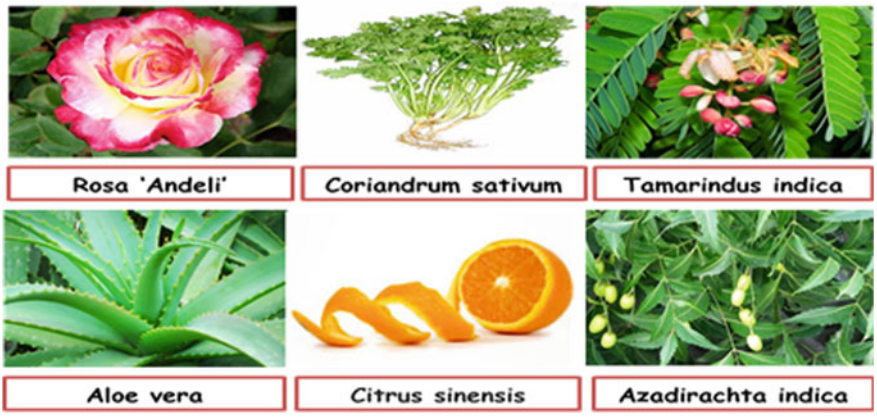

Fig. 4 Plant sources for the biosynthesis of nanoparticles

2016), *Eucalyptus globulus* (Ali et al. 2015), *Saraca indica* (Perugu et al. 2016), *Rosa 'Andeli'* (Suárez-Cerda et al. 2015) and etc., are used to the phytochemical synthesis of AgNPs (Table 1).

The control over the shape and stability of NPs preparation is depending on the nature of plants. This is observed by the fast synthesis of stable Au nano triangles at higher concentration utilizing leaf extract of tamarind as a reductant (Ankamwar et al. 2005a). Also, using leaf isolated of *Aloe vera* as a reductant for the synthesis of Au and AgNPs has been attained in crystalline triangular shape. For the fluid chloroauric particles were utilized as the forerunner for the planning of Au NPs through the Aloe Vera plant extricates. The size and state of the Au nano triangles (50–350 nm) were constrained by changing the amount of concentrates. The important role in the form of Au nano triangles were battled by the moderate decrease of fluid Au ions ($HAuCl_4$) together with the shape-directing effects of carbonyl compounds of the plant extract. Otherwise, aqueous Ag ions ($AgNO_3$) after incubated with *Aloe Vera* extract gave spherical AgNPs. The brown-red colour and also pale yellow colourise shows the development of Au and AgNPs (Chandran et al. 2006).

Nanoparticle Synthesis Using Microorganisms

Microorganisms have been used to prepare NPs extracellularly and also intracellularly. The synthesis of extracellular method, the microbes are culturing for 1–2 days placed in a rotating shaker in optimal conditions, after that the culture was centrifuged to eliminate the biomass. The filter-sterilized metal salt solution was added to the supernatant and incubated to form NPs, which was examined by the colour change of culture medium. After the incubation period, the reaction mixture could be centrifuged, washed with solvent (water/ethanol/methanol) and removed large particles. Finally, the NPs are collected in the form of a bottom pellet. In the intracellular synthesis of NPs, the microbes are cultured under defined growth period. Finally, the biomass was collected by centrifugation, washed and dissolved carefully with sterile water. Like to extracellular synthesis method, The NPs synthesis could be observed by the colour changes of the culture medium. Afterwards the incubation, the biomass was eliminated by centrifugation. These steps have been supported to break down the cell wall and permit the NPs to be released (Singh et al. 2016).

In current research, bacteria, including *Pseudomonas deceptionensis* (Jo et al. 2015), *Brevibacterium frigoritolerans* (Singh et al. 2015a), *Bacillus methylotrophicus* (Wang et al. 2015), *Weissella oryzae* (Singh et al. 2015c), and *Bhargavaea indica* (Singh et al. 2015b, c) (Table 2) are explored for Ag and AuNP synthesis. Several micro-organisms are conveyed for MNPs synthesis, including *Bacillus*, *Pseudomonas*, *Escherichia*, *Rhodobacter*, *Aeromonas*, *Streptomyces*, *Plectonemaboryanum*, *Enterobacter*, *Brevibacterium*, *Klebsiella*, *Shewanella*, *Lactobacillus*, *Sargassum*, *Pyrobaculum*, *Corynebacterium*, *Weissella*, *Rhodococcus*, *Tricho-derma*, *Desulfovibrio*, *Rhodopseudomonas*, and others (Gowri et al. 2019).

The monodisperse Au NPs are prepared by alkalotolerant *Rhodococcus* sp., while used to great biological conditions are basic and a little increased temperature conditions (Ahmad et al. 2003a). Bacterial synthesised of Au NPs by Southam and Beveridge, whereas readily has been precipitated in the bacterial cells through incubation

of the cells by Au^{3+} ions (Southam and Beveridge 1996). Lengke et al. reported the synthesis of Au NPs using Au^{+}-thiosulfate and Au^{3+}-chloride complexes. Au NPs have been formed in several shapes like cubic, spherical, and octahedral by filamentous cyanobacteria and analysed the role of microorganism in their formation of Au NPs (Lengke et al. 2006).

The synthesis of extracellular of Au NPs was obtained by using the fungus actinomycete *Thermomonospora* sp. and *Fusarium oxysporum* respectively. They testified prepared Au NPs by fungus Verticillium sp followed by the intracellular method (Ahmad et al. 2003b; Mukharjee et al. 2002).

2.1.2 Other Metallic Nanoparticles

Other than the Au and AgNPs, some of the NPs are played a significant role in several biological applications including platinum (Pt), Palladium (Pd), Nickel (Ni), Copper (Cu), Lead (Pb), Iron (Fe), Zinc (Zn) etc.

Nanoparticle Synthesis Using Plants Sources
Water-soluble isolated chemicals from plants to reduce the metal ions in short time. The leaf isolated are extensively used as a made from preparation of metal and metal oxide NPs, due to the containing of many phytochemical, which act as a reducing agent (Yadi et al. 2018). Various characteristic metal NPs formed by plants are listed in Table 1.

Platinum Nanoparticles (Pt NPs)
Pt NPs are used in biomedical applications together with NPs different metals, an alloy, center shell, or bimetallic nanocluster structure (Gao et al. 2007). Yolk-shell nanocrystals of FePt@CoS2 are demonstrated to be harder in murdering HeLa cells as related to cisplatin (Kim and Song 2009). Persimmonleaves soup gathered and dried, were kept at room temperature. Bubbled leaf broth extracts were emptied and put away at 4 °C for multi-week. PtCl62-particles was decreased by adding leaf concentrate to the fluid arrangement of $H_2PtCl_6.6H_2O$ under reflux. The temperature impact on the response rate and size has been considered between 25 and 95 °C. The coming about Pt NPs arrangement was separated by persistent centrifugation at 15,000 rpm for 20 min, in DD water (Song et al. 2010).

Palladium Nanoparticles (PdNPs)
As a result of numerous applications of Pd NPs in the area of biotechnology, biomedical and medicine, the methods for synthesizing these NPs are broadly expanded newly. *Filicium decipiens* leaves were dried, ground and mixed with distilled water to get the extract. The leaf extract mixed with $PdCl_2$ solution and separated in a dark place at room temperature for some days (Sharmila et al. 2017). In order to achieve the *Cinnamomum camphora* leafs broth, was mixed with deionized water. The solution was boiled and filtrated. PdNPs an applicable volume of the concentrated $PdCl_2$

solution had been added to the filtrate and saved in a dark place at 30 C to get a quasi-spherical PdNPs (Yang et al. 2010).

Nickel Nanoparticles (Ni NPs)

Ecological uses of Ni NPs within the field of adsorption of harmful dyes and inorganic pollutants play a significant role in the sanitation of atmosphere (Pandian et al. 2015). Generally, Ni NPs used in the area of biomedicine because of their good anti-inflammatory and antibacterial activities (Angajala and Subashini 2014). Nickel nitrate solution was mixed with urea solution. Then, methanolic extract of *Calotropis gigantean* has been added in the mixture, pH scale was in tune to 10 and the colour of the solution was light green. Then the mixture was vigorously shaken and kept at 80 °C for 90 min. There has been a distinct colour change light to dark green after the reducing reaction. The samples were dried at 100 °C for 2 weeks were obtaining Ni NPs crystals (Din et al. 2018).

Butea monosperma petals were washed, dried and crushed. Then, the crushed petals have been mixed with deionized water and follow the reflux condensation. Afterwards plant isolated was filtered and the kept at 4 °C. The Salt of ($Ni\,NO_3.6H_2O$) solution slowly added the plant extract and held in reserve the solution for 3 h. The colour of the solution was changed during the reaction. The finishing observation of colour change was confirmed by the formation of NPs. The produced NPs centrifuged and Ni NPs were separated (Tayade et al. 2018).

Copper Nanoparticles (Cu NPs)

The jackfruit (*Artocarpus heterophyllus)* leaves were composed, washed and dried out. The dried leaves powder has been sieved and mixed with distilled water at 60 °C for 10 min and filtered to get the aqueous extract (Sharon et al. 2018). Then, the synthesized Cu NPs, an Erlenmeyer flask having copper sulphate ($CuSO_4$) was magnetically stirred for 3 h. After this, the aqueous extract of *Artocarpus heterophyllus* was added with $CuSO_4$ at room temperature and was consequently stirred for 24 h (Harne et al. 2012). The Copper sulphate solutions have been prepared by the various molarities of 1, 5, 10 and 20 mM were mixed with the flower, leaf, fruit rind, and seeds extracts of *Punica granatum*. Post incubation of the copper sulphate solution with plant extracts, the formation of a dark yellow colour showed specifying the creation of Cu NPs (Padma et al. 2018a, b).

Nanoparticle Synthesis Using Microorganisms

The preparation of NPs using microbes is eco-friendly, cost-effective, non-toxic and requires high energy for physiochemical synthesis. Microorganisms can collect and detoxify substantial metals because of the nearness of different reductive proteins, which can lessen the metal salts into MNPs with tight size dissemination and, in this way, less polydispersity. The metal-safe qualities, proteins, peptides, chemicals, lessening cofactors, and natural materials have critical jobs by going about as diminishing specialists. Besides, this guide in giving normal topping to incorporate the NPs, consequently hindering the collection of NPs and helping them to stay perpetual for quite a while. In addition, when contrasted with microbes, organisms have

expanded resiliences, and take-up abilities for metals, especially regarding the high divider restricting the capacity of metal salts with parasitic biomass for the high return creation of NPs (Singh et al. 2016).

Heavy metals are known to be hazardous to the life of microorganisms. Chemical detoxification and energy dependent ion flow from the cell by membrane proteins makes the microbes more resistant to most harmful metals. Changes in the solubility also play a key role in microbe resistance. Konishi et al. reported that Pt NPs are acquired using metal ion reducing bacterium *Shewanella algae*. Within 60 min aqueous $PtCl_6^{2-}$ ions were reduced into Pt by the resting cells of *S. algae* at room temperature and neutral pH, during which lactate acted as the electron donor. In the periplasm, about 5 nm of Pt NPs were situated. Sinha and Khare demonstrated that Hg NPs were synthesized by *Enterobacter* sp. Cells (Liu et al. 2011). The culture conditions (pH 8.0 and lower concentration of mercury) promote the synthesis of uniform-sized 2–5 nm, spherical, and monodispersed intracellular Hg NPs. With hydrogen as electron donor, an aerobic hyperthermophilic microbe, *Pyrobaculum islandicum*, was able to reduce several heavy metals as well as U(VI), Tc(VII), Cr(VI), Co(III), and Mn(IV). It is predicted that the sulfate-reducing bacteria, *Desulfovibrio desulfuricans*, and metal ion-reducing bacteria, *S. oneidensis* synthesises the Pd NPs.

In nature, microbe's resistance to most harmful heavy metals is because of their chemical detoxification also as due to energy-dependent ion flow from the cell by membrane proteins that perform either as ATPase or as a chemiosmotic cation or proton antitransporters. Alteration in solubility also plays a role in microbic resistance (Konishi et al. 2007). Konishi and coworkers told that Pt NPs have been attained using the metal ion-reducing bacterium *Shewanella algae* (Sinha and Khare 2011). Resting cells of *S. algae* was able to reduce aqueous $PtCl_6^{2-}$ ions into Pt at room temperature and neutral pH within 60 min while lactate was donated as the electron donor. Pt NPs of about 5 nm was situated in the periplasm. Sinha and Khare demonstrated that Hg NPs were synthesized by *Enterobacter* sp. Cells (Liu et al. 2011). The culture conditions (pH 8.0 and lower concentration of mercury) promote the synthesis of uniform-sized 2–5 nm, spherical, and monodispersed intracellular Hg NPs. *Pyrobaculum islandicum*, an anaerobic hyperthermophilic microbe, was testified to reduce several heavy metals as well as U(VI), Tc(VII), Cr(VI), Co(III), and Mn(IV) with hydrogen as the electron donor (Kashefi and Lovley 2000). The Pd NPs might be synthesized by the sulfate-reducing bacteria, *Desulfovibrio desulfuricans*, and metal ion-reducing bacteria, *S. oneidensis* (De Windt et al. 2005; Yong et al. 2002) are also itemised in Table 2.

2.2 Alloy Nanoparticles

A metal has been prepared by combining two or more metallic elements, mainly to give more strength. Alloy NPs are of more attention through their applications in biological, catalysis, electronics, coatings and as optical materials (Senapati et al.

2005; Zheng et al. 2010a). The first scientific report in this field synthesis protocols of Au and AgNPs alloy by Michael Faraday in 1857 (Arvizo et al. 2012).

Alloy Nanoparticles Synthesis Using Plants

In the Au and Au\Ag alloy NPs are prepared by using the leaf essential oil of *Coleus aromaticus* (C.a). The boiling HAuCl4 solution (pH 7) and the $AgNO_3$ solution was added slowly along stirring, after that the addition of the diluted (C.a) oil. the colour varying from colourless to light violet, and further to dark yellow this is exposed the development of bimetallic NPs is indicated by 1:1 (Au: Ag) molar ratio. The technique is carried out for varying molar ratios. The colour was varying from golden yellow to dark red. This is all shows the formations of Au and Ag alloy NPs are polydisperse, displays flat and plate like structure (Vilas et al. 2016). The bimetallic Au core-Ag shell NPs are prepared by using the *Azadirachta indica* leaf extract act as reducing agent to reduce the Au^{3+} and Ag^{+} ions. The NPs are in the range 50–100 nm also spherical in nature (Shankar et al. 2004).

The nano trimetallic alloy of Au–Ag–Sr has been successfully synthesised by using *Coriandrum sativum* root extract with the aqu. solution of Au^{+}, Ag^{+}, Sr^{+}. The NPs are found of size 70 nm and spherical in shape. When the extract was added, the colourless solution turns into blue colour, when heated its changed dark. This is indicated as the formation of trimetallic alloy (Au Ag, Sr) NPs. The shape of the NP generated as well as the size of the particle. Also, the trimetallic alloy used to the gas sensing application studies showed sensing of gases like ethanol, methanol, acetone and ammonia (Binod et al. 2018).

Nanoparticle Synthesis Using Microorganisms

Senapati et al. have been testified *F. oxysporum* were utilized to the arrangement of Au–Ag alloy and said that the discharged cofactor NADH demonstrates a noteworthy job in the creation of Au–Ag compound NPs (Senapati et al. 2005). TEM and Fluorescence microscopic characterizations point out that the Au–Ag alloy NPs were chiefly produced via an extracellular method and mostly existed in the form of asymmetrical polygonal NPs. Sawle et al. confirmed the biosynthesis of Au–Ag alloy NPs by the fungal strains *Fusarium semitectum* and reported that the NPs are fairly stable for several weeks (Dasaratrao Sawle et al. 2008). Using the microbes of *Fusarium oxysporum* Au–Ag alloy has been synthesised in the range of 8–14 nm and Spherical in nature. *Neurospora crassa* was used to the synthesis of Au–Ag alloy NPs were mainly produced via an Intra and extracellular Ag, Au and bimetallic Au–Ag in a Quasi-spherical Shaped (Castro-Longoria et al. 2011).

2.3 Oxide Nanoparticles (MONPs)

Oxide NP is a very important kind of compound NP synthesized by plants and microbes. These pull in numerous fields because of the potential mechanical utilizations of these mixes. MONPs are extensively used in several applications such

Table 3 Biosynthesis MONPs by natural plants

S. No.	Plants	MONPs	Particle size (nm)	Shape	References
1.	*Tea*	FeO_2	20–40	Irregular spherical	Kuang et al. (2013)
2.	*Aloe vera*	TiO_2	32	Tetragonal	Rao et al. (2015)
3.	*Gloriosa superba* L.	CeO_2	5	Spherical	Arumugam et al. (2015)
4.	*Aloe barbadensis Miller*	ZnO	25–40	Spherical	Gunalan et al. (2012)
5.	*Malva sylvestris*	CuO	5–30	Spherical	Awwad et al. (2015)
6.	*Green Tea*	ZnO	54	Hexagonal wurtzite	Dhanemozhi et al. (2017)
7.	*Citrus aurantifolia*	ZnO	50–200	Spherical	Ain Samat and Md Nor (2013)
8.	*Carissa edulis*	ZnO	50–55	Flower	Fowsiya et al. (2016)
9.	*Carica papaya*	ZnO	11–26	Hexagonal, prism, buds and flower	Sharma (2016)

as, medicine, catalysis, energy storage, sensor and information technology. During this section, we tend to analysis the biosynthesized oxide NPs from the 2 features: (1) Magnetic oxide NPs. (2) Nonmagnetic oxide NPs. Furthermost of the specimens of the magnetotactic bacterium used for the synthesis of MO (magnetic oxide) NPs and biological systems for the establishment of the nonmagnetic oxide NPs are encapsulated in Tables 3 and 4.

2.3.1 Magnetic Oxide Nanoparticles

Recently Magnetic oxide NPs are one of the advanced novel materials, remarkable to their one of a kind miniaturized scale setup and properties like superparamagnetic and high coercive power, and their prospect for wide applications in organic partition and biomedicine fields. Attractive NPs like Iron oxide NPs FeO_2, Fe_2O_3 (maghemite) and Fe_3O_4 (magnetite) are known to be biocompatible. They are effectively examined for focused malignant growth treatment undeveloped cell arranging and control, guided quality treatment, magnetically responsive drug delivery therapy, immunoassays, DNA analysis, cell labelling, tissue repair, detoxification of biological fluids and magnetic resonance imaging (MRI) (Fan et al. 2009).

Nanoparticle Synthesis Using Plants

The 3 distinct types of tea separates from green tea, oolong tea, and dark tea are used

Table 4 Biosynthesis of MONPs by Microorganisms

S. No.	Microorganisms	MONPs	Particle size (nm)	Shape	References
1.	Yeast cells	Fe_3O_4	–	Wormhole-like	Zhou et al. (2009)
2.	*Saccharomyces cerevisiae*	Sb_2O_3	2–10	Spherical	Jha et al. (2009c)
3.	*Fusarium oxysporum*	TiO_2	6–13	Spherical	Bansal et al. (2005)
4.	*Fusarium oxysporum*	$BaTiO_3$	4–5	Spherical	Bansal et al. (2006)
5.	*Fusarium oxysporum*	ZrO_2	3–11	Spherical	Bansal et al. (2004)
6.	*Shewanella oneidensis*	Fe_3O_4	40–50	Rectangular, rhombic, hexagonal	Perez-Gonzalez et al. (2010)
7.	*Magnetospirillum magneticum* AMB-1	Fe_3O_4	20	Cubo-octahedral	Amemiya et al. (2007)
8.	*Lactobacillus* sp.	$BaTiO_3$	20–80	Tetragonal	Jha and Prasad (2010)
9.	*Lactobacillus* sp.	TiO_2	8–35	Spherical	Jha et al. (2009a)

to give an unpredictable round organized iron oxide (FeO_2) NPs union with the scopes of 20–40 nm size (Kuang et al. 2013). Jeevanandam et al., recorded the concentrates of Wheat extricate from *Sorghum* sp., grape seed proanthocyanidin, plantain strip remove, grape marc and banana strip cinder separate (Jeevanandam et al. 2016) are utilized to create iron oxide NPs additionally incorporate into Table 3.

Nanoparticle Synthesis Using Microorganisms

Since 2000 a number of new magnetotactic bacteria have been found in various aquatic environments. In the comparison between synthesized intracellular magnetic particles of the artificial and bacterial method. The bacterial technique could without much of a stretch scatter in fluid arrangements since they are secured by natural layers that basically contain phospholipids and proteins (Fowsiya et al. 2016). So it has superior magnetic properties. In generally uncultured magnetotactic bacteria's are commonly grow at 30 °C and below. Most extreme surely understood refined magnetotactic microbes was mesophilic and couldn't to develop above 30 °C. Zhou et al. described that magnetic Fe_3O_4 NPs with mesoporous structure was synthesized by yeast cells as a template through coprecipitation method (Zhou et al. 2009). Several magnetic oxide NPs are itemised in Table 4.

2.3.2 Nonmagnetic Oxide Nanoparticles

Other than the magnetic oxide NPs, Nonmagnetic oxide NPs also studied including TiO_2, CuO, ZnO, CeO_2 Sb_2O_3, SiO_2, $BaTiO_3$, and ZrO_2 NPs.

NP Synthesis Using Plants
Titanium oxide nanoparticles (TiO_2 NPs) and Cerium oxide (CeO_2) nanoparticle

Generally TiO_2 NPs used for many applications such as medicinal applications, catalysis, electrical ceramics, electric conductors and etc. Due to their limited band hole, while used in photocatalytic properties. TiO_2 NPs are prepared using leaf extract of *Aloe Vera* washed and cut into small pieces. Formerly added distilled water and boiled to get an extract then filtered. Titanium Chloride ($TiCl_4$) has been dissolved in water. Added extract dropwise with constant stirring up to attain the pH of the solution turn into 7.0. The Ti NPs were dried and calcined (Rao et al. 2015). Another one important non-magnetic NPs is CeO_2 and CeO_2 act as a semiconductor metal oxide with 3.19 eV band gap energy, it has been used in several applications like a catalyst, bio-imaging, antimicrobial activity and sunscreen cosmetics. This metal oxide is prepared by varied physical and chemical approaches. For example; plant extract, egg, and honey are used to synthesize CeO_2 NPs (Arumugam et al. 2015).

Copper Oxide Nanoparticles (CuO NPs)
CuO NPs were synthesised by leaf extractions of *Aloe barbadensis Miller* (Gunalan et al. 2012) and *Malva sylvestris* (Awwad et al. 2015). *Punica granatum* fresh peels were also used for the preparation of CuO NPs. The fresh dried powder was mixed with water and boiled while waiting for the solution changed to yellow in colour. The copper acetate powder was dissolved in the DD water subsequently the *P. granatum* extract was mixed to the solution to produce the CuO NPs (Fig. 5). The green colour of the solution was change into brown, which displays the development of monodispersed Cu NPs (Padma et al. 2018b).

Zinc Oxide Nanoparticles (ZnO NPs)
Nowadays Zn Oxide NPs have to pay more attention in a research field as a result of their important and necessary roles as catalysts, superconducting materials, ceramic resistors, gas sensors, biological fields and energy sector. Moreover, ZnO NPs are low-cost and safe; they also have sensible anti-microbial activity, in addition to many other applications (Murugesan et al. 2018). The green tea leaf was also used for the synthesis of ZnO NPs by dried and crushed power. The powder has been mixed with distilled water. Then, the zinc acetate used as precursor, dissolved in water to obtained zinc acetate solution. The concentrate was included into the arrangement and dried well to get a white ZnO NPs at that point dried up to acquire NPs (Dhanemozhi et al. 2017). ZnO NPs were synthesized with different process and method including greener method using, pyramid-like ZnO NPs are synthesised by *Citrus aurantifolia* (Ain Samat and Md Nor 2013), J. Fowsiya et al. attained flower shaped ZnO NPs by using *Carissa edulis* (Fowsiya et al. 2016) and S. C. Sharma found the various

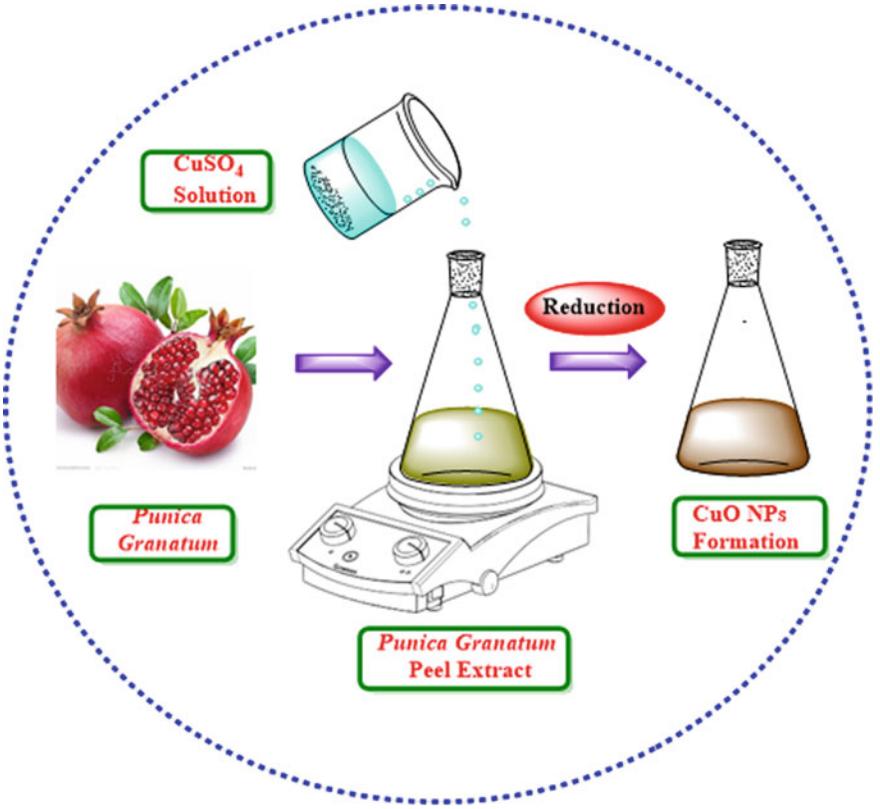

Fig. 5 CuO nanoparticles formation by natural plants

shapes of hexagonal to prism to nanobuds to nanoflowers of ZnO NPs by *Carica papaya* milk extracts (Sharma 2016).

Nanoparticle Synthesis Using Microorganisms

Usual chemical synthesis of NPs is hampered by the high cost, quality and toxicity of the method steps. Therefore, the chance of using microbes to biosynthetize these compounds is attracting a lot of attention, as these eco-friendlier systems permit a better downstream purification method—and so, quite promising results are discovery. Most microbe-produced NPs are of metallic nature. Metal NP deposition is caused by reduction of a metal salt employing the redox machinery within the cell. Cations that are found within the microbic surroundings participating in metabolic processes are reduced and precipitated by some microorganism as NP (Tanzil et al. 2016). Jha and co-workers discovered a green low-cost and reproducible *Saccharomyces cerevisiae* based biosynthesis of Sb_2O_3NPs (Jha et al. 2009c). The synthesis was performed in a room temperature. Investigation showed that Sb_2O_3 NPs are spherical in nature and having a size of 2–10 nm (Jha et al. 2009c). Bansal et al. widely applied the Fungus of

F. oxysporum to produce SiO_2 and TiO_2NPs from aqueous anionic complexes SiF_6^{2-} and TiF_6^{2-}, respectively (Bansal et al. 2005) and also prepared quasi spherical ZrO_2 and tetragonal $BaTiO_3$ NPs from *F. Oxysporum* having a size of 4–5 and 3–11 nm (Bansal et al. 2004, 2006).

2.4 Metal-Sulfide Nanoparticle

A variety of CdS NPs is prepared by Cadmium nitrate and sodium sulfide utilized as a source of the precursor, over varying the reaction conditions the quantity of banana peel isolated (banana extracts, concentration, temperature and solution pH). The prepared CdS colloid shows physically powerful fluorescence spectrum (Zhou et al. 2014). Mani et al., synthesised ZnS NPs using methanol extracts of plants such as *Tridax procumbens*, *Phyllanthus niruri*, and *Syzygium aromaticum* by a simple co-precipitation method and it has good antimicrobial activity and photocatalytic degradation properties. Similarly, the extract of *Syzygium aromaticum* mediated ZnS NPs showed excellent photocatalytic dye degradation Properties (Sathishkumar et al. 2018) (Fig. 6).

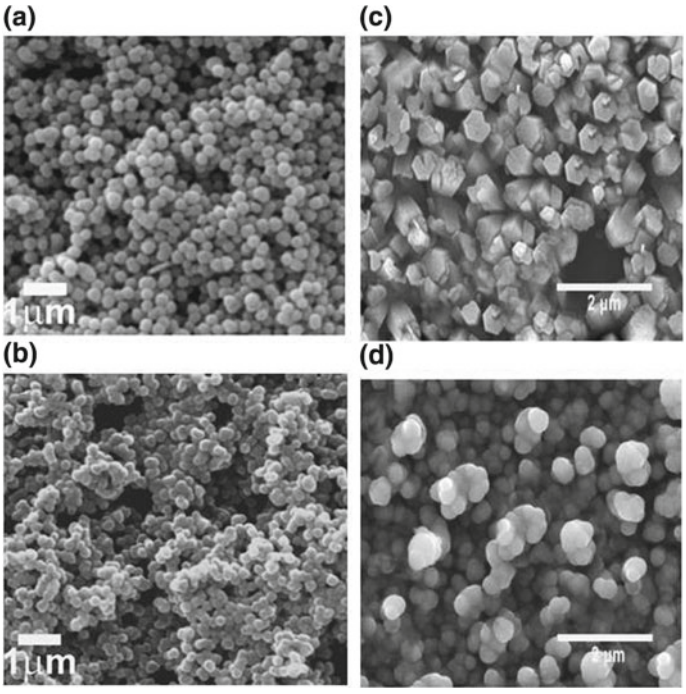

Fig. 6 SEM image of **a** ZnS, **b** T: ZnS, **c** P: ZnS, **d** S: ZnS (Sathishkumar et al. 2018)

ZnS and CdS NPs were synthesised through various sizes at ambient temperature. Zinc acetate, zinc chloride, cadmium acetate cadmium nitrate and sodium sulfide are used as precursor for Zn, Cd and sulfide ions. ZnS and CdS NPs are prepared by *Ligustrum vulgare* leaf isolated has been utilized as a capping and dispersing agent. The optical band gap for ZnS and CdS NPs was found to be 4.5 and 2.7 eV.

Furthermore, enzyme based extracellular preparation of CdS, ZnS, PbS, and MoS_2 by the fungus *F. oxysporum* while exposed to an aqueous solution of metal sulphate (Ahmad et al. 2002). The CdS nanocrystals were synthesised intra-cellular by the *E. coli* while used to cadmium chloride and sodium sulfide (Sweeney et al. 2004). Extracellular synthesised CdS NPs were shaped using a protein, cysteine desulfhydrase, secreted by *Rhodopseudomonas palustris*. Bacterial intracellularly synthesis of ZnS NPs using a *Rhodobacter sphaeroides*, by secreted enzymes such as sulfurylase, sulfate permease, and sulfite reductase (Saratale et al. 2018). Then, the PbS Nanoparticles was reported by using immobilized *Rhodobacter sphaeroides* in the size of 10.5 nm. In this study, the synthesis of extracellularly PbS NPs influences by the culture time and applying for large-scale applications of advantageous immobilized biomass strategy (Bai and Zhang 2009). Using the marine yeast of *Rhodosporidium diobovatum* yielded a stable intracellularly PbS NPs (Seshadri et al. 2011).

2.5 Nanoceramics and Composites

In biosystems, a multiplicity of bio-sources helps to form organic/inorganic composites like metal carbonates, phosphates are well-ordered morphology by the use of biopolymers such as protein, plant extracts and microbe cells. Moreover, $PbCO_3$, $CdCO_3$, $SrCO_3$, $Zn_3(PO_4)_2$, PHA, HAp (hydroxyapatite), nano polymers and CdSe NPs have been synthesized by plants and microbes (Tables 5 and 6).

Table 5 Biosynthesis of metal-sulfide and nanoceramics and composites nanoparticles by natural plants

S. No.	Plants	MNPs	Particle size (nm)	Shape	References
1.	Banana	CdS	1.48	Spherical	Zhou et al. (2014)
2.	*Tridax procumbens*, *Phyllanthus niruri*, and *Syzygium aromaticum*	ZnO	165.9, 203.1 and 218.4	Spherical	Sathishkumar et al. (2018)
3.	*Curcuma longa*	HAP	10–25 width and 50–80 length	Rod	Kumar et al. (2018)
4.	*Pineapple*	Cellulose	50–60	Fibre	Cherian et al. (2010)

Table 6 Biosynthesis of metal-sulfide and nanoceramics and composites nanoparticles by microorganisms

S. No.	Microorganisms	MONPs	Particle size (nm)	Shape	References
1.	*Fusarium oxysporum*	CdS	5–20	–	Ahmad et al. (2002)
2.	*Escherichia coli*	CdS	2–5	Spherical, elliptical	Sweeney et al. (2004)
3.	*Rhodobacter sphaeroides*	PbS	10.5 ± 0.15	Spherical	Bai and Zhang (2009)
4.	*Rhodosporidium diobovatum*	PbS	10	–	Seshadri et al. (2011)
5.	*Desulfobacteriaceae*	ZnS	2–5	Spherical	Labrenz et al. (2000)
6.	*Fusarium oxysporum*	$SrCO_3$	10–50	Needlelike	Rautaray et al. (2004)
7.	*Fusarium oxysporum*	$Zn_3(PO4)_2$	10–80 × 80–200	Rectangular	He et al. (2009)
8.	*Fusarium oxysporum*	CdSe	9–15	Spherical	Kumar et al. (2007)
9.	*Bacillus megaterium and Cupriavidus necator*	PHB	100–125	–	Ram Kumar Pandian et al. (2009)
10.	*Fusarium oxysporum*	$PbCO_3$, $CdCO_3$	120–200	Spherical	Sanyal et al. (2005)

Govindaraj et al., have been synthesised green and low cost of nano HAP-flakes using *Moringa oleifera* leaves for the application of bone repair treatment (Govindaraj and Rajan 2016). Sumathra et al., was prepared the nano HAP by pectin extracted as a template from the bio waste of citrus fruit peel. The synthesised nano HAP composite was used for the biomedical applications of dental, orthopedic and tissue engineering (Sumathra et al. 2017). Govindan et al., reported the HAP nanorods synthesis and it having nanocrystalline characteristics by *Curcuma longa* tuber and eggshell as a calcium source for the treatment of bone infections in orthopedics with antibacterial activity (Kumar et al. 2018).

Bibin Mathew Cherian et al., also isolate nano cellulose fibres from pineapple leaf (PALF) by steam explosion method. Resulted in the isolation of PALF having width in the range of 5–60 nm (Cherian et al. 2010). Djalal Trache et al., evaluated that various lignocellulosic sources of cellulose nanocrystals fibers (<100 nm) (CNCs) by woody plants, non-woody plants and agricultural residues. CNCs are being discovered for a numeral of innovative applications for their crystalline nature. The degree of size and crystallinity of the crystalline regions be influenced with the natural source of the cellulose and the isolation process. The degree of crystallinity may differ from

nearly 50% in plants, 60% in bacterial cellulose, 80% in tunicates and up to 90% in some algae (Trache et al. 2017).

$SrCO_3$ crystals are attained by fungi was incubated using aqueous Sr^{2+} ions (Rautaray et al. 2004). D. Rautaray et al. assumed that secretion of proteins in the enlargement of the fungus *Fusarium oxysporum* was in authority for controlling the morphology of strontianite crystals. $Zn_3(PO_4)_2$ nanopowders have been produced by yeasts as templates (He et al. 2009). Kumar et al. exhibited the extremely luminescent CdSe quantum dots have been prepared by *F. oxysporum* at RT (Kumar et al. 2007). Sushobhan Pradhan concentrated on an improved and innovative method for the synthesis of poly (3-hydroxybutyrate) (PHB) by two microorganisms such as *Bacillus megaterium* and *Cupriavidus necator* (Ram Kumar Pandian et al. 2009).

3 Mechanisms of Nanoparticle Formation

Now a day, MNPs have more consideration of scientists for their wide-ranging application to innovative technologies. So we need a complete knowledge of the mechanisms involved in the NPs biosynthesis was essential to direct the size, shape and crystallinity of NPs. Biosynthesis of NPs also involved, alkaloids, flavones, anthracenes, quinones, proteins, many enzymes and etc. (Jeevanandam et al. 2016).

The mechanism of metal NPs synthetic mechanism is proposed in different ways. One of the important and acceptable mechanisms is the reducing of metals oxides, sulphide, chloride to metal and metal oxides. Huang et al. (2007) and Kesharwani et al. (2009). proposed that carbohydrates, proteins (amino groups) and the presence of functional groups (ether, carbonyl and alkene) derived from plant extracts to reduce the metal ions and enable the formation of MNPs. Therefore, it might be determined that plant extract compounds are the corresponding source for the synthesis of MNPs by natural source plant (Li et al. 2007). Li et al. (2007) and Mukherjee et al. (2012) suggested that the formation of MNPs through reduction followed by stabilization consist of proteins (MW ~ low and high) mixture of starch and some plant phytochemicals as declared in Fig. 7a, b. Makarov et al. (2014), reported that the metal ions were reduced by plant extracts and further they are coated by plant source for reduce the size by three phase mechanism which is shown in Fig. 7c. Two steps were carried out in activation phase; metal ions reduction and nucleation. In the growth phase, the NPs stability increases, and in the termination phase, the final shape of NPs was formulated (Si and Mandal 2007). Moreover, for MO formation, before growth process, phytochemicals or atmosphere oxygen binds with metal ions. Figure 7d summarise the concise mechanism for the formation of MONPs (Gowri et al. 2019; Mukunthan and Balaji 2012; Akhtar et al. 2013; Malik et al. 2014).

Ahmed et al. synthesized AgNPs from Ag ions using the extract of *Ocimum basilicu* in which the enol form to keto form conversion of luteolin and rosemarinic acid helps the AgNPs formation (Ahmad et al. 2010). Moreover, certain flavonoids are bind with some metal ions such as Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Al^{3+}, Pb^{2+}, Cr^{3+}, and Co^{2+} by chelation (Makarov et al. 2014). As flavonoids involve in both reduction

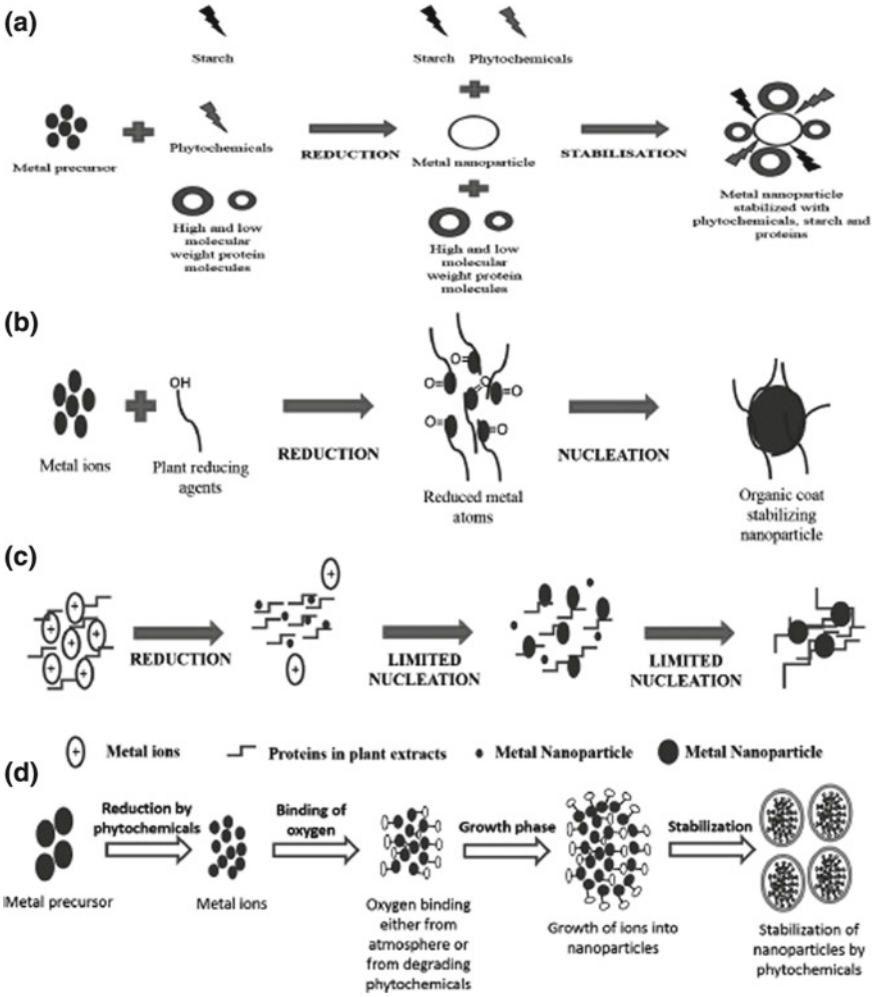

Fig. 7 Mechanism of nanoparticle formation by natural plants (Makarov et al. 2014)

and chelation, they are expected to be concerned in nucleation, growth and stabilization process steps leading to NP formation. However, there is no information to confirmation that a specific phytochemical was answerable for the synthesis of NPs. Furthermost extract of some plant leaf have utilized for MONPs synthesis in which the flavonoid content was high when compared to others. Flavonoid stabilized NPs also reduced harmfulness of the nano-formulation. Besides, flavonoids

have antioxidant, anti-inflammatory, hepatoprotective, anticancer, antibacterial, and antiviral properties (Jeevanandam et al. 2016).

Nanoparticle Formation by Microorganisms

Then, the NP synthesis of microorganism through is bioreduction. In the bioreduction mechanism myriads of proteins, carbohydrates and biomembranes are played a vital role in the processes of microbial bioreduction (Narayanan and Sakthivel 2010). NPs are made by bioreduction leads to aggregation of the metal ions on cell wall surface. This maybe happens by the strong electrostatic interaction of positively charged enzymes present at the cell wall with metal ions (Bansal et al. 2004). With the help of some reducing agents such as NADH or nitrate dependent reductase, microbial cell reduces metal ions (Mandal et al. 2006). The yeast, membrane bound oxidoreductases and quinones are played a significant role in the process. The pH sensitive oxidoreductases works in low pH, oxidase gets induced whereas a high pH induces the reductase (Ramezani et al. 2010). Besides protein assays showed that the responsible factor for the biosynthesis process is NADH-dependent reductase. It gets electrons and converts the NADH to NAD^+. Then, the reductase was oxidized by the reduction of metal ions (Senapati et al. 2005). The in vitro synthesis of 10–25 nm Ag hydrosol using NADPH-dependent nitrate reductase using Fusarium oxysporum was reported recently. Duran et al. explained that mechanistic feature, napthoquinones and anthraquinones some quinine derivatives also used in AgNPs formation (Narayanan and Sakthivel 2010).

In the fungus, the proteins and reducing agents are stabilized the NPs. At least four fungal proteins are associated with NPs. Conversely, fluorescence spectra show the native protein was not affected when it bound on the NPs surfaces (Duran et al. 2007). One of the essential enzymes was in charge for the reductions in certain microorganisms are nitrate/nitrite dependent reductase. In Fusarium oxysporum, this enzyme was bind with quinine, reduced the metal ion (Ramezani et al. 2010). The detoxification mechanisms occurred in the yeast cells through the molecules of glutathione (GSH) and two metal-binding ligands groups are major contributed. GSH with c-Glu-Cys-Glystructure is an essential tripeptide concerned in yeasts, bacteria, plants and animals metabolic processes (Praphakar and Rajan 2017).

4 Drawbacks of Biosynthesised Nanoparticles

Biosynthesised NPs have a lot of advantages, but there also some drawbacks. Utmost of the biosynthesis methods for NPs, with plant-mediated synthesis, are still need to develop. The most important tasks in NPs synthesis are stability, morphology, size, monodispersity, and aggregation of NPs (Iravani 2011). Hence, active control of the particle size and monodispersity need to be broadly studied. Compared with other physical and chemical approaches, synthesis of NPs by microorganisms is a slow process since it is take several hours or few days. Still, there are a no results on industrial level production of NPs using as a bio-organisms (Mittal et al. 2013). Even

though the disadvantages, Plant-mediated synthesis possesses to be a hopeful method in scale up processes of less harmful NP production as they are environmentally benign (Dreher 2004), inexpensive (Iravani 2011), and deliver NPs are controlled size compared to other biosynthesis methods (Makarov et al. 2014).

5 Biological Applications of Nanoparticles

5.1 Drug and Gene Delivery Agents

The intention of novel drug delivery system is to attempt the maximum therapeutic effect and controlled release at the target site at the right time in a safe manner. In the case of targeted carriers, it should find the way through blood barriers and then to reach targeted sites. Their internalization into the cells were carried out by endocytotic and transcytotic transport mechanisms (Fadeel and Garcia-Bennett 2010). Due to the small size, NPs can easily travel into the blood barrier and penetrate the cells and deliver the drugs at the desired place. Moreover, the extended high surface area to volume ratio of NPs show enhanced biodistribution and pharmacokinetics of drugs and thereby reduce the toxicity (Poorani et al. 2017). For poor water soluble drugs, nanocarriers act as a promising candidate to improve the solubility and render them suitable for parenteral administration. The therapeutic agent's stability was systemically increased by nanocarriers (Vinothini et al. 2019b) (Fig. 8).

The well-known biocompatible magnetic NPs such as Fe_3O_4 and Fe_2O_3 have been widely investigated in various biomedical applications such as gene therapy, DNA analysis, sorting and manipulation of stem cells and drug delivery (Fan et al. 2009). Xiang L. et al. investigated the toxic profile of magnetosomes from *Magnetospirillum* gryphiswaldense via in vitro to mouse fibroblasts and conclude that the magnetosomes were non-toxic to mouse fibroblasts (Xiang et al. 2007). Recently, Meng et al. analysed the native bacterial magnetic particles influencing nature on mouse immune response (Meng and Tian 2010). From the results, the native bacterial magnetic particles did not show any effect on mouse immune response and hence magnetosomes have been considered as a potential candidate for tumor treatment. Sun et al. reported that the doxorubicin loaded magnetosomes ability at the tumor site. From the observation, magnetosomes exist execute therapeutic action at the target site (Sun et al. 2007).

Gold nanoparticles (AuNPs) have long been used as a therapeutic agent due to its stability, function tenability, high surface to volume ratio and unique size. The surface of the AuNPs can easily conjugate with ligands having functional groups such as thiol, amine which shows high affinity on AuNPs surface (Giljohann et al. 2010). The synthesis of AuNPs via chemical method has been widely studied. To the best of our knowledge there are no investigations of biosynthesized AuNPs for drug and gene delivery.

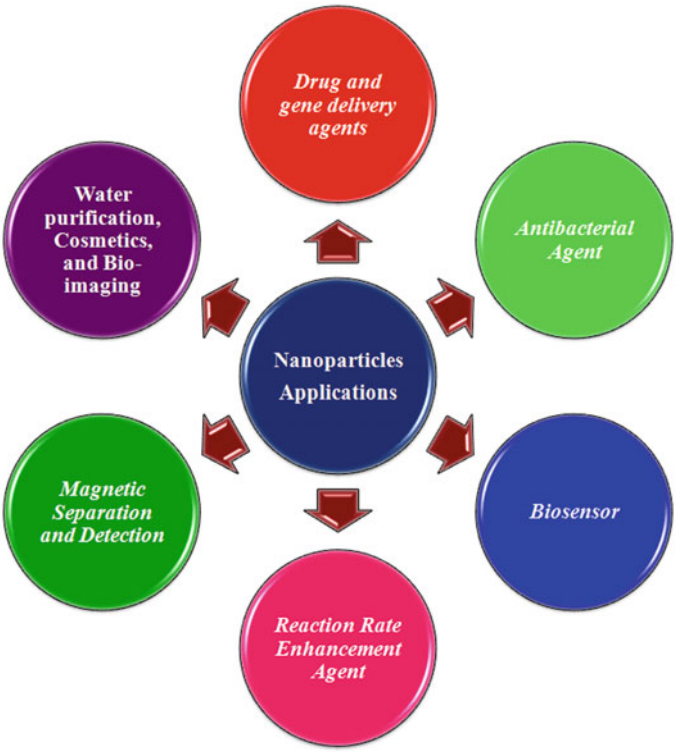

Fig. 8 Applications of biosynthesized metal nanoparticles

Silver nanoparticles (AgNPs) possess interesting antiviral, antifungal, antibacterial and antiinflammatory properties. Kalishwaralal et al. reported that the anti-angiogenic potential of silver nanoparticles by using *Bacillus licheniformis*. AgNPs shows better inhibition of cells via PI3K/Akt-dependent pathway in BRECs (Kalishwaralal et al. 2009).

5.2 Antibacterial Agent

The resistance nature of microorganisms against multiple antibiotics induces the formation of new antibiotic. Silver nanoparticles prepared by using fungus Trichoderma viride results the formation of stable AgNPs with size <50 nm. The anti-microbial properties of as prepared AgNPs were evaluated against gram positive and gram negative bacteria (Fayaz et al. 2010). The anti-biotic effects of therapeutic antibiotics were systemically increased in the presence of AgNPs. The combination of antibiotics with AgNPs has much effect against microbes and this will help to promote new antibiotic formulations. Duran et al. formulated AgNPs by using *Fusarium*

oxysporum which can inhibit the microorganism growth especially Staphylococcus aureus in textile fabrics (Duran et al. 2007). Ahmad et al. reported AgNPs prepared by using culture supernatants of *Klebsiella pneumoniae* and improve the effect of antibiotics when it combined with AgNPs (Rai et al. 2009). Moreover, the AgNPs also have the property of disrupting the cell membrane of pathogenic organisms. Sondi et al. reported that the action of cell membrane breaking and the mechanism of inhibition of protein synthesis by AgNPs (Sondi and Salopek-Sondi 2004). The high concentration of AgNPs were ruptured the cell quickly when compared with lower concentration (Kasthuri et al. 2009). *Rhizophora apiculata* reduced AgNPs shows lower number of bacterial colony compared with $AgNO_3$ reported by Anthony et al. (2011). Kaviya et al. analysed the anti-bacterial activity of *Citrus sinensis* peel extract reduced AgNPs against *Escherichia coli*, *Pseudomonas aeruginosa* (gram-negative) and *Staphylococcus aureus* (gram-positive) and proved the effectiveness of AgNPs (Kaviya et al. 2011). Consequently, Krishnaraj et al. prepared AgNPs by using Acalypha indica plant leaf which shows better efficiency to reduce the water borne pathogenic bacteria with minimum concentration (Mohanpuria et al. 2008). Palladium and copper nanoparticles also show antimicrobial activity against *E. coli* (Krishnaraj et al. 2010).

5.3 Biosensor

The interesting properties (optical and electronic) of nanoparticles can be induced their applications in the field of biosensor. Selenium nanoparticles with 50–400 nm exhibited in spherical shape and which can be used as enhancing materials for building horseradish peroxidase biosensor. Due to the good adhesive ability and biocompatibility, Se nanoparticles show well electrocatalytic activity towards the H_2O_2 reduction (Wang et al. 2010). Hence, Se nanoparticles will be a promising candidate to detect H_2O_2 in various fields. Au–Ag alloy prepared by Zheng et al. from yeast cells has the ability to detect vanillin and thus used to fabricate vanillin sensor (Zheng et al. 2010a). AuNPs could increase the enzyme activity of glucose oxidase. Based on this aforementioned information AuNPs based glucose oxidase biosensor was developed by Zheng et al. (2010b). Haes et al. designed triangular AgNPs coated glass substrate. This biosensor was used to detect biotin-streptavidin interactions (Haes and Van Duyne 2002) and two biomolecules related to Alzheimer's disease (Haes et al. 2004). Recently, AgNPs based biosensors also used in the cancer detection (Zhou et al. 2011). Furthermore, the silica-coated nanosilver biosensors were used for the bovine serum albumin detection (Sotiriou et al. 2010).

5.4 Reaction Rate Enhancement Agent

Due to their large surface area, nanoparticles were used as catalysts in various reactions to improve the rate of the reaction. For enhance the microbiological reaction rates, magnetic nanoparticles are the best choice. In addition to the good catalytic activity, magnetic nanoparticles can easily disperse. Shan et al. carried out the desulfurization of dibenzothiophene reaction by using coated microbial cells of *Pseudomonas delafieldii* with magnetic Fe_3O_4 nanoparticles (Jain and Pradeep 2005). Due to the large surface area, it can easily bind with the surface of the cells.

5.5 Magnetic Separation and Detection

Biological molecules conjugated magnetic particles are attractive materials which are used as a biological label. Antibodies immobilised bacterial magnetic particles are used to detect molecules such as environmental pollutants, hormones and detergents (Shan et al. 2005). Based on the xenoestrogens competitive reaction, some xenoestrogens were easily detected by using antibodies immobilised bacterial magnetic particles. When compared with typical plate method, this procedure was completed within 15 min could make this method as a developed one. The surface of the antibodies immobilised bacterial magnetic particles modified with aminosilanes were used in DNA extraction.

5.6 Other Applications

The applications of nanoparticles have been widely investigated in some other fields such as water purification (Nagaraj et al. 2018), cosmetics, bio-imaging and tissue engineering. AgNPs coated surfaces were used as antimicrobial coatings in water purification (Jain and Pradeep 2005). In bio-imaging, folic acid associated CdTe QDs were used to imaging cancer cells (Bao et al. 2010). The cell uptake in macrophages, AgNPs anchored iron oxide NPs were easily detected by two-photon imaging (Kumar et al. 2010). The gold, silver and platinum NPs are widely investigated in various products. New dimensional metallic nanoparticles are used in various commercial applications (Kokura et al. 2010). AgNPs were used in the preparation of ointments and creams used for burns and open wounds (Becker 1999).

6 Conclusion

Biosynthesised NPs by plants and microbes is believed to be much safer, clean, inexpensive, nontoxic, high efficient and eco-friendly compared to others. Plant extracts have several chemical compounds such as proteins, carbohydrates, alkaloids, flavonoids, terpenoids and oils which used for medicinal applications and also act as reducing and capping agents for MNPs synthesis. The methodology employed by using microorganisms including yeast, bacteria, fungi, and actinomycetes are classified into intracellular and extracellular synthesis depending on the location of NPs are formed. The shape, size, and size distribution of MNPs have been controlled by optimization of reaction parameters such as temperature, pH, and ratio and concentration of plant extract to the metal salt, substrate concentration, and exposure time to substrate of microbs it will be possible to attain a huge amount of stable, and small size NPs. Besides, the lack of knowledge which chemical components are answerable and the fundamental mechanisms for the synthesis, reduction, and stabilization of biological NPs. Particularly in terms of biocompatibility, it is significant to realize how active groups present in the bio sources attach to the NP surface, and which active groups are involved, to formed NPs with greater efficacy. Biosynthesized MNPs are used to several applications such as biomedical, antimicrobial, drug and gene delivery agents, tissue engineering, agriculture, bioinsecticides, biosensor, catalyst, etc. The various studies showed that biosynthesized MNPs by using plant extract have higher antibacterial activity than MNPs chemically synthesized. We hope that carrying out of these methodologies on a large scale and their commercial applications have been used for several fields will take place in the coming years.

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An Overview of Nanotoxicological Effects Towards Plants, Animals, Microorganisms and Environment

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Abstract In recent years, nanotechnology has reached the limelight of research in applications of medicine and technology. Due to its onset, huge varieties of nanoparticles possessing significant characters are synthesized with broad application fields. Even though these particles are infesting our present life; conflictual views regarding their medical and biological effects are debatable. The non biodegradable nature and nanosize are the alarming features of the nanoparticles that confront potential threats to both environment and biomedical field on its expanding usage. NPs synthesized from heavy metals like lead, mercury and tin are proclaimed as stringent and stable compounds for degradation, hence results in environmental biohazards.

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The extensive applications of silver nanoparticles in biosensing, cosmetics, medical devices, food and clothing products inflates its human exposure and obviously resulted in toxicity (short and long term). In vitro studies revealed various cytotoxic effects in the cells of mammals such as brain, liver, lung, skin, reproductive organs and vascular system. Furthermore, ingestion, inhalation or injection of nanoparticles in intraperitoneal region resulted in toxic effect of multiple organs inclusively brain. Accounting the metal nanoparticles biohazardous effects like ROS (Reactive oxygen species) generation, DNA damage, protein denaturation and lipid peroxidation has been proved on carbon based nanoparticles, organic lipid based nanoparticles, mineral based nanoparticles, nano diamonds, nano composites, etc. Although, nanotechnology has become an advent field of research nowadays, it is importing significant environmental and health hazards thus couldn't be beneficial to both society and economy.

Keywords Nano particles · Toxicity · Nano composites · Bioeconomy · Human health

1 Introduction

Existence of nanoparticles (NPs) is uncertain, over million years ago and their employment by humans is about thousands of years. Because of the accelerated human capacity in nanoparticle synthesis, enough attention has been directed on this type of particles. Due to their compelling potential of usage in wide areas like electrical industry, pharmaceuticals, cosmetics, medical and environmental applications, their respective investments are also growing worldwide (Guzman et al. 2006). The imperative fact about nanotechnology is the consideration of scientists as the lucid step of science to integrate biology, chemistry, physics, medicine and engineering (Chen and Mao 2007; Dahl et al. 2007; Vo-Dinh 2007; Janata 2008; Stewart et al. 2008).

The applications of nanotechnology has inclined greatly from the laboratory to economic market with huge interest scientifically through pharma industry. The particles right from the distinct nano to sub-micron sized were engaged widely in food, pharmaceuticals and cosmetics industries. In pharmaceutical industries nanoparticles were employed adversely as carriers of drug delivery, imaging, diagnostic agents of oncology and in diabetes. In the flourishing field of pharmaceuticals the nanotechnology are engaged with great potential through oral, dermal and injectable routes. As per FDA, 25 nanoparticles has been approved to use in enormous drug delivery systems, which implies its competency in treating diseases (infectious and non infectious). Nanomaterials are worn in numerous forms like nanotubes, nanomembranes, nanoparticles, nanofibers, liposomes, nanofilms etc. In pharmaceutical industries, lipid based nanoparticles (nanolipidsomes, lipid nanoparticles, nanoemulsions, lipid nanocapsules, lipid polymer nanoparticles), dendrimers, nanoshells and fullerenes were extensively studied in drug delivery systems of both academics and industries.

Apart from these, some emerging particles includes, metal nanoparticles, nanodiamonds, carbon nanotubes, graphene nanoparticles and quantum dots, they were used in diagnosis, drug delivery and imaging so as to achieve decisive targeting upon organs and cells.

The probable toxicity of metals that are accounted herein was well rooted since roman times and not new. Pedanius Dioscorides, the greek physician has previously described the probable effects of metals like mercury (Caley 1928), lead oxide (Osbaldeston and Wood 2000), copper silicate (Wisniak 2004), poisonous effects of Arsenic in yellow and red sulfur mines as referred by Strabo (Cilliers and Retief 2000) and demise of Alexander, the great as a consequence of drinking contaminated water of River Styx (Atkinson and Truter 2009). In the initial part of the present century, toxicity of metals and toxic effects of the excessive tiny particles were explored (Donaldson et al. 2001).

Expulsion of nanoparticles from consumer's body is pivotal. It is vital to determine the exceeding nanoparticles to overcome adverse effects (Kantiani et al. 2010). It was determined from the ancient times that dose of poison was ample to evoke a response. Nonetheless, size, physicochemical properties and mode of entry of nanoparticle will influences to determine persistence, hazard threat and biotoxicity so as to formulate and implement safety patterns (Scott-Fordsmand et al. 2014). The unwelcome consequences of the nanoparticle exposure are health ailments due to cytotoxicity, genotoxicity, cancer and autoimmune diseases.

The primary concern regarding the employment of metals within living organisms is their corroding and degrading ability results in diminished toughness, disintegration and weakening of accounted implants. Their activity would be diminished by the curtailing effects of biocompatibility and escalation of toxic effects (Burugapalli et al. 2016; Khodaei et al. 2016). The components like dissolved oxygen, soluble carbonates, nitrogen and electrolytes along with some physiological fluids (proteins, enzymes, organic acids and macromolecules), secretory compounds of inflammatory and fibrotic cells are responsible for progress of metal degradation which are made possible by the inhabitance of stress, strain and frictional forces.

Concerning on the biosafety of health and environmental issues into account the risk factors of NPs should be assessed prior to its application. Additionally, engineered nanoparticles could be released into the water bodies during the manufacturing and utilization processes unaviodably. Some environmental factors includes UV radiation, dissolved organic matter, ionic strength and pH could possibly react with the NPs, then the converted NPs make toxic effects on the concerning environment (El Badawy et al. 2010; Levard et al. 2012).

The undenied biocompatibility nature of NPs were contemporarily swamped off by the biotoxicity effects. Understanding of their properties relating to biological responses is vital so as to understand the flawless usage of nanoparticles. The mechanism of the nanoparticle reckons on respective factors like composition, chemical functions, shape along with its exclusive size and charge (Goodman et al. 2004; Roiter et al. 2009; Simon-Deckers et al. 2009; Xiao et al. 2012; Silva et al. 2014). Moreover, probable risk of nanoparticle resides on its respective particle size below 100 nm (nanoparticle). Based on the nanoparticle nature (metal or magnetic), the

breakdown mechanism that arise within the body results in unpredictable and noteworthy toxic effects. Since, NPs are involving in numerous catalytic and oxidative mechanisms in vivo, it is very hard to predict. Nanomaterials that exposing reactive surfaces with very high surface area are attractive for specific objectives. Beddoes et al. (2015) has conferred from both in vitro and in vivo examination of human cells along with membranes and succeedingly addressed that (a) Efficient translocation of nanoparticle through the membrane that resulting in cellular damage has been made possible by small NPs, whilst nanoparticles of large size displays active cellular uptake without toxic effects, (b) Disruption of membrane integrity was made by nanoparticles of positive charge rather than negative charged particles.

The nanomaterial field is very extensive with diverge toxicity, in the present review, few nanoparticle effects were highlighted as examples to predict the disturbances in biological systems. The study of nanoparticle toxicity towards any biological substances (animals and plants) are known as nanotoxicology, which comprises in vitro studies using cell lines of human or animal, in vivo experiments using human volunteers and animals, along with epidemiological data regarding the pollution of particle and studies of workers those who are exposed to nanoparticles (during welding, mining, etc.). Applications of nanoparticles are being inflated nowadays in agriculture field in the form of agrochemicals.

Toxicity induced by nanoparticle involves in evolution of oxidative stress (free radical or liberation of reactive oxygen species (ROS), genetic damage, inflammation and suppression of cell division which results in apoptosis. In addition, ROS stress accounts for fibrosis, inflammation, genotoxicity followed by carcinogenesis through the liberation of adverse cytokines. Above all the vital mechanism of toxicity resides on the reactive oxygen species generation, such that free radical possess detrimental impacts over biomolecules (DNA, lipids, proteins). Numerous biological mechanisms like endocytosis, phagocytosis with its processing (antigen presentation on MHC class molecules) and passive diffusion reckon on the particle size of nanoparticles (gold, silver, nickel, titanium, carbon nanotubes). The large surface area of NPs contributes to few toxic indications of biological molecules that confers oxidation results in DNA damage than the larger particles with similar size (Gatoo et al. 2014). The factor that contributes the difference between nanoparticle and large particle composed of same material are the quantum effects and its respective surface (Buzea and Pacheco 2017). The nanosized material displayed diverge properties (physical, chemical and mechanical) rather than the bulk sized particles. As a consequence of the proportion of atoms found exposed on the surface of the nanoparticle correlated with the interior surface escalates results in boost up of its physical (increased surface area along with volume ratio, and shortened melting point) and chemical properties (higher chemical reactivity). By the cause of the small size of the nanoparticles (gold, palladium and platinum), the electrons confined and possess quantized spectrum of energy, producing quantum size effects like magnetic moments.

The nanomaterials are prone to contaminate the vulnerable water ecosystem directly or indirectly so as their possible toxicity to aquatic biota should be evaluated. Adverse effects like inhibition of algal growth, behavioural changes associated with severe mortality rate in water fleas (*Daphnia* species), damage in fish brain cells and

changes in molecular biomarkers were explored. But, interaction of aquatic biota with nanomaterials, and their respective destiny in water is least recognized still. Along with the coastal progression, NiO nanoparticles separated during welding has turned into the vital sources of coastal pollution (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans). These NiO nanoparticles can be enforced as risk factors for the environment and health of human. The risk associated with inhalation of NiO nanoparticles by mammals were well established in in vitro assays (Oyabu et al. 2007).

Even though the nanoparticles acquire huge beneficial applications in the fields of agriculture, environment, medical diagnosis and treatment, some hazardous effects were also observed in animal models, human, plants, and water bodies of the environment. The fundamental complication explored by the nanoparticles are their ability to enter into the cells and concludes in cytotoxicity to inhibit their growth and development respectively. This chapter probes the biohazardous effects of different nanoparticles towards various hosts and habitat. Analysing those detrimental effects of nanoparticle would grant a wide view upon commercialization of nanoparticles in the field of agriculture, medicine and environment.

2 Factors Responsible for the Toxicity of Nanoparticles

The shape, size, surface charge, crystallinity, aggregation and surface coating of NPs are some of the factors responsible for the toxicity of nanoparticles.

The shape dependent toxicity is associated to the metal nanoparticles (gold, silver, nickel, titanium) and engineered particles (Carbon nanotubes). The nanoparticle entry into the cell by endocytosis and phagocytosis have serious impact due to the shape of the nanoparticles. For example spherical shaped nanoparticles are very prone to endocytosis when compared to other shapes of nanoparticles (Gatoo et al. 2014). Investigations revealed that the shape of the particle could affect the cellular level. K^+ ion channel blockage is three times higher by rod shaped Single walled nanotubes (SWNTs) than the spherical shaped C_{60} fullerene (Park et al. 2003). ZnO nanorods are confirmed to be more cytotoxic than the spherical ones (Hsiao and Huang 2011).

The size of nanoparticle is also inevitable in cytotoxic effects. Asbestos fibres of <2 μm size could cause asbestosis, whereas asbestos with <5 μm size cause mesothelioma and 10 μm sized asbestos would results in carcinoma (Lippmann 1990). Similarly 15 mm length TiO_2 fibres are more toxic than the 5 mm length fibres, which cause inflammatory response by alveolar macrophages in mice. Long multi walled carbon nanotubes (MWCNTs) can cause inflammatory response in abdominal cavity of mice than the small MWCNTs (Poland et al. 2008).

The surface charge of the nanoparticle employs vital impact over toxicity. The charge of the nanoparticle directs huge interactions like selective absorption, blood brain barrier integrity, plasma protein binding and membrane permeability. For example, negative charge carrying mammalian cell membranes improves interaction with cationic particles with the cells to a terrific degree than the negative or neutral

nanoparticles. Nonetheless greater cationic charge results in serious toxicity through hemolysis and aggregation of platelets (Gatoo et al. 2014). Silica NPs which carry positive charge are shown to induce ROS than the silica NPs with negative or neutral charge (Bhattacharjee et al. 2010).

Several studies have reported that TiO_2 (anatase form) could results in toxicity thereby inducing DNA damage with higher lipid peroxidation in the presence of light whereas the rutile form couldn't results in any toxic effects (Gurr et al. 2005).

Aggregation of particles could also conveys toxicity. Aggregation of the particles mostly imparts on the size, surface charge and particle composition. For example, aggregated carbon nanotubes (CNTs) will have more cytotoxic effect than the dispersed ones (Wick et al. 2007).

The physiochemical properties of NPs like surface charge, chemical, magnetic, optical and electric charge can altered by the surface coating of the particles. These changes can eventually results in interactions with biomolecules to produce significant nanoparticle toxicity. For example, the presence of oxygen radicals with heavy metals and ozone on surface of nanoparticles results in ROS formation and triggers cell inflammation. In certain cases the surface coating is essential to subside the NP toxicity. For example, essential coating in quantum dots made them non toxic because of their hydrophobic metal core and toxic heavy metals like cadmium (Talkar et al. 2018).

3 Nanoparticles in Agriculture Field

3.1 Phytotoxicity of Nanoparticles

The efficient uptake of nanoparticle is very specific depending on the plants. The factors involved in uptake are type and physicochemical properties of the nanoparticle, species and substrate of the plants (Arruda et al. 2015; Zuverza-Mena et al. 2017). Translocation of nanoparticle within the plants are made by establishing complexes between root exudates and transporter proteins (Yadav et al. 2014). Roots could intake tiny nanoparticles through its pores (5–20 nm size) present in epidermal cell wall of roots (apoplast) (Deng et al. 2014). Larger particles will be blocked so that small particles pass the cell walls results in capillary forces as a result of osmotic pressure and finally reaches endodermis by diffusing through the apoplast (Lin et al. 2009; Deng et al. 2014). In plants, nanoparticles can be uptaken by symplastic pathway through the plasma membrane inner side. Migration of nanoparticles to neighbor cells occurs through 20–50 nm (diameter) plasmodesmata channels (Deng et al. 2014). One more possible way of nanoparticle entry is foliar via pores of stomata and could be translocated to other parts along with roots (Hong et al. 2014). Nanoparticles (silver, zinc oxide, iron oxide, ceria and titania) with huge range of size and composition can interact with plants by means of internalization into leaves (Chichiricco and Poma 2015). The nanoparticles react with organelles of cell and contributes in oxidative stress,

metabolic transformations and genotoxicity (Deng et al. 2014). Even though few nanoparticles exposes positivity on one extreme, also urges negative consequences on another extreme. For Example CeO nanoparticles (500 mg/kg) exposed to barley could boost up shoot biomass (300%) but no grain formation was possible (Rico et al. 2015).

3.2 Detrimental Effects of Nanoparticle on Biochemical Traits of Plants

Even though the plant nanoparticle interaction brings some beneficial effects, huge studies are found available in indicating the detrimental effects of nanoparticle towards plants. The detrimental effects upon biochemical traits involves in ROS generation, lipid peroxidation, decline transpiration rate, disruption in mitosis, cell wall breakdown, diminished content of chlorophyll and cutback photosynthesis (Tripathi et al. 2017). Exposure of carbon-based nanoparticles (CNTs, C_{60}) results in cellular toxicity of rice, onion and spinach respectively (Chen et al. 2010; Shen et al. 2010; Begum and Fugetsu 2012). TiO_2 exposure produces stress in cucumber (Servin et al. 2013). Exposure of NiO nanoparticles in tomato triggers stress which was followed by mitochondrial and cell damage (Faisal et al. 2013). TiO_2 exposure produces chloroplast damage and hence photosynthetic rate of spinach was also decreased. In green peas the chlorophyll is greatly affected by ZnO nanoparticles (Mukherjee et al. 2014).

3.3 Unfortunate Outcomes of Nanoparticles on Plant Morphological Changes

The morphological changes of plants include germination index (germination rate and time), biomass of shoot and root, morphology of root tip, root elongation, etc. (Deng et al. 2014). The phytotoxic nanoparticles includes gold, silver, copper oxide, zinc oxide, carbon nanotubes and alumina which produce detrimental effects on roots and shoots (Ghodake et al. 2011; Begum and Fugetsu 2012; Begum et al. 2012; Burklew et al. 2012; Dimkpa et al. 2013; Deng et al. 2014; Feichtmeier et al. 2015). Exposure of ZnO in soybean plant affects formation of seeds (Yoon et al. 2014). Gold nanoparticles exposure in tobacco plant urges necrosis in tissues (Sabo-Attwood et al. 2012). CNTs inclusion is found to be phytotoxic against cucumber, lettuce and red spinach by decreasing length of roots and shoot, at the same time no unfavourable effects were recognised in soybeans and chilli (Begum et al. 2014). By virtue of nanoparticle absorption by roots, numerous NPs contributes adverse effects to seedling during roots and shoot elongation. Nanoparticle phytotoxicity pertinent to inhibition of growth reveals biomass reduction, decrease in germination

and growth of leaf, reduced elongation of root, decreased root biomass, change in root tip morphology, and shoot growth, flowering delay and yield decrease (Tripathi et al. 2017). Silver nanoparticles exposure results in stunted germination of corn and rice (Pokhrel and Dubey 2013; Thuesombat et al. 2014) followed by reduction of mitotic index and fragmentations of chromosomes in onion (Kumari et al. 2009). Growth of rice, soybean, corn and cabbage plants were adversely inhibited by the exposure of ZnO nanoparticles (Lin and Xing 2007; Boonyanitipong et al. 2011; Xiang et al. 2015). Carbon-based nanoparticles like C_{60} and CNTs urges biomass reduction in zucchini (Stampoulis et al. 2009), followed by delay in flowering and diminished harvest (Lin et al. 2009). TiO_2 exposure towards corn brings about inhibition in growth of leaf with damage of DNA damage (Asli and Neumann 2009; Castiglione et al. 2011).

3.4 Genotoxic Effects of Nanoparticles in Plants

Because of tiny size, NPs can migrate into cells and evoke genetic response of plants. Numerous metal nanoparticles like, Ag, CuO, CeO, TiO_2, ZnO and CNTs triggers genotoxicity against huge plant varieties (Fava beans, Soybean, Buckwheat, Ryegrass, Radish, Tobacco, Onion) (Kumari et al. 2011; Atha et al. 2012; Burklew et al. 2012; Chichiricco and Poma 2015; Ghosh et al. 2015). Genotoxic effects of nanoparticle comprises mitotic index reduction, fragmented sticky chromosomes, gene alteration, chromosomal aberrations, damage of DNA structure and decline viability of cell (Tripathi et al. 2017). These effects were observed in garlic, onion and buckwheat as a consequence of ZnO exposure (Kumari et al. 2011; Shaymurat et al. 2012; Lee et al. 2013). Exposure of CuO to buckwheat and radish results in genotoxic effects (Atha et al. 2012; Lee et al. 2013). Various chromosomal aberrations (breaking of chromosome and nuclear blebbing) were resulted by titanium oxide nanoparticle exposure (Pakrashi et al. 2014). Accumulation of CNTs in onion plants ascertained both cytotoxic and genotoxic consequences, which includes alteration in morphology of cells, affecting function of mitochondria and membrane integrity, damage of DNA and chromosomal aberrations (Ghosh et al. 2015). CeO nanoparticles causes adverse effects in intake of nutrition along with genetic alterations of wheat, rice and cucumber (Hong et al. 2014; Rico et al. 2014; Zhao et al. 2014).

3.5 Depletion of Growth Nutrients in Plants Due to Nanoparticles

The plants and plant products like fruits are being consumed mainly for its nutrients and minerals. Exposure of nanoparticles could also results in altered nutrient content, flavor of fruit, performance of growth and antioxidant capability (Deng et al. 2014;

Petersen et al. 2014; Antisari et al. 2015). Hence, usage of agrichemicals composed of nanoparticles would affect nutrients of various crops like rice, soybean, corn, cucumber and tomato (Rico et al. 2013; Antisari et al. 2015; Zhao et al. 2014, 2015). Numerous metal nanoparticles (TiO, Ag, Co, Fe_3O_4, CeO_2 and Ni) exposure to tomato plants displays depletion of compounds like Mg, P and S (Antisari et al. 2015). Exposure of CeO_2 nanoparticles in rice harvest grains resulted in negotiable nutrition values which includes least amount of starch, antioxidants, glutelin, iron, lauric and valeric acid (Rico et al. 2013). Nanoceria exposed cucumber plants would produce fruits with altered Mo micronutrient, sugar, phenolic contents along with fractionation of protein (Zhao et al. 2014). Exposure of nanoceria to corn plant urges decreased yield and curtail calcium translocation to kernals provided by cob (Zhao et al. 2015). ZnO nanoparticles exposure to corn plants produce subtle effects on altered nutrient contents, and reduced photosynthesis as a result of chlorophyll content consequently reduction in yield (49%) (Zhao et al. 2015).

3.6 Transgenerational Effects in Plants by Nanoparticles

Nanoparticles can got concentrated within tissues of roots, seeds, fruits and leaves. Uptake of nanoparticles by seeds has revealed to produce transgenerational effects over few plants (Lin et al. 2009; Wang et al. 2013). The nanoparticles could be disseminated to the progenies of plants through seeds even without exposure of nanoparticles externally. C_{70} could be found in the rice plants even after second generation as black aggregates adjacent to vascular system of stems and leaf tissues (Lin et al. 2009). The second-generation tomato plants obtained after exposure of ceria nanoparticles to parent plants were found to be uncertain with decrease in biomass, declined transpiration of water and greater ROS amount (Wang et al. 2013). The impact of nanoparticles on plant is given in Fig. 1.

4 Nanoparticles on Humans and Animals

4.1 BioToxicity of Nanoparticles in Humans and Animals

The nanoparticles are inappropriate in some extent that some are beneficial agriculturally, nevertheless those are internalized within crops and toxic towards human and laboratory animals by some extent. The toxicity of nanoparticle on animals relies on their size that helps in entering into the organisms, reach circulatory system, translocation to various organs like brain, kidneys, spleen, liver finally enter cells and organelles (Buzea et al. 2007). Those adverse effects are correlated with inflammation and discrete diseases including cancer. Even though the nanotoxicology is

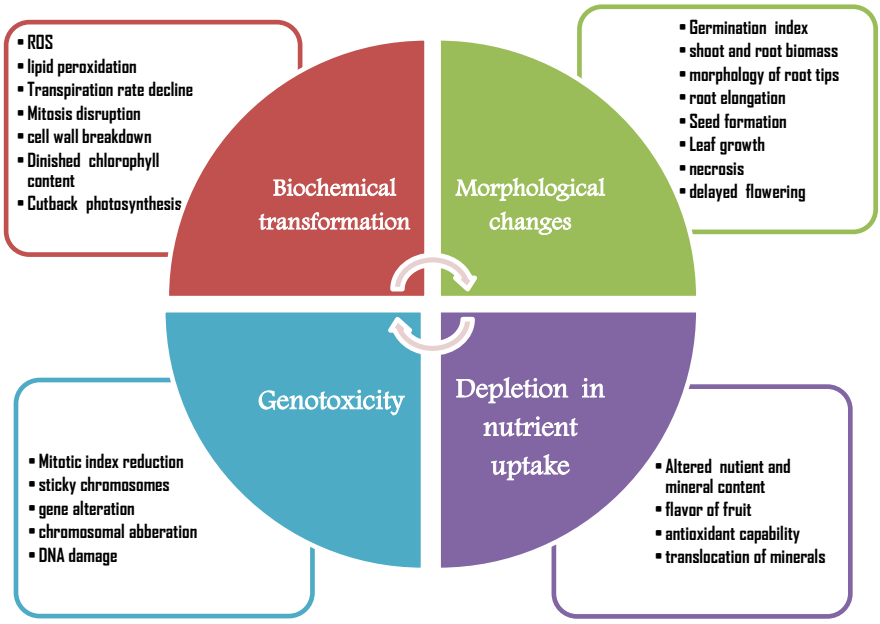

Fig. 1 Impacts of nanoparticles on plant growth, physicochemical and genetic activities

rather a new discipline, plenty of epidemiological investigation on toxicity of environmental nanoparticle towards human are elderly available. Some of the toxic effects of various nanoparticles tested against huge animal models and human cell lines were tabulated (Table 1).

4.2 Factors Affecting Biotoxicity of Nanoparticles—Physicochemical Characteristic

The determination of nanoparticle toxicity confides on the physico-chemical properties like shape, size, composition, porosity, hydrophobicity, surface area, aggregation, magnetic properties and electric charge (Buzea et al. 2007; Li et al. 2015; Silva et al. 2015; Schlinkert et al. 2015; Teske and Detweiler 2015). Same compound derived nanoparticles would exhibit diverse toxicity based on its distinction in size, surface charge and functionalization. Nanoparticles with similar size but with different material composition would also exhibit diverse toxicities obviously. Smaller size nanoparticles will have greater toxicities than the larger ones (Buzea et al. 2007). Simultaneously NPs (Titanium oxide) with same material composition but with varied crystalline forms (rutile and anatase forms) could exerts divergent properties and toxicity. Titania in rutile form (200 nm) would induce oxidative damage to DNA and cytotoxicity in bronchial epithelial cells of human, on the other hand anatase

form of titania could not (Gurr et al. 2005). Few NPs displays both hydrophobic and hydrophilic properties (Garcia-Ivars et al. 2015) which are modulated by the employed coating substances (Podila and Brown 2013) such as polyethylene glycol (PEG) provides hydrophilicity for the accompanying nanoparticle (Kettler et al. 2014). Charges of NPs either positive or negative charge is responsible to react with different biological systems (Gatoo et al. 2014; Salatin et al. 2015). For example NPs with positive charge would be attracted towards cell membranes carrying negative charge and results in cellular intake, which couldn't be made possible by nanoparticles with negative and neutral charge (Kettler et al. 2014). Investigations also revealed

Table 1 Biotoxicity of nanoparticles against model animals and human cell lines

Nanoparticle employed	Animal model/cell line	Toxic effects	Reference
SWCNTs	Rats	Interstitial inflammation and lesions	Lam et al. (2004)
	Kidney cells of human embryo	Cell proliferation inhibition Cell adhesive ability decrease	Cui et al. (2005)
	Lung fibroblast of chinese hamster (V79)	DNA damage	Kisin et al. (2007)
	Fibroblast cells of Mouse embryo	DNA damage	Yang and Watts (2005)
	Epithelial BEAS 2B cells of human	DNA damage	Lindberg (2009)
MWCNTs	Mouse embryonic stem cells	DNA damage	Zhu et al. (2007)
C_{60} fullerenes	Human lung adenocarcinoma	DNA polymerase inhibition (size dependent) Enhanced cytotoxicity	Song et al. (2012)
Citrate capped AgNPs	Rats	Induction of microvessel vascular endothelial cells inflammation Integrity of blood brain barrier affliction	Trickler et al. (2010)
AgNPs	Sprague Dawley rats	Locomotory activity diminishing Injury of central nervous system	Zhang et al. (2013)

(continued)

Table 1 (continued)

Nanoparticle employed	Animal model/cell line	Toxic effects	Reference
	Rats	Histopathological alterations (kidney, liver) swollen epithelium with cytoplasmic vacuolization Basement membrane thickening Mitochondrial cristae destruction Endosomes and lysosomes filled with AgNPs	Sarhan and Hussein (2014)
	Mice	Reduced hemoglobin content RNA transcription inhibition-red cell precursors Downregulation of hemoglobin level-fetal anemia Retardation of embryonic development	Wang et al. (2013)
	Human hepatoma cells	Cytotoxicity	Kim et al. (2009)
CoO	primary human immune cells	toxicity mediated with oxidative stress	Chattopadhyay et al. (2015)
MgO NPs	Vein endothelial and microvascular endothelial cell of human	Toxic effects on cells, oxidative stress	Ge et al. (2011), Sun et al. (2011)

that higher charge (positive and negative) imparts increased endocytic uptake mediated by receptors than the nanoparticles with neutral charge (Kettler et al. 2014). Toxicity of nanoparticles confides on internalization within the cells, such that gold nanoparticles with cationic property are toxic than the nanoparticles with anionic property (Goodman et al. 2004).

4.3 Mode of Internalization of Nanoparticles into Humans

By virtue of its tiny size, NPs could be ingested, inhaled or penetrated via the skin. The smaller nanoparticles will have higher accumulation within tissues (Sonavane et al. 2008). Accumulation of NPs within the body sites is resolved by its respective

composition and functional groups on surface. By means of the gastrointestinal and respiratory systems, the nanoparticles could hastily reaches circulatory and lymphatic system respectively (Landsiedel et al. 2012). It was revealed from various studies that the nanoparticles inhaled would accumulate in the lungs, some of them could reach alveoli based on their respective size and physicochemical properties, could also be systemic by translocating to other organ. These nanoparticles were found available in various parts like heart, brain, liver, spleen, thyroid, kidney, colon, bones along with lymphatic system and circulatory system (Johnston et al. 2010; Khlebtsov and Dykman 2011; Landsiedel et al. 2012; Anderson et al. 2015; Bruinink et al. 2015; Davidson et al. 2015; Geiser and Kreyling 2010; Gosens et al. 2015). Various types of NPs were found in the blood of many diseased patients (Gatti and Montanari 2006). Those nanoparticles combine with the plasma present in the circulatory system and results in the formation of protein corona which determine its toxicity and translocation. Later on, the nanoparticles reach and thereby acquire within various organs and tissues of heart, brain, liver, kidney, spleen, lymph nodes, bone marrow (Landsiedel et al. 2012; Sonavane et al. 2008).

The NPs enter through ingestion reach the gastrointestinal tract and are partially eliminated through feces, few get absorbed and found systematically (Hillyer and Albrecht 2001). It was evident from studies of animal model (in vitro and in vivo) that the nanoparticles could pass the placenta and reach fetus so as resulting in detrimental effects to pregnancy and fetus (Melnik et al. 2013; Semmler-Behnke et al. 2014; Snyder et al. 2015).

4.4 Cytotoxicity of Nanoparticles Towards Animals

Gold nanoparticle cytotoxicity depends on cell specificity and its coating upon surface respectively (Cheng et al. 2013; Schlinkert et al. 2015). These NPs internalized within the cells as a result of surface functions and by locations in mitochondria and lysosomes (Cheng et al. 2013), nuclei (Ojea-Jimenez et al. 2012) and vacuoles (Khlebtsov and Dykman 2011). In vivo studies of NPs in macrophages (spleen and liver kupffer cells) shown severe inflammation and liver cells apoptosis (Cho et al. 2009). Overexposure of silver nanoparticles in the form of wound dressings or drugs to humans undergo a condition called argyria (blue-gray discoloration of skin) associated with adverse toxic effects on liver (Christensen et al. 2010; Hadrup and Lam 2014). Exposure of silver nanoparticles would results in cardiac dysfunction in chicken, malformation of heart in fish and formation of thrombus in rats (Yu et al. 2016). Some studies represented that lungs and liver are the main targets of AgNPs exposure (Sung et al. 2008; Takenaka et al. 2001). Exposure of AgNPs on rat liver cells deplete antioxidant glutathione, decreased mitochondrial membrane potential and elevated ROS mediated by oxidative stress of liver cells (Hussain et al. 2005). Titanium oxide nanoparticle exposure develop arrhythmia in rats because of their direct contact with cardiac tissue (Savi et al. 2014). Titanium NPs on rodents heart tissue results in myocarditis, arrhythmia, vascular dysfunction, cardiac damage with

dysfunction, and some inflammatory responses (Yu et al. 2016). Degradation of DNA is possible with generation of oxygen species after copper nanoparticle exposure. As a result of in vivo experiments using mice, exploration of copper NPs translocate to organs like spleen, kidney and liver and finally results in inflammation of the respective organs (Magaye et al. 2012). Affirmatory effects of cerium oxide NPs on various cell lines would results in reactive oxygen species (ROS) and apoptosis (Mittal and Pandey 2014; Gagnon and Fromm 2015). Magnetic nanoparticles (Fe, Co and Ni) can be used in vivo imaging for diagnosis, more liable for aggregation, and finally results in inflammation followed by immune responses (Markides et al. 2012). Intravenous administration of ultrasmall supermagnetic iron oxide nanoparticles (USPION) in mice boost up blood clot formation followed by cardiac oxidative stress (Nemmar et al. 2016). Elicitation of nickel nanoparticles produce severe cytotoxic effects like oxidative stress which was followed by cell death (Magaye et al. 2012). Silicon nanoparticles could produce adverse cytotoxic effects on diverse human cell types like epithelial cells, platelets, microvascular endothelial cells, umbilical vein endothelial cells and aortic vessel cells (Yu et al. 2016). Exposure of carbon nanotubes (CNTs) in rodents produce consequences like thrombus formation, damage of placenta vessel, vasorelaxation, endothelial and cardiac dysfunction (Yu et al. 2016). Cytotoxic effects were observed in huge range of cell types like smooth muscle cells, blood cells, aortic endothelial cells, umbilical vein endothelial cells and microvascular endothelial cells of human (Yu et al. 2016). Even though, lack of action on cell viability or migration was observed, the platinum nanoparticle (Pt NPs) exposure produce some extent of activity in triggering toxicity towards primary keratinocytes and diminished metabolism of cells (Konieczny et al. 2013). Cytotoxic effects of Pt NPs resulted in accumulation in lysosomes and liberation of Pt^{2+} (Asharani et al. 2010).

4.5 Toxicity of Nanoparticles in Organ Development

Exposure of titanium oxide nanoparticle towards animal models would results in reduction of sperm production, alteration of neurobehaviour, abnormality in brain development of fetus, small fetuses, deformation of fetus and mortality (Savi et al. 2014).

4.6 Immunogenic Responses of Nanoparticles

Accumulation of AgNPs in the organs of immune system were followed by multiple organ (thymus, spleen, liver and kidney) damage (Wen et al. 2017). The reduced cell viability of alveolar macrophages and epithelial cells of lungs are possible on AgNPs exposure (Soto et al. 2007) Oxidative stress along with alveolar macrophage toxicity was observed in AgNPs by Carlson et al. (2008). Titanium oxide exposure

would cause toxicity which comprises effects of immune system (Savi et al. 2014). Exposure of ZnO NPs on rats and mice results in cardiac inflammation and apoptosis (Yu et al. 2016). Long term exposure of cobalt NPs were accompanied with immune system related health effects, skin, lungs and thyroid gland (Simonsen et al. 2012).

4.7 Genotoxic Effects of Nanoparticles

Injection of gold nanoparticles on rat samples (spleen and liver) produce changes in gene expression and results in lipid metabolism, defense response, detoxification, circadian rhythm and cell cycle (Balasubramanian et al. 2010). Genotoxic effects, because of chromosomal breakage is made possible by the exposure of nanoparticles to human (Wen et al. 2017). Silver nanoparticle exposure in chicken cause genotoxic effects (Yu et al. 2016). Intraperitoneal injection of AgNPs in mouse results in cytotoxic effects upon brain which are mediated by apoptosis, neurotoxicity with oxidative stress and change in genetic expression (Rahman et al. 2009). In addition to cytotoxic effects, some genotoxic effects were also observed in numerous cell lines as a result of titanium dioxide nanoparticles exposure (Gurr et al. 2005; Coccini et al. 2015; Yu et al. 2016). Zinc nanoparticle exposure to mice and rats were more probable to produce DNA damage (Yu et al. 2016). Administration of USPION intravenously to mice promote DNA damage (Nemmar et al. 2016). Exposure of nickel nanoparticle would bring about genotoxic effects as a result of cytotoxicity (Magaye et al. 2012). Alike production of cytotoxic effects by silicon nanoparticles, genotoxic effects were also possible on human cell types like, epithelial cells, aortic vessel cells, platelets, microvascular endothelial cells and umbilical vein endothelial cells (Yu et al. 2016). Genotoxic effects were observed followed by cytotoxic effects in huge range of cell types like smooth muscle cells, blood cells, umbilical vein endothelial cells, aortic endothelial cells and dermal microvascular endothelial cells of human (Yu et al. 2016). Exposure of platinum NPs displayed more detrimental effect on the stability of DNA (Konieczny et al. 2013).

4.8 Tumorigenesis in Animals by Nanoparticles

Nanoparticle could be vital for greater than 6 months within the body (Lin et al. 2015). Persistence for a longer time in the body would cause tissue inflammation injury and finally results in various diseases including cancer. The metallic nanoparticles residence in tissues aids tumorigenesis (Sighinolfi et al. 2016). Various studies are available to demonstrate the accumulation of nanoparticles within the tissues of patients infected with numerous diseases like pulmonary embolism, deep vein thrombosis, Hodgkin's lymphoma, prostrate cancer, renal failure, colon cancer, ulcerative colitis, emphysema, lung cancer, liver necrosis, asthma, stroke and Crohn's disease (Ballestri et al. 2001; Gatti and Rivasi 2002; Gatti 2004; Gatti and Montanari 2006;

Iannitti et al. 2010; Roncati et al. 2015a, b). Exposure of copper dust or fumes on copper smelter workers would expand cancer risk (Magaye et al. 2012). Dermal exposure and inhalation of magnetic nanoparticle (copper and nickel) results in cancer, lung fibrosis and skin allergies (Magaye et al. 2012). A detailed study on toxicity of CNTs revealed that its exposure would result in fibrosis and granulomas as consequences of carcinogenic and genotoxic effects (Aschberger et al. 2010).

5 Biohazards of Nanoparticle on Environmental Concern

The nanoparticles and their products enter the environment inevitably by means of washing, recycling and disposing (Kohler et al. 2008). The natural ecosystems are contaminated directly by the discharge of waste water and powder nanoparticles into the atmosphere. Unintentional release of AgNPs also results due to the activities like sampling, leaking and accidental release during transportation (Yu et al. 2013). Elemental silvers are found available in various forms as native silver (Leblanc and Lbouabi 1988; Lu et al. 2012) and as blends with metals like gold (Electrum) (Saunders et al. 2008; Denditius et al. 2011). Recently, AgNPs are extensively used in our day to day life with disinfectant sprays, outdoor paints, odour free socks, antimicrobial plastics and textiles. These nanoparticles reach the environment through scrapes, ageing of the materials and periodic washing of the materials. Liquid products like sprays and disinfectant are very rapid in entering the environment than the particles fixed to a solid cast like textiles and paints. The nanoparticles reacted to the sewage treatment plants could be used as fertilizers for agriculture land so as to reach the terrestrial system or groundwater system as leachate. Once the silver nanoparticles get released into the atmosphere, it could transport, disseminate and alter into various forms. The humans are exposed to nanoparticles through breathing, skin contact or eating. Environmentally exposed nanoparticles are associated with several neurodegenerative diseases (Alzheimer's disease, dementia and Parkinson's disease) (Calderon-Garciduenas et al. 2016; Chin-Chan et al. 2015; Gonzalez-Maciel et al. 2017). It was evident from the recent studies that the environmental polluted nanoparticle can translocate to the brain (adults and child) then enter cells and organelles and finally results in cellular damage with neurotoxicity (Gonzalez-Maciel et al. 2017). The exposure of AgNPs are manifested to affect huge number of aquatic and terrestrial habitats. They invade the embyos of zebrafish and results in growth interruption and abnormality. Acute and chronic studies deals with biological toxicities available based on organisms like algae, cladoceran and freshwater fishes. Over all, some iron nanostructures (iron oxide, ferrihydrite, lepidocrocite, hematite, maghemite, magnetite) are also naturally available in aquatic and terrestrial ecosystem (marine, rivers, lakes, springs, soils and sediments) and could possibly results in cytotoxic effects accompanied with ROS (Guo and Barnard 2013).

6 Hazardous Effects of Nanoparticles Towards Aquatic Organisms

The toxicity of various species in the aquatic system were studied by various researchers, among them the dominant species is fish followed by crustaceans, crabs and algal species. Their respective growth, organ development and reproductive behavior which were analysed by various studies and were tabulated (Table 2). The results revealed their lethality, behavioural change, toxicity and related stress. The environmental impact of a nanoparticle resides on various physical, chemical and biological parameters like shape, size, surface structure, surface charge, chemical composition, solubility aggregation and dispersion of nanoparticles (Navarro et al. 2008).

6.1 Cytotoxic Effects of Nanoparticles Against Algae

Nanoparticles are curious because of their high surface which can adsorb to pollutants, thereby alters its bioavailability along with the pollutants, and hence toxic to algae. Few heavy metals (Zn, Co, Cu, Ni, Pb and Cd) possess some detrimental effects towards algal growth, cell division, photosynthesis and primary metabolites elimination. The cytotoxicity is influenced by factors responsible for its conceivable mechanisms. Exposure of TiO_2 nanoparticles would entraps the algal cells thereby reducing the availability of light and subsidise toxicity to the algal cells (Aruoja et al. 2009). When compared with dark conditions, greater cytotoxicity was observed under light conditions. *Scenedesmus obliquus* produce serious ROS and increased membrane permeability while exposed to TiO_2 nanoparticles (Cherchi et al. 2011). Hazeem et al. (2016) reported that ZnO nanoparticles impose obscure effects upon marine algae thereby affecting its growth and chlorophyll a content during early stages of its growth respectively. Exposure of CuO nanoparticles produce adverse impacts on morphological, biochemical and physiological algae processes. The production of ROS, oxidative stress results in biomolecules (protein and lipids) damage, and finally reduced activity of glutathione activity was also happened (Melegari et al., 2013, Babu et al. 2014). As the concentration of CuO nanoparticles increased, metabolic activity of the cells were also decreased (Melegari et al. 2013) followed by damage of photosynthetic pigments in the presence of light meanwhile alters photosynthesis (Gouveia et al. 2013). The extent of DNA damage increases with higher concentration of CuO nanoparticles (Babu et al. 2014). Moreover, Cu NPs were found available in the cell membranes of algal cells at various sites while investigating the lipid peroxidation of cell membranes (Manusadzianas et al. 2012; Melegari et al. 2013). The GO exposure towards *Raphidocelis subcapitata* brought some adverse effects like oxidative stress and membrane damage (Nogueira et al. 2015). The pristine graphene exposure will results in inevitable disruption of cell wall and cell swelling (Pretti et al. 2014). The toxicity of nC_{60} nanoparticle was

Table 2 Toxicity of AgNPs on fresh water organisms

Nanoparticles employed	Organisms	Toxicity observed	Reference
AgNPs	*Danio rerio*	Increased rate of operculum movement and surface respiration, shows respiratory toxicity	Bilberg et al. (2012)
	Oryzias latipes	Multiple malformations during embryo development Decreased optic cup pigmentation, exophthalmia, abnormal finfold, anal swelling, head reduction and pericardial edema.	Wu et al. (2010)
	Pimephales promelas	Enter the embryos Induction of concentration-dependent increase in larval abnormalities, mostly edema	Laban et al. (2010)
	Zebrafish embryos	Heart rate- drop Increased mortality rate Delay in embryo hatching Organ deformities	Sutherland et al. (2010), Becker et al. (2011)
	Zebra fish	Oxidative stress, DNA damage and tumor formation risk	Asharani et al. (2008), Becker et al. (2011)
	Algae	Inhibition of photosynthesis	Tuominen et al. (2013)
C60 fullerenes	*Daphnia magna*	Elevated lipid peroxidation-cephalic ganglion and gills	Zhu et al. (2006)
Carbon nanotubes with fats	*Daphnia* sp.	Acute toxic effects	Roberts et al. (2007)
Carbon nanotubes	Fresh water crabs	Increased mortality	Templeton et al. (2006)
CNFs	*Klebsormidium flaccidum*	ROS production Promotion of physical damage to cells and inhibition of algae proliferation Induction of change in morphology, cell death	Munk et al. (2015)

(continued)

Table 2 (continued)

Nanoparticles employed	Organisms	Toxicity observed	Reference
GO	Green algae *Raphidocelis subcapitata*	Induction of ROS and film damage, results in toxic effects and the density of algae	Nogueira et al. (2015)
SWCNTs	*Chlorella vulgaris* *Raphidocelis subcapitata*	Restrain of growth	Sohn et al. (2015)
GONS, GOQD	*Chlorella* sp.	Reduced permeability of the cell Plasmolysis Increase of oxidative stress Mitochondrial membrane damage Inhibition of cell division and chlorophyll biosynthesis	Ouyang et al. (2015)
ZnO	*Chlorella vulgaris*	Distortion in morphological features Reduction in cell viability	Suman et al. (2015)
TiO_2 and C_{60}	*Daphnia magna*	Accumulates within digestive tract and other body parts	Becker et al. (2011), Johnston et al. (2012)
	Earthworm	Delay in reproduction	Hund-Rinke et al. (2012)
TiO_2	*Scenedesmus obliquus*	Production of ROS and increase in membrane permeability	Cherchi et al. (2011)
Nano zerovalent iron (nZVI)	Earthworm	Increased rate of mortality	Sevcu et al. (2011), Becker et al. (2011)
Nanodiamonds	Zebra fish	Malformations in embryo	Lin et al. (2016)
	Xenopus laevis	Increased embryo malformations decreased embryo survival rate	Marcon et al. (2010)
	Daphnia magna	Chronic toxicity of high concentrations resulted in reproduction inhibition and 100% mortality	Mendonça et al. (2011)
MgO	Zebrafish embryos	Inhibition of embryo hatchability	Ghobadian et al. (2015)

contributed by absorption and aggregation of the particles over the algal surface thereby hindering Mg^{2+} channels and triggering photosynthetic toxicity (Tao et al. 2016). The vital factor responsible for Au nanoparticle toxicity are bioavailability and biotoxicity. The electric charge present on its surface, for example positive charge functional group can employ toxicity on algae as it combined with the algae. The secondary factor that assimilate toxicity is its smaller hydrodynamic particle size (Garcia-Cambero et al. 2013). The exposure of AgNPs towards algae results in toxic effects including membrane adhesion, alteration in permeability and ion transport thereby expanding the porosity of cell, interruption in phosphate management of cell and DNA synthesis inhibition and ROS formation (Klaine et al. 2008). After exposure of AgNPs, deformation of algal cells from spindle to round was happened, ultimately results in cell lysis and collapse. The dose of the AgNPs is responsible for the severity of toxicity and algal viability, even more ionic silver exhibits extreme algal toxicity than AgNPs (He et al. 2012).

6.2 *Nanoparticles Effects on Terrestrial Species*

The released nanoparticles could be deposited in the terrestrial ecosystem like sewage and soil matrix thereby absorbed so as to interact soil organisms finally results in toxic effects. The routes of nanoparticle exposure includes nutrition absorption, body surface contact and through water. As per the observation of Yin et al. (2011) AgNPs coated with gummi arabicum inhibited the growth and morphological damage was triggered on *Lolium multiflorum*. Few terrestrial animals like nematodes and earthworms were chosen as the models for toxicity evaluation of AgNPs in soil due to high permeability of their skin. Exposure of AgNPs to *Eisenia fetida* earthworm resulted in growth and reproductive toxicity (Shoults-Wilson et al. 2011). Feeding *Acheta domesticus* (House cricket) with nanodiamond supplemented diet affected the insect development with oxidative damage followed by feeding disturbances (Karpeta-Kaczmarek et al. 2018). Some soil microbes which were exposed by AgNPs are found to be extremely sensitive and toxic.

6.3 *Cytotoxic Effects of Nanoparticles Against Beneficial Microbes and Protozoa*

Microorganisms are the unique nitrogen fixers and animal degraders found in nature, by the mean time these microbes were located at the end of the food chain to complete the cycle. Silver nanoparticles (AgNPs) has been acknowledged because of its antimicrobial (antibacterial and antifungal) activity so as to be used as agrochemicals extensively. Correspondingly, the AgNps sustenance in soil would have consequence upon beneficial microbiota of soil like nitrogen fixing bacteria, consecutively affects

physicochemical characteristics of both plants and soil (Anjum et al. 2013). Additionally the AgNPs interact with the bacterial cells and are toxic that finally results in death of microbes like *E. coli* (Lok et al. 2006). Exposure of inorganic nanoparticles like TiO_2, SO_2 and ZnO produce toxic effects upon bacteria which found to increase in the presence of light (Lovern and Klaper 2006). Death of microbes resulted by the cell membrane damage are made possible by the exposure of carbon nanomembranes (CNMs), could also vitally confides upon harmness to ecosystem, human health and finally resides in loss of biodiversity (Chen et al. 2017). Exposure of TiO_2 nanoparticles to *Saccharomyces cerevisiae* under dark condition results in toxic effect (Kasemets et al. 2009), whereas TiO_2 exposure towards *E. coli* and *Bacillus subtilis* results in growth inhibition (Erdem et al. 2015). CNMs also possibly induce ROS associated with lipid peroxidation, DNA damage, protein denaturation and finally cell death. The cells of protozoa could able to absorb carbon nanotubes resulting in accumulation within the mitochondrial cells (Zhu et al. 2006).

7 Future Perspectives

The experimental data which reveals the possible interaction between plant root and the nanoparticle is needed. Since the nanoparticles are widely consumed by human, their physical, chemical and biological interactions have to be studied keenly. Hence more prudential idea about interactions of nanoparticle with cells and their respective toxicity will be recognized. There is no detailed data regarding the consequences resulting from the chronic exposure of the nanoparticles to both environment and living beings.

8 Conclusion

Even though the utilization of nanoparticles has elevated in consumer and economical aspects, some detrimental consequences were also being faced by plants, animals, human and environment. This chapter explored the fragmentary views of discrepancy in practicing nanoparticle based on its toxicity and environmental hazards. If the nanoparticles are used as agrichemicals to boost up soil fertility some phytotoxic effects were also ascertained, which results in various diseases related to human and animals. Over exposure of nanoparticles to the environment will also pose catastrophic risks to the organisms residing in the environmental habitat.

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Synthetic, Natural Derived Lipid Nanoparticles and Polymeric Nanoparticles Drug Delivery Applications

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Abstract In modern therapeutic field, the delivery of drugs to the desired site is a crucial bottleneck that needs to be addressed for efficacy and potency of the administrated drug. The recent advancements in the field of nanotechnology has enabled researchers to deliver the drug and other diagnostic agents without unfavorable effect in human. Though drug delivery system (DDS) is highly advantageous, the clinical success rate depends on the appropriate carrier molecules which precisely recognize the target site for the release of drug and its biocompatibility. To overcome this concern both synthetic and naturally derived lipid-based nano carriers are the preeminent option as it is biocompatible, non-toxic, enhances the bioavailability of poorly absorbed drugs, drug release modulation flexibility, improved drug loading capacity and stability. Similarly, several bioinspired synthetic polymeric nanomaterials shown advantage of controlled release with less toxic effects, better encapsulation and grand bioavailability. In this chapter, we discussed about the broad spectrum of lipids (synthetic and natural) and polymeric nanoparticles (synthetic and natural) for potential drug delivery applications.

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1 Introduction

A grasp over the recent decade in therapeutics divulges the rapid evolutions in drug and its delivery processes. Nano-based drug delivery played a major role in driving these advancements in the field of therapeutics by industries as well as academic institution. These insurgences resulted in accumulation of scientific data including huge data-ware house, several patents and scientific papers from all round the globe in nanoparticles-based drug delivery. With all the cumulative manhours on research and development with the enormous research fund pumped in nanoparticles for drug delivery has enhanced this field to its current state and these churns out the immense potential of nanoparticles as therapeutic agent. The pharmaceutical industry was all focused on different dose, novel compounds and drug repurposing as the patents are expiring with issues like non-specificity, inadequate dissolvable medication there arose a paradigm shift towards alternative drug delivery system. With all these consideration and advancements in the field of nanoparticle based drug delivery has made significant difference and attention towards this field.

Various research and development towards different diseases with the latest improvement in drug discovery has resulted in several drug candidates with certain challenges which need to be surpassed. First such challenge is the solubility of the drug which reduces the bioavailability that leads to lower therapeutic efficacy (Merisko-Liversidge and Liversidge 2008, 2011). Nanoparticles based formulation of drugs can overcome this problem which has seen enhance bioavailability with other pronounced benefits like biocompatibility, surface functionalization, high encapsulation ability and biodegradability (Merisko-Liversidge and Liversidge 2008, 2011; Bawa 2008). Nanoparticles has several advantages while used in parenteral drug delivery like counter acting the aggregation of compounds which are usually seen in microparticles, their smaller size can boost the circulation of drugs when administered systematically. These enrich the therapeutic value of the drugs compared to the traditional one, which is very useful for treating cancers where the ability to pass through the vasculature of tumor cells and have a focused impact on disease (Gulati and Gupta 2011).

The vital importance for various commercial medications is first pass digestion. This denotes their low bioavailability and shrunken efficiency at the target site. In such circumstances, nanoparticle drug delivery has upper hand over the traditional one due to its balanced site-specific delivery. Other than its specificity, numerous other enhancements like reduced side effects, improved bioavailability with encapsulation of drugs, diminished dosage associated toxicities and amazing patient consistence (Bawa 2008; Merisko-Liversidge and Liversidge 2008; Jain et al. 2011a, b).

Owing to its small size, nanoparticles has proven ability to efficiently surpass various natural barrier prevail in our system (Fig. 1). Though nanoparticles has the

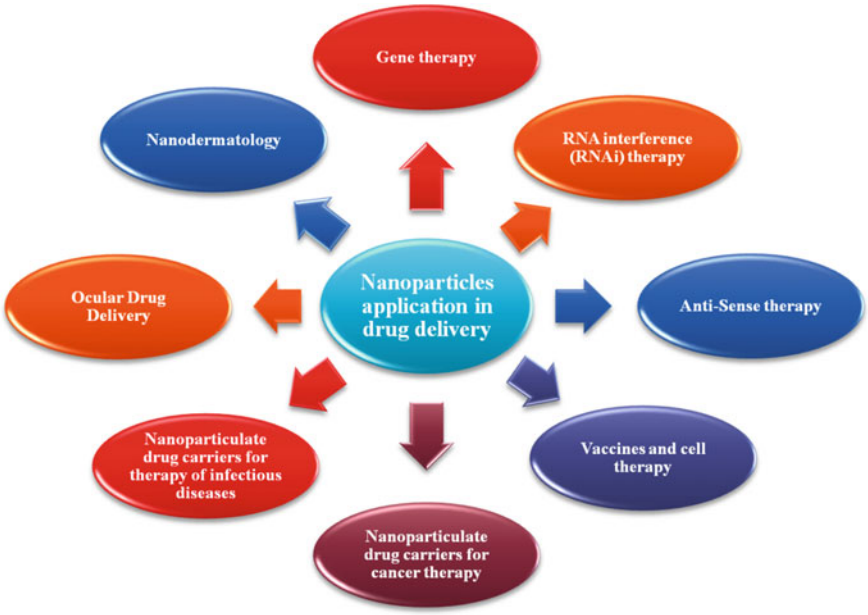

Fig. 1 Schematic presentation of different applications of polymeric nanoparticles

ability to saturate blood brain barrier which can be used to treat various brain diseases but the BBB is extremely impermeable that hinders the bioavailability results in poor therapeutic. As nanoparticles could by pass the barriers, innumerable publications have revealed the feasibility of them targeting on central nervous system (Caldorera-Moore et al. 2010). Compared to traditional dosage, nanoparticles owing to its size offer lesser dosage which favorable. The variable size of these nano-structures vastly impact the release profile of encapsulated drug candidate thus the formulator can regulate the medication on its target site (Jain et al. 2009, 2011a, b). Nanoparticles were examined and found to be malleable systems to encapsulate, transport drug molecules as well as bio-compounds (DNA, siRNA) for nucleic acid therapeutics, imaging and as diagnostic operators for site precise transport and detection (Jain et al. 2009, 2011a, b).

2 Lipid-Based Nanoparticles for Targeted Drug Delivery

Various researchers are currently working on nanotechnology especially in medicine field for drug delivery. Wide ranges of materials are under examination for drug delivery and more precisely for cancer therapy. A fascinating part is that several nanoscale drug delivery devices have been developed. Different particulate system those that biocompatible are biopolymers, oils and lipids which has the ability to increase oral

bioavailability of drugs by enhancing drug permeability or asphyxiating the first-pass effect (Harde et al. 2011). Nano scale particles has several advantages over the tradition at drug delivery system as their nano sizes with distinct physico-chemical properties, safeguard of drugs against harsh pH, moisture, enzymatic actions, improved bioavailability, less dosage, persistent circulation period, enhanced intracellular permeation and selective drug delivery makes it a promising candidate as drug carriers. They are used as carriers for diverse molecules like DNA, RNA, peptides, proteins, antibodies, etc. (Saha et al. 2010; Tagami and Ozeki 2017). Several nano particles are used as drug carriers and also for diagnostic purposes including polymeric nano particle (Cheng et al. 2015; Masood 2016), hydrogel (Hoffman 2012; Hamidi et al. 2008), nanotubes, nanowires (Bianco et al. 2005; Peng et al. 2014; Karimi et al. 2015), nano crystals (Müller et al. 2006; Junghanns and Müller 2008), liposomes (Allen and Cullis 2013; Narayan et al. 2016), lipid nanoparticles (Schwarz 1999; Battaglia and Gallarate 2012) and dendrimers (Majoros et al. 2008). With all the above mentioned nano-particle, lipid based nano particles have numerous advantages over other nanoparticles as it is biocompatible, can penetrate physiological barriers (Blood-Brain barrier) because of its high lipophilicity with ease of formation, cheaper and scalability for mass production making it an effective delivery system (Naseri et al. 2015; Tapeinos et al. 2017).

Lipid nanoparticles are the best carrier molecules for oral drug delivery as it offers diverse physiochemical properties with high biocompatibility and oral bioavailability (Das and Chaudhury 2011). Furthermore, these lipids based nano particles have numerous ways of constructive effect over the drug delivery process such as improved membrane permeability, higher solubilization ability, hindering efflux transporters, averting drug precipitation because of intestinal dilution, reducing CYP enzymes, increasing chylomicron production and lymphatic transport (Gursoy and Benita 2004; Porter et al. 2007; O'driscoll and Griffin 2008).

Lipid carriers are broadly classified based on its preparation techniques, physicochemical properties as niosomes, liposomes and solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) (Table 1). Niosomes are vesicles made of derivatives of cholesterol and non-ionic surfactants, they are capable of transporting both lipophilic and hydrophilic molecules, moreover are cost-effective and substantially stable over liposomes (Abdelkader et al. 2014; Moghassemi and Hadjizadeh 2014). Liposomes are engineered from natural phospholipids and cholesterol with core aqueous region (Puri et al. 2009; Akbarzadeh et al. 2013). Currently, numerous formulations of liposome have been approved to use treat different disease conditions like acute fungal infection (Ambisome), treat various cancers (doxorubicin formulates Doxil, Lipodox and Myocet), pain relievers (morphine sulfate DepoDur), inactivated antigens for vaccine purpose like hepatitis A virus (Epaxal), Influenza virus (strains A & B) (Inflexal) and several others are in pipeline for approval (Chang and Yeh 2012). Liposome applications are restricted because of the, minimal encapsulation competence specifically for the hydrophilic drugs and quick uptake exclusion by reticuloendothelial system (RES) (Naseri et al. 2015).

Solid Lipid Nanoparticles (SLN) are made up of lipids in an aqueous dispersion prepared by emulsifying agents for stabilization (Cacciatore et al. 2016; Tapeinos

Table 1 Overview of SLN and NLC nanoparticles which were used for several drug delivery applications

Type	Drug delivered	Targeted disease	Reference
SLN	Compritol and precirol	Bone diseases	Dolatabadi et al. (2014)
NLC	Compritol 888 ATO	Lung cancer	Hu and Jia (2010)
SLN	Stearic acid	HIV infections	Negi et al. (2013)
NLC	Glycerol monostearate	Pulmonary Delivery	Li et al. (2010)
SLN	Compritol 888 ATO	Schizophrenia	Silva et al. (2012)
NLC	Glycerol palmitostearate	Antitumor	Videira et al. (2012)
SLN	Glyceryl monostearate	Solid tumors	Miao et al. (2013)
NLC	Palmitic acid	Pulmonary drug delivery of insulin	Liu et al. (2008)
SLN	Docosahexaenoic acid	Cancer	Mussi et al. (2013)
NLC	Cetyl palmitate	In vitro drug release	Tofani et al. (2016)
SLN	Compritol and precirol	Inflammation in rheumatoid arthritis	Chawla and Saraf (2012)
NLC	Precirol ATO 5	Pulmonary application	Pardeike et al. (2011)
SLN	Efavirenz (EFV)	Antiretroviral (ARV) drug	Makwana et al. (2015)
SLN	Compritol 888 ATO, tripalmitin, and cacao butter	Human immunodeficiency virus (HIV)	Kuo and Chung (2011)
NLC	Glyceryl Palmitostearate	Anti-breast cancer	Sun et al. (2014)
SLN	Stearic acid	Bacterial infection	Wang et al. (2012)

et al. 2017). These arenanospheres crafted in solid lipid core by employing photon correlation spectroscopy (PCS) with particle diameter between 50 and 1000 nm range (Battaglia and Gallarate 2012). The lipid utilized are mostly intricate glyceride mixtures, triglycerides or waxes which retains its solid shape at both ambience and human physiological temperature that are alleviated by appropriate surfactants (Wissing and Müller 2003; Wissing et al. 2004; Kumar et al. 2012). SLN are fascinating lipid-based drug-delivery carrier molecule, substitutes for the traditional colloidal carrier like polymeric micro and nanoparticles, liposomes and emulsions (Kumar et al. 2012). Wide range of particle size can be prepared varied from 50 to 1000 nm with the drug molecule in it and moreover they are made of biodegradable and biocompatible which doesn't need organic solvents for their assembly. The main advantage of SLN is that they overcome all the pitfalls which other particles can't perform.

SLN is similar to liquid lipid based nanoemulsions but possesses solid lipid core which reduces the mobility of drugs contrasted to oily phase, improves controlled drug release in the system. SLN stability can be drastically enriched by supplementing it with surfactants coatings (Müller et al. 2002a, b; Pardeshi et al. 2012; Martins et al. 2007). SLN could be produced as powders that can be coated into tablets, pellets

or capsules which will enhances the drug delivery process (Puri et al. 2009). There are certain short comes in SLN such as low drug loading capacity which results from densely packed lipid crystal networks. This provides less space to the drug molecules to accommodate and drug expulsion after polymeric transition in the course of storage with relative highwater content of dispersion. The drug molecule may solubilize in lipid melt, miscibility of drug with lipid, polymorphic state of lipid matrix and structure of solid matrix.

NLCs are made up of diverse lipid molecules i.e. amalgamation of different natured lipids (both liquid and solid lipids) that provides the necessary room to accommodate more drug molecules as it forms imperfect crystals compared to SLN. NLCs retain its solid state both in ambience/physiological body temperatures though it possesses liquid lipid by controlling the constituent of it. NLCs tackle all the challenges where other nano particles failed to perform. NLCs has all the more than SLN as it is less toxic to human system, biodegradation capability, protection of drug molecules against worst environment (internal & external), controlled release of drug and eliminating organic solvents in the process of production.

2.1 Types of NLC

NLCs can be classified into three major types based on the various composition, fabrication process as

a. The imperfect type
b. The amorphous type
c. Multiple oil-in solid fat in water

Imperfect NLC are composed of lipids with different ratio such as numerous fatty acid, glycerides which affects the crystal order (imperfection) that results as positive outcomes of increased drug load capacity which can be further improved by introducing various amalgamation of glycerides by, varying carbon chain length and saturation. Whereas in amorphous type structureless matrix is created by combining special lipids like iso-propyl myristate or hydroxy octacosanyl hydroxyl stearate with solid lipid. These modification enables the NLC to occur in amorphous state instead of ordered one which inhibits drug expulsion subsequently forming β modifications throughout storage. Multiple O/F/W (oil-in-solid fat-in-water) type has several nano-sized liquid oil cubicles spread out in solid matrix that improves the drug solubility and increased drug loading capability. Additionally, the drug discharge is extended due to the compartments surrounded by solid lipid matrix (Müller et al. 2002a, b; Üner 2006; Jaiswal et al. 2016).

Alternatively, in case of hydrophilic drugs, lipid molecules are conjugated with the drug where the functional group of both lipid (Carboxylic acid) and drug (amine group) are conjugated through carbodiimide. NLCs are composed of aqueous dispersion lipid as of in SLNs, few of the lipids are oleic acid, sesame oil, almond oil, cetiol, corn oil, peanut oil, olive oil, Mygliol 812, sesame oil (Tapeinos et al.

2017), soybean oil, Tegosoft, Speziol, EOL NF, Tegosoft M, Lphosphatidylcholine (PC), P, Suppocire NC and soy lecithin. Similarly some of the surfactants used for NLCs fabrication are Tween 20, Pluronic F68, Tween 80, Myrj™ 59, Cremophor EL, Eumulgin SML, Span 85, Speziol TPGS Pharma and N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethyl-ammonium chloride (DOTMA). NLCs are prepared with the above mentioned solid and liquid lipids in the ratio from 4:1 to 1:4, surfactant are varied in concentration [0.25–6% (w/v)] and the concentration of total lipid are varied in the range of 1–30% (w/v) (Pardeike et al. 2011).

The key bottleneck problem in synthesis of lipid-based nanoparticle is its distinctive lipid nature that deterred the physicochemical characterization of nanoparticles by straight forward and usual techniques. Consequently, lipid nanoparticles have to be characterized by sophisticated technique like transmission electron microscopy (TEM). Moreover, the images illustrated shows inadequate information about the lipid nanoparticles appearance with their stability in the course of storage is also uncertain. These nanoparticles can only be preserved using cryoprotectant which hinders the formerly compromised physicochemical properties to a greater extend. Even the in vivo imagining of LP is been a debatable issue as tracking dyes that lipophilic in nature hamper labeling by binding to the tissues surrounding supposed to be lipid accumulation and production sites which results in false positives. But fortunately, these vital challenges are be overcome step-by-step with the advancements in technology.

3 Polymeric Nanoparticles (PNPs)

In recent years, PNPs are widely utilized as biomaterials due to their great qualities as far as a broad structure's variety, simple elaboration and design, good biocompatibility and notable bio-imitative characteristics. Explicitly in distinct drug delivery regulation, PNPs had a stamped job as they can carry therapeutics directly into the suggest location in human body, with incredible efficiency. Favorable circumstances and attributes of PNPs are numerous as pursues (Kayser et al. 2005). PNPs efficacy and bioavailability, they demonstrate an amazing upgrade over intravenous and oral administration routes. Likewise, in connection to tranquilize conveyance, PNPs can be effectively incorporated into different exercises, for example, tissue building. What's more, they transport dynamic fixings to a focused-on organ or tissue with the predetermined fixation and bestow steadiness and longer movement span for unstable dynamic fixings.

Also, PNPs can be considered as a potential possibility for vaccines conveyance, cancer therapy, and focused on target antibiotics conveyance as per the polymer decision and ability to alter medicate discharge from PNPs. The ideal necessities for PNPs conveyance framework configuration are to productively control their shallow

element and particulate size to control penetration, change dissolvability, upgrade flexibility and oversee cures discharge design from PNPs to gain the chose particular activity and the assigned target place at a coveted time and level (Bennet and Kim 2014).

3.1 Types of PNPs

PNP is an aggregate articulation and can be modified for some polymeric nanoparticle frames, it very well may be more limited for two noteworthy composes; nanospheres and Nanocapsules and nanocapsules. Nanocapsules are going about as drug stores, because of their vesicular structure, in which the held dynamic pharmaceutical fixings are saved in a fluid or nonaqueous fluid center put in the vesicle depression and encased by the solidified polymeric shell. Then again, nanospheres can be portrayed as a solid/mass of grid polymers. As such, any nanosphere may depicted as a whole polymeric circular mass, as result drug molecules might be caught inside the circle focus or ingest at the mass surface (Couvreur et al. 1995; Rao and Geckeler 2011).

3.2 PNPs Preparation

To expand PNPs, numerous materials and components should have been created together to detail PNPs of the desired highlights and specifications. The fundamental component used for PNPs formulation is the polymer. As per polymer source of origin, two kinds of polymers utilized for PNPs formulation; synthetic polymers and natural polymers. There is not any more favored classification than alternate as the polymer decision step is relying upon many difficult factors to outline the PNPs of essential qualities. The commonly used natural polymers in PNPs formulation are Albumin, gelatin, chitosan and sodium alginate (Fernández-Urrusuno et al. 1999; Kaul and Amiji 2004; Kriegel and Amiji 2011; Luppi et al. 2011; Ahmed and Khalid 2014). Furthermore, a few synthetic polymers normally utilized for PNPs gathering like polylactides (PLA) (Zambaux et al. 1998; Edlund and Albertsson 2002; Musumeci et al. 2006), polyglycolides (PGA) (Nagavarma et al. 2012), poly (lactide co-glycolides) (PLGA) (Derakhshandeh et al. 2007; Demento et al. 2009; Kalaria et al. 2009; Grabrucker et al. 2011; Danhier et al. 2012), polyorthoesters (POE) (Leong et al. 1985), polycaprolactone (PCL) (Wilczewska et al. 2012), polyanhydrides (Saroja et al. 2011), polyglutamic acid (PGA) (Kamaly et al. 2012), polycyanoacrylates (González-Martín et al. 2000), poly (malic acid) (PMLA) (Kamaly et al. 2012), poly (vinyl alcohol) (PVA) (Kalaria et al. 2009), poly (N-vinyl pyrrolidone) (PVP) (Bhavsar and Amiji 2007), poly (methyl methacrylate) (PMMA) (Reis et al. 2017; Thickett and Gilbert 2007), poly (methacrylic acid) (PMAA) (Lohmeyer et al. 1980), polyacrylic acid (PAA or Carbomer) (Asua 2004; Wang and Kuo 2008), polyacrylamide (PAM) (Brannon-Peppas and Blanchette 2004) and polyethylene

glycol (PEG) (Kaul and Amiji 2002; Shah et al. 2010). The majority of the previously mentioned synthetic polymers can be utilized securely. The polymers decided for PNPs preparation ought to be biocompatible and biodegradable for versatility fulfillment objective.

3.3 Distinct Advantages of Nanostructured Lipid Carriers

The common conventional measurement forms, the novel PNPs carriers provide a few beneficial uses and advantages. A standout amongst the most fundamental advantages they having nano-range size as this element grants them the capacity to allow through a numerous barriers and capillaries and to circle around the body distinctive chambers till achieving their allocated tissues (Lai et al. 2014). The smallest capillary in the human body has a diameter across of around 5–6 μm, thusly PNPs with under 1 μm diameter will allow a simpler systemic transportation (Hans and Lowman 2002). Additionally, the high surface zone (nano size) takes into account a bigger contact zone between the nanoparticle and the natural target and warrants for a fast adsorption rate. The small size PNPs characterized by great stability nature in suspension PNPs are normally organized through an unconstrained modern self-get together amid, the therapeutic compounds are ensnared inside the PNP center (Pridgen et al. 2014). Controlling self-get together conditions and picking particular polymer composes can empower a decent outline malleability by permitting the alteration of physicochemical properties (surface charge, size, hydrophobicity) and drug discharge parameters (controlled, prompt, sustained) (Kamaly et al. 2012). The PNPs surface properties can be altered by utilizing various polymer end groups or coupling specific polymers to PNPs planned (Valencia et al. 2013). These kinds of PNPs, numerous cancer therapies with extensive cytotoxicity impacts appeared with small doses can be controlled by supported release. The modern perspective, such an issue considered a weakness in any detailing development set out toward the market, that transferring from the research bench scale to the generation scale with holding similar highlights, pharmaceutical and clinical results of formulation. PNPs sealed that they likewise had another critical accomplishment which is the presence of methods which allow the production of substantial batches of reproducible mode (Vauthier and Bouchemal 2009). To accomplish every one of these necessity, the utilization of PNPs as a drug delivery carrier has been strongly examined.

3.4 PNPs for Oral Drugs Delivery

The oral drug delivery executions, PNPs are the major innovation technology fields that is as of late being effectively studied (Mo et al. 2014). Thinking about the oral route, the entry of macromolecules, drugs and PNPs to circulation are overseen and controlled essentially by intestinal epithelium. Small intestine tract surface territory

is amplified especially because of the overwhelming microvilli and villi and which is fundamental role in GIT drug ingestion. The transcellular as well as paracellular transport systems are illustrating to the greater part of NPs translocation in the intestinal epithelium (Chen et al. 2011; Alai et al. 2015). By utilizing PNPs, a few drugs with lacking sufficient bioavailability or in vivo harmful impacts can be conveyed efficiently and securely (De Jong and Borm 2008). The protein or peptide active ingredients can't be out of the unpleasant situations of the gastrointestinal tract because of their unfathomably weakness to disposal and degradation by natural enzymes (Danhier et al. 2012). Likewise, pH sensitive PNPs are observed to be an extremely encouraging tool for oral delivery of medications, solely for poor water-solvent meds and peptide/protein drugs (Wang and Zhang 2012). Along these lines, their capture into such PNPs will upgrade their bioavailability and guarantee protection (De Jong and Borm 2008). If there should arise an occurrence of long term impact of treatment is essential and for diminishing the dosing recurrence point, PNPs can be balanced for supported discharge. Moreover, nano drug carriers that can protect drugs from indignity and convey them to planned destinations inside the GIT may empower more proficient and maintained drug delivery (Ensign et al. 2012).

Because of their small size, NPs have sufficiently extensive surface regions for association with epithelial surfaces (He et al. 2012). Form previews reports, the multifunctional PNPs achievement was a promising proof, when PNPs applied to numerous therapeutics for oral delivery purposes like fluorescent yeast extract as a biocompatible dye, Epirubicin, Clotrimazole Econazole, Lamivudine, Paclitaxel, Estradiol and N-thiolated β-lactam antibiotics (Pandey et al. 2005; Turos et al. 2007; Bhardwaj et al. 2010; Dev et al. 2010; Taylor et al. 2010; Tariq et al. 2015). An additional achievement and tremendous accomplishment of PNPs for oral conveyance course is the PNPs and micelles work for insulin oral delivery. Especially, these PNPs prevailing to save insulin from decay and help insulin take-up by means of a transcellular or potentially paracellular pathway (Krauland and Bernkop-Schnürch 2004; Deutel et al. 2007; Mo et al. 2014; Alai et al. 2015). Fundamental little interfering ribonucleic corrosive (siRNA)—PNPs conveyance by means of the oral course was likewise investigated which would speak to an energizing anticipation of siRNA medications idea for inflammatory conditions therapeutics (Xu et al. 2012; He et al. 2013).

3.5 PNPs for Central Nervous System

A standout amongst the most generous difficulties in the field of nanotechnology is the medication conveyance to the brain and central nervous system (CNS), as the investigation and outlining of NCs ready to cross the blood brain barrier (BBB) are issues of an incredible interest (Tosi et al. 2016). Surprisingly BBB was discovered that both academic and modern industrial research projects and plans on neuroscience shows just 1% or even less of BBB crossing and focusing on purposes (Federoff 1999). The examination and improvement of procedures for drug delivery to cross

the BBB are relied upon to be more engaged and tended to. The CNS represents to a particular test that challenging medication focusing on and conveyance. The BBB impressively preclude the bypassing of foundationally coursed therapeutics and the extracellular network of cerebrum frustrates viability length and the conveyance of privately conveyed cures. PNPs embody a promising suggestion to these obstructions. Over the most recent couple of decades, major research endeavors have illustrated that PNPs can be expounded and intended for useful therapeutics conveyance to the CNS fundamentally and locally. Numerous clarifications can represent the system of PNPs take-up and their capacity to move drugs into the brain (Kreuter 2012, 2014).

3.6 PNPs Applications in Cancer Therapy

Nano carriers use in traditional chemotherapy is perceived and a great deal of NCs has been accepted by FDA for more extensive utilization (Panzarini et al. 2013). Beginning the earliest starting point of nanotechnology, liposomes were of the principal NCs-tranquilize conveyance frameworks that had been contemplated and utilized in cancer therapy. Then again, PNPs were arranged with the most recent generations outlined and changed for anticancer delivery. The PNPs targeted delivery can overcome inconveniences going with the utilization of ordinary anticancer cures, including low water dissolvability, quick disposal, and an insufficient selectivity, causing a wide danger for typical tissues and lessening the anticancer amount achieved the cancer tissues (Ashley et al. 2011). The PNPs exploitation for oral anticancer medications conveyance has extended and given careful consideration. Anticancer medications entanglement inside PNPs protects them from efflux transporters and the nano-sized range of PNPs quickens its passageway via biological membranes (Sun et al. 2010). Moreover, the polymer shell manages tranquilize shield against the body enzymes (Li et al. 2009; Naahidi et al. 2013). Then again, enzymatic degradation, dispersion and hydrolysis are conceivable mechanisms for anticancer discharge from PNPs (Edlund and Albertsson 2002). Moreover, PNPs demonstrated to can possibly improve tranquilize stability, draw out remediation result, corrected cell take-up and diminish degradation and additionally metabolism (He et al. 2012; Kalaria et al. 2009). Recent improvements in nanotechnology have uncovered numerous kinds of focusing on methodologies for expanding drug amassing into the tumor while confining the unwanted toxicity to normal typical cells, as the PNPs intended for targeted drug delivery systems (TDDS) can improve the tissue particular anticancer active ingredients conveyance through either a active or passive targeting tumors denied of influencing non-cancerous areas (Maeda 2001; Yu et al. 2012).

3.7 PNPs Applications in Gene Delivery and Therapy

Nucleic acid therapy grasps groundbreaking inclination in upgrading clinical issue for various chronic and acute diseases. These types of approach are focused on two divergent speculations: the primary theory is tied in with displaying target genes as

plasmids or oligonucleotides to enhance or influence proteins expression and the second speculation is for giving siRNA or antisense oligonucleotides to exasperate the focused-on genes function, and start silencing (Xu et al. 2012). Change of successful and safe vectors for nucleic acid delivery to the tissue has been a fundamental deterrent to clinical understanding of this extremely exciting test hypothesis (Hoag 2005). Gelatin kind B-based PNPs were the primary set up to be planned and arranged as frameworks for gene conveyance inferable from their EPR impact (Kaul and Amiji 2002). These gelatin PNPs showed that they could be focused to the tumor passively subsequent foundational presentation of PNPs and they were viewed as a productive route for hereditary treatment of cancer (Kaul and Amiji 2004). while passive targeting offers some favored fixation in tumor region and allow an intracellular dispersion, there are different kinds of tumors that don't have adequate vasculature or these PNPs may not penetrate profoundly into such tumor mass. Accordingly, active targeting on might be favored for these tumor types. One case of active targeting on relied upon human epidermal receptor (HER) is eminent as focusing on peptide functionalized type B gelatin PNPs system (Magadala and Amiji 2008). This considerable arrangement of conveyance was eminent as nanoparticles-in-microsphere oral system (NiMOS) which DNA-gelatin PNPs were furthermore caught in PCL microspheres to keep them shielded from enzyme-induced or pH-mediated disintegration of the payload protected inside the polymeric network in the GIT (Jones et al. 1997). Probably, NiMOS framework productively conveyed the siRNA and DNA plasmid into the intestinal cells and completed the gene conveyance by means of oral route (Kriegel and Amiji 2011). For oral organization of vascular endothelial development factor (VEGF) siRNA (siVEGF) and Survivin shRNA- expression pDNA (iSur-pDNA) for focused and synergistic hepatoma treatment, the adjusted Galactose trimethyl chitosan-cysteine (GTC) conjugates are another promising PNPs composed and settled as of late (Han et al. 2014; Xu et al. 2012). The investigation on quality therapeutics will run further with all the more understanding and advanced conveyance approaches. With expanded concentration and further advancement, PNPs for gene therapy, focusing on and transport will eventually prompt better therapeutics for some sicknesses and probably that would enhance more inadequacies and defeat additionally challenges gone up against by many promising cures alone before.

4 Conclusion

The development of nanoparticle-based drug delivery systems (NDDS) demonstrated an amazing advancement and different huge and imaginative innovations have been investigated and explored for as far back as six decades. During recent decades, different sorts of natural inorganic composites have been produced for an assortment of medications to give maintained medication releasing over a more extended period to enhance better therapeutic efficiency through expanding bioavailability,

reducing multiple dosing and decreasing the odds of side-effects and reduced cytotoxicity to cells and organs. One of the most encouraging approach is the utilization of biodegradable nanoparticles in medication conveyance, for example, lipid nanoparticles, polymeric nanoparticles, as drug carriers. Because of their capability to encapsulate medications and input capacity, they are competent to convey drugs to various parts of the body. The capability of lipid nanoparticles for the drug delivery of diverse therapeutic agents has been effectively established. This carrier has a number of points of interest over different nanoparticles which make it valuable in drug delivery. The suitability of the lipids and different surfactants is the real obstacle in their commercialization. These type drug carriers permit the properties of the drug being carried to be covered up and afterward it very well may be discharged, under controlled circumstance, in the coveted target territory. They permit drug protection biological degradation and against chemical linked to the organization course. In this manner, the physical and chemical characteristics of the transporters themselves oversee the kinds of utilization. Due to the huge adaptability of SLN-based frameworks, as well as the other positive characteristics of SLN, this moderately new class of drug carriers has immediately been adopted for the conveyance of a variety of anticancer compounds. From this chapter it is indicated that in current years polymeric, lipid nanoparticle based targeted drug delivery systems have been made more improvement compare to other targeted drug delivery systems.

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Lipid and Polymeric Nanoparticles: Drug Delivery Applications

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Abstract Recently, among the novel nanocarriers investigated for drug delivery lipid and polymeric nanoparticles have gained big interest due to their safety and potency. In the last decades, lipid nanoparticles presented by solid lipid nanoparticles SLNs and their newer generation known as nanostructured lipid carriers NLCs provided a promising alternative to traditional colloidal drug carriers. Furthermore, polymeric nanoparticles are innovative systems and used widely to incorporate active ingredients and replace conventional vehicles. Polymeric nanoparticles have been shown to be highly effective in drug delivery, imaging, therapy, and theranostic applications. This chapter will mainly focus to give information about the structure, the properties, the advantages, the constituents and the methods of preparation of lipid and polymeric nanoparticles and emphasize the application of these nanoparticles in drug delivery. Furthermore, it will cover recent studies dealing with the therapeutic applications of these two types of nanoparticles.

1 Introduction

In the last years, it has been found that developing new drug entities is not sufficient anymore to guarantee a good advance in drug therapy. The successful results obtained experimentally are not generally accompanied by good results in vivo or in the clinical studies. The causes of this disappointing results could be the rapid drug metabolism which leads to low drug concentration in the target site, the poor water solubility of the drug in the prepared formulations, and the extensive distribution of the drug causing high toxicity (Mehnert and Mader 2001). To overcome these problems realistic drug delivery systems are being extensively investigated. In the last decades, some approaches are oriented to develop Nanosized drug delivery systems (Fang et al. 2013). Nanoparticles are largely explored for their applications

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in therapy. They are often referred as nanocarriers, these nano-sized particles have many advantages compared to the other drug delivery systems. Mainly nanocarriers are characterized by: (a) providing an enhancement in the solubility of poorly soluble drugs (b) providing a modified release (controlled and sustained release) for the incorporated therapeutic agents (c) increasing the stability of the encapsulated drugs chemically or physically (d) delivering higher drug concentrations to the target sites thanks to the Enhanced Permeation and Retention (EPR) effect, and (e) when they are modified with cell-specific ligands they attend a targeted therapy (Goyal et al. 2016; Awat et al. 2006).

Nanoparticles or in other words nanomedicines or nanotherapeutics include different colloidal drug nanocarriers, this nanocarriers in general could be classified as lipid-based and polymeric nanocarriers (Awat et al. 2006; Fang et al. 2013). Lipid-based nanocarriers are sub-divided into particulate, vesicular and emulsion-based nanocarriers, while polymeric nanocarriers are classified as particulate, capsular, and self-assembled nanocarriers (Rahman et al. 2015). Among the lipid based nanocarriers, microemulsions are known as isotropic dispersions composed of lipid, surfactant and cosurfactant with high drug permeation (Gupta and Moulik 2008; Rahman et al. 2015). Liposomes are the most massively studied type of the lipid-based drug nanocarriers. Liposomes are bilayered vesicles mainly composed of natural or synthetic phospholipids. Liposomes vary in their structure from unilamellar to multilamellar vesicles according to which the vesicle size change from nanometer to several hundred micrometers (Wagner and Vorauer-Uhl 2011; Manconi et al. 2011). The liposomes are a highly innovative system with a high performance in the academic research, they have also a huge success in cosmetology and in the pharmaceutical market. Liposomes as nanovesicular drug delivery system and its applications are explained in the Chapter "Biosynthesized Nanoparticles and their Biological Applications" form this book. Liposomes were introduced by the end of the 1960s by Bangham and since this many efforts were performed to develop other nanocarriers that could match the benefits of liposomes with better improvement in the performance. This concern is committed to lipid nanoparticles as a delivery system having a solid matrix able to compete with liposomes and even outperform them (Müller 2007).

This lipid-based nanocarriers are presented by Solid lipid nanoparticles (SLNs) which are mainly formed by dispersing lipids in a surfactant containing aqueous solution and, they have a size ranging from 50 to 1000 nm (Rawat et al. 2008; Martins et al. 2007; Puglia and Bonina 2012). SLNs have many advantages like: their ability to enhance biocompatibility, they proceed relatively high surface area and a high drug loading capacity, they extend the release of the drug and promote its stability. These properties make the SLNs a promising carriers over conventional polymeric nanocarriers (Rawat et al. 2008; Martins et al. 2007) besides nanostructured lipid carriers (NLCs) which are the second generation of lipid nanoparticles composed of both liquid and solid lipids and providing very high biocompatibility and surface area (Xia et al. 2007).

Compared to polymeric nanoparticles, lipid nanoparticles have lower toxicity due to the natural and biological origin of their components (Fang et al. 2013). Inversely,

Polymeric nanoparticles consist of biodegradable macromolecular material from natural, semisynthetic or synthetic origin or could be non-biodegradable synthetic polymers. Thus, polymeric nanoparticles have limited use due to the polymer cytotoxicity and deficiency of a convenient large scale production techniques. However, formulations based on lipid nanoparticle are not available in commercial preparations and even they are in limited use in research. The reason for this is that they need more progress in their stages of development, and considering their safety and technological practicability further investigations are indispensable (Alvarez-trabado et al. 2017).

In this chapter we would like to show the current advances in lipid and polymeric nanoparticles in drug delivery and their therapeutic applications.

2 Lipid Nanoparticles

2.1 General Characteristics, Structure and Advantages

With regards to the other lipid based nanocarriers, lipid nanoparticles present different features: they are biodegradable and biocompatible due to the generally recognized as safe (GRAS) substances used in their preparation (Puglia et al. 2015), they are highly stable both biologically in mediums with excessive enzymatic metabolism and physically during their storage (Gao and McClements 2016; García-Fuentes et al. 2003), their physicochemical properties could be modulated depending on the desired requirements (Hao et al. 2011), they are produced with solvent free techniques and could be sterilized by autoclaving, thus lipid nanoparticles are considered as a promising carriers for industrial production (Alvarez-trabado et al. 2017).

In this part the advantages, the special features and the applications of lipid nanoparticles as drug delivery systems are explained. Solid lipid nanoparticles SLNs are the first generation of lipid nanoparticles which were introduced at the beginning of the 1990s parallelly by Dr. Jörg-Stefan Lucks and Prof. Rainer H. Müller and (from Kiel University in North Germany) and Prof. Dr. Maria Gasco (from Turin University in Italy). SLNs were developed by using high pressure homogenization by the two German scientists, while Prof. Gasco prepared the SLNs by microemulsion technique (Müller 2007). These researchers have focused to develop an alternative carrier to emulsions, liposomes and polymeric nanoparticles and named it SLNs (Małgorzata and Moritz 2016). SLNs is an alternative system to conventional colloidal carriers like emulsions, liposomes and polymeric nanoparticles. They associate the benefits of these conventional carriers and avoid their major problems (Müller et al. 2000).

SLNs are colloidal drug delivery system mainly prepared using a solid matrix and are obtained from oil in water O/W nanoemulsions but instead of the liquid oil a solid lipid is used in their preparation. These lipids are solid at both body and room temperatures and have a high physiological tolerance. The lipids used are dispersed in surfactants containing aqueous medium. Using lipids and preventing the use of

organic solvents in addition to the possibility to obtain a large scale production are the main advantages of SLNs. Also, SLNs can improve the stability of drugs by protecting them from the environment, enhance their bioavailability and proceed a controlled release profile for the active agent (Table 1) (Fang et al. 2013; Kumar et al. 2014; Weber et al. 2014). This later can be interpreted by the use of solid lipids rather than liquid oils which lowers the mobility of the drug due to the solid nature and consequently a controlled drug release could be provided (Mehnert and Mader 2001). Additionally, the matrix medium formed of solid lipids is stabilized by surfactant. During the solidification of the lipid matrix the surfactant is got immobilized on SLNs surface (Kumar and Randhawa 2013). Regarding to liposomes, SLNs can provide more stable formulations and extended drug release and possess no problems in sterilization, also compared to nanoemulsions which have unpredictable drug release profile, SLNs can give better profiles compared to other nanocarriers (Kumar and Randhawa 2013). SLNs are widely used to encapsulate cosmetic active ingredients (Pardeike et al. 2009) and biologically active food components (Patel and San Martin-Gonzalez 2012). In particular, these lipid nanoparticles show important potential as suitable drug delivery system (Gastaldi et al. 2014; Kim et al. 2009; Hao et al. 2011; Patel and San Martin-Gonzalez 2012; Wissing et al. 2004; Małgorzata and Moritz 2016).

However, SLNs present some drawbacks (Table 1) like their gelation tendency which is not predictable, in addition, polymorphic structure changes and the crystalline structure of the solid lipids is responsible of a low drug incorporation. Thus, another system known as nanostructured lipid carriers NLCs was developed to solve the problems of SLNs. NLCs have a special nanostructure of the matrix because they are prepared by a controlled mixing of the liquid oil with solid lipids which provides a larger space to locate higher concentrations of the drug. This new generation is able to avoid the limitations of SLNs like the low drug loading capacity and drug expulsion when storing the SLNs (Müller et al. 2002a; Fang et al. 2013; Puglia and Bonina 2012). NLCs are prepared using solid lipids and to increase the molecular disorganisation of the lipid lattice liquid lipids are also added. This novelty is responsible of increasing the loaded drug amount and preventing the expulsion of the incorporated agent during the storage (Müller et al. 2002a). Although the overall structure of NLCs mostly contains up to 30% of liquid lipids still the solid status is preserved in this case even though the lipid matrix crystalline formation is absent.

NLCs are classified into three different types depending on the nanostructure of the lipid which are: imperfect type, structureless type and multiple types (Müller et al. 2002a; Alvarez-trabado et al. 2017). The preparation of Imperfect NLCs consists of mixing solid lipids with the liquid ones to prevent the crystallization process. Structureless NLCs are composed of a solid lipid like hydroxyoctacosanylhydroxystearate which is in amorphous state after being solidified. However, multiple NLCs are formed by blending high quantity of solid and liquid lipids (Alvarez-trabado et al. 2017) the structure of SLNs and NLCs is presented in Fig. 1.

Compared to SLNs, NLCs have a higher loading capacity and a firmer incorporation of the drug in the matrix in storage. In SLNs, the highly purified solid lipids used in their preparation constitute a perfect crystal structure and leave just restricted space

Table 1 Advantages and disadvantages of SLNs

Advantages	Reference
No organic solvents used in their preparation	Goyal et al. (2016), Silva et al. (2011), Mehnert and Mader (2001)
Biodegradability, biocompatibility	Hee et al. (2012), Goyal et al. (2016)
Physico-chemical stability	Ferreira et al. (2013), Goyal et al. (2016), Puglia and Bonina (2012)
Low toxicity due to well tolerated excipients	Małgorzata and Moritz (2016), Date et al. (2007), Puglia and Bonina (2012)
Large industrial scale production at relatively low cost, in addition to the low cost of excipients	Puglia and Bonina (2012), Radomska-Soukharev (2007)
Improving cell/tissue tolerance due to the use physiologically acceptable lipids	Date et al. (2007)
Entrapping both lipophilic and hydrophilic active ingredients by utilizing different methods of preparation	Hee et al. (2012), Date et al. (2007)
Drug protection from chemical and enzymatic degradation	Kumar et al. (2014), Małgorzata and Moritz (2016)
Drug release can be modulated by the type of SLN, for example SLNs with drug enriched shell provide a burst release behavior whether SLNs with drug enriched core can sustain the drug release	Date et al. (2007), Radomska-Soukharev (2007)
Can provide drug targeting and controlled drug delivery	Date et al. (2007), Puglia and Bonina (2012)
Due to SLNs small size, they possess a high specific surface area, narrow size distribution. They are also spherical in shape and have favorable zeta potential	Gasco (2007)
SLNs based formulations are compatible with all the routes of administration due to non-toxic excipients used in their formulation	Kumar and Randhawa (2013), Mehnert and Mader (2001)
Possibility of sterilization	Mehnert and Mader (2001)
Disadvantages	**Reference**
Drug expulsion phenomena due to polymorphic transition during storage	Freitas and Müller (1999), Puglia and Bonina (2012), (Müller et al. 2002a)
Limitation in drug loading capacity	Freitas and Müller (1999), Puglia and Bonina (2012), Müller et al. (2002a), Almeida and Souto (2007)
Storage should be in refrigerated condition	Małgorzata and Moritz (2016)
Initial burst effect of incorporated drug	Małgorzata and Moritz (2016), Lobovkina et al. (2011)
Relatively short circulation time	Małgorzata and Moritz (2016)

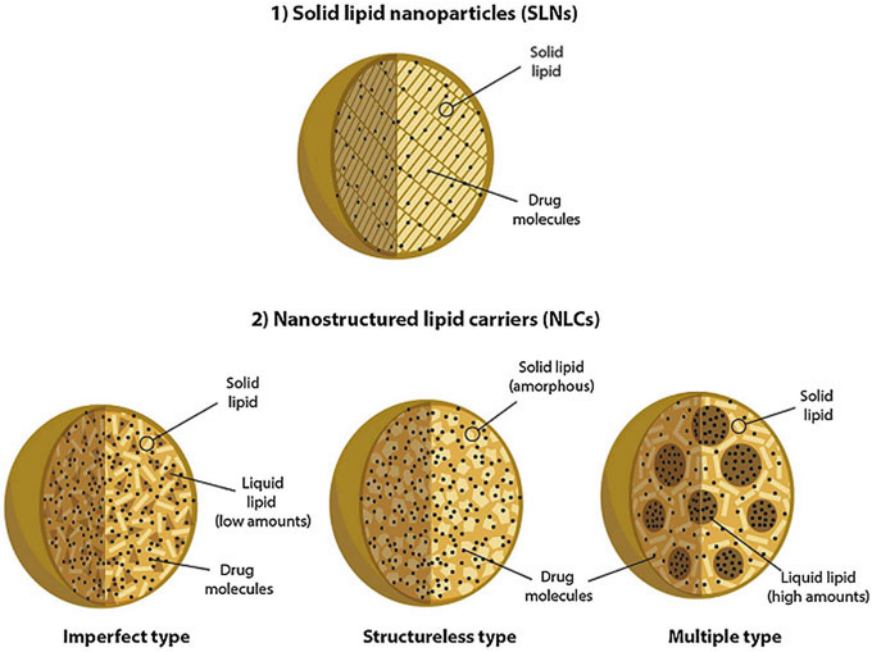

Fig. 1 Schematic representation of different types of lipid nanoparticles used in drug delivery. From Alvarez-trabado et al. (2017) with permission

to hold the drug resulting in a limited drug loading capacity of SLNs and also drug expulsion during the shelf life. Whereas in NLCs the lipid mixtures used for their preparation have very different structures causing an alteration of forming a perfect crystal lattice. The imperfections in the matrix of NLCs provide larger space to hold the active ingredient as amorphous cluster or in molecular state. As conclusion, the imperfectness in the crystalline structure of NLCs is responsible of its perfectness as a carrier (Bunjes et al. 1996; Müller et al. 2007).

As we mentioned above, SLNs are composed of solid lipids which form a perfect crystal lattice and leave small space to incorporate the drug. However, using lipid mixture of liquid and solid lipids alters the construction of this perfect crystal lattice. The active ingredient is holded in amorphous clusters provided by the imperfection in the matrix structure. NLCs were also proposed to be formed of a solid lipid matrix enclosing oil droplets and they have a morphology which is not necessarily spherical. The hypothesis proposed by Jores et al. showed that the NLCs structure is presented as solid platelets with oily phase between this solid platelets and the emulsifier layer (Jores et al. 2005; Fang et al. 2013).

2.2 Ingredients for Lipid Nanoparticles

Appropriate selection of bulk material is crucial due to its effect on the final physicochemical and pharmaceutical characteristics of the developed formulation. The type of lipid used and its properties such as the hydrophobicity, crystallization and the shape of lipid crystals, the concentration of the lipid and its physical state are important parameters having a big impact on the formulation of nanoparticles (Alvarez-trabado et al. 2017; Mehnert and Mader 2001). The lipids used in preparing lipid nanoparticles could be solid or liquid depending on the type of the formulation, and are summarized as below:
Solid lipids: used in lipid nanoparticles

- Triglycerides: esters of glycerol and three fatty acids, many triglycerids are used to prepare lipid nanoparticles like trilaurin (Dynasan® 112), tricarpin, tripalmitin (Dynasan® 116), trimyristin (Dynasan® 114) and tristearin (Dynasan® 118). For a better stability of the lipid nanoparticle longer hydrocarbon chain length of the lipid is preferred. In addition it is recommended to use a mixture of lipids having different chain length to enhance the drug incorporation and to avoid the re-crystallization of lipids (Bunjes et al. 1996; Souto et al. 2010; Alvarez-trabado et al. 2017).
- Partial glycerides: a blend of mono, di and tri-glycerides, they are selected when the drug to be added in SLNs has low aqueous solubility.
- Fatty acids: such as stearic acid, it could be used also in association with Compritol® and the mixture showed high loading capacity, improved stability and a high drug loading capacity (Kalam et al. 2010).
- Steroids: such as cholesterol which is largely used in liposomal preparations, it is used in lipid nanoparticles production because it is well tolerated.
- Waxes: using waxes was reported to give physically more stable lipid nanoparticles although it lowers the drug incorporation compared to glycerides (Jenning and Gohla 2000).

Liquid lipids:

Represented by oils with short to medium hydrocarbon chain possessing a melting point lower than the temperature of the body. A mixture of 70:30 up to 99.9:0.1 of solid lipids to liquid lipids is used in NLCs production. Examples of liquid oils used in NLC s preparation include oleic acid, castor oil, squalene and Mygliol® especially for ocular drug delivery (Patel et al. 2012; Alvarez-trabado et al. 2017).

Cationic lipids:

They contain one or two hydrocarbon chains or a steroidal structure and a positive charge on the head group. Examples of cationic lipids include DOTAP (1,2-dioleoyl-3-trimethylammoniumpropane) or DDAB for ocular drug delivery. This positively charged lipids in lipid nanoparticles interact with the negative charge of the cornea. Quaternary ammoniums like benzalkonium chloride, cetyl trimethylammonium bromide (CTAB), benzethonium chloride and cetylpyridinium chloride are also cationic

lipids used in ocular drug delivery (Lallemand et al. 2012; Alvarez-trabado et al. 2017).

SLNs are prepared by dispersing 0.1–30% w/w of solid lipids in aqueous media and their stabilization is provided by using 0.5–5% (w/w) of surfactant. However, for NLCs a mixture of solid and liquid lipids in a ratio of 70:30 to 99.9:0.1 is used in their preparation. Triglycerides, fatty acids, waxes and partial glyceride are the main lipids used to produce both SLNs and NLCs. Surfactants are used to improve the stability of lipid nanaoparticles by preventing particle agglomeration. The selection of the surfactant type is dependent on the administration route. For example, if the lipid nanoparticle is intended to be given parenterally the number of surfactants to be used is limited, whilst when it is intended to be administered topically a wide number of surfactants could be used (Puglia and Bonina 2012). For dermal preparations, when SLNs and NLCs are intended to be given topically they are prepared using glycerol palmitostearate (Precirol® ATO 5), glycerol behenate (Compritol® 888 ATO) or the wax cetyl palmitate. Liquid lipids like medium chain triglycerides (Miglyol® 812) and oleic acid are utilized to prepare NLCs. Surfactants are added at a concentration of 0.5–5% for dermal preparation to enhance the formulation stability such as polysorbate 80, poloxamer 188, lecithine, TegoCare® 450 (polyglycerol methylglucose distearate), Tyloxapol, Miranol® Ultra C32 (sodium cocoamphoacetate) or saccharose fatty acid ester (Schäfer-korting et al. 2007).

2.2.1 Ingredients for SLNs

The excipients used to prepare SLNs are generally lipids having low melting points and are mostly available in human body and in foodstuff. This lipids stay solid both at body and room temperatures. Organic solvents are not used in SLNs production and the excipients used are approved by the FDA under Generally Recognized as Safe (GRAS) situation. The lipids and surfactants used in preparing SLNs intended to be administered orally or dermally have been already approved by the regulatory authorities for the conventional dosage forms like tablets and creams. If SLNs are intended to be used parenterally glycerides are chosen because they are formed of fatty acids and used already for parenteral administration (Gasco 2007; Kumar and Randhawa 2013).

Three main constituents are used to produce SLNs which are lipids, surfactants and water. Many lipids could be used for the preparation of SLNs including highly purified triglycerides such as tristearin, complex glyceride mixtures, steroids like cholesterol, fatty acids such as stearic acid and even waxes like cetyl palmitate. To stabilize the lipid dispersion the use of surfactants is generally necessary (Mehnert and Mader 2001; Goyal et al. 2016; Müller et al. 2000; Date et al. 2007). Several surfactants were used to improve the stability of SLNs such as poloxamer 188, polysorbate 80, polyglycerol methylglucose distearate, sodium cocoamphoacetate and saccharose fatty acid esters (Schäfer-korting et al. 2007; Wu and Guy 2009). It has been proved that combining several emulsifiers (regarding to their charge and molecular weight) is able effectively to prevent particle agglomeration (Mehnert and Mader 2001).

Additionally, SLNs have been prepared using high amount of lecithins, para-acyl-calix-(4)-arenes and amphiphilic cyclodextrins (Müller et al. 2002b; Schubert et al. 2005; Dubes et al. 2003; Shahgaldian et al. 2003; Date et al. 2007).

2.2.2 Ingredients for NLCs

NLCs are composed of lipids, water and surfactants. Both solid and liquid lipids are used to construct the inner core of NLCs. The main solid lipids used are: glyceryl palmitostearate (Precirol® ATO 5), glyceryl behenate (Com-pritol® 888 ATO), triglycerides (such as tristearin), fatty acids (such as stearic acid), waxes (such cetyl palmitate) and steroids (such as cholesterol). These lipids are used in preparing SLNs, they are solid at room temperature and their melting point is high. Liquid lipids used to prepare NLCs are medium chain triglycerides like Miglyol® 812 (Jenning et al. 2000b; Fang et al. 2013). Other oils such as isopropyl myristate, propylene glycol dicaprylo-caprate (Labrafac®), paraffin oil, squalene and 2-octyl dodecanol are used too. Fatty acids like linoleic acid, oleic acid and decanoic acid are oils playing the role of liquid lipid in addition they have permeation enhancement properties when used topically. Tocols are being investigated as oils for NLCs due to their characteristics like the good stability and capacity to be produced on large scale. Natural oils extracted from plants could be used too as liquid lipids in NLCs (Constantinides et al. 2004; Tsai et al. 2012; Averina et al. 2010, 2011; Fang et al. 2013). Surfactants used in preparing NLCs as stabilizers could be hydrophilic such as polysorbates, Pluronic F68 (poloxamer 188), sodium deoxycholate and polyvinyl alcohol. Amphiphilic or lipophilic surfactants are used too like lecithin and Span 80. Additionally, polyethylene glycol was added in some studies to the shell of NLCs to increase the circulation time and avoid the uptake by reticuloendothelial system (Zhang et al. 2008a; Fang et al. 2013; Schäfer-korting et al. 2007; Gu et al. 2011).

2.3 Preparation Methods of Lipid Nanoparticles

Since the discovery of lipid nanoparticles in the nineties of the last century several preparation methods were described. Mehnert and Mäder elucidated four techniques: high shear homogenization and ultrasound, high pressure homogenization, solvent emulsification/evaporation and microemulsion method (Mehnert and Mader 2001; Üstündağ-Okur et al. 2014; Alvarez-trabado et al. 2017). Additionally, a double emulsion method W/O/W has been reported to prepare SLNs loaded vancomycin to enhance its ocular penetration (Yousry et al. 2016), and a quasi-emulsion solvent diffusion technique has been used to prepare positively charged SLNs loaded idebenone (Leonardi et al. 2015). Further methods such as coacervation, supercritical fluids, electrospray, spray drying and membrane contactor although not widely used, they have been reported too to prepare lipid nanoparticles (Alvarez-trabado et al. 2017). In the following section, we discussed each of these methods.

Mainly, the preparation techniques for both SLNs and NLCs are almost the same. Firstly, the solid lipids or the lipid mixture (solid and liquid lipids) are proceeded by a melting process, then the active or the cosmetic ingredient is added to this melt of lipids and would be dispersed by high speed stirring in a stabilizer or surfactant containing aqueous solution having the same temperature. The resulted pre-emulsion is subjected to homogenization using a high pressure homogenizer to give a hot oil in water nano-emulsion. The cooling of this nanoemulsion causes the crystallization of the oil droplets and the formation of lipid nanoparticles with a solid matrix and the resulted structure depends on the raw material used even SLNs or NLCs (Müller et al. 2007).

So the preparation of lipid nanoparticles is based on one common step which is the formation of O/W nanoemulsion then the dispersed lipid phase of this nanoemulsion is solidified. The nanoemulsion formation is the crucial step when it is wondered to prepare lipid nanoparticles having a small particle size and low polydispersity index. For example, when SLNs are given parenterally the precursor nanoemulsion is mostly homogenized by high shear homogenization, high pressure homogenization and ultrasonication. The high shear homogenization and ultrasound methods are rapid and not expensive although they have some disadvantages. In high shear homogenization, the quality of the dispersed preparation is limited by the presence of microparticles and ultrasonication method can lead to metal contamination. These disadvantages are the result of prolonged time of processing. To solve these problems the two methods could be associated as short cycles of high shear homogenization and ultrasonication. The most widely used method to prepare lipid nanoparticles is the high pressure homogenization which can be hot or cold homogenization. Among the high pressure homogenization methods, hot homogenization is the most used and it is suitable even for heat labile compounds because the time of exposure to heat is relatively short. But in this technique, the incorporation of hydrophilic drugs is difficult because they distribute into the aqueous phase while the homogenization is performed. However, the cold homogenization is used to prepare lipid nanoparticles incorporating drugs with high water solubility or high heat sensitivity (Puglia and Bonina 2012; Schäfer-korting et al. 2007; Müller et al. 2002b). Besides the above methods, other methods are available like the microemulsion technique, the emulsification solvent evaporation technique and the solvent diffusion method. These techniques avoid the use of any temperature but the solvent utilized in the preparation of lipid nanoparticles is difficult to be removed (Puglia and Bonina 2012). Figure 2 shows the most frequently used methods for SLNs preparation.

2.3.1 High Pressure Homogenization

This method is technically feasible that's why it is often used to prepare lipid nanoparticles. It has been used to prepare nanoemulsions intended to be used parenterally and then it was utilized to produce SLNs. It can be used for large scale production and could yield 11 thousand liters per hour (Muller et al. 2011; Alvarez-trabado et al.

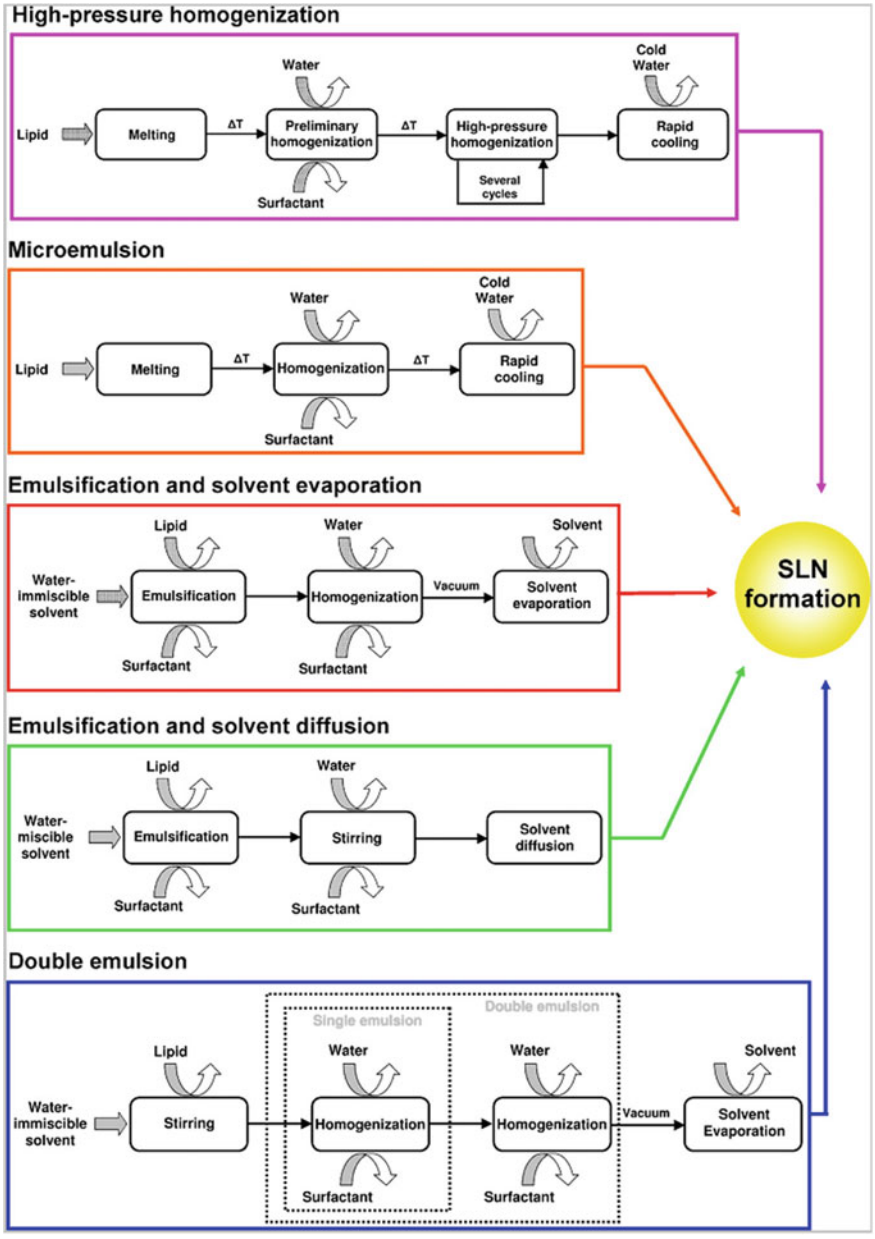

Fig. 2 The most frequently used methods for SLNs preparation. From Małgorzata and Moritz (2016) with permission. Mainly, the preparation techniques for both SLNs and NLCs are almost the same, the final structure depends on the raw material used even SLNs or NLCs

2017). However, in this method a big stress is applied on the formulation mechanically and thermodynamically. High pressure homogenizer is composed mainly of a chamber filled with the pressurized pre-formulation (almost 100–200 bar), and by forcing this pre-formulation to pass through a micrometer sized tight gap the particles size is reduced to the sub-micrometer range due to its exposition to high degree of shear stress and cavitation forces (Mehnert and Mader 2001; Kumar and Randhawa 2013; Alvarez-trabado et al. 2017). The lipid content is from 5 to 10% presenting no issues to the homogenization technique, even though the homogenization process to lipid nanodispersion was successful with a high lipid concentration until 40% (Lippacher et al. 2000; Mehnert and Mader 2001). The high pressure homogenization is subdivided into two types the hot and the cold homogenization. For both types, the drug should be dissolved or dispersed into the lipid melt before processing (Alvarez-trabado et al. 2017; Mehnert and Mader 2001).

A. **Hot homogenization**

Could be considered as homogenizing an emulsion, it is realized at a temperature above the melting point of the lipid. Firstly, high shear mixer apparatus (Ultra-Turrax) is used to prepare a pre-emulsion from the lipid melt containing the drug and a water based surfactant phase at the same temperature. Here, it is requested to achieve droplet size in the range of few micrometer because the quality of the final nanoparticles depends largely on the quality of the pre-emulsion.

As we mentioned above the high pressure homogenization is accomplished at a temperature above the lipid melting point, and generally increasing temperature leads to smaller particle size resulting from decreasing the inner phase viscosity (Lander et al. 2000; Mehnert and Mader 2001). In hot homogenization, at the beginning, a nanoemulsion is produced as a result of the liquid status of the lipid, then solid particles are produced when the nanoemulsion is subjected to cooling to the room temperature or even below. The lipid tendency to crystallize might be retarded because of the small particle dimension and the existence of surfactants or emulsifiers, this can lead to a sample presented as a supercooled melt for long time for example for months (Mehnert and Mader 2001).

B. **Cold homogenization**

It is performed with solid lipids and it could be considered as a high pressure grinding of a suspension. Temperature should be controlled effectively to avoid the liquefaction of the solid lipids as the temperature increases during the homogenization process. The cold method was introduced to solve the hot methods problems where the drug is degraded due to the high temperature, also the drug could distribute into the aqueous phase when the hot homogenization is performed, in addition, the crystallization of the nanoemulsion is complicated and may lead to different changes or supercooled melts (Ganesan and Narayanasamy 2017; Seyfoddin et al. 2010; zur Mühlen et al. 1998; Mehnert and Mader 2001).

The preparation of lipid nanoparticles using cold homogenization method resembles to hot homogenization method in the first step which is common presented by

solubilizing or dispersing the active ingredient in a melted form of the bulk lipid at approximately 5–10 °C above melting point of the lipid, but the other steps are different (Müller et al. 2000). The lipid melt that contains the active ingredient is subjected to fast cooling using dry ice or liquid nitrogen. As a result, the drug is homogeneously distributed within the lipid matrix due to this high rate of cooling. The solid lipid that contains the active ingredient will pass through a milling process to form microparticles using ball or mortar mil. The size range obtained will be in the range 50–100 um. The low temperature makes the lipid more fragile and favorize the particle size reduction. Then, a dispersion of the solid lipid microparticles in a solution of a chilled surfactant or emulsifier is prepared. This pre-suspension is homogenized below or at room temperature (Alvarez-trabado et al. 2017; Mehnert and Mader 2001; Ganesan and Narayanasamy 2017). The forces of cavitation are sufficiently strong breaking lipid micro-particles into solid lipid nanoparticles, this technique prevents lipid melting and minimizes the migration of the drug to the aqueous medium (Müller et al. 2000).

Homogenization can lead to temperature elevation, thus to prevent the lipid from melting in this step, the homogenization temperature and the melting point of the lipid should be sufficiently different (Wu and Guy 2009). In cold homogenization, generally the resulted particle size is larger and its distribution is wider in comparison with the hot homogenization method. The cold method although it protects the drug from exposing to high temperature, still it can't totally prevent it in the first step where the lipids and the drug mixture are melted (Mehnert and Mader 2001; Fang et al. 2013; Ganesan and Narayanasamy 2017).

2.3.2 The Microemulsion Technique

In microemulsion method, a microemulsion is added to water and the lipid phase is precipitated and form small particles (Gasco 1993). This method is appropriate for heat sensitive drugs. Additionally, no organic solvent is involved in the process (Alvarez-trabado et al. 2017). The feasibility of method is high regarding the large scale production (Müller et al. 2000). The lipid is melted almost 10 °C above its melting point, after that the active ingredient is solubilized in this melt. Then, the lipid melt containing the drug is added to a surfactant based aqueous phase of the same temperature (could contain a cosurfactant if needed). The system is agitated to form an oil in water micro-emulsion. Then, the micro-emulsion formed is cooled down to 2–3 °C with mixing. The cooling process is performed even with an ice bath or by the dispersion of the microemulsion droplets drop wise in a cold aqueous medium. As a result, the lipid crystallizes due to the change in temperature suddenly and lipid nanoparticles are formed (Alvarez-trabado et al. 2017; Wu and Guy 2009; Güngör et al. 2015). It was found that the addition of the emulsion to the cooled aqueous medium in a high rate leaded to lipid nanoparticles with a small particle size and low polydispersity index (Marengo et al. 2000; Alvarez-trabado et al. 2017).

2.3.3 Solvent Emulsification/Evaporation

This method of preparing lipid nanoparticles is based on precipitating an oil in water emulsion in an aqueous phase. It is realized by solubilizing the lipid in an appropriate organic solvent which should be not miscible with water. After that, the lipid solution is emulsified in water. Then, the solvent is evaporated and immediately nanoparticles are produced because of the precipitation of the lipid in the non evaporated water (Alvarez-trabado et al. 2017; Mehnert and Mader 2001). Cholesterol acetate loaded lipid nanoparticles were prepared using this technique, where lecithin and sodium glycolate were used as emulsifiers, and the resulted lipid nanoparticles were about 25 nm (Sjöström and Bergenståhl 1992). Tripalmitin loaded nanoparticles were also prepared using this technique. The particle size obtained was 30–100 nm depending on the surfactant and the cosurfactant type and the lipid amount. Particles were smaller when bile salts (cosurfactant) and low lipid concentrations were used (Alvarez-trabado et al. 2017).

As advantage, this method avoids any thermal stress in the entire procedure, however, using an organic solvent remains as an important disadvantage (Alvarez-trabado et al. 2017; Mehnert and Mader 2001).

2.3.4 Solvent Emulsification–Diffusion Technique

In this technique, an organic solvent is saturated with water and utilized to solubilize the lipids. After that, the emulsification of the resulted solution is performed with an aqueous phase saturated with organic solvent with vigorous mixing. The addition of water to the obtained emulsion produce lipid nanoparticles, then the organic phase is diffused into the continuous phase. The resulted nanoparticles dispersion is subjected to purification by ultrafiltration method using a dialysis membrane of approximately 100,000 KDa (Mao et al. 2003; Kumar and Randhawa 2013).

2.3.5 Solvent Injection Technique

Here, water miscible solvent is utilized to solubilize the lipid and the resulted lipid solution is injected through an injection needle into a surfactant containing aqueous medium. The lipid amount, the nature of the solvent, the lipid solution injected amount, the viscosity and the diffusion of the lipid solvent phase into the water medium are critical points affecting this method (Schubert and Muller-Goymann 2003; Kumar and Randhawa 2013).

2.3.6 O/W Microemulsion Breaking Technique

The lipid containing the active ingredient, the surfactant and the cosurfactant were mixed together at the melting point of the lipid. Then, a dispersion of the resulted

microemulsion in aqueous medium is prepared at 2–10 °C, and a diluted SLNs dispersion was formed. This diluted dispersion is due to the high amount of surfactant and cosurfactant. Thus, ultrafiltration or cryodessication were used to concentrate the SLNs. A large scale preparation method was also developed, consisting of a device with a thermochamber, a pneumatic piston and a needle. A high pressure and temperature and a tight gauge needle favorized the formation of small sized SLNs (Marengo et al. 2000; Kumar and Randhawa 2013).

2.3.7 W/O/W Double Emulsion

It is selected when encapsulating hydrophilic substances is required. In this method no thermal stress is applied so it could be applied with temperature sensitive compounds. Using of organic solvents is the principal disadvantage of this technique. The preparation of W/O/W double emulsions consists of two steps. Firstly, a water in oil emulsion is produced by the emulsification of a hydrophilic drug containing water in a lipid containing organic solvent by sonication. Then, the resulted primary emulsion is added to a surfactant containing aqueous phase and its emulsification to a water-in-oil-in-water emulsion is performed by sonication too. The organic solvent is vaporized by stirring the emulsion and leads to the production of lipid nanoparticles. Sonication time was reported to affect the zeta potential, the size and its distribution of lipid nanoparticles (Yousry et al. 2016; Alvarez-trabado et al. 2017; Gallarate et al. 2009; Kumar and Randhawa 2013).

2.3.8 Quasi-emulsion Solvent Diffusion (QESD)

In this technique, the lipid and the active ingredient are solubilized in a blend of organic solvents, after that the resulted solution is injected into a surfactant containing water. An adequate mixing for 15 min is performed using high shearing at 0 °C. Then the dispersion is subjected to sonication where hydrophilic compounds spread out to the aqueous phase and the lipid precipitate to form lipid nanoparticles. At last, the organic solvent is evaporated by leaving the preparation stirred for one day at 25 °C. Generally, the formulation prepared with this technique represent optimum physicochemical characteristics (Leonardi et al. 2015; Alvarez-trabado et al. 2017).

2.3.9 Ultrasonication

Ultrasonication energy is utilized in reducing the particles size. Ultrasonication is associated with high pressure homogenization to produce homogenous particles (Seyfoddin et al. 2010; Pradhan et al. 2015b; Kumar and Randhawa 2013).

2.3.10 Super Critical Fluid Technique

Lipophilic active ingredients are soluble in supercritical carbon dioxide, so this supercritical fluid associated with ultrasonic energy have been used to produce lipid nanoparticles (Kumar and Randhawa 2013).

2.3.11 Membrane Contactor Technique

Here, the lipid is pressed to pass through a membrane contractor. This passage is realized above the lipid melting point. Water is circulating beyond membrane pores to carry the resulted lipid droplets which will be cooled at room temperature (Charcosset et al. 2005; Kumar and Randhawa 2013).

2.3.12 Electro-Spray Technique

In this method, electrodynamic atomization is used to form SLNs with small particle size and low polydispersity index in the form of a powder (Trotta et al. 2010; Kumar and Randhawa 2013).

2.4 Drug Incorporation in Lipid Nanoparticles

Many models have been proposed to describe the incorporation of the drug in SLNs (Fig. 3). According to one of the models, the drug could be dispersed in the lipid matrix or exist as amorphous clusters and this is known as the homogenous matrix model. In another model, the active ingredient is present in the outer lipid shell and lipids are in the core and this model is known as outer lipid shell model. Another model suggests that the drug exists in the core surrounded by lipid layer and the model is known as drug enriched core model (Müller et al. 2000; Mehnert and Mader 2001; Date et al. 2007). Several drugs with different lipophilic character were incorporated into SLNs such as: pilocarpine, doxorubicin, cyclosporine, aciclovir, vitamin E palmitate, progesterone, timolol, hydrocortisone, camptothecin, azidothymidine palmitate, etc. (Mehnert and Mader 2001).

The burst release of the active ingredient is a drawback faced with SLNs. This effect is due to a phase separation in the structure of SLNs. Because of the cooling step in SLNs preparation which leads to lipid crystallization rapidly like a drug free core and leave an outer shell enriched with the active ingredient (zur Mühlen et al. 1998; Müller et al. 2002a; Kumar and Randhawa 2013). Even though, the prolonged release is still possible with SLNs and was reported in literature (Yang et al. 1999; Jenning et al. 2000a; Müller et al. 2002a). This extended release could be achieved by controlling the preparation steps, for example using low surfactant amount, applying low heat such as in cold homogenization. However, the avoidance of the burst release

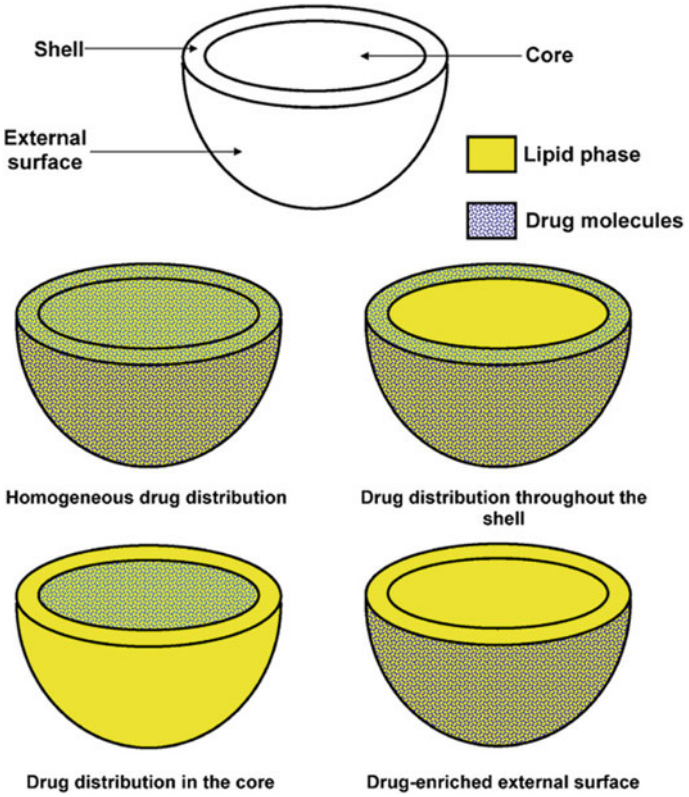

Fig. 3 Schematic representation of drug distribution in SLN matrix. From (Małgorzata and Moritz 2016) with permission

from SLNs for some drugs such as etomidate was impossible although changing the preparation steps. Thus, the application of the NLCs technology is able to provide more feasible and effective extended release profile (Müller et al. 2002a). Regarding to NLCs, retinol was the first drug incorporated into NLCs with a loading capacity around 1% using Compritol. When a blend of Compritol and Miglyol 812 was used, the amount of the drug incorporated increased to 5% (Müller et al. 2002a).

2.5 Physicochemical Characterization of Lipid Nanoparticles (After Preparation)

The physicochemical characteristics of lipid nanoparticles even SLNs or NLCs play a key role in formulating lipid nanoparticles based drug delivery systems. The characterization of lipid nanoparticles include determining: particle size, particle shape, zeta

potential, drug loading capacity, drug release behavior, crystallinity of the system, the stability and the attitude in biological medium. Additionally, lipid nanoparticles characteristics could be studied by X-ray, differential-scanning calorimetry, nuclear magnetic resonance and Raman spectroscopy (Fang et al. 2013; Małgorzata and Moritz 2016).

2.5.1 Stability of Lipid Nanoparticles

SLNs suffer from the burst release of the active ingredient due to a phase separation phenomenon. The crystallization of the lipid in the cooling phase leaving a a drug enriched outer shell and drug free core is responsible of this burst release in SLNs (Müller et al. 2002a) (Fig. 4).

The stability of SLNs could be maintained by controlling the parameters affecting their stability such as lipid type, surfactant concentration and storage circumstances. Polymorphic transition of lipids during storage is the lipid modification from the state with lower stability to a highly stable crystal lattice. In triglycerides, three different crystal modifications exist: α (alpha), β′ (beta prime) and β (beta). In the cooling step in lipid nanoparticles preparation, the lipid is crystalized into less stable α modification. However, during the storage, it changes to more stable β modification. This modification is related to the properties of the lipid. Adding electrolytes causes a destabilization of the lipid nanoparticle dispersion presented by a β′ modification of the lipid causing gel formation and increased size. A high amount of surfactant provides an intense covering at the interface which could affect the stability and the crystallinity of lipid nanoparticles by forming surface mediated crystal growth (Weber et al. 2014; Freitas and Müller 1999; Helgason et al. 2009; Kumar and Randhawa 2013).

Drug expulsion during storage has been recognized for years mainly from suppositories, because the lipid tends to transform to a more stable perfect β-modification. A high degree of perfection in the crystal structure will leave just small space for incorporating the active ingredient causing active ingredient expulsion during storage. SLNs suffer from this disadvantage. When stored after their preparation more

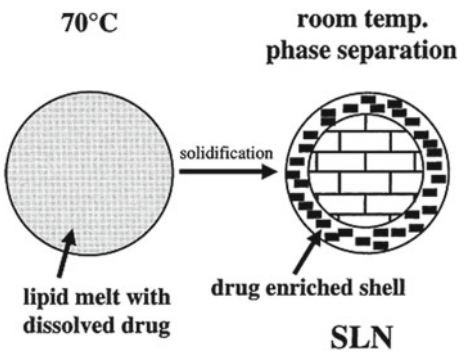

Fig. 4 Phase separation process during the cooling step in SLN production forming a drug enriched shell responsible of the burst release in SLNs. From Müller et al. (2002a) with permission

perfect crystalline β-modifications occurs causing drug expulsion and the formation of active ingredient crystals. Also, when extremely ordered lipid crystals are formed a limited drug loading capacity results. This perfect lipid crystals is favorized when the molecules of the lipid are similar. For example, lipid nanoparticles prepared using tristearine with high purity (Jenning et al. 2000a; Bunjes et al. 1996; Müller et al. 2002a).

Thus, NLCs technology arise as a promising alternative to solve the mentioned limitations of SLNs. For a high drug loading capacity it is recommended that the space between fatty acid chains of the glycerides should be larger enough, in addition imperfect crystal structure permits a higher drug accommodation. Increasing the space between fatty acid chains could be realized by the use of a glyceride type formed of fatty acid with very diverse structure such as using fatty acids having different chain length, saturated and unsaturated acids. A high crystal imperfection can be obtained by blending liquid lipids with solid ones which results in higher drug accommodation (Fig. 5) (Müller et al. 2002a; Pardeike et al. 2009). Thus, NLCs were prepared using a mixture of solid and liquid lipids. This NLCs are solid but not crystalline, because the lipid particles when cooled transform to solid state but not crystalline. This solid status is confirmed by NMR analysis, in addition to DSC analysis which should show an absence of any melting mechanism (Agrawal et al. 2010; Müller et al. 2002a).

It is important to mention that the drug is more soluble in liquid lipids than in solid lipids. Thus, in SLNs preparation, the active ingredient is more soluble in the melted lipid than in the final particles where the lipid becomes solid. Using a high drug amount in the melted lipid can cause an instantaneous expulsion of the drug when the formulation is cooled or diluted. According to this, the type III of NLCs was prepared where the solid matrix of the lipid nanoparticle is formed of very small oily liquid nano-compartments. The drug is more soluble in this compartments leading to a higher drug loading capacity (Fig. 6). But the solid lipid matrix is still surrounding these compartments and allow an extended drug release to be achieved (Seyfoddin et al. 2010; Müller et al. 2002a). These nano-compartments in the NLCs are produced during the phase separation step due to a miscibility gap. As example, when compritol is blended with a high amount of Miglyol 812 to prepare lipid nanoparticles with

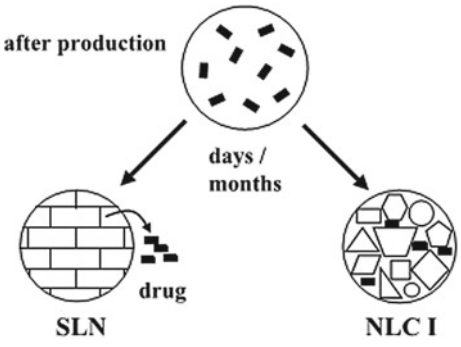

Fig. 5 Crystallisation process during storage to perfect crystal in SLN (left) and unchanged remaining NLC I structure with imperfections (right). From Müller et al. (2002a) with permission

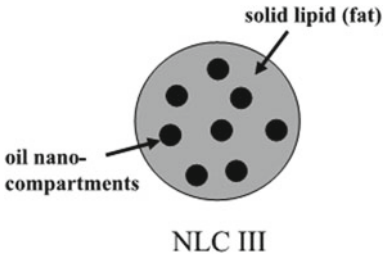

Fig. 6 The proposed structure of multiple type NLC (oil-in-solid fat-in-water, O/F/W). From Müller et al. (2002a) with permission

hot homogenization. One miscible phase is formed during the hot step. However, the two lipids separate in the cooling step and miglyol droplets precipitate and could be confirmed by DSC where peaks of crystallization would appear. In fact, there is no separation of solid lipid particles and liquid lipid droplets in the final structure and NMR and DSC analysis can confirm it, actually the compartments of the liquid lipid are in association with the solid lipid matrix (Müller et al. 2002a).

2.6 Applications of Lipid Nanoparticles in Drug Delivery

Lipid nanoparticles behavior in in vivo depends on the route of administration and how this nanoparticles interact in the body such as their distribution process and their metabolism by enzymes. The material utilized in the preparation of lipid nanoparticles are physiologically compatible lipids. Thus, the transport and the metabolism mechanisms of this lipids already exist in the body. The main enzymes which degrade lipid nanoparticles are lipase distributed in many tissues. Lipase is responsible of the cleavage of the ester bond into glycerides or glycerin and free fatty acids, and requires an activating O/W interface which causes the opening of the catalytic center (Poovi and Damodharan 2018; Mehnert and Mader 2001).

The effectiveness of SLNs as a drug delivery system was tested via several routes of administration. Different routes such as oral, duodenal, parenteral, ocular, transdermal and topical were investigated (Kumar and Randhawa 2013). The applications of NLCs are that of an active ingredient nanocarrier. NLCs are given by several administration routes like oral, parenteral, ocular, topical, transdermal and pulmonary routes. The parenteral and the topical routes are the most studied routes regarding NLCs (Fang et al. 2013).

2.6.1 Lipid Nanoparticles for Parenteral Administration

Parenteral administration of active ingredients can ensure a fast action, high bioavailability but drugs are cleared fast, thus it is needed to be given frequently. Therefore,

the administration of most drugs proceeding a relatively low solubility should be performed using special vehicles that can solve the problems met in parenteral administration. Lipid nanoparticles are able to provide a controlled release profile and enhance the drug solubility in plasma. Drugs incorporated into lipid nanoparticles are cleared from the plasma depending on the size and the surface characteristics of these lipid nanoparticles. The metabolism of lipid nanoparticles administered parenterally is similar to that of chylomicrons. When injected, the triglyceride containing lipid nanoparticle adsorbs Apo-lipoproteins from high density lipoprotein, so that lipid nanoparticles are metabolized by lipoprotein lipase (Göppert and Müller 2005; Kumar and Randhawa 2013).

SLNs can be given intravenously due to their small size. They could be utilized for targeting active ingredients into different organs. SLNs are eliminated by the liver and the spleen in a similar way such as the other colloidal particles. Drug targeting in tumor tissues could be enhanced by avoiding the reticuloendothelial system by using polyoxyethylene or utilizing block polyoxyethylene polypropylene copolymers such as Pluronic F188 where the lipophilic part of the molecule constitutes the matrix of the nanoparticle and the hydrophilic part presented by poly-oxyethylene block constitutes coating on the particle (Wissing et al. 2004). Consequently, an increase of drug accumulation in cancer cells occurs (Chen et al. 2002), improved antimicrobial effect of drugs (Bargoni et al. 2001) and an improvement in the brain delivery of antitumor drugs to bypass the blood brain barrier was achieved (Wissing et al. 2004). Unloaded SLNs also can pass the blood brain barrier when given by intravenous route, it has been reported that the stealth drug loaded SLNs given by IV to lab animals crossed the blood brain barrier more than commercial drug solutions did. The amount of stealth active ingredient was increased compared to conventional preparations leading to the improvement of its pharmacokinetic properties (Zara et al. 2002; Gasco 2007).

NLCs are also an appropriate system for parenteral administration. They provide a controlled release of the drug and protect it from deterioration. When given intravenously, nanoparticles are rapidly uptaken by mononuclear phagocytic system presenting an obstacle that prevents the use of lipid nanoparticles to deliver drugs to locations other than reticuloendothelial system. To avoid this phagocytic uptake, Pluronic F68 and polyethylene glycol were used to provide a modification of the surface of lipid nanoparticles (Joshi and Müller 2009; Fang et al. 2013; Esposito et al. 2012). NLCs are also used in cancer treatment. They provide a sustained release of the antitumor agent, and enhance its cytotoxic effect and chemical stability. Camptothecin and topotecan were formulated successfully in NLCs and showed a high cytotoxic effect and cellular uptake in melanoma and leukemia cells (Huang et al. 2008; Souza et al. 2011; Fang et al. 2013). Buprenorphine is analgesic drug suffering from the excessive first pass metabolism and is given only parenterally or by sublingual route. Therefore, it was formulated into NLCs using cetyl palmitate and linseed oil as solid and liquid lipid respectively. The sustained release of the drug was achieved by incorporating buprenorphine prodrugs in NLCs (Wang et al. 2009).

2.6.2 Lipid Nanoparticles for Oral Administration

SLNs could be given orally in the form of aqueous dispersions or could be transformed to a conventional dosage forms such as tablet, capsule, pellet and powder in sachet. To produce tablets, the aqueous SLNs dispersions are utilized in the place of the granulation liquid in the granulation procedure, or by transferring SLNs into powders (spray dried) and adding the powder to the tablet blend. The SLNs dispersion could be utilized to increase the wettability during the extrusion procedure in the preparation of pellets. SLNs spray dried powder could be used to fill hard gelatin capsules or even could be added to liquid polyethylene glycol 600 to form soft capsules (Müller et al. 2000). However, the high acidic character and ionic strength in stomach may produce aggregated particles. It is also supposed that food can affect the SLNs performance (Mehnert and Mader 2001).

Cyclosporine A was incorporated in SLNs designed for oral administration. The mean particle size of the resulted nanoparticles was around 157 nm, and the entrapment efficiency was high (96.1%). The drug containing SLNs was given orally to three young pigs and was compared to Cyclosporine A commercial product Sandimmun Neoral/Optoral®. Plasma levels of the commercial product showed a peak above 1000 ng/ml in just two hours. However, the drug loaded SLNs revealed similar lower variation in plasma levels without the initial peak seen with the commercial product (Fig. 7). Regarding the area under the curve AUC, drug loaded nanoparticles avoided undesirable effects by preventing plasma drug levels from exceeding 1000 ng/ml. As conclusion, SLNs can be used as a promising carrier for the improvement of the oral delivery of Cyclosporine A by providing low variability in drug bioavailability and preventing the first peak of Sandimmun® formulation (Müller et al. 2006).

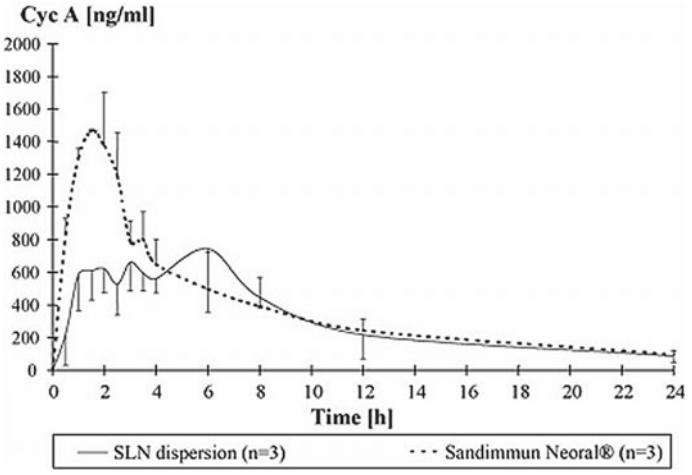

Fig. 7 Mean plasma profiles after oral administration of Sandimmun Neoral® versus Cyclosporine A-loaded SLN dispersion, standard variations (n = 3). From Müller et al. (2006) with permission

NLCs are also used for oral delivery of active ingredients to enhance their bioavailability and plasma levels. This lipid nanoparticles can prevent drug degradation in GIT. The aqueous solubility of poorly soluble active ingredients such as the antidiabetic repaglinide was enhanced by entrapment in NLCs (Poonia et al. 2016).

In addition, it was found that BCS class-II drugs are ideal candidates to be entrapped in lipid nanoparticles (SLNs/NLCs). This entrapment is responsible of decreasing side effects, lowering the first pass metabolism and enzymatic metabolism, in addition to enhancing the water solubility. Acitretin was formulated in NLCs and showed a high effectiveness in treating psoriasis with lower adverse effects. Buspirone HCL was also formulated in SLNs and provided a higher bioavailability compared to the conventional oral preparations (Poovi and Damodharan 2018).

2.6.3 Ocular Delivery of Lipid Nanoparticles

The conventional dosage forms applied to treat ocular diseases such as eye drops suffer from the low corneal absorption and the fast clearance due to the nature of the ocular barrier (Fig. 8). Thus, nanocarriers specifically lipid nanoparticles are investigated for their use in ocular drug delivery. It was found that this system as modified eye drops provided better therapeutic effectiveness with a safe profile (Souto et al. 2010; Alvarez-trabado et al. 2017).

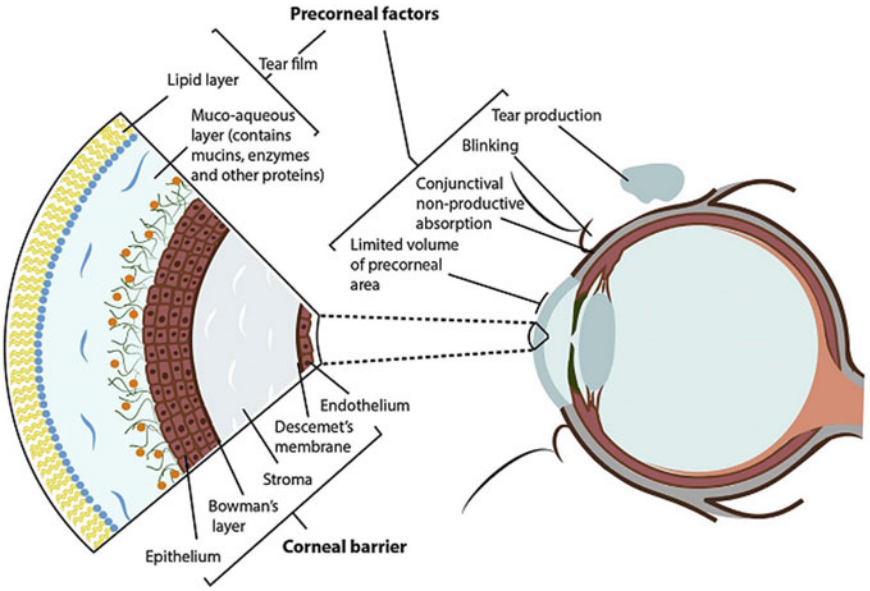

Fig. 8 The ocular barriers that should be overcome by any drug delivery system to exert a pharmacological effect after its topical administration. From Alvarez-trabado et al. (2017) with permission

Drugs having high lipophilicity proceed high permeability to corneal epithelium but this drugs are difficult to be formulated. Therefore, colloidal drug delivery systems may ensure controlled and sustained release of the active ingredient into the target tissue, reduce the dosing frequency and bypass ocular blood barriers. SLNs have high viscosity responsible of increasing the retention time of the active ingredient in the eye and improving its permeability through the cornea, in addition they provide a controlled release profile.

Miglyol 840 as the liquid lipid and Precifac ATO 5 were used to prepare Cyclosporine A containing NLCs and the nanoparticles were investigated for their permeability on human and rabbit cornea. It was found that NLCs improved the topical bioavailability of Cyclosporine A and can be considered as biocompatible and potential nanocarrier to enhance the ocular delivery of Cyclosporine A (Shen et al. 2010).

2.6.4 Pulmonary Delivery of Lipid Nanoparticles

SLNs are suitable for pulmonary administration, this was demonstrated by nebulizing SLN dispersion with Pari-Boy then collecting the resulted aerosol droplets to analyze the size of SLNs. It has been shown that the size distribution before and after nebulizing SLN dispersion was relatively similar with very small aggregation that could be considered insignificant for pulmonary delivery (Müller et al. 2000; Weber et al. 2014).

NLCs like SLNs have many benefits when given by pulmonary route. Both of the lipid nanoparticles have bioadhesive characteristics due to their small size and high lipophilicity leading to prolonged residence time in lungs. When the particle size of these nanoparticles is lower than 500 nm, they possess a high deposition in pulmonary epithelium which results from the increased diffusion mobility (Peter and Kim 2000; Fang et al. 2013). In addition, they provide a controlled release for the entrapped drugs resulting in a prolonged therapeutic effectiveness and inhalation intervals (Pardeike et al. 2011; Fang et al. 2013).

2.6.5 Topical and Transdermal Delivery of Lipid Nanoparticles

The development of lipid nanoparticles was mainly to give them parenterally, they are physically stable and highly tolerable by patients. However, their intravenous application is challengeable because the size of the nanoparticle should be absolutely controlled. This system is used to achieve a retarded and controlled release of drugs, and in brain and liver targeting too. Furthermore, lipid nanoparticles are used widely to deliver drugs into skin (Schäfer-korting et al. 2007).

Besides liposomes, SLNs and NLC s are widely investigated (Schäfer-korting et al. 2007), SLNs could be considered as the next candidate for skin delivery after liposomes. Like liposomal preparations, SLNs are prepared using highly tolerable excipients and they have a small particle size that gives them adhesive character

responsible of film formation on the skin surface. In addition, the matrix of SLNs is solid, thus they enhance the chemical stability of drugs and provide a modified drug release profile. Pharmaceutical products registration is easier for SLNs compared to liposomes, because liposomes cannot be quantified during their storage. However, SLNs are simply quantified in a cream by determining their melting points by DSC. The change in the melting behavior of the SLNs containing cream gives an indication of their stability when stored. Due to this character of SLNs, new topical products are prepared based on SLNs to reach the market. Both SLNs and NLCs possess an adhesive property similar to other nanoparticles and form an adhesive film on the skin. This film can recover any impaired defensive lipid film on the skin, and can provide an occlusive effect too (Dingler et al. 1999; Müller et al. 2000).

Occlusive products improve the hydration of the skin and enhance its drug permeation. Occlusive effect is responsible of water retention in the skin leading to a stratum corneum swelling and enhanced drug permeation (Wissing et al. 2001).

Stratum corneum is composed of intercellular lipids, and lipid nanoparticles applied into the skin surface can exchange their lipids with the stratum corneum (Müller et al. 2007). Lipid nanoparticles can deliver drugs by hair follicles. The follicular route is related to sebum secreting glands. The sebum is formed of a blend of lipids presented by triglycerides, squalene and wax. Thus, this medium can entrap lipid nanoparticles due to the similarity of their components with sebum lipids. The lipids present in lipid nanoparticles enhance the penetration via follicular and sebaceous route. Moreover, Lipid nanoparticles lower the skin irritation caused by some drugs by avoiding the contact directly between the skin and the drug (Fang et al. 2013).

Cyclosporine A was loaded in SLNs and studied for its skin penetration. SLNs were produced using hot homogenization technique. The resulted SLNs were about 73 nm in size and have a surface charge of 16 mV. The in vitro penetration studies in murine skin revealed that cyclodporine A loaded SLNs have 2 folds higher skin permeation efficiency compared to Cyclosporine oil mixture. In addition, the topical application of the drug loaded SLNs resulted in an improvement of atopic dermatitis symptoms in murine model represented by a decrease in T helper 2 cell related interleukins (IL-4 and IL-5), it was concluded that cyclosporine loaded SLNs are an effective carrier to treat skin disorders (Kim et al. 2009).

In another study, Fluocinolone acetonide was entrapped into NLCs using a modified microemulsion method. The resulted nanocarriers showed high stability and the drug release followed a higuchi release kinetic (Fig. 9). Furthermore, higher drug deposition in epidermis and dermis was observed with drug loaded NLCs compared to free drug suspension (Fig. 10). Thus, the drug accumulation in epidermal and dermal layers minimizes the systemic absorption of the drug and the corresponding systemic adverse effects (Pradhan et al. 2015a; Güngör and Rezigue 2017).

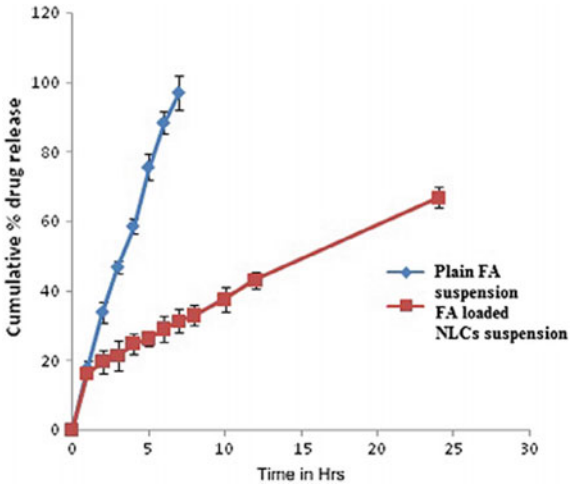

Fig. 9 Drug release profile from Fluocinolone acetonide loaded NLCs suspension and pure Fluocinolone acetonide suspension. From Pradhan et al. (2015a) with permission

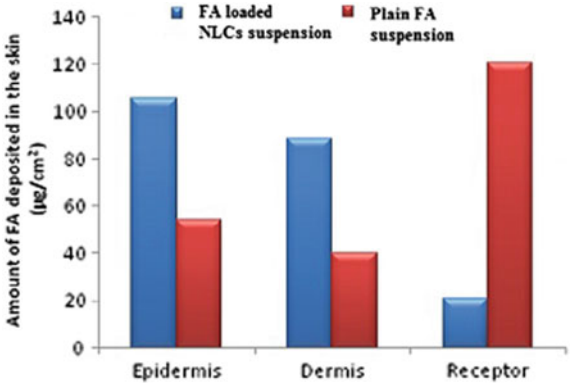

Fig. 10 In vitro skin accumulation study of Fluocinolone acetonide loaded NLCs suspension and pure Fluocinolone acetonide suspension. From Pradhan et al. (2015a) with permission

2.6.6 Cosmetic Applications of Lipid Nanoparticles

The use of lipid nanoparticles in the field of pharmaceutical dosage form possess few dissimilarities from their use in cosmetics, and the main differences could be related to technology based aspects. Moreover, cosmetic products are introduced to the market faster than pharmaceutical products because the regulations guiding them are fewer.

Lipid nanoparticles have several benefits in cosmetics. They improve the chemical stability of agents having a high tendency to be oxidized or hydrolyzed. Lipid nanoparticles were used to entrap several cosmetic agents such as: retinol and retinoids, vitamin E, ascorbyl palmitate, lutein, lipoic acid, carotene and some sun filters. The selection of the type of the lipid is extremely important in the preparation of lipid nanoparticles because it is responsible of the solubilization and the retention of chemical agents in the lipid matrix in storage (Puglia and Bonina 2012). It is

important to notify that entrapping cosmetic agents and modulating their release is more flexible by using NLCs. Natural products from plants are used in preparing cosmetic NLCs as the core materials (Fang et al. 2013).

Lipid nanoparticles possess UV reflecting characteristics provided by the solid nature of these nanocarriers. Furthermore, due to the biocompatible lipids utilized to produce lipid nanoparticles, they have higher safety compared to other systems containing UV blockers (Mehnert and Mader 2001). The crystalline lipid particles cause a scattering of UV light leading to protection of the skin form the UV radiations (Müller et al. 2002a). Lipid nanoparticles (SLNs and NLCs) are also used cosmetically to release insect repellent, to prolong the release of perfumes and to stabilize sensitive chemicals by their solid matrix such as stabilizing retinoids (Jenning and Gohla 2001; Müller et al. 2002a).

The cosmetic advantages of using lipid nanoparticles (SLNs and NLCs) could be summarized as: the formation of a film on the skin surface and controlled occlusion, the improvement of chemical stability of active agents, providing skin hydration in vivo, the enhancement of dermal bioavailability of actives and providing skin targeting with improvement in physical stability of topical formulations (Müller et al. 2007).

The first NLCs introduced to the market for cosmetic use is NanoLipid Restore CLR® in 2016. NLCs were formed of black currant seed oil as the liquid lipid and carnauba wax as solid lipid (Müller et al. 2007). NanoLipid Q 10 CLR® is the second product introduced to the market containing the antioxidant coenzyme Q10. This product was used as an antiaging cosmetic product (Fang et al. 2013).

2.6.7 Lipid Nanoparticles in Gene Therapy

SLNs and NLCs are used to treat genetic and non-genetic diseases as safe non-viral vectors for gene transfection. Lipid nanoparticles can bypass the challenges associated with gene delivery. They avoid the principal biological barriers of cell transfection presented by the nucleases degradation activity, cell internalization, intracellular traffic and selective targeting to an appropriate type of cells. Lipid nanoparticles are also prepared using physiologically suitable ingredients, their large scale production is possible, their sterilization and lyophilization is possible too and they possess high stability during storage.

Due to these features, lipid nanoparticles are considered as promising delivery system for proteins, enzymes, nucleic acids and DNA (Pozo-rodríguez et al. 2009; Marcato and Durán 2008; Fang et al. 2013). They are used to treat of infectious and ocular diseases, cancer and lysosomal storage disorders. (Pozo-rodríguez et al. 2016). The development of a new non-viral vector was performed by the modification of NLCs with cetylated polyethylenimine (PEI) (Zhang et al. 2008b).

SLNs were used the first time for gene therapy by Olbrich et al. (Olbrich et al. 2001) and since that they are largely investigated in this field. As we mentioned before, surfactants are used in SLNs production as a surrounding layer on the surface of the nanocarrier. In gene delivery, surfactants having positive charges form cationic

SLNs which are used to interact electrostatically with nucleic acids (Pozo-rodríguez et al. 2016; Pozo-rodr et al. 2007).

If the nucleic acid already is bounded to a positively charged components, anionic SLNs could be used in transfection. Nucleic acid condense with SLNs due to their electrostatic interaction, this improves the nucleic acid motility and prevent any enzymatic degradation. The ratio between lipid nanoparticle and nucleic acid determines the properties of the final vector. There should be an equilibrium between condensation level responsible of the protection and the ability to release the nucleic acid in the site of action. NLCs are also used in gene therapy. They can bind to nucleic acids by electrostatic interaction and in the same time encapsulate a lipophilic active ingredient in their core. This approach was also applied with SLNs. The role of lipid nanoparticles (SLNs and NLCs) as promising nucleic acid delivery system was studied by many researchers (Pozo-rodríguez et al. 2016; De Jesus and Zuhorn 2015; Sakurai et al. 2000).

It is important to notify that cationic SLNs intended to be used for DNA and RNA transfection should be prepared with solvent diffusion or emulsification methods which are safe for DNA and RNA without damaging them (Carbone et al. 2012; Kumar and Randhawa 2013).

3 Polymeric Nanoparticles

Polymeric nanoparticles have gained great interest in nanotherapy for both therapeutic and diagnostic agents. They are biocompatible, able to bypass biological barriers and can be used in targeting. Therefore, they showed effectiveness in many applications. Polymeric nanoparticles were mostly investigated for their particle size to control biological interactions. Their size depends on their applications and generally it is between 1 and 1000 nm. Particle size highly affect the biodistribution and cellular uptake of polymeric nanoparticles. The shape of the polymeric nanoparticle also has arised as an important property of polymeric nanoparticles which have a crucial role in controlling the interface between nanoparticles and biological systems (Meyer and Green 2016; Zhang et al. 2013).

Polymeric nanoparticles are colloidal solid nanocarriers formed of matrices of polymers with acceptable biocompatibility where the drug could be introduced by adsorption, entrapment or covalent attachment. In general, polymers such polyalkylcyanoacrylates (PACA) and poly(d,l-lactide-*co*-glycolide) (PLGA) are chosen to prepare polymeric nanoparticles because they are biodegradable and biocompatible. In addition polysaccharides and macromolecules such as gelatin, albumin and chitosan were used to produce polymeric nanoparticles (Date et al. 2007; Lockman et al. 2002; Couvreur and Vauthier 2006). The in vivo performance of polymeric nanoparticles is related to their size, surface characteristics, composition, concentration and their hydrophilic or lipophilic nature (Date et al. 2007).

3.1 Advantages of Polymeric Nanoparticles

Advantages and properties of polymeric nanoparticles include:

- Considering effectiveness and absorption, they possess a better enhancement compared to IV and oral routes.
- Regarding to drug delivery, the integration of polymeric nanoparticles into other functions for example tissue engineering can be achieved.
- They can transport drugs to the target site at a specific amount with enhanced stability and prolonged action especially for volatile drugs.
- They are promising carriers to deliver vaccines, antitumor drugs, and targeting antibiotics according to the polymer type and possibility of adjusting the release of the drug from the polymeric nanoparticle.
- Their features can be controlled such as the size to control their infiltration. Their solubility can be adjusted too and thus controlling the release rate of the active ingredient is possible. Therefore, they are designed to deliver active ingredients specifically to the target tissue at the appropriate rate and time (Bennet and Kim 2014; El-say and El-sawy 2017).
- Their nano-sized dimention is responsible of imparting them with the capacity to pass through many barrier types and capillaries, before they reach their target they circulate from one compartment to another in the body. Additionally, they possess a high surface area due to their very small size, this increases the contact space between polymeric nanoparticles and targets and improves the absorption rate. They are also highly stable in suspensions due to their tiny size (Lai et al. 2014; El-say and El-sawy 2017).

3.2 General Types of Polymeric Nanoparticles

Polymeric nanoparticles expression is mainly used for many polymeric nanoparticulate forms, but it is more related to two principal types which are nanospheres and nanocapsules.

Nanocapsules have a vesicle like structure and act as drug reservoir. The active ingredient is located in a hydrophilic or lipophilic core of the nanocapsules which is surrounded by solid polymeric shell. Nanospheres are formed of a solid polymeric matrix. It is a polymeric spherical mass where active ingredient is incorporated in the center or adsorbed at the surface (Rao and Geckeler 2011; El-say and El-sawy 2017). A graphical draw of polymeric nanoparticles types is shown in Fig. 11 (El-say and El-sawy 2017).

The core of polymeric nanocapsules is generally an organic solvent. The polymers used to prepare the shell of nanocapsules are poly-L-lactide, poly-(ε-caprolactone), poly-(glycolic acid), polyvinyl acetate, poly-(lactide-co-glycolide),

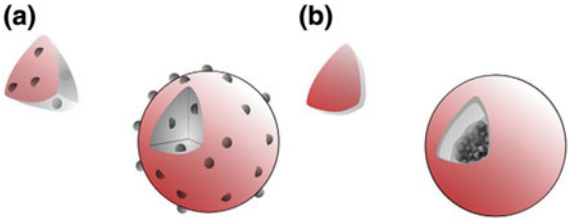

Fig. 11 Polymeric nanoparticles different types: nanospheres (**a**) and nanocapsules (**b**). From El-say and El-sawy (2017) with permission

poly-(ethylcyanoacrylates), poly-(butylcyanoacrylates), poly-(alkylene adipate), cellulose acetate phthalate, poly-(ε-caprolactone)-block-poly-(ethylene glycol), poly-(methyl methacrylate), and polystyrene. Polymeric nanocapsules are prepared by involving interfacial polymerization in situ surrounding a droplet, or by interfacial nanodeposition of a preformed polymer. In interfacial polymerization, the process is performed on the surface of the droplet where an emulsion is prepared generally before polymerizing the shell (Wu and Guy 2009). In interfacial nanodeposition, a blend of the polymer containing organic phase and a surfactant containing aqueous phase favorizes the deposition of the polymer in the shell. When a nanosized droplet of oil is formed, the polymer precipitation at the interface with the aqueous medium is realized and nanocapsules are formed and stabilized by surfactants (Tiarks et al. 2001; Lambert et al. 2000; Wu and Guy 2009). The preparation methods of polymeric nanoparticles are explained in the later section. Nanocapsules have been used as carriers for several drugs as topical formulations such as: retinoids, beta-carotene, lidocaine, diclofenac, vitamin E, antioxidants and UV protectors. Their core possesses a high loading capacity with low quantity of polymers. Nanocapsules are more strong in comparison to liposomes because their shell is a covalent linkage of polymers (Wu and Guy 2009).

Nanospheres are composed of a polymer matrix where the active ingredient is dispersed homogenously. They are prepared by interfacial polymerization, emulsion polymerization and solvent evaporation methods (Wu and Guy 2009). Nanospheres are used widely to achieve a controlled release of drugs. For example, cyclosporine A incorporated into chitosan nanospheres have shown an efficient cyclosporine A concentration on eye surface and avoided any systemic absorption, fast clearance, local irritation and blurred vision (De Campos et al. 2001; Wu and Guy 2009).

3.3 Polymers Used to Prepare of Polymeric Nanoparticles

Polymeric nanoparticles are mainly composed of polymers. Two categories of polymers exist depending on their origin and no one of these categories is more favorable. The selection of the polymer is related to the required properties of the polymeric nanoparticle (El-say and El-sawy 2017).

3.3.1 Natural Polymeric Nanoparticles

Chitosan, sodium alginate, albumin, and gelatin are the most widely utilized natural polymers to prepare polymeric nanoparticles (El-say and El-sawy 2017; Ahmed and El-say 2014; Kaul and Amji 2004).

Chitosan obtained from chitin is a linear polysaccharide present in the exoskeletons of crustaceans. Chitosan is positively charged in acid to neutral mediums because it possesses an amino group that protonates. Therefore, chitosan is soluble in water and have bio-adhesive character due to its positive charge which binds to the negative charge of mucoproteins. This binding is helpful in prolonging the circulation time of drugs bounded to chitosan, and leads to an enhancement in drug bioavailability (Goyal et al. 2016; Wang et al. 2011). Chitosan nanoparticles are prepared by ionic or covalent cross linking, polymerization, precipitation or self-assembly techniques. The size of the resulted polymeric nanoparticles depend on the preparation method and varies from 20 to 800 nm. Chitosan drug complex resulted in general from the electrostatic interaction between the negatively charged drug and the positively charged chitosan (Cheng et al. 2010; Kim et al. 2006; Goyal et al. 2016).

Alginate obtained from algae is a negatively charged polysaccharide. It is a versatile biopolymer used in different applications. Alginate is a linear non-branched polysaccharide with different proportions of a-L-guluronic acid and B-D mannuronic residues. Sodium alginate is a natural polymer with a pH dependent gel forming capacity. By hydrating alginic acid an acid gel with high viscosity is formed because of the intermolecular binding. The physical entrapment of water molecules in the alginate matrix is responsible of the gelation, in the same time water is free to migrate. This properties is used in different applications of alginate gels for encapsulation and immobilizing cells (Jain and Bar-Shalom 2014).

Albumin is a natural biocompatible and biodegradable protein applied largely in drug delivery. Because of its high binding ability, it is widely used as vehicle in drug delivery. A wide range of molecules and substances including drugs, peptides, vaccines, genes, and antibodies can be efficiently bounded to albumin matrix that acts as a versatile vehicle (Karimi et al. 2016; Goyal et al. 2016).

3.3.2 Synthetic Polymeric Nanoparticles

The consistency from batch to batch may be a lacking property in natural polymers. This problem makes the preparation of nanoparticles with good reproducibility hard. Therefore, synthetic polymers are synthesized with a batch to batch consistency and a high purity and provide a regular drug release from nanoparticles. Polymeric nanoparticles prepared from synthetic polymers encapsulate mostly small lipophilic drugs. However, it is a big challenge to protect the biological effect of proteins or peptides when polymeric nanoparticles are manufactured because of using volatile organic solvents in their preparation (Goyal et al. 2016; Panyam and Labhasetwar 2003). Synthetic polymers are also utilized to prepare polymeric nanoparticles presented by polyglycolides (PGA), polylactides (PLA), poly (lactide co-glycolides)

(PLGA), polycyanoacrylates, polycaprolactone (PCL), polyethylene glycol (PEG), polyanhydrides, polyorthoesters (POE), poly (*N*-vinyl pyrrolidone), polyglutamic acid (PGA), (PVP), poly (malic acid) (PMLA), poly (vinyl alcohol) (PVA), polyacrylamide (PAM), (methyl methacrylate) (PMMA), poly (methacrylic acid) (PMAA) and polyacrylic acid (PAA or Carbomer) (Nagavarma et al. 2012; El-say and El-sawy 2017). The synthetic polymers mentioned above are safe. They are biocompatible and biodegradable which means that they are not proceeding any biological toxicity or antigenicity (El-say and El-sawy 2017).

Initially, polymeric nanoparticles were prepared using non-biodegradable polymers like poly (methyl methacrylate) (PMMA), polystyrene, polyacrylamide and polyacrylates. The design of non-biodegradable based polymeric nanoparticles is performed that nanoparticles should be cleared rapidly with urine or feces, and any accumulation or distribution in tissues should be avoided because their excretion or degradation is difficult. Their removal could be provided by physical ways too (Burman et al. 2009). Non-biodegradable polymer based nanoparticles were used in drug delivery and wound healing, but inflammation and chronic toxicity were the main reasons for shifting to the use of biodegradable polymers. Biodegradable polymers compromise the previously explained natural polymers like gelatin, alginate, chitosan and albumin, and also synthetic polymers like poly(ε-caprolactone) (PCL), poly(lactide) (PLA), poly(lactide-co-glycolide) copolymers (PLGA) and poly(amino acids). Biodegradable polymers are associated with lower toxicity and higher biocompatibility with the possibility of affecting the release profile of active ingredients (Banik et al. 2016).

Some of the biodegradable synthetic polymers presented by: tyrosine-derived polymers, aliphatic polyesters, and poly(ε-caprolactone) (Goyal et al. 2016) are explained below.

A. **Tyrosine-derived polymeric nanospheres (TyroSpheres)**

These nanospheres are trademarked as Tyrospheres™ and have been developed in Rutgers University by New Jersey Center for Biomaterials, they are used widely in drug delivery. Tyrospheres or Tyrosine derived polymeric nanospheres are prepared form a group of completely degradable ABA type triblock copolymers formed of the hydrophilic poly (ethylene glycol), oligomers of desaminotyrosyl-tyrosine alkyl esters, and other different natural dicarboxylic acids like suberic acid, succinic acid, sebacic acid or adipic acid. Tyrospheres result from the spontaneous self-assembly of tyrosine derived copolymers in water, they generally have a diameter around 70 nm (Fig. 12) (Goyal et al. 2016; Sheihet et al. 2007; Zhang et al. 2013).

B. **Poly (lactic-co-glycolic acid) nanoparticles**

Poly (lactic-co-glycolic acid) is a biocompatible and biodegradable polymer used largely in drug delivery, it is included in many pharmaceutical dosage forms and medical devices approved by FDA. Poly (lactic-co-glycolic acid) exists in the market with several molecular weights and copolymer ratios. It is used to encapsulate even hydrophobic or hydrophilic drugs, it is able to modify the surface for site specific

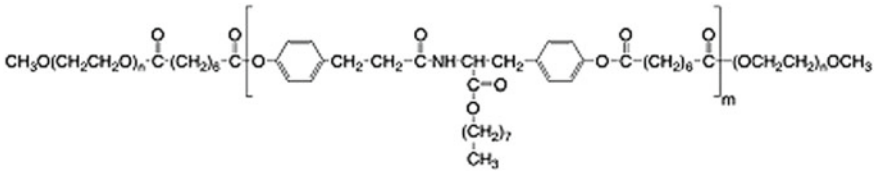

Fig. 12 Structure of the tyrosine-derived, ABA-type poly(ethylene glycol)-b-oligo(desaminotyrosyl-tyrosine octyl ester suberate)-b-poly(ethylene glycol) triblock copolymer (DTO-SA/5 K) used in the preparation of TyroSpheres. From Kilfoyle et al. (2012b) with permission

drug delivery. Additionally, this polymer is used to formulate nanoparticles by many techniques like solvent diffusion, solvent displacement, emulsification–evaporation and phase-inversion. The incorporation of poly(lactic-co-glycolic acid) nanoparticles into gel structures by the use of hydroxyl propyl methy cellulose (Carbopol®) can be performed for enhancing the penetration of active ingredients (Kapoor et al. 2015; Goyal et al. 2016).

C. **Poly(ε-caprolactone) nanoparticles**

Poly(ε-caprolactone) is a synthetic polymer used to prepare nanoparticles. It is biocompatible, biodegradable, and possess appropriate rheological behavior presented by low glass transition temperature. This polymer degraded slowly compared to other polyesters because of its semi-crystalline nature. Thus, generally it is modified to modulate its biodegradable character to meet the requirements of targeted-clinical applications (Goyal et al. 2016; Dash and Konkimalla 2012; Shahin and Lavasanifar 2010).

3.4 Preparation Methods for Polymeric Nanoparticles

Polymers are prepared either from already existing polymers or by direct polymerization process. The methods used to prepare polymeric nanoparticles from existing polymers are solvent evaporation, supercritical fluid technology, salting out and dialysis methods. Polymeric nanoparticles could be prepared from monomers by polymerization techniques like controlled radical polymerization, emulsion, mini-emulsion, micro-emulsion, surfactant-free emulsion, and interfacial polymerization. The mostly used techniques are explained below (Rao and Geckeler 2011; Nagavarma et al. 2012; El-say and El-sawy 2017). Figure 13 represents a schematic representation of the different methods used to prepare polymeric nanoparticles.

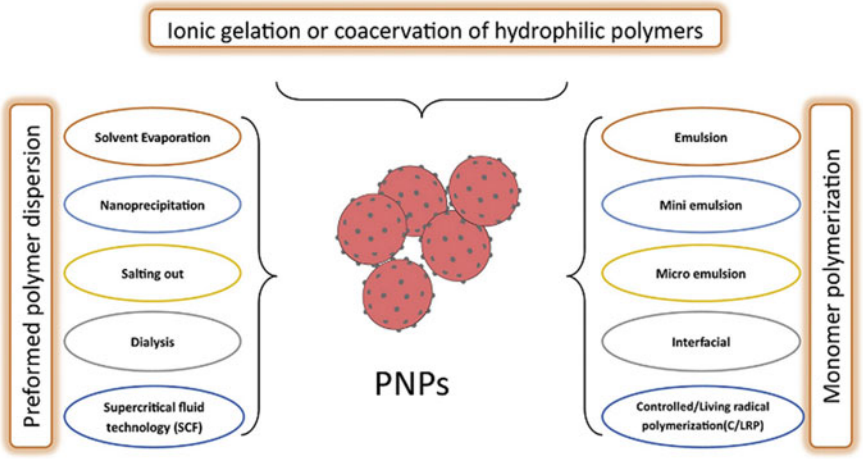

Fig. 13 Different methods used for the preparation of polymeric nanoparticles. From El-say and El-sawy (2017) with permission

3.4.1 Dispersion of Preformed Polymers

Many techniques were developed to produce polymeric nanoparticles by dispersion of readily preformed polymers.

Solvent Evaporation

Polymeric solution is formed in volatile solvents producing emulsion. Chloroform and dichloromethane were previously used but now ethyl acetate replaced them due to its higher safety (Anton et al. 2008). Then the transformation of the emulsion into nanoparticle suspension is achieved by evaporating the solvent, allowing the polymer to diffuse through the emulsion continuous phase. This technique is the most widely used in literature to prepare polymeric nanoparticles by dispersing existing polymers. In traditional techniques, two methods are utilized to form emulsions, preparing single emulsion like oil in water emulsion or double emulsion like W/O/W. These techniques use ultrasonication or high speed homogenization. After that, the solvent is vaporized under reduced pressure or by continuously stirring at room temperature. Then after collecting solidified nanoparticles by ultracentrifugation, they are subjected to washing with water to eliminate surfactant-like additives. At last, the product is lyophilized. In general, the oily phase is formed of the polymer solubilized in the organic solvent, whether the water phase is formed of the stabilizer containing aqueous phase (El-say and El-sawy 2017; Rao and Geckeler 2011).

Salting-Out

In the previous method of polymeric nanoparticles preparation, organic solvents were used. These solvents are toxic environmentally and physiologically. Thus, a modification of the emulsion technique involving salting out mechanism was introduced, it prevents the use of chlorinated solvents or surfactants. The emulsion is formed by emulsifying a polymer solution (the solvent should be miscible with water like acetone) in an aqueous phase. High concentrations of salt (like magnesium acetate and magnesium chloride) or sucrose are used for the salting out effect in water (Galindo-rodriguez et al. 2004; Rao and Geckeler 2011).

Nanoprecipitation

It is known also as solvent displacement method. It was developed by Fessi et al. (1989). The technique consists of interfacial deposition of a polymer after displacing a semipolar solvent (should be miscible with water) from a hydrophobic solution. The fast diffusion of the solvent to the non-solvent phase causes a decrease in the interfacial tension between the solvent and the non-solvent phases. The lowering in interfacial tension causes an increase of the surface area and small droplets of organic solvents are formed. Nanoprecipitation method compromises three constituents, the polymer, the non-solvent of the polymer and the solvent of the polymer. An organic solvent miscible with water mainly acetone is used as the solvent of the polymer and is generally easily removed by evaporation (Dalpiaz et al. 2009; Mishra et al. 2010; Rao and Geckeler 2011).

Dialysis

This method gives small and narrow distributed polymeric nanoparticles. In this technique, the polymer solubilized in an organic solvent is put in a dialysis tube having an appropriate molecular weight cut off. Thus, the solvent is displaced inside the membrane and the polymer aggregates progressively because of losing its solubility and forming a suspension of homogenous particles (Fessi et al. 1989; Rao and Geckeler 2011).

Supercritical Fluid Technology

The use of supercritical fluids in preparing polymeric nanoparticles appeared to avoid the organic solvents involved in the previous methods due to their environmental safety (York 1992). To prepare polymeric nanoparticles two different techniques are used, rapid expansion of supercritical solution into liquid solvent and rapid expansion of supercritical solution. In rapid expansion of supercritical solution, a solution of the solute in supercritical fluid is formed then the expansion of this solution is performed

through an aperture or a fine nozzle to ambient air. Then the pressure is dropped in this expansion causing a homogenous nucleation and the formation of well dispersed particles. In the rapid expansion of supercritical solution into liquid solvent, here the critical solution is expanded into a liquid solvent and not ambient air. This liquid solvent prevent particles form growing in the expansion jet and nanoparticles could be obtained primarily (Nagavarma et al. 2012; Rao and Geckeler 2011).

3.4.2 Polymerization of Monomers

Emulsion Polymerization

This method is largely utilized to prepare polymeric nanoparticles from monomers. Water is used as dispersion medium representing high safety for the method and good controlling of the heat removal in the polymerization process. Depending on the use of surfactants the method could be divided into two different techniques: conventional and surfactant-free emulsion polymerization (El-say and El-sawy 2017; Thickett and Gilbert 2007).

A. **Conventional emulsion polymerization**

The constituents used principally in this method are the surfactant, hydrophilic initiator agent, monomer with low aqueous solubility and water. Firstly, the monomer is dissolved in the continuous phase using the surfactant. The initiation begins when the dissolved monomer interacts with the initiator such as an ion or a free radical. High energetic radiations can used to convert the monomer into initiation radicals. Gamma radiations, strong visible light or UV can be employed. Phase isolation and nanoparticles formation can happen after or before the polymerization reaction. When the reaction is finished, the formed polymeric nanoparticle are almost 100 nm having many polymer chains that are embedded in each single nanoparticle (Reis et al. 2006; El-say and El-sawy 2017).

B. **Surfactant-free emulsion polymerization**

This method avoids the use of surfactants, thus it received great interest. Monomers (principally acryl or vinyl monomers), hydrophilic ionisable initiator and deionized water are used in this process. Ionisable initiators help to stabilize the polymeric nanoparticles.

In surfactant free emulsion polymerization, the nucleation and particle growth mechanisms were suggested as homogeneous nucleation and micellar like nucleation. The solubility of the monomer in water governed the difference between the two mechanisms. The degree of the nucleation homogeneity and the monomer concentration influence the characteristics of polymeric nanoparticles like particle size and the stability of the nanoparticles (El-say and El-sawy 2017).

Mini-emulsion Polymerization

The constituents used in this technique are: the monomer blend, water, surfactant, initiator and costabilizer. Unlike the emulsion polymerization, this method uses a low molecular weight component as a costabiliser and utilizes a high shear mechanism such as ultrasound. To reach a steady state miniemulsions a high shear mechanism is needed, they are stabilized critically and possess an interfacial tension higher than zero (Wang et al. 2007; Rao and Geckeler 2011).

Micro-emulsion Polymerization

This method consists of adding a hydrophilic initiator to an aqueous phase of thermodynamically stabilized microemulsions formed of swollen micelles. From this thermodynamically stable form the polymerization process begins and depends on high amount of surfactants possessing an interfacial tension approaching zero (at the interface between oil/water). Therefore, the high quantity of surfactant used results in the total coverage of particles. In the initial phase, the formation of polymer chains occurs just in some droplets because this initiation cannot happen in all micro-droplets. After that, the elastic and the osmotic effect of the chains causes destabilizing of the weak microemulsions leading to increased particle size and empty micelles formation, resulting in a second nucleation. Most of the empty micelles in the final product contain latexes (5–50 nm in size) (Rao and Geckeler 2011; Qiu et al. 2001).

Interfacial Polymerization

This method is widely used to produce polymeric nanoparticles. The method consists of dissolving two reactive monomers in two liquids, one is the continuous phase and the other is the dispersed phase, then step polymerization is conducted. The reaction happens mainly at the interface of the two phases. This technique is easy and thus it is chosen and used in many applications such as encapsulating of pharmaceutics and preparing conducting polymers (Nagavarma et al. 2012; Karode et al. 1998).

3.5 Application of Polymeric Nanoparticles in Drug Delivery

By using Polymeric nanoparticles as a drug delivery system many features can be achieved such as:

- A controlled release of the drug from the polymeric nanoparticles to its target site of treatment can be obtained.

- Sensitive molecules could be encapsulated and stabilized such as DNA, RNA and proteins.
- They give the possibility for the modification of the surface using ligands.
- They enhance the in vivo and in vitro stability of encapsulated agents (Banik et al. 2016).

Polymeric nanoparticles used in controlled drug delivery possess several advantages like: the selectivity in targeting, the controlled release and the enhancement of stability by protecting drugs, in addition to extending the circulation half-life (Cheng et al. 2013; Banik et al. 2016). In this context, polymeric nanoparticles are widely used in the treatment of many disorders.

3.5.1 Polymeric Nanoparticles Application in Oral Drug Delivery

The oral administration of polymeric nanoparticles is largely studied in literature. The intestinal epithelium is responsible of the permeability and the absorption of nanoparticles or active ingredients into the blood stream. The large surface area of the small intestine due to the presence of villi and microvilli has a major role in the drug absorption in the gastrointestinal tract. The paracellular and/or transcellular routes represent the mechanisms of translocation of nanoparticles through the epithelium of the intestine (Alai et al. 2015; Chen et al. 2011). For oral administration of polymeric nanoparticles, the polymers used to prepare these nanocarriers have to be biocompatible and biodegradable possessing high safety profile (Hrkach et al. 2012). The surface of polymeric nanoparticles can be attached to antibodies, peptides or even molecular targeting ligands to boost the interaction of polymeric nanoparticles with cellular receptors (Yu et al. 2012; El-say and El-sawy 2017).

Polymeric nanoparticles were used to enhance the oral absorption of insulin. Many biodegradable hydrophilic and hydrophobic polymers were investigated. The insulin embedded in polymeric nanocarriers is protected from the enzymatic degradation and the pH change. Polymeric nanoparticles can also modify the insulin release profile. In addition, the polymer structure could be modified with the purpose of modulating the physicochemical characteristics of the polymeric nanoparticles by providing pH-sensitivity, mucoadhesion and penetration enhancement capacity to improve insulin oral absorption. Polymeric nanoparticles based on chitosan, alginate, dextran and other polymers were used to deliver insulin orally (Mo et al. 2014).

Polymeric nanoparticles are used in nano-oncology as delivery vehicles for oral chemotherapeutic drugs. They showed an important role in treating colon cancer. Many in vivo and in vitro studies for the delivery of cancer chemotherapeutic agents by using several oral polymeric nanoparticles are reported in literature (Agrahari and Mitra 2016). Curcumin is an oral anti-proliferative agent that induces apoptosis in cancer cells. It possess very low solubility and bioavailability restricting its clinical use. Thus, Chaursia et al.; have developed polymeric nanoparticles to improve its bioavailability and effectiveness in treating colon cancer in vitro and in vivo. Eudragit E 100 which is a cationic copolymer was used as vehicle, and curcumin

loaded Eudragit E 100 polymeric nanoparticles were prepared to enhance curcumin low bioavailability. To study the in vitro cytotoxicity of curcumin loaded Eudragit E 100 polymeric nanoparticles, sulphorhodamine B assay was used. Anti-tumor effectiveness and pharmacokinetic parameters were performed after oral administration in Wister rats and colon-26 tumor-bearing mice. The resulted drug loaded polymeric nanoparticles gave acceptable particle size and entrapment efficiency. In vitro cytotoxicity results were given in 50% cell growth inhibition and showed approximately 19-fold decrease with drug loaded polymeric nanoparticles in comparison with the free drug. The oral bioavailability of the curcumin loaded polymeric nanoparticles showed approximately 91-fold and 95-fold increase in Cmax and AUC-12 h respectively with polymeric nanoparticles compared to free curcumin. In vivo results showed enhancement in the effectiveness with drug loaded polymeric nanoparticles compared to free drug in terms of tumor volume, body weight and survival rate. This study indicated the potentiality of polymeric nanoparticles to enhance the oral bioavailability and anti-proliferative effect of low water soluble anticancer agents (Chaurasia et al. 2016).

3.5.2 Polymeric Nanoparticles Application in Cancer Treatment

The encapsulation of anti-proliferative agents in nanoparticles provides a controlled release of these agents and thus improves their effectiveness. Polymeric nanoparticles also minimize the toxic effect of anticancer drugs to the normal cells and enhance their solubility in water. Zhao et al. have encapsulated Docetaxel in polymeric nanoparticles. A new copolymer, D-α-tocopheryl polyethylene glycol 1000-block-poly-(β-amino ester) (TPGS-b-PBAE) was prepared to overcome the multidrug resistance by the synergistic effect of the P-glycoprotein inhibiting activity of D-α-Tocopheryl polyethylene glycol succinate (TPGS), and the pH-sensitive behavior of poly (β-amino ester) (PBAE). The resulted drug loaded polymeric nanoparticles have shown good stability at pH 7.4, they dissociate and release the active ingredient at pH 5.5. The cell cytotoxicity against both drug-resistant A2780/T cells and drug-sensitive human ovarian A2780 was enhanced by the prepared drug loaded polymeric nanoparticles. In addition, P-glycoprotein inhibition results indicated an elevation in the fluorescence intensity of rhodamine 123 and intracellulare ATP levels were reduced. As result, polymeric nanoparticles were able to improve the efficacy of anticancer drugs in Multidrug resistant tumors (Zhao et al. 2013).

Many companies have tried to introduce polymeric nanoparticles into the market, Accurins™ was developed by BIND THERAPEUTICS. It is a polymeric nanoparticulate system that contains docetaxel and microtubular inhibitor used in prostate cancer. The nanoparticles aimed to deliver the active ingredient to tissular, cellular and molecular level. The company's objective is to produce a controlled release of the encapsulated agents and minimize the side effects on normal cells. The in vivo preclinical tumor studies showed a high effectiveness and improvement in toxicity patterns (Hrkach et al. 2012; Banik et al. 2016). Therefore, polymeric nanoparticles are promising delivery systems to be the next generation in treating and enhancing

the quality of life of cancer patients, as they overcome the drawbacks of traditional chemotherapeutic agents (Banik et al. 2016).

3.5.3 Polymeric Nanoparticles Application for Central Nervous System

The delivery of drugs to the central nervous system and to the brain is a big challenge. Nanocarriers are able to bypass the blood brain barrier. The blood brain barrier prevent the penetration of drugs given systemically. In addition the extracellular matrix of the brain cease the efficacy and the distribution of the administered drug. Therefore, polymeric nanoparticles are considered as a good approach to overcome these limitations (Kreuter 2014; Salvalaio et al. 2016; El-say and El-sawy 2017). Polymeric nanoparticles are used to treat neurodegenerative diseases like Parkinson and Alzheimer's disease because they can overcome the blood brain barrier.

Trapani et al. have prepared polymeric nanoparticles encapsulating the neuromediator dopamine to assess its capacity to pass the blood brain barrier. Chitosan based nanoparticles were put with dopamine at different concentrations. X-ray photoelectron spectroscopy was used to study the absorption of dopamine on the external surface of nanoparticles. There was an enhancement in the transport across MDCKII-MDR1 cell line with dopamine loaded chitosan nanoparticles compared to the control and the neurotoxicity was found low after three hours by measuring reactive oxygen species. Dopamine loaded polymeric nanoparticles given intraperitoneally to rats have shown dose dependent enhancement in striatal dopamine output in in vivo microdiaysis studies. Therefore, chitosan nanoparticles were successful in the brain delivery of dopamine and could be helpful in treating Parkinson's disease (Trapani et al. 2011).

In another study, to overcome the problems associated with the blood brain barrier and treat Alzheimer's disease, Zhang et al. have developed PEGylated poly (lactic acid) (PLA) polymer based dual-functional nanoparticles. Two types of peptide TGN and QSH were targeted and screened by phage display. The conjugation of these peptides was done on the surface of nanoparticles. TGN mainly can target the ligands in the blood brain barrier, whereas QSH possess high affinity to the principal constituent of amyloid plaque known as Ab1-42. An enhancement in the delivery to the amyloid plaque and a precise targeting were obtained using these polymeric nanoparticles in the brain of mice model with Alzheimer's disease. The dual targeted delivery system was found safe in MTT experiments using bEnd.3 and PC 12 cells. It can be concluded from this study that polymeric nanoparticles can be used as versatile targeting delivery system to treat Alzheimer's disease (Zhang et al. 2014a).

3.5.4 Polymeric Nanoparticles Application in Cardiovascular Diseases

Another field where nanoparticles showed promising results is in treating cardiovascular disorders, like atherosclerosis, coronary artery disease and other diseases

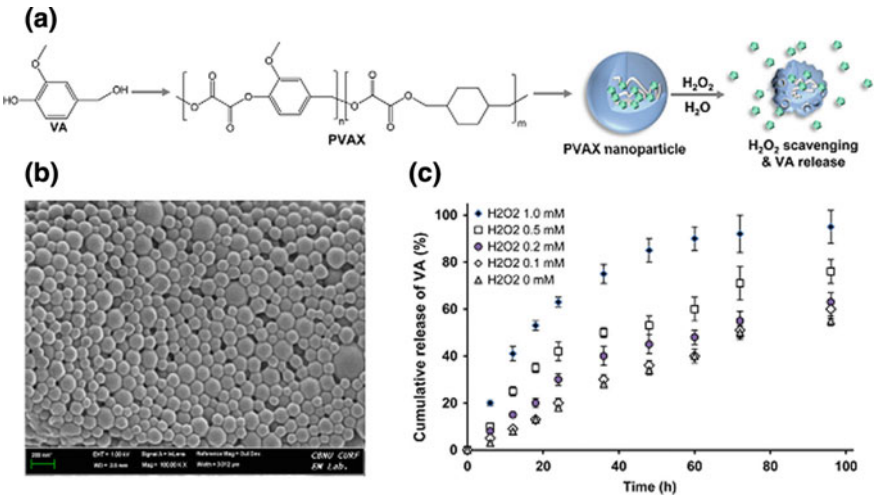

Fig. 14 Characterization of H_2O_2-responsive PVAX nanoparticles. **a** A schematic representation of H_2O_2-responsive PVAX nanoparticles. **b** A Scanning Electron Microscope image of PVAX nanoparticles. **c** Release kinetics of VA from PVAX nanoparticles at different H_2O_2 molar concentrations. Values are mean ±S.D. (n = 3). From Kwon et al. (2016) with permission

(Banik et al. 2016). One of the most popular circulatory disorders is the peripheral artery disease where narrow arteries cause limited blood circulation to lower extremities. The leg muscles of patients with peripheral artery disease contain high levels of reactive oxygen species, which is responsible of the disease progression. Thus, Kwon et al. developed H_2O_2-responsive polymer PVAX (polyoxalate containing vanillyl alcohol) responsible of scavenging H_2O_2 and liberate vanillyl alcohol VA possessing antioxidant and anti-inflammatory effect (Fig. 14). PVAX polymeric nanoparticles improved the expression of angiogenic inducers like vascular endothelial growth factor and platelet endothelial cell adhesion molecule in human umbilical vein endothelial cells. The upregulation of platelet endothelial cell adhesion molecule and the vascular endothelial growth factor by polymeric nanoparticles revascularized and restorated the blood flow to ischemic tissue. It can be concluded that H_2O_2-responsive PVAX nanoparticles was successful to treat ischemic disorders like peripheral artery disease (Kwon et al. 2016).

3.5.5 Polymeric Nanoparticles Application for Topical Drug Delivery

Polymeric nanoparticles are effective in protecting labile active ingredients from degradation. By providing a controlled release they lower the toxic effects of drugs. In addition, they improve drug permeation through the skin by producing a concentration gradient. The topical administration of drug loaded polymeric nanoparticles into skin have several advantages:

- They improve the percutaneous absorption of drugs, principally drugs with low aqueous solubility by enhancing their concentration gradient through the skin.
- They enhance the stability of drugs.
- They lower the irritating effect of drugs.
- They have the capacity of delivering drugs directly to the site of disease and lower their systemic absorption (Zhang et al. 2013).

Natural polymeric nanoparticles are formed of polymer present in nature like gelatin, alginate, chitosan and albumin. These polymers are extracted and purified by different steps. They are used in the formation of hydrogels thus, they are ideal candidates to for proteins, peptides, oligonucleotides and hydrophilic active ingredients (Zhang et al. 2013). Gelatin nanoparticles incorporating pilocarpine HCl or hydrocortisone were prepared and evaluated for topical administration to the eye (Vandervoort and Ludwig 2004). Chitosan is a widely used natural biodegradable cationic polymer in topical delivery into skin. It is formed of glucosamine units and possess antiinflamatory, antioxidant and antimicrobial characteristics. Due to the previous properties chitosan is an appropriate polymer in the formulations used to treat skin diseases. At physiological pH, chitosan is positively charged due to the protonation of the amine group. Thus, it could be used for encapsulating negatively charged drugs (Zhang et al. 2013).

Matos et al. have prepared minoxidil sulphate loaded chitosan nanoparticles to provide sustained drug release into hair follicles for the treatment of alopecia. Tripolyphosphate was used as crosslinking agent with low molecular weight chitosan. The particles size and shape were confirmed by scanning electron microscopy. Particles were in nanometer range with highly positive zeta potential and good encapsulation efficiency. Minoxidil sulfate release through cellulose membrane was 5 times more extended from polymeric nanoparticles compared to free drug solution (Fig. 15a). Drug accumulation in hair follicles was found 2 times more than its accumulation from drug solution in in vitro skin permeation experiments (Fig. 15b). Thus, minoxidil loaded polymeric nanoparticles can be considered as an efficacious carrier to hair follicle for treating alopecia (Matos et al. 2015). Also, chitosan based nanoparticles are able to deliver active ingredients into nasal, ocular and intestinal epithelium (Zhang et al. 2013).

Natural polymers mostly are not consistent from batch to batch and are different in the level of their purity too, thus the reproducibility in the prepared polymeric nanoparticles using natural polymers may be affected and so the release profile of the drug. Due to these issues, polymeric nanoparticles are often formed of synthetic polymers. Inversely, synthetic polymers could be purified to high degree and are consistent from batch to another, the release of the drug can be modulated both for hydrophilic and lipophilic drugs. The most widely used synthetic polymers are either biodegradable aliphatic polyesters like and poly(ε-caprolactone), polylactides (PLA), poly(lactide-co-glycolide) copolymers (PLGA), or non biodegradable polymers like polyacrylates and poly(methyl methacrylate) (Zhang et al. 2013).

Poly(lactide-co-glycolide) copolymers (PLGA) are widely used in preparing polymeric nanoparticles for topical delivery. They are biodegradable and when

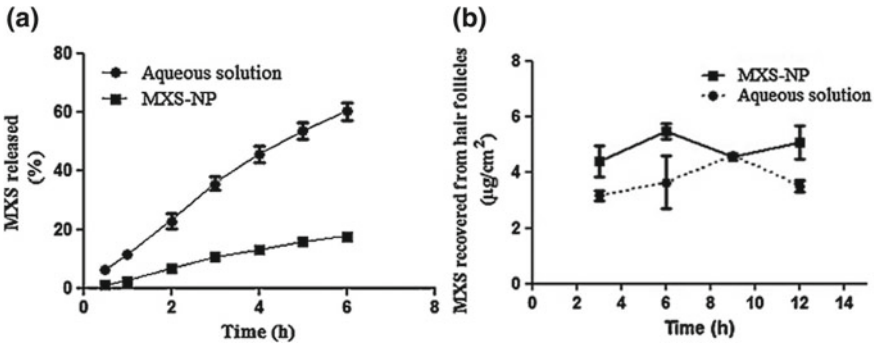

Fig. 15 **a** Release profile of minoxidil sulphate MXS from minoxidil sulphate loaded chitosan nanoparticles MXS-NP formulation compared to an aqueous solution of the drug (control formulation), both containing 1.25 mg/mL of the drug and pH 5.5. From Matos et al. (2015) with permission. **b** Minoxidil sulphate MXS recovery from hair follicles after 3, 6, 9 and 12 h of topical application of minoxidil sulphate loaded chitosan nanoparticles MXS-NP formulation compared to the control formulation, both containing 1.25 mg/mL Minoxidil sulphate, at pH 5.5. From Matos et al. (2015) with permission

degraded form glycolic and lactic acid eliminated from the body by citric acid cycle (Zhang et al. 2013). Takeuchi et al. prepared minoxidil-encapsulated poly(lactide-co-glycolide) nanoparticles for prolonging the drug release for the delivery to hair follicles to treat alopecia. W/O/W solvent evaporation and sonication technique was used to prepare the drug loaded polymeric nanoparticles. In vivo percutaneous absorption studies were performed using C3H/He mice. Minoxidil loaded polymeric nanoparticles showed three times more drug accumulation in stratum corneum and 2.5 times more accumulation in hair follicles compared to drug solution (Takeuchi et al. 2018).

Poly(ε-caprolactone) is also a synthetic polymers used largely due to its mechanical properties, biocompatibility and biodegradability. Additionally, it has a semicrystalline structure that delays its degradation in comparison to amorphous polyester structures (Zhang et al. 2013). Badri et al., prepared indomethacin loaded polycaprolactone nanoparticles to be applied topically for its analgesic and anti-inflammatory action in the treatment of inflammatory disorders. Polymeric nanoparticles were prepared by nanoprecipitation method. The hydrodynamic diameter of the prepared drug loaded polymeric nanoparticles was from 220 to 245 nm with high encapsulation efficiency around 70%. SEM and TEM analysis showed that the polymeric nanoparticles were spherical with smooth surface. Confocal laser scanning microscopy confirmed that polymeric nanoparticles are able to penetrate the skin. As result, Indomethacin loaded polymeric nanoparticles can be considered as an effective carrier in treating inflammation with lowering side effects of indomethacin (Badri et al. 2017).

Polymeric nanoparticles prepared using non biodegradable polymers were also investigated for percutaneous absorption. Turos et al., prepared polyacrylate nanoparticles by conjugation with a b-lactam antibiotic in water by emulsion polymerization. The resulted nanoparticles were around 40 nm, and possessed a high in vitro antimicrobial characteristics (Turos et al. 2007; Greenhalgh and Turos 2009).

Polymeric nanoparticles for acne treatment

Reis et al. developed azelaic acid loaded poly (lactide-co-glycolide) nanoparticles for acne treatment. Azelaic acid loaded polymeric nanoparticles were prepared by emulsification process and poloxamer 188 was used as the stabilizer. The results showed that by modifying formulation parameters polymeric nanoparticles with high loading capacity and controlled drug release were obtained. Toxicity profile was similar for both free drug and drug loaded polymeric nanoparticles. As conclusion, azelaic acid loaded polymeric nanoparticles were able to induce a controlled drug release to follicular unit (Reis et al. 2014).

TyroSpheres™ also are able to self assembly with lipophilic drugs and maintain the activity of the loaded drug. Hydrophobic drugs were delivered successfully to the skin using TyroSpheres™. When paclitaxel was encapsulated into TyroSpheres™, the hyperprolifiration of keratinocytes was inhibited, and paclitaxel was accumulated in epidermal layer of the skin with no systemic absorption and systemic side effects. Thus, TyroSpheres™ can be considered as promising carriers to treat skin disorders such as psoriasis (Kilfoyle et al. 2012a; Zhang et al. 2013).

It is important to notify that polymeric nanoparticles are not able to cross the skin stratum corneum. The drug release from polymeric nanoparticles is slow to the upper skin layers. Thus, polymeric nanoparticles are used in the cosmetic field, they provide a protection of the encapsulated agents and avoid the absorption of sunscreen products by forming a film on skin surface. Although the potential and the effectiveness of polymeric nanoparticles in this field, there is still limited number of polymeric nanoparticles based products reaching the market. The reasons of this limitation could be due to the difficulty to scale up the complicated preparation techniques and the cytotoxicity of some polymers (Wu and Guy 2009).

3.5.6 Polymeric Nanoparticles Application in Ocular Delivery

Gatifloxacin suffers from low residence time in the eye and recrystallization after administration. Thus, it was incorporated into cationic polymeric nanoparticles. The drug loaded polymeric nanoparticles were prepared using a mixture of 50:50 Eudragit® RL and RS as the cationic polymer. The optimized formulation showed 46% drug loading capacity, with optimum physicochemical characteristics and low cytotoxicity. The release rate of the drug from this formulation wad prolonged. The antimicrobial activity was also investigated and showed prolonged antimicrobial drug activity (Duxfield et al. 2016).

3.5.7 Polymeric Nanoparticles Application for Gene Delivery and Therapy

Gene therapy showed an important potency to help curing patients suffering from several diseases. But its biggest hurdle is to find an efficacious and safe systemic

delivery system. Even though viral vectors are considered promising to achieve high transfection rate, these systems have high immunogenicity which limits their further evolution. Therefore, alternatively polymeric nanoparticles given orally and systemically showed elevated trans-gene expression. In one study, nanoparticles based on type B gelatin were investigated for passive and active tumor targeting and transfection by the use of reporter and therapeutic plasmid DNA. In addition, nanoparticles-in-microsphere oral systems were found effective in the delivery of reporter and therapeutic gene components in GIT. Also, small interfering RNA (siRNA) is used for silencing genes, and here both gelatin nanoparticles and nanoparticles in microsphere were successful to deliver siRNA systemically and orally (Xu et al. 2012).

The gene therapy by using siRNA and polymeric nanoparticles is considered an effective therapy compared to traditional preparations used to treat serious diseases like cancer, pulmonary, ocular and neurodegenerative disorders. But, different parameters with regards to stability, effectiveness and formulation should be regulated to obtain a product acceptable for the market and clinical practice. The requirements needed to achieve an ideal carrier for siRNA include size, charge, stability, the relevance of surface functionalization, biocompatibility, biodegradability and non-immunogenicity (Gallas et al. 2013). Gene therapy can succeed if the carrier used was able to perform many functions in precise periods in the delivery stages. Polymers are considered as appropriate candidate serving as nucleic acid carrier with extended applications in gene therapy (Howard 2009; Kang et al. 2012).

A cationic charge of the polymer based carrier allows the formation of a stable complex of nucleic acids which protects them from enzymatic degradation by nucleases. However, the cationic nature of the conventional polyplexes may lead to toxic effects in the cell. Thus, PEGylation of siRNA carriers can solve the toxicity problem faced with polyplexes. PEGylation enhances the stability of the carrier in blood stream. The functionalization of hydrophilic polymers can be conducted with hydrophobic moieties to achieve derivatives with amphiphilic nature capable to self assembly and form nanocarriers like micelles or nanoparticles. Biodegradable polyester derivatives (like PLGA) are mostly employed to obtain the hydrophobic derivatization. Non viral polymers that showed effectiveness in carrying siRNA are mostly polysaccharides such as chitosan and inulin (INU), synthetic polyamines such as polyethylenimine (PEI) and polyaminoacids such as a,b-poly(N-2-hydroxyethyl)-d,l-aspartamide (PHEA) and poly-L-lysine (PLL) (Cavallaro et al. 2017).

An example of using polymeric nanoparticles in gene delivery in literature is provided here. Han et al., have developed Galactose modified trimethyl chitosan-cysteine (GTC) conjugates for the oral administration of Survivin shRNA-expression pDNA (iSur-pDNA) and vascular endothelial growth factor (VEGF) siRNA (siVEGF) to treat hepatoma. The prepared iSur-pDNA and siVEGF loaded GTC nanoparticles have shown acceptable stability in biological fluids and an enhancement in intestinal absorption in comparison to free genes. The polymeric nanoparticles (GTC nanoparticles) were accumulated in tumor tissue and silenced the Survivin and VEGF expression when given by oral route to tumor bearing mice model. In addition, apoptosis was enhanced, angiogenesis was prohibited and tumor was regressed. The codelivery

of the two genes iSur-pDNA and siVEGF provided synergism on inhibiting tumor growth in vivo and proliferation of cells in vitro compared to single gene therapy. It can be concluded that polymeric nanoparticles can be used for the oral administration of genes in cancer treatment successfully (Han et al. 2014).

3.5.8 Polymeric Nanoparticles Application in Imaging

Polymeric nanoparticles are used for the delivery of fluorescent contrast agents. They possess many features such as: (1) their nanosized dimension helps endocytosis and cell probing, (2) they have high drug loading capacity, (3) they could be modified with several molecules on their surface like targeting receptor molecules, (4) they also have a theranostic capacity to act both as detector and treater of the targeted disease (Srikar et al. 2014; Banik et al. 2016).

Polymeric nanoparticles used in molecular imaging have even covalent bonds or they encapsulate contrast agent within the polymer matrix. In the covalent type, the contrast agent is covalently bounded with the polymeric structure then the nanoparticles is prepared by using traditional techniques. But this type suffer from the uneven distribution of contrast agent, in addition the loading capacity of contrast agent in minimal. The encapsulated type possesses higher loading capacity and evenly distributed contrast agent by entrapping the contrast agent physically with polymers matrices. These polymeric nanoparticles can be used in cancer diagnosis and treatment, they are used in molecular imaging techniques such as Magnetic Resonance Imaging, optical imaging and X-Ray Computed Tomography (Banik et al. 2016).

A. ***Magnetic Resonance Imaging MRI***

Contract agent can be physically encapsulated into the polymeric nanoparticles for MRI. An example is explained here. The diagnosis for acute pancreatitis is related to serum enzyme analysis and imaging methods which are not sufficiently sensitive and specific. Thus, gadolinium loaded nanoparticle based MRI nanoprobes were prepared for the diagnosis of acute pancreatitis. The synthesis of gadolinium diethylene triamine pentaacetic fatty acid (Gd-DTPA-FA) nanoparticles was performed by conjugating gadolinium acetate and DTPA-FA ligand. In in vitro and in vivo experiments, the prepared nanoparticles Gd-DTPA-FA were found biocompatible with low cytotoxicity. It was concluded that the synthesized MRI contrast agent is effective and has high specificity for the early detection of acute pancreatitis (Zhang et al. 2014b). Polymeric nanoparticles can also be covalently linked to contrast agents. Shalviri et al., prepared a multifunctional terpolymeric system used for molecular imaging and to deliver the active ingredient doxorubicin. The results suggested that the new terpolymeric system represents a theranostic platform for enhancing contrast MRI of vascularization and tumor structure and also to deliver doxorubicin (Shalviri et al. 2013).

B. ***Optical Imaging***

This method is largely utilized in molecular imaging due to its non-invasive nature. Many polymers are utilized for encapsulating indocyanine green and near-infrared fluorescence dyes for optical imaging in human. In one study, Indocyanine green nanoparticles were prepared by electrostatic interaction of Indocyanine green and dextran based block copolymers acting like near-infrared theranostic nanoparticles. The activation on nanoparticles can be performed from an "OFF" to an "ON" state of near-infrared fluorescence in intracellular medium and utilized in NIR imaging and also photothermal treatment (Liu et al. 2013).

C. ***X-Ray Computed Tomography***

An example of the use of polymeric nanoparticles in X-Ray Computed Tomography is the preparation by Yin et al., of biocompatible poly(iohexol) nanoparticles. Nanoparticles were developed by cross linkage of mPEG-polylactide with the polymer. In in vivo X-ray computed tomography, nanoparticles are used as contrast agents. Poly(iohexol) nanoparticles gave a 36-fold increase in the computed tomography contrast in comparison to small contrast agents. This improves the imaging techniques and suppress the need for multiple administration (Yin et al. 2013).

3.5.9 Polymeric Nanoparticles Application to Deliver Theranostics

A theranostic possess both therapeutic and diagnostic properties. Using theranostics, the administration of the appropriate dose of the active ingredient to the appropriate place at the appropriate time can be provided. This helps to image the disease extension, and to monitor the real time drug effectiveness. Three parameters should be available in the theranostic: it should deliver contrast agent, bioactive entity, and should well distribute them (Banik et al. 2016). Theranostic nanoparticles were developed to provide simultaneously a diagnosis for the disease, to deliver the active ingredient to the target site with minimum toxicity, and to monitor the treatment. The enhancement of the specific targeting to tumors of the contrast agent and antitumor agents is considered a big challenge. Kim et al. developed a tumor homing chitosan based nanoparticles for both cancer diagnosis and treatment (Fig. 16). These nanoparticles were found stable in plasma, deformable and rapidly uptaken by cancer cells. This increases the targeted delivery to tumor and reduces the drug distribution into normal cells. The theranostic nanoparticles were developed by labeling them with Cy5.5, which is a near infrared fluorescent dye for imaging, in addition the loading antitumor agent paclitaxel was done too. The results in SCC7 tumor-bearing mice showed that paclitaxel-loaded-Cy5.5 labeled theranostic nanoparticles enhanced the tumor homing ability and minimized the non specific distribution to normal tissues (Kim et al. 2010).

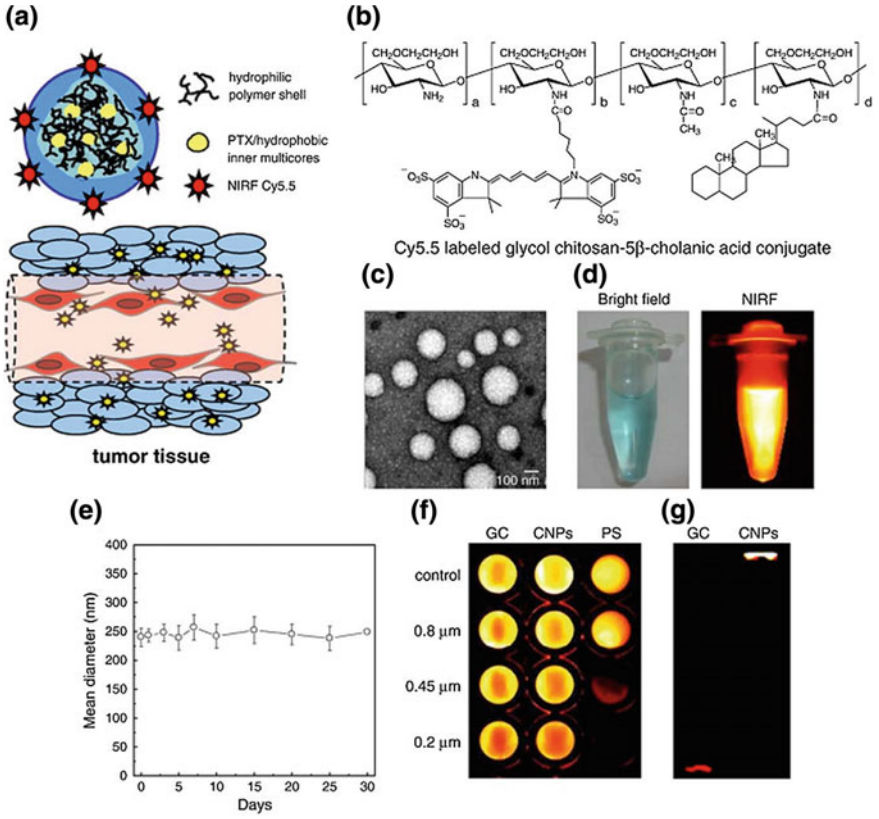

Fig. 16 **a** Diagram representing the concept of using theragnostic nanosparticle for cancer imaging and therapy. The theragnostic chitosan-based nanoparticles (CNPs) could preferentially accumulate at the tumor tissue by the enhanced permeation and retention (EPR) effect because of their stability in blood, deformability, and fast cellular uptake (PTX: paclitaxel). **b** Chemical structure of the glycol chitosan conjugates labeled with Cy5.5, a near-infrared fluorescent (NIRF) dye, and modified with hydrophobic 5β-cholanic acid. **c** A TEM image of Cy5.5-labeled CNPs (1 mg/ml) in distilled water. **d** Bright field and NIRF images of the Cy5.5-labeled CNPs in PBS. The NIRF image was obtained using a Cy5.5 filter set (ex = 674 nm, em = 695 nm). **e** Using dynamic light scattering, time dependant size distribution of Cy5.5-labeled CNPs in PBS at 37 °C was confirmed. **f** Filtration of water-soluble glycol chitosan (GC), CNPs, and polystyrene (PS) beads through filters of different pore sizes (0.8, 0.45 and 0.2 μm). The amount of each particle passed through the filters was quantified through NIRF intensity of the filtrate. **g** In vitro stability of the Cy5.5-labeled CNPs was determined using an SDS-PAGE test. Cy5.5-labeled GC polymers and CNPs were incubated in 10% serum for 6 h at 37 °C and their migratory positions were monitored using a Cy5.5filter set. From (Kim et al. 2010) with permission

3.5.10 Polymeric Nanoparticles Application to Stabilize Enzymes

To solubilize and stabilize enzymes, a chelating agent as an additive could be added to the aqueous phase. In addition, several procedures of immobilization were performed to stabilize enzymes. Polymeric nanoparticles have the ability to transport and stabilize enzymes (El-say and El-sawy 2017). Watanabe and Ishihara have prepared polymeric nanoparticles for sequential enzymatic reactions by the combination of a phospholipidic polymer shell with a core formed of polystyrene. In this combination, the incorporation of the active ester sites and the polar sites of phospholipid was done in the phospholipid polymer backbone by the use of 2-methacryloyloxyethyl phosphorylcholine and an active ester monomer. The immobilization of choline oxidase, acetylcholinesterase and horseradish peroxidase-labeled IgG onto the nanoparticles was done for the sequential enzymatic reactions. The addition of acetylcholine chloride, choline chloride, and tetramethylbenzidine as substrates into the nanoparticle preparation was performed. Then, the conversion of acetylcholine chloride to choline chloride was realized. After that, choline chloride was oxidized using choline oxidase, and the formation of hydrogen peroxide as an enzymatic degradation product was realized. The authors showed that the diffusion pathways of enzymatic products and the localizations of immobilized enzymes were interesting. Thus, nanoparticles are able to facilitate sequential enzymatic reactions (Watanabe and Ishihara 2006).

3.5.11 Polymeric Nanoparticles Application to Activate and Inhibit Autophagy

Autophagy is a defending mechanism for the cell. In this mechanism, the sequestration of protein molecules and organelles into vesicular structures happened. Then the lysis of these vesicles is realized when they fuse with lysosomes. Amplified autophagy is a process of fighting for cancer cells and can be a goal for drugs to treat cancer (El-say and El-sawy 2017).

Polymeric nanoparticles can affect the autophagy process. A selective compartmentalization of polymeric nanoparticles can happen in the cell. Polymeric nanoparticles are able to modify the routes of autophagy by inhibiting the signals resulting from oxidative stresses.

An example of the use of polymeric nanoparticles is the study performed by Shi et al. Because folate and its conjugates has a role in facilitating the delivery of antitumor drug to folate receptor-positive tumor cells, this binding specificity is used to enhance drug delivery to tumor cells. The group prepared folic acid-decorated bovine serum albumin conjugated carboxymethyl-β-cyclodextrin nanoparticles able to entrap Gefitinib. The resulted nanoparticles were nanosized and spherical. Gefitinib loaded polymeric nanoparticles can induce apoptosis of Hela cells by the elevation of the expression of caspase-3 and the inhibition of autophagy by the decrease in the expression of LC3. It was concluded that folic acid can be considered as a good molecule for targeting and Gefitinib loaded polymeric nanoparticles can be

considered as a promising approach to treat tumor cell having over expression of folate receptors (Shi et al. 2014).

3.6 Polymeric Micelles as Polymeric Nanoparticles and Their Applications in Drug Delivery

Despite of the development achieved in drug delivery, designing an appropriate carrier to deliver drugs with low aqueous solubility is still challenging. Micelles formed of surfactants have been used to solubilize drugs with low aqueous solubility. But, these surfactants are suffering from the high critical micelle concentration and the related low stability. Therefore, polymeric micelles were developed presenting higher stability in blood stream and higher tolerability. In addition to their low toxicity and high loading capacity, they provide controlled drug release and prolonged therapeutic potential (Ahmad et al. 2014). When polymeric micelles are present in water, their hydrophobic moiety is excluded from water. Polymeric nanoparticles possess a special core shell structure where hydrophobic part encapsulates hydrophobic agents, proteins or DNA. The hydrophilic moiety of polymeric micelles has an interesting role because of its brush like structure that permits the hydrophilic moiety to keep the hydrophobic moiety safe from biological invasions. Additionally, proteins adsorption on micelles could be minimized by the hydrophilic shell (Ahmad et al. 2014; Cho et al. 2014). Polymeric micelles are formed by dispersing a block copolymer having hydrophobic and hydrophilic moieties. In addition, cross linking the core of micelle can enhance its stability. Polyethylene glycol PEG is widely used to provide a stealth character as a hydrophilic shell. Polymeric micelles possess a passive targeting to tumor cells due the low lymphatic drainage to tumor cells and the high tumor vascularization resulting in an enhanced penetration and retention (EPR) effect of the micelles (Englert et al. 2018).

Due to polymeric micelles features they are considered as potential candidates to treat cancer. Many anti-tumor drugs such as Genexol®-PM, NK105 and SP1049C were studied in clinical trials (Banik et al. 2016). Genexol-PM is paclitaxel loaded polymeric micelle having the approval in South Korea to treat non-small cell lung cancer and breast cancer. Its main components are paclitaxel and low molecular weight amphiphilic diblock copolymer, monomethoxy poly (ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA). Genexol-PM have been shown to possess higher tolerability compared to Taxol and mush lower toxicity. The tolerated dose of Genexol-PM is two to three times that of taxol. The size of the polymeric micelle is approximately 24 nm (Fig. 17a) and its zeta potential is almost −8 mV. Paclitaxel is released in a first-order release kinetic (Fig. 17b). In vivo experiments showed that Genexol-PM is more efficacious as radiosensitizer compared to taxol. Additionally, after 6 h of administration, it was proved that Genexol-PM gave mush lower exposure of paclitaxel to normal lung cells than taxol (Werner et al. 2013).

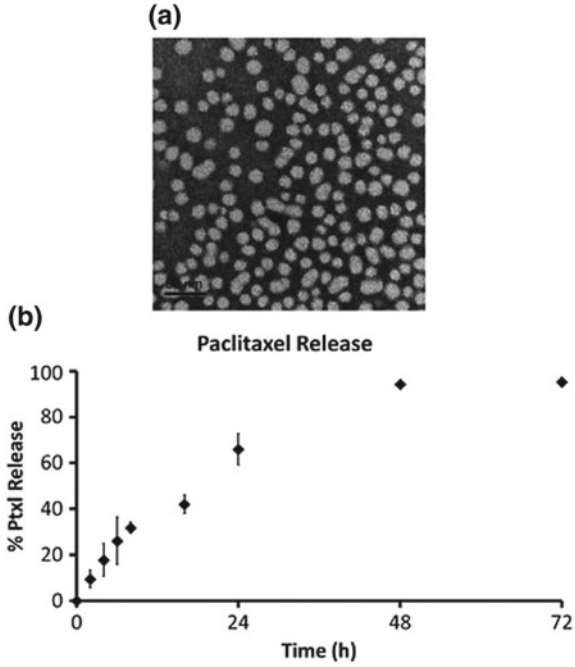

Fig. 17 **a** Transmission electron microscopy image of Genexol-PM showing a monodispersed population of particles with a narrow size distribution of 23.0 ± 4.5. **b** Drug-release curve of Genexol-PM. Genexol-PM releases paclitaxel (Ptxl) in a first-order release kinetic. Genexol-PM was incubated in phosphate buffered saline at 37 °C. From Werner et al. (2013) with permition

Estrasorb™ is a micellar formulation of estradiol and it is an FDA approved micelle. It is used in treating moderate to severe vasomotor symptoms of menopause topically. The transdermal delivery is known to avoid first pass metabolism and the gastrointestinal side effects, which stabilizes plasma levels for 8 to14 days. Beyond this example, other micellar preparations in the late stages of clinical trials are being explored (Bobo et al. 2016).

In addition, due to the enhancement in chemical stability and drug solubility provided by polymeric micelles, they are utilized as drug and gene delivery system via different routes of administration like: parenteral, nasal, oral, ocular and topical/transdermal routes. Polyethylene glycol is the most extensively used hydrophilic block because in water the dense brush of polyethylene glycol provides solubilizing effect (Güngör and Rezigue 2017). Polymeric nanoparticles are used also for topical administration into skin. Lapveta et al., prepared tacrolimus loaded polymeric micelles using methoxy-poly(ethylene glycol)-dihexyl substituted polylactide (MPEG-dihex-PLA) diblock copolymer with biodegradability and biocompatibility. The drug deposition in epidermis and upper dermis was studied. Tacrolimus permeation from this carrier was investigated in human skin. It was found that tacrolimus accumulation from the polymeric micelles was significantly much higher compared to the marketed product Protopic® ointment. The penetration routes for the polymeric micelles were investigated using confocal laser scanning microscopy. Micelles were found deposited in hair follicles. As conclusion, tacrolimus loaded polymeric

micelles are more effective carriers in the targeted delivery of tacrolimus to hair follicle compared to its marketed ointment (Lapteva et al. 2014; Güngör and Rezigue 2017).

4 Conclusion

The choice of drug carrier is fundamental for drug delivery to exert maximum effectiveness with lowest side effects. Among the novel nanocarriers investigated for drug delivery lipid and polymeric nanoparticles have gained big interest recently thanks to their safety and potency. The current chapter summarized recent advances in drug delivery by lipid and polymeric nanoparticles.

Lipid nanoparticles presented by solid lipid nanoparticles SLNs and their newer generation known as nanostructured lipid carriers NLCs provided a promising alternative to traditional colloidal drug carriers. In SLNs, the highly purified solid lipids used in their preparation constitute a perfect crystal structure and leave just restricted space to hold the drug. Whereas in NLCs the lipid mixtures used for their preparation have very different structures causing an alteration of the formation of a perfect crystal lattice. Thus, the imperfectness in the crystalline structure of NLCs is responsible of its perfectness as a carrier. Lipid nanoparticles can be produced in laboratory and on large scale. SLNs and NLCs were proved to be well tolerated drug delivery systems. Their effectiveness as a drug delivery systems was tested via many routes of administration. Several routes including parenteral, oral, pulmonary, ocular, topical and transdermal were investigated. However, formulations based on lipid nanoparticles are not available in commercial preparations and even they are in limited use in research. They need more progress in their stages of development, and considering the safety and technological practicability further investigations are indispensable.

Polymeric nanoparticles have gained great interest in nanotherapy for both therapeutic and diagnostic agents. They are biocompatible, able to bypass biological barriers and can be used in targeting. Therefore, they showed effectiveness in many applications. Polymeric nanoparticles used in controlled drug delivery possess several advantages like: the selectivity in targeting, the controlled release and the enhancement of stability by protecting drugs, in addition to extending the circulation half-life. In this context, polymeric nanoparticles are largely used in the treatment of many disorders. Currently, a variety of polymeric nanoparticles are investigated in both preclinical and clinical studies. Polymeric nanoparticles have been shown to be highly effective in therapy, imaging, drug delivery and theranostic applications. The upcoming progress in nanomedicine, especially through polymeric nanoparticles, will enhance the drug effectiveness and show an improvement in the diagnosis, treatment, and prevention of health problems. However, Polymeric nanoparticles consist of biodegradable macromolecular material from natural, semisynthetic or synthetic origin or could be prepared using non-biodegradable synthetic polymers. Thus, polymeric nanoparticles have limited use due to the polymer cytotoxicity and deficiency of a convenient large scale production techniques.

As conclusion, more animal and clinical studies should be performed to promote the future application of both lipid and polymeric nanocarriers in drug therapy. Unfortunately, despite the use of biocompatible and biodegradable components, the possible toxicity from these nanoparticles cannot be ignored due to the very small size of these carriers and their large surface area. Additionally, for both lipid and polymeric nanoparticles, the mechanisms of enhancing drug effectiveness are not totally implicated. Thus, further exploration of these mechanisms should be extensively elucidated.

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Biodegradable Natural Polymeric Nanoparticles as Carrier for Drug Delivery

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Abstract Nanotechnology has been a very interesting and led to the significant progress in a biomedicine field such as controlled drug and gene delivery. Nanoparticles have been used for drug delivery because of their efficiency and in particular, biodegradable nanoparticles are now being continuously explored because of their versatility and properties like good bioavailability, very less toxicity and enhanced encapsulation. These carriers play an efficient role in cancer therapy and controlled delivery of drug molecules to the target site. The present chapter focuses on the reason for using nanoparticles as drug carrier, various methods used for polymeric nanoparticles synthesis, and various applications of biopolymers-based nanoparticles in biomedical field.

Keywords Biopolymeric nanoparticles · Preparation methods · Drug loading · Drug release · Biomedical applications

1 Introduction

Nanotechnology is a science that deals with extremely small particles in the range of nanometres. Polymeric nanoparticles are nanoparticles that are prepared from either synthetic or natural polymers. Nanotechnology in general has a wide range of applications ranging from agriculture, textiles, forensics, space and medicine. They have become an important area of research in the field of targeted drug delivery. Conventional methods of drug delivery have been characterised as less effective when compared to these nanoparticle–drug formulations. This is because there are several

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challenges in the chemical methods that include poor bioavailability, adsorption into the intestines and targeted delivery of the drugs (Hans and Lowman 2002).

In the late 20th century, nanoparticles were used for drug delivery through oral administration. But this encountered several problems, one of which was the size limit of the particles to cross the intestinal lumen. The therapeutic effect of the drug was also very low because the nanoparticles-drug formulation was cleared off very soon by phagocytosis, following intravenous administration (Hans and Lowman 2002). Another very important nanosized particle that has been considered for targeted drug delivery is liposome. Liposomes are prepared from lipids and the major advantage is that they provide protection for the drug stored in their core (Soppimath et al. 2001). However biodegradable nanoparticles have shown several advantages over liposomes as efficient drug delivery system because of a greater stability and controlled release of drug on target cells.

In the recent years, the interest in using nanoparticles for targeted drug delivery has increased significantly. They also have a huge advantage over other larger microparticles because of their ease of application through intravenous delivery. The size, in terms of diameter, of the smallest blood vessels in the body is in the range of 5–6 μm. Therefore, to ensure that no aggregates are formed while delivering the drug through the blood vessels, the size of the encapsulated drug has to be lesser than 5 μm, which is possible by using nanoparticles (Lemoine and Préat 1998).

A large number of drugs can be delivered using nanoparticles as carriers, via a number of routes. Hence, there are a large number of methods to deliver the drug to various parts and organs of the body. This requires several methods of synthesis of the nanoparticle–drug formulation. Once a method is set up, it is then optimised to maximise the drug carrying capacity and the efficiency of targeted drug delivery.

Although, the benefits that nanoparticle have provided to biomedicine field, there are some applications remain challenging; particularly, real time monitoring of cellular level actions, reach the specific target to the action site or efficient drug delivery to the target cell. In this connection, the design of multifunctional nanoparticles (Fig. 1) could significantly improve already existing nanoparticle characteristics and help to overcome these challenges (Sanvicens and Marco 2008). The main advantages of multifunctional nanoparticles that can combine with an antibody or peptide as specific targeting moiety, quantum dots or magnetic nanoparticles as imaging agents for imaging, polyArg peptide TAT as biocompatible polymer for a cell-penetrating agent, stimulus-sensitive agents for control bioavailability and reduces the toxicity and a stabilising polymer to ensure biocompatibility (mostly polyethylene glycol used) and the Integration of therapeutic compounds into nanoparticles with diagnostic agents refers to the theranostic nanoparticles. Examples of such nanotechnology products approved by the Food and Drug Administration (FDA) for human applications are based on iron oxide, gold, protein, liposomes and synthetic polymers (Xia et al. 2009; Davis et al. 2010). Simultaneously, the same multifunctional particle can be modified with an imaging agent to monitor the drug transport process, a function to evaluate the therapeutic efficacy of a drug, a specific cellular penetration moiety and a therapeutic agent (Torchilin 2006).

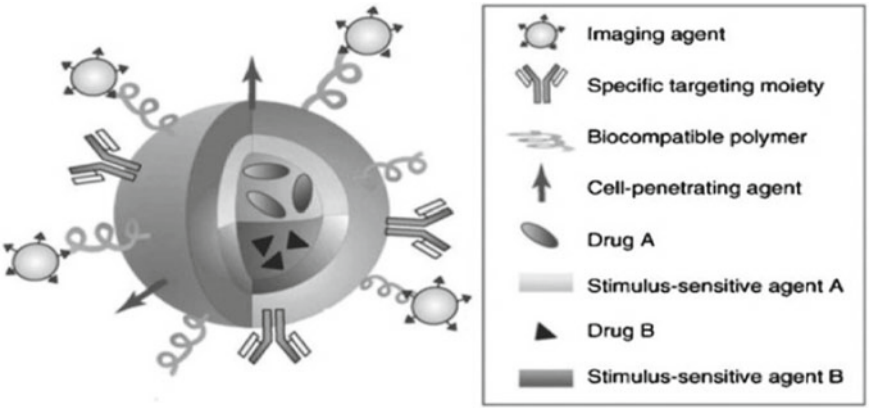


Fig. 1 Multifunctional nanoparticles for drug delivery. Adopted from Sanvicens and Marco (2008)

2 Methods for Synthesis of Polymeric Nanoparticles

There are several methods employed to synthesize nanoparticles and coat drug particles onto them for drug delivery. These nanoparticles find tremendous application in the field of medicine in the domain of targeted drug delivery. They pose several advantages over other methods of drug delivery because they are non-toxic, biodegradable, stable in the blood and do not cause too much of damage (Reis et al. 2017).

The general synthesis and encapsulation of the drug occurs in one of two methods as shown in Fig. 2. In the recent past, several works have been carried out by experimenting various methods of synthesizing different nanoparticles for drug delivery.

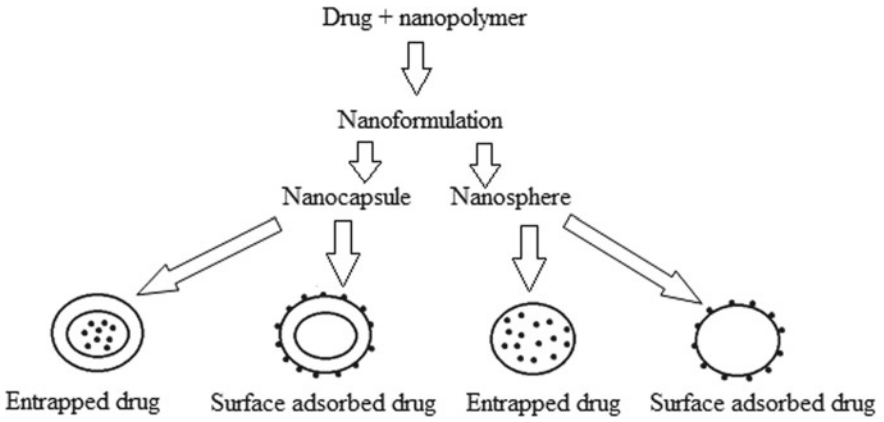


Fig. 2 Methods of formulation of nanoparticles

According to the structure of the biodegradable nanoparticles, they can be classified as nanocapsules and nanospheres. The drug particles can either be adsorbed on the surface or entrapped within the nanocarriers (Fig. 2).

According to Hans and Lowman (2002), some of the main factors that the selection of the polymer to be used to make synthesize nanoparticles out of are:

(1) Size of the nanoparticle required
(2) Properties of the drug to be encapsulated by the polymeric nanoparticle
(3) The surface features of the nanoparticle
(4) Degree of bioavailability of the drug and therefore the polymer
(5) Drug release profile of the nanoparticle.

There are some common methods of preparation of nanoparticles are:

- Nanoparticles obtained by the polymerization of a polymer
- Interfacial polymerization
- Interfacial polycondensation
- Nanoprecipitation

  Solvent displacement and interfacial deposition
  Emulsification/solvent diffusion
  Salting out with synthetic polymers
  Emulsification/solvent evaporation.

- Production of nanoparticles from natural polymers
- Desolvation of macromolecules.

3 Nanoparticles Obtained by the Polymerization of a Polymer

3.1 Emulsion Polymerization

This is one of the fastest and easiest methods of preparing nanoparticles. Based on the use of organic or continuous phase, this method can be classified into two. In the organic phase methodology, a monomer is dispersed into an emulsion or into any material in which the monomer is insoluble. One of the first nanoparticle to be prepared in this method was the polyacrylamide nanosphere (Ekman and Sjöholm 1978; Lowe and Temple 1994). To prevent aggregation in the starting stages of polymerisation, surfactants were used (Kreuter 1991). This is also one of the major reasons that this method is no longer much popularly used. The organic phase methodology requires the use of initiators, surfactants, organic solvents etc., which are then eliminated from the finally formed particles. Due to the non biodegradable nature of polyacrylamide, several different polymers were dispersed into emulsions and nanoparticles were made.

The other method is the aqueous continuous phase of preparation of nanoparticles. In this method, the nanoparticles are formed when the polymer is dispersed into a continuous aqueous solution. There are no surfactants needed in this method and there are several ways to initiate the process. One is by the collision of the polymers with initiators in the aqueous solution which may be an ion or free radical. Another method of initiation is the use of high energy particles. Once initiated, chain growth occurs from the initiated monomer, when the other monomers collide with the initiated particle. Termination of this polymerization finally leads to the formation of nanoparticles (Vauthier et al. 2003).

One very good example of a polymeric nanoparticle prepared using emulsion polymerisation is PMMA where the monomer is MMA. This can encapsulate a variety of vaccines and drugs like doxorubicin, insulin, ampicillin and progesterone. On the other hand, in the organic phase, polyacrylamide can be used to encapsulate enzymes. Other polymers that can be used to prepare nanoparticles using emulsion polymerisation are polyethylcyanoacrylate, polystyrene and polyacrolein among others.

4 Interfacial Polymerisation

This method has the major advantage of having extremely rapid polymerisation—that is this occurs in seconds. Initiation of this process is done by the ions in the medium (Reis et al. 2017). When a monomer is slowly allowed to dissolve in a solvent and then this is extruded through a needle into a stirred aqueous solution, nanoparticles are spontaneously formed as they come in contact with the initiating ions present in the water. There are also several other advantages in using this method of polymerisation. There are no toxic components used in the making of the nanoparticle. The only ingredient is the monomer and hence there is no difficult purification required.

In interfacial polymerisation, there is another advantage of drug encapsulation having high efficiency, like insulin has 95% efficiency (Reis et al. 2017). The only disadvantage of this process is that it is time consuming and it is a difficult process.

5 Interfacial Polycondensation

Nanoparticles made form polymers can also be made from interfacial polycondensation of lipophilic monomers, in the presence or absence of surfactants. In this case, the drug carriers were smaller than 500 nm. A modified interfacial polycondensation method was also employed for the preparation of nanoparticles.

6 Nanoprecipitation

Nanoprecipitation is the basic process that can be used to explain the formation of nanoparticles using all the processes.

This is a technique that requires two solvents that are miscible with each other, but the polymer should be miscible only with one. The drug and the polymer are dissolved completely in the first solvent and then following this, nanoparticles are made to precipitate by diffusion into the second polymer in which the polymer is insoluble. Once this is done, the second solvent is evaporated causing the resulting nanoparticles made of polymer holding the entrapped drug.

6.1 Emulsification/Solvent Evaporation

This process occurs as a two step process. In the first stage, the polymer along with drug (which is mixed in a water non miscible solvent) are stabilised in water or an aqueous solution. The second stage involves the solvent evaporation, which causes the polymer to precipitate and form nanoparticles (Fig. 3).

When an organic solution of the polymer containing the dissolved drug is dispersed into an aqueous solution into nanodroplets, the polymer precipitates into

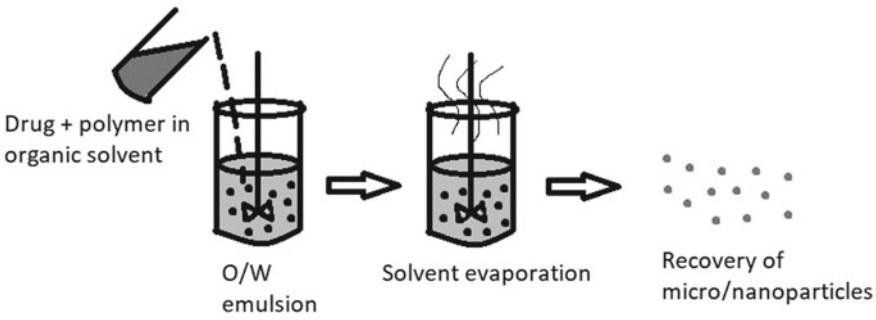


Fig. 3 Schematic representation of the emulsification/solvent evaporation

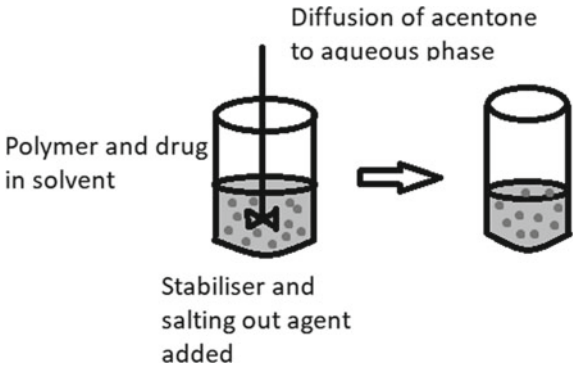


Fig. 4 Schematic representation of the scaling out method

nanoparticles in which the drug is uniformly distributed. The solvent is then evaporated using high temperature under pressure with continuous stirring (Soppimath et al. 2001).

Some of the frequently used polymers for the method of emulsification/solvent evaporation are cellulose, ethylcellulose, PLGA among others. Examples of drugs encapsulated in this method are testosterone, loperamide, nucleic acids, praziquantel and tetanus toxoid (Navni et al. 2018).

6.2 Scaling Out with Synthetic Polymers

This method of salting out is a modification of the Emulsification/solvent displacement method. The polymer which is to be used as a nanocarrier and the drug are dissolved in a solvent. This solvent is then emulsified in an aqueous solution in the presence of salting agents which causes the polymeric nanoparticle to salt out and precipitate (Fig. 4). The aqueous solution is then removed and this leads to the collection of the nanoparticles formed.

6.3 Solvent Displacement and Interfacial Deposition

Both the methods are similar in the fact that they are based on emulsification of the organic phase containing the polymer into an external phase which is the aqueous solution. In interfacial deposition, the only form of nanoparticles that can be formed is nanospheres (Fig. 5).

Solvent diffusion can occur either in the presence or absence of surfactants. The preformed polymer precipitates and the organic solvent diffuses into the aqueous solution. The polymer is first dissolved in a solvent which is water miscible. This phase is then dispersed into a stirred aqueous solution. This causes the polymer to

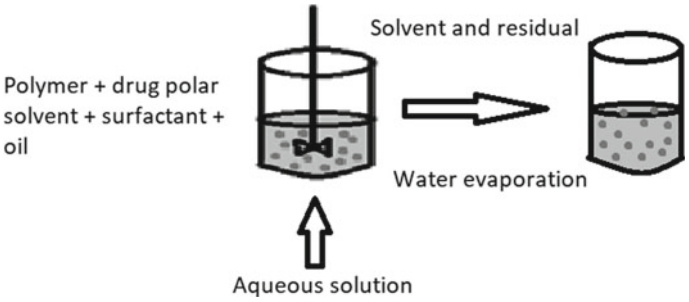


Fig. 5 Schematic representation of solvent displacement and Interfacial deposition

deposit in the interface between the water and the organic solvent. This leads to the formation of suspension (Quintanar-Guerrero et al. 1998).

In the first step of this process, the polymer, containing the drug, is dissolved in the organic solvent. This is then dispersed into an aqueous solution. Following this, the water is evaporated and the nanoparticles are formed. A major disadvantage of this technique is that it requires a solvent which is miscible with water. Hence, choosing of the drug and polymer system is very difficult. The solvent of the polymer and the non-solvent must both be miscible. So, the drug entrapment efficiency also depends on above mentioned factor (Irache et al. 2005).

6.4 Emulsification/Solvent Diffusion

This technique is also carried out in two steps. The first step involves the dispersion of the polymer which is to carry the drug into an organic solvent which is partially soluble. Next this has to be saturated by dilution with water to facilitate the formation of nanoparticles from it. This phase of polymer-water is then emulsified by dispersing into an aqueous solution. This causes the polymer to precipitate leading to the formation of the nanoparticles (Fig. 6). Followed by, the solvent is removed by evaporation to obtain the nanoparticles.

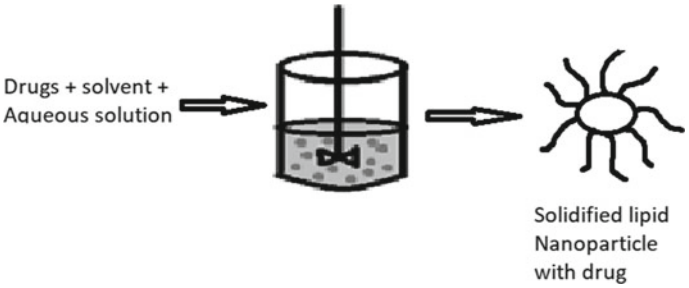


Fig. 6 Schematic representation of emulsion/solvent diffusion

Table 1 List of polymers and solvent used for the various methods of synthesizing nanoparticles

Methods	Polymer	Solvent used
Solvent diffusion	PLGA	Acetone
	PLGA	Propylene carbonate
	PHDCA	THF
	PLG-PEG	MC
Solvent displacement	PLA	Acetone
Nanoprecipitation	PLGA	Acetonitrile
Salting out	PLA	Acetone
Ionic gelation	Chitosan	TPP
Interfacial deposition	PLGA	Acetone
Multiple emulsion	PLGA	Ethyl acetate
	PLGA	DCM
	PLGA	Acetone
Solvent evaporation	PLA-PEG-PLA	DCM
	PEO-PLGA	MC

DCM Dichloromethane; *MC* Methylene chloride; *PVP* Polyvinylpyrrolidone; *PHDCA* Poly(hexadecylcyanoacrylate); *THF* Tetrahydrofuran; *SB-PVA-g-PLGA* Sulfobutylated PVA, graft, PLGA

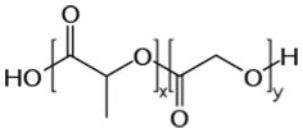
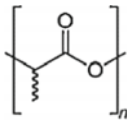
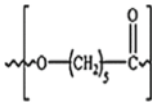
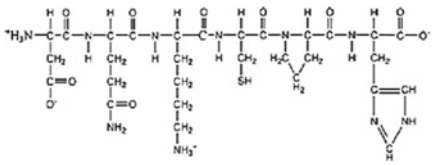
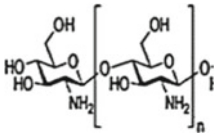
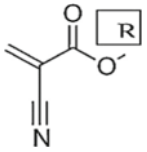
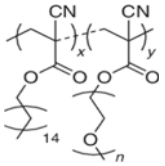
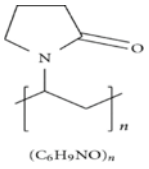
Most commonly used polymer for this technique involves PLGA an PLA, and the drugs encapsulated include plasmid DNA, doxorubicin, cyclosporin loaded gelatin and indocyanine.

There are various polymers and solvents used for preparation of nanomaterials listed in Table 1 and biodegradable polymers used to prepare nanoparticles for drug delivery are listed in Table 2 with chemical structure.

7 Drug Loading

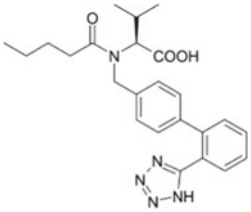
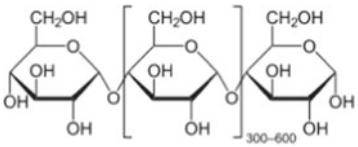
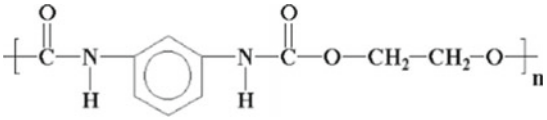
One of the most important reasons why nanoparticles are successful in the medical field as drug carriers is because they have a very high loading capacity. This ensures that the amount of carrier required to deliver the same amount of drug when compared with other carriers is much lesser. This drug loading into the nanoparticle can be carried out in one of two ways: (1) by introducing the drug at the time of synthesis of the nanoparticle or (2) after the production of nanoparticles by adsorption. It has been shown that the loading of the drugs through incorporating in the beginning as more efficient than by adsorption after the formation of the nanoparticle (Losa et al. 1991).

Table 2 List of biodegradable polymers that can be used to make nanoparticles for drug delivery

Bioplymers	Chemical structure
PLGA	
PLA	
Polycaprolactone	
Gelatin	
Chitosan	
Polyalkyl-cyanoacrylate	
PHDCA	
PVP	

(continued)

Table 2 (continued)

Bioplymers	Chemical structure
Albumin	
Starch	
Polyurethane	

Drugs can be loaded onto the nanoparticle by incorporating it into the solution that contains the prepared nanoparticles. They can also be loaded initially while preparing the nanoparticles by mixing it into the solvent that contains the monomer and the necessary surfactants. The amount of the drug loaded onto the nanoparticle and the type of interaction between the drug and the nanoparticle can be found by studying the chemical structure of the drug and the nanoparticles. The best method to do is to understand the isotherm that it follows. Adsorption isotherms are extremely useful in studying this. There are several adsorption isotherms, in which solid solutions follow linear sorption isotherms and colloidal systems follow Langmuir S type isotherms. Most of the nanoparticles are colloidal in nature and hence it is extremely difficult to directly estimate the amount of drug loaded onto the nanoparticles. For this, the most efficient method would be to make use of gel filtration or ultra-centrifugation to separate the loaded nanoparticles from the unbound drugs in the solution. (Soppimath et al. 2001)

From the amount of drug that is bound to the nanoparticle, it is possible to estimate the encapsulation efficiency, which is defined as the ratio of the amount of drug bound to the total amount of drug that is used for the production of the nanoparticle. It is represented as encapsulation efficiency (EE) (Kumari et al. 2010).

$$EE\ (\%) = \frac{Amount\ of\ drug bound\ to\ the\ nanoparticles - Untrapped\ nanoparticles}{Amount\ of\ drug\ bound\ to\ the\ nanoparticles} \times 100$$

To understand loading of the drug, the example of albumin is explained in brief.

Albumin has several drug binding sites and this serves as a major advantage as this leads to high binding capacity of various drugs to albumin. In case of water-soluble drugs, they can be loaded in either one of the two ways: by incorporation into the solution of the monomer before the process of polymerisation or in the final step of synthesis after the formation of the nanoparticle. A third method is also available in case of albumin, which involves addition of the water-soluble drug into a glutaraldehyde solution prior to the formation the nanoparticles. For this it was found out that the most efficient method was employing the second method of adsorption of drugs onto the nanoparticle after the complete synthesis of the polymeric nanoparticle, albumin (Elzoghby et al. 2012).

8 Drug Release

Release of drug is as important as the mechanism of drug loading. This is because it is based on this that the efficiency of the drug loaded nanoparticles' application is understood. Therefore, there is a necessity to understand the mechanism of the drug release from the nanoparticle onto the target site. For this, there are five methods of drug release that can be summarised as: (1) desorption of the drug that is bound to the surface of the nanoparticle onto the target, (2) diffusion through the matrix of the nanoparticle, (3) diffusion through the polymer wall of the nanocapsules, (4) erosion of the nanoparticle matrix, (5) a combination of the erosion-diffusion process (Kumari et al. 2010).

The kinetic analysis of the drug release can be explained as a function of an exponential equation as follows:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

Here, C is the concentration of the drug remaining in the nanoparticle at time t and A and B are constants that are characteristic of the system. A is for a diffusion control system, B is for an erosion control system, and α and β are rate constants (Kumari et al. 2010).

The rate of drug release is dependent on solubility, diffusion of the drug, biodegradation of the nanoparticle, loading efficiency of the drug and the size of the nanoparticles. Larger the particle, greater the initial burst release of the drug and the drug loading capacity is directly proportional to the burst and release of the drug that is encapsulated on the nanoparticle.

9 Applications of Nanoparticles in the Biomedical Field

9.1 Tumour Targeting

Because of the property of being colloidal in nature, nanoparticles pose several advantages like having a high loading capacity and having the stability to control size. There is also the most important advantage of preventing the drug that is encapsulated from metabolising (Nobs et al. 2006).

If the nanoparticles coated with the drug is large, then they have the advantage of higher drug's therapeutic index. This is because they can circulate inside the bloodstream for a longer time. However, prolonged time of circulation can itself be a major disadvantage because it can lead to the accumulation of the nanoparticle along with the tumour. The main purpose of sing nanoparticles as carriers is targeted drug delivery. Hence to enable this, there are two methods of targeting the tumours: (1) direct targeting, (2) pre-targeting method. In the former method of direct targeting method, the nanoparticles are coupled covalently with the ligand. This means that the nanoparticle and the ligand are administered together. In the latter method of pre-targeting, the ligand is not coupled directly to the nanoparticles. The ligand is administered first and following a time lag, the nanoparticles are administered. This time delay between the administration of the ligand and the nanoparticle allows for the ligand to first get localised in the tumour (Nobs et al. 2006).

According to several studies, tumour vasculature has permeability to macromolecules of diameter up to 600 nm. In a certain study, biodegradable polycaprolactone nanoparticles, prepared for targeted drug delivery of the drug tamoxifen on a tumour and the uptake of the biodegradable nanoparticles was observed in the estrogen receptor positive human breast cell line (Ud Din et al. 2017).

9.2 Oral Delivery

Oral delivery of therapeutic drugs is an important problem and several studies have been devised to develop strategies to improve the oral delivery of therapeutic molecules such as drugs and vaccines. Oral delivery using polymeric nanoparticles as carriers for these therapeutic molecules could increase the oral bioavailability of the drug.

This is because polymeric nanoparticles have several characteristics that pose as advantages. Their submicron size with large surface area helps in giving them a better trait than larger molecules. This allows them to encapsulate the protein/drug and protect them from the harsh conditions of the gastrointestinal tract. Hence this method is suitable because of the particular properties of the polymeric nanoparticles like stability against the harsh conditions of the gastrointestinal tract, drug release properties and the protection ability of the drugs that are encapsulated.

One of two methods can be used to improve the transport of the drug encapsulated nanoparticle in the transmucosal membrane: (1) Modification of the surface property of the nanoparticles, (2) Adsorbing the drug to the nanoparticle (Des Rieux et al. 2006).

Studies showed that oral delivery of the drug enoxaparin for the treatment of tumours was possible when it was encapsulated in a biodegradable polymeric nanoparticle like chitosan. Effective results were obtained from both in vivo and in vitro studies (Bagre et al. 2013).

9.3 Gene and Vaccine Delivery

There are two methods of delivery of nucleic acids in gene therapy. One is the viral gene delivery method and the other is the non-viral method. In general, viral methods are not used to a great extent. This is because viral methods of gene delivery pose safety threats. So non-viral methods are more popular in usage as they are chosen based on biodegradability and biocompatibility (Gu et al. 2014). Nanoparticles containing the adsorbed or encapsulated drug can also be used to deliver vaccine. This is better because this reduces the frequency of immunization required.

For example, Chitosan which has high bio-absorption into the nasal mucosa can be used to continuously deliver antigens into the body (Kim and Kang 2008).

9.4 Brain Targeting

The blood brain barrier (BBB) separates the brain from the blood circulation. It is composed of impermeable endothelial cells and hence prevents the transport of water-soluble molecules across it. Hence, the delivery of drugs to the central nervous system is a matter of huge concern.

Nanoparticles however can infiltrate the blood brain barrier and hence can be used to deliver drugs to targeted regions of the brain. One of the main advantages of using nanoparticles to deliver drugs to the brain is that it protects the characteristics of the drug while delivering it (Chakraborty et al. 2009). Therefore, nanoparticles are being used to deliver drugs to the brain due to them being safe and cost effective. However more studies are required before implementation.

10 Conclusions

Nanoparticles are promising in the field of drug delivery because of its various advantages like controlled release, submicron size, biocompatibility, biodegradability and stability in the blood. However, they also pose disadvantages like exposure to phagocytosis and low drug loading capacity. They can be implemented for targeted drug

delivery in one of the many forms: oral, injectable and implantable formulations. Their characteristics make them superior over other conventional methods of drug delivery. Hence, if a successful nanoparticle is synthesized using one of the above-mentioned methods to encapsulation of drug, it can be used as an excellent system for drug delivery. Also, more studies are required to develop proper functional nanoparticles as drug delivery systems. But, it may become a possible one to attain stable and effective formulations of nanoparticles in the near future.

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Quantum Dots Application in Biomolecules Interaction and Bioimaging

Ellappan Vaishnavi and Rajalingam Renganathan

Abstract The semiconductor nanocrystals known as quantum dots (QDs) have become a potential candidate for next generation fluorophores. Attention in QDs research is focussed on a wide range of applications like sensor, photovoltaic cells, catalysis, biolabelling, early cancer detection, etc. The unique morphology, tunable optical properties, photostability and flexibility for surface modification by biomolecules of QDs has provoked the scientific community to unravel many problems in biological processes. In view of these scientifically advantageous and distinguishable characteristics of QDs, an overview of research on the interaction of Cadmium telluride QDs with biomolecules and bioimaging applications and limitations of QDs is presented in this chapter.

1 Introduction

Nanoscience, a hybrid discipline of science, has triggered many scientific discoveries in the 21st century. These small sized materials behave smarter as compared to microscale materials (Binns 2010). Unusual properties make the nanoparticles applicable in a variety of fields including medicine, sensor technology, optoelectronics, or photovoltaics, etc. Nanomedicine an emerging field of nanotechnology, which plays a crucial role in combating infections, monitoring, trapping and preventing the body from illnesses (Pelaz et al. 2012). In the midst of nanoparticle research, there is intense focus on quantum dots (QDs) due to their size tunable optical properties. These novel semiconductor nanocrystals have

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become serious contenders for the manufacture of atomic scale machines in various biomedical applications which includes cell labelling, gene therapy, drug delivery, biosensor, etc., (Donegan 2013).

These engineered nanoscale devices can control diseases at the molecular level. This chapter is an overview of the basics of QDs and their role as fluorescent material for various biological applications (Howes et al. 2010). Though there are many successes of QDs in clinical applications, limitations to overcome their toxic effect have been discussed.

1.1 Overview of Quantum Dots

1.1.1 History of QDs

The nineteenth century laid the foundation for synthesis of colloidal suspensions of semiconductors. Russian Physicist, Alexei Ekimov and Alexander Efros in 1980, observed quantum confinements in PbS embedded glass matrix (Ekimov and Onushchenko 1981) The term "Quantum dots" was coined by Mark Reed and he stated the manipulation of zero dimensional materials in his paper (Reed 1993).

> Nanotechnologists can now confine electrons to pointlike structures. Such "designer atoms" may lead to new electronic and optical devices.
>
> —Mark A. Reed

Later, Louis E. Brus from Columbia University, synthesised nanocrystals in colloidal solution which led to the birth of QDs (Brus 1986). In 1993, Norris, Murray and Bawendi described a new method for the synthesis of mono-dispersed colloidal semiconductors (Murray et al. 1993). Systematic development in QDs chemistry followed after continuous research for decades. In order to improve the quality of optical property of CdX, the core/shell (CdSe/ZnS) nanocrystals capped with organic ligands were synthesised successfully by L. Brus and co-workers at Bell Laboratories. Shortly, thereafter, more core/shell nanocrystals were synthesised. Biocompatibility of the QDs was achieved only after development of water soluble QDs. A. Rogach from the University of Hamburg reported a technique to make water soluble QDs in 1996. "Quantum Dot Corporation" then developed and commercialised QDs in 1998. As a groundbreaking application of QDs, researchers commercially released "Sony XBR X900A series of flat panel televisions" and Kindle fire HDX in 2013. These two products employed QDs technology. In 2016, Samsung SUHD TV QLED manufacturer demonstrated the usage of QDs in modern TV. (Patent P1, Video link and articles A1, A2 were provided in the reference part). According to various innovative researches, QDs have been touted to be next breakthrough in medicinal field (refer the article reference A3).

1.1.2 Basis of Quantum Dots

Quantum dots are nanocrystalline material composed of alternative arrangement of group II–VI, III–V elements with size smaller than exciton Bohr radius (~7 nm). Typical dimensions of QDs range from 2–10 nm containing 10 to 50 atoms. CdSe and CdTe quantum dots exhibit good optical stability in visible to near IR region (Mattoussi et al. 2000; Wang et al. 2004). Other QDs (PbS, ZnTe and ZnSe etc.) (Allen and Bawendi 2008) are available, but show less quantum efficiency and are also difficult to prepare in a competitive time scale (Alivisatos 1996).

1.1.3 Quantum Confinement Effect

Interesting properties of QDs are realised by exploring the quantum confinement effect. The light hitting the bulk semiconductor results in charge separation between valence band and conduction band. The energy difference between the bands is well-known as the band gap energy of the semiconductor. Gradual transition of this bulk material to condensed matter results in restricted mobility of charge carriers in space. On comparison with continuous band gaps of bulk materials, discrete energy levels result because of the quantum confinement and hence these nanocrystals are also termed as QDs. Consequently confinement effects (Fig. 1) endow zero dimensional QDs with unique size-dependent optical, chemical, electronic and magnetic properties, (El-Sayed 2004; Eychmüller 2000).

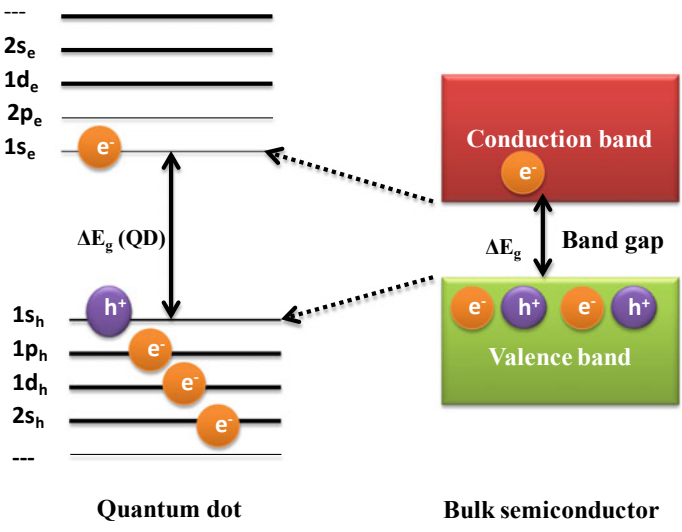


Fig. 1 Discrete electronic states in a zero-dimensional semiconductor nanostructure

Similar to the movement of an electron in a box, as the size of the box decreases, its excitation energy as well as its kinetic energy increases. Likewise, as the size of the particle becomes smaller than the Bohr radius, the band gap energy is enlarged. Hence, energy of the band gap absorption (color) and those of the emission is amplified and become sensitive to the size of the particles. Bulk Cadmium telluride has a band gap of 1.5 eV and exciton Bohr radius of 7.3 nm. Due to strong confinement of this zero dimensional charge carriers, CdTe QDs should have enhanced non-linear optical properties, which can be helpful in X-ray detectors and electro-optics (Nirmal and Brus 1998; Wu et al. 2003).

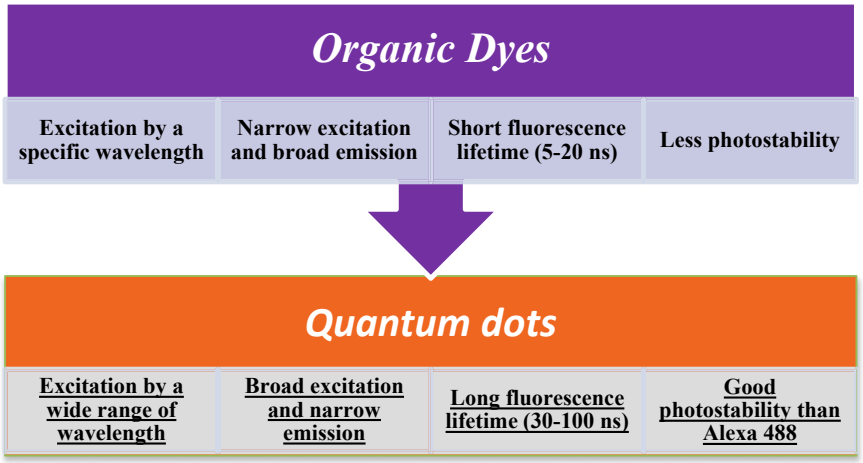
1.1.4 Organic Dyes Versus Quantum Dots

Over the past two decades, organic chemistry has broadened the application in molecular probes for biomolecules, sensing, cell imaging, biophysics, etc. In comparison to common dyes, QD holds attractive optical properties (Resch-Genger et al. 2008; Wang and Chen 2011). These fluorescent quantum dots are typically composed of group II to VI and III to V elements, e.g. Cd, Ag, Hg, P, Pb, Te, Se etc. (Resch-Genger et al. 2008). Under the same excitation wavelength, different QDs can simultaneously emit different colors, hence these multicolor QDs probes can be utilised for tracking and imaging applications. QDs possess broad excitation spectra together with narrow emission spectra. The quantum dots have molar extinction coefficients in the order of $0.5–5.0 \times 10^6\ M^{-1}\ cm^{-1}$, which is almost 10–50 times larger than that of classical organic dyes. As a result, the QDs are able to absorb photons 10–50 times more than organic dyes at the same excitation photon flux, leading to a major enhancement in the probe brightness (Dey and Rao 2011). By varying the composition, surface ligands and particle size the fluorescence can be tuned from UV to near IR region. The superior photobleaching of QDs enabled them for highly sensitive detection and long observation times in fluorescence microscopy (Alshehri et al. 2017).

Fascinating optical properties of these semiconductor nanocrystal make them an alternative material over traditional organic fluorophores Alexa 488 (Resch-Genger et al. 2008) (Scheme1).

2 Synthesis of Semiconductor Quantum Dots

In early 80's, Norris and Bawendi synthesised CdX (X-S, Se, Te) with narrow size distribution, high quantum yield and good photostability (Murray et al. 1993). The most popular synthesis method for CdSe QDs is organometallic approach (injecting precursor and ligand in oxygen free environment, at high temperature) (Murray et al. 1993). Peng produced CdSe and CdTe quantum dots using CdO precursor (Peng et al. 2007, 2010; Peng and Peng 2000). In addition to the aforementioned synthesis protocol, there is also a procedure in which nucleation and growth are concomitant ("heating-up method") and preliminary growth can be supplemented by a second

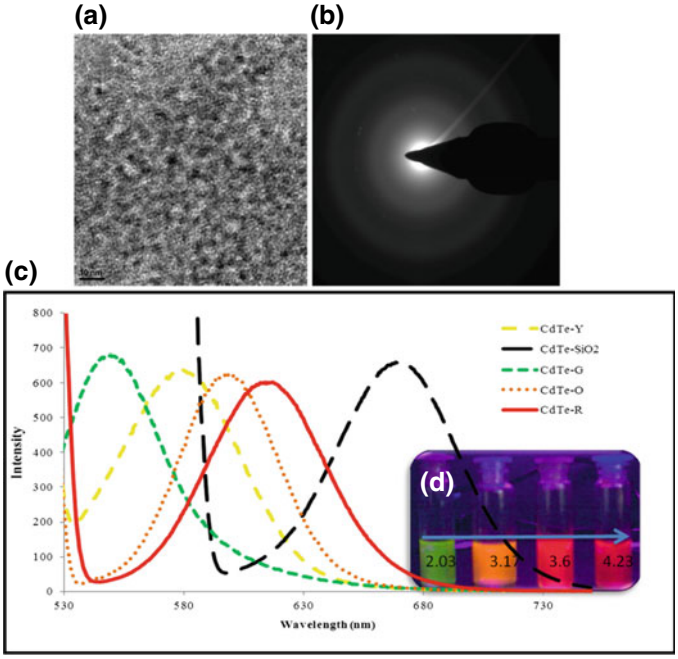


Scheme 1 Illustrating the advantages of quantum dots over organic dyes

injection, a procedure called Ostwald ripening, where larger crystals are formed at the expense of small crystals. The heating process is better suited for large scale production of QDs and therefore is the subject of widespread research. Core-shell systems have been developed to increase quantum yield and stability. The size of the shell is controlled by the addition of the concentration of shell precursor at lower temperatures. The above method highlights the use of core/shell materials (CdTe/ZnS) with QY of 50–100% (Reiss et al. 2009). Several methods are known to generate QDs with varied size, e.g., by the use of gamma and UV rays, electrochemical reactions and solvo-thermal reactions. The uniform particle size of CdTe QDs can be measured using TEM (Fig. 2a) and their corresponding SAED ring patterns of polycrystalline structures were shown in Fig. 2b. Figures 2c, d clearly demonstrate the size dependent optical behaviour of CdTe quantum dots (Vaishnavi et al. 2015).

2.1 Surface Functionalisation Strategies of Quantum Dots

Surface functionalisation plays an eminent role concerning the properties of QDs namely stability and quantum yield, etc. The high surface area of nanocrystals makes them unstable in colloidal solution, consequently, the surface passivation by capping layer is necessary. Ligands bind strongly with the surface of nanomaterials via electrostatic, chemisorption or hydrophobic interactions (Talapin et al. 2001). CdTe QDs with high luminescence are prepared using trioctylphosphine/trioctylphosphine oxide (TOP/TOPO) synthesis at high temperature (Yu et al. 2011). This thermolitic method produces only hydrophobic QDs, which limits their application in biological systems. Transfer of QDs in water is highly motivated for biological applications. Ligand exchange methods utilise various surface modifiers which includes small ligands



(Vaishnavi et al. 2015)

Fig. 2 **a** HRTEM image and **b** SAED pattern of red emissive CdTe quantum dots. **c** Size dependent emission behaviour of green, yellow, orange and red MPA-CdTe and CdTe-SiO_2. **d** Photo showing the image of emission of quantum dots when exposed to UV light and the growth of the particle diameter is shown by an arrow

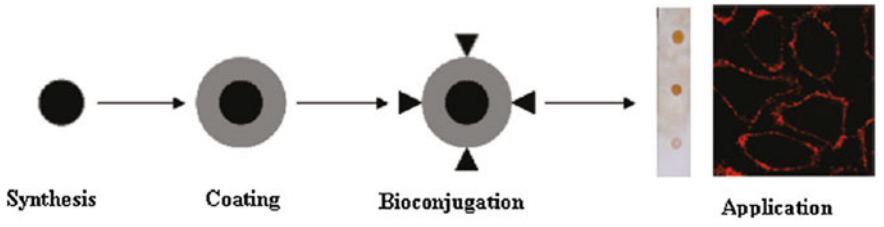
(phosphine, amine, thiol), (Gaponik et al. 2002; Peng et al. 2007), polymers, (Colvin et al. 1994; Tessler et al. 2002), amphiphilic polymers, dendrimers, polysaccharides, micelles, amino dextranes, calixarenes or cyclodextrins etc. (Sheng et al. 2006), (Brus 1998). Thus extensive literature exists on the phase transfer method for preparing QDs in organic/aqueous interface. Moreover, tailoring of QDs using silica coating achieved excellent solubility and functionalization to conjugate with biomolecules. The uniform size silica coatings are carried out following micro emulsion or reverse micro emulsion strategies. The core-shell material exhibits good photostability and less cytotoxicity for biological use. The modern era motivated the fabrication of QDs in planar surface and substrate by means of self-assembly and soft lithography patterning methods. This nanocrystal immobilised structures widen their application in sensor technology (Kim et al. 2013; Wang and Chen 2011).

2.2 Biofunctionalisation of QDs

It is noted that bare QDs are impossible to use in biological applications. Since the QDs are synthesised in water-insoluble strategies, the surface has to be modified by a hydrophilic coating for use in a biological system. QDs with its large surface area-volume ratio, extraordinary reactivity and strong interaction with macromolecules draws a lot of attention in biomedical applications (Hamada et al. 2013).

Scheme 2 showing the steps for bio-conjugations and imaging. Antibodies, aptamers, and small molecules are cross-liked to water-soluble QDs to make them bio-compatible to biological targets and hence can be utilised for drug delivery and or diagnosis of diseases. QDs can be modified by mixing the thiol exchange with biomolecules containing a carbohydrate, phospholipid, protein and peptide containing cysteine residue and nucleic acid etc. Initial coating of biomolecules on the QDs surface is obtained after incubation, which results in stable covalent bonds. The thiol groups can anchor the surface of QDs, while the COOH groups outside the surface renders the formation of stable amide bonds with amine groups of various biomolecules with the aid of coupling reagents, like carbodiimide and N-hydroxysuccinimide (NHS). Second, the chosen approach is exploiting streptavidin modified QDs since they can be linked to biotin-tagged biomolecules. Table 1 represents the various bio-conjugated-QDs for targeting. Further, electrostatic interactions between the macromolecules such as proteins, peptides and the QDs surface can also offer the easiest way for coating and modification.

A variety of organic functional groups can be easily attached to QDs encapsulated in silica shell. To date, various affinity reagents are used to modify QDs which recognize, diagnose the cancer and target drug delivery vesicles. This is an important aspect being exploited in the use of QDs for different application as sensitizers in PDT of cancer and as donor in FRET. QDs have the ability to conjugate to protein and have been used as probes for bioimaging applications. Due to their favourable optical properties, QDs have replaced dyes in many applications. QDs offer versatility with regard to charge carrier multiplication leading to multiexcitons and it is advantageous to efficiently separate them via electron transfer. QDs-protein conjugates are useful as sensors, catalysts and thus find utility in biomedical research, clinical diagnostics and treatments. It is to be noted that QDs have been used in early cancer imaging and detection with remarkably better advantage than the current techniques like



Scheme 2 Describing the steps involved in the bio-conjugation of bare nanoparticles and imaging application

Table 1 The various QDs-bio-conjugate tag for targeting applications (Wang and Chen 2011)

Modified molecule	Type of QD (stabilizer)	Conjugating configuration	Cell type/target
Peptide			
TAT[a]	CdSe/ZnS (streptavidin)	Biotin-streptavidin interaction	A549 (lipid-raft-mediated macropinocytosis)
TAT	CdSe/ZnS (TOPO[b])	Ligand exchange of cysteine-TAT	HepG2 (perinuclear region/lysosome)
$(His)_8$-Trp-Leu-Ala-Aib-Ser-Gly-$(Arg)_8$-amide	CdSe/ZnS (–COOH)	Metal-affinity driven self-assembly	COS-1, HEK293/T17 (endolysosome)
Allatostatin (AST1, APSGAQRLYGFGL-NH_2)	QD605 (streptavidin)	Biotin QD streptavidin interaction	A431 (argalanin receptor mediated endocytosis)
Chlorotoxin (CTX)/dendrotoxin-1 (DTX-1)	ITK QD 525/655 (NH_2-PEG, Invitrogen)	N-succinimidyl iodoacetate and 2-iminothiolane reaction	C6 glioma cells (potassium channel)
Protein			
Lectin	CdSe (–COOH)	NHS[c]-EDC[d] reaction	Leukemia cells (specific target oligosaccharides)
Alpha-fetoprotein antibody	CdSe/ZnS (thioglycolic acid)	NHS-EDC reaction	HCC cell line HCCLM6 (Alpha-fetoprotein)
Cholera toxin B	CdSe/2nS, CdTe/CdSe/ZnS	EDC reaction	NIH 3T3, hMSC, MDSC, M21, MH15 (gangliosides)
Aptamer			
AS141I, TTA1, MUC-1	QDs 605,655,705 (–COOH, Invitrogen)	EDC reaction	PC-3, HeLa, C6, NPA (intracellular)
GBI-10	CdSe (polyamidoamine dendrimer))	EDC reaction	U251 glioblastoma cells (membrane)

(continued)

Table 1 (continued)

Modified molecule	Type of QD (stabilizer)	Conjugating configuration	Cell type/target
TLS9a	QDs (streptavidin)	Biotin-streptavidin interaction	Mouse liver hepatoma cell (membrane)
Anti-PSMA aptamers A9	CdTe (biotinylated PEG)	Avidin-biotin interaction	LNCaP and PC3 cells (PSMA)
Carbohydrate			
N-(2-aminoethyl) gluconamide hydrochloride	CdTe/CdS (–COOH)	NHS-EDC reaction	HeLa (intracellular)
Hyaluronic acid	CdSe/CdS/ZnS (N-(2-aminoethyl)-6, 8-dimercaptooctanamide, amine-DHLA)	Electrostatic interaction	HeLa (HA receptor CD44)
D-galactose	CdSe/ZnS (–COOH)	NHS reaction	HepG2 (intracellular)
Lactose	CdSeS/ZnS (TOPO)	1-thiol-βD-lactose ligand exchange	Leukocytes (β_2 integrin) (CD1 lb/CD18)
Other small molecule			
HaloTag protein ligand	QD655 (streptavidin, Invitrogen)	Biotin-streptavidin interaction	COS7 (HaloTag protein ligand mediated membrane labeling)
GPI/cRGD	CdSe/ZnCdS (cysteine)	NHS-EDC reaction	Prostate cancer cell (PSMA)/melanoma cell (integrin $\alpha_v\beta_3$)

(continued)

Table 1 (continued)

Modified molecule	Type of QD (stabilizer)	Conjugating configuration	Cell type/target
Hoechst 33342	CdTe (N-acetylcysteine)	Electrostatic interaction	
Polyarginine-pyrenebutyrate	QDs655 (Invitrogen)	Biotin-streptavidin linkage	BS-C-1 monkey kidney cells (pyrenebutyrate increased uptake in cytosol)
β-CD-L-Arg	CdSe/ZnSe (–COOH)	Electrostatic interaction	ECV-304 (cytoplasmic localization)
Folate	InP/ZnS (–COOH)	NHS-DCC[e] reaction	KB cells (folate receptor)
	QDs (NH_2-PEG)	NHS-DCC reaction	KB cells (folate receptor)

[a]Human immunodeficiency virus-1 transactivator protein
[b]Trioctylphosphine oxide
[c]N-hydroxysuccinimide
[d]N-(3-dimethylaminopropy1)-N′-ethylcarbodiimide
[e]N,N′-dicyclohexylcarbodiimide

ultrasound and CT scans. These methods cannot visualise cancer in its early stages, but QDs can. QDs have been used in the detection of brain tumour.

2.3 Merits of QDs in Biological Assays

The increased optical activity of QDs provides innumerable avenues of applications in life sciences and biotechnology. QDs can resist degradation as compared to other imaging probes, permits cell tracking for longer periods of time and creates new pathways for molecular interactions. QDs afford good contrast for imaging techniques with an electron microscope as scattering increases. The small size gives them great versatility by allowing them to be injected in liquid mixtures, fabrics, and polymer matrices. In comparison with conjugates formed from organic fluorescent dyes like rhodamine, QD probes are less expensive, twenty times as brighter, and a hundred times more stable against photobleaching.

3 Application of Quantum Dots in Medical Diagnosis

The size (2–100 nm) similarity of QDs and biomolecules makes them ideal candidates in nanomedical applications (Jamieson et al. 2007). In general, nanomedicine exhibits different response to light, electronic, and magnetic irradiations compared to traditional drugs. Hence, nanomedicine established multifarious merits in the biological characteristics for therapeutics and site-specific drug delivery, which defeats the boundaries of molecular imaging and drug/gene delivery normally experienced in the modern era. For e.g., QDs will improve the stability of drugs, shield drugs from degradation, protract the distribution and target searching time, diminish the side effects, enhance the drug delivery and metabolic process in cells/tissues. Research encompassing these concepts has been reported (Guo et al. 2010; Ozkan 2004).

The distinctly characterised optical features of QDs have made them applicable not only in basic studies like fluorescence labelling of biological species but also in life science as probes for molecular level detection, sensor (Chen et al. 2014; Eastman et al. 2006), cell imaging (Ballou et al. 2004), and in vivo imaging (Klostranec and Chan 2006; Li and Zhu 2013).

3.1 Fluorescence Resonance Energy Transfer Analysis

Fluorescence resonance energy transfer (FRET) is a phenomenon involving transfer of energy from a donor to an acceptor provided the distance between them is less than the critical radius known as the "Forster radius" (Lakowicz 2006). The suitability of FRET distance between QDs as donor and organic dyes, rare earth metals,

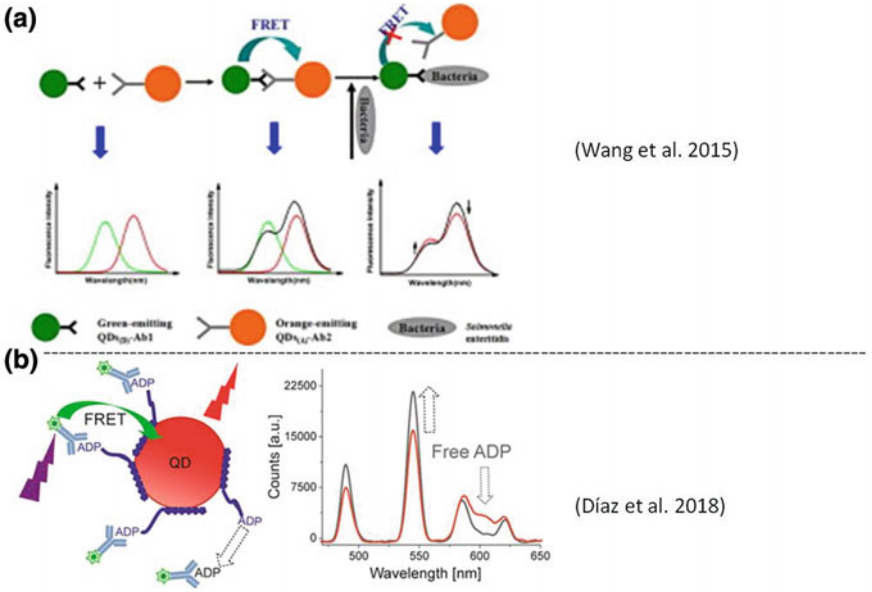


Fig. 3 **a** Illustrate the receptor conjugated QDs analysing of *S. enteritidis* on eggshell. Reproduced from Wang (2015), Copyright from Elsevier publications. **b** FRET donor-acceptor model between ADP modified His6-peptide conjugated to QDs surface and antibody labelled–terbium (Tb) and that selectively sensing ATP verse ADP, Terbium (Tb)-labelled antibody (Ab) that selectively recognizes ADP versus ATP

(Díaz et al. 2018) receptors as acceptors has enabled monitoring of protein binding interactions, quantification of the conformational change, assay of enzyme activity (Shi et al. 2007), nucleic acids, peptides (Frasco and Chaniotakis 2009), vitamins (Vaishnavi and Renganathan 2013) and immune-assays of QDs conjugated to biological molecules (Jiang et al. 2009). Normal cells can be distinguished from cancerous breast cells using QD FRET assays (He et al. 2018). The dynamics of telomerisation, DNA replication, sensing of DNA cleavage has been studied making use of QDs biosensors (Jiang et al. 2009; Patolsky et al. 2003). Figure 3a, illustrates the quantum dot receptor conjugate for analysing (bacteria) *S. enteritidis* on eggshells (Wang et al. 2015). Time resolved-FRET (TR-FRET) bioassay constructed for selective recognition of 10 nM ADP involving energy transfer between the biocompatible QD and antibody labelled Terbium (Fig. 3b). The Role of QDs is to improve energy transfer based biosensors for regular assay and applications.

3.2 Live Cell Labelling

The brighter and stable quantum dots are mostly preferred for cell labelling, targeting and imaging applications (Jin et al. 2011; Smith et al. 2004). QDs access the cellular

membrane without the use of any probes. The most challenging aspect of cytoplasmic translocation of QDs is solved by many techniques (Jaiswal et al. 2004). For example: facile uptake, receptor mediated and nonspecific endocytosis, electroporation and micro injection. While electroporation disturbs the phospholipid bilayer and causes aggregation of QDs, micro injection of QDs into cells avoids such undesirable factors. Thus intracellular organelle labelling is feasible but time consuming and only leads to aggregation of QDs. For this reason, scientists sought to find methods that were short and less time consuming and reduced aggression on QDs. The QDs successfully rendered for labelling HeLa cells, human epidermal growth factor receptor 2 (HER2), antibody, etc. (Wu et al. 2002; Fu et al. 2006). Serotonin labelled CdSe nanocrystals were used to probe the transporter membrane protein in living cells. Peptide labelled NIR emitting QDs were used for imaging tumours in living cell.

3.3 Cellular Tracking and Imaging

In 1998, Bruchez and Nie applied QDs in fluorescence imaging of biological species (Bruchez et al. 1998). Multiplexed imaging and recognition of proteins and genes enabled by QD-transferrin conjugate was useful for real time visualisation of metastasis staging of cancer in live animals (Woodward et al. 2011; Liu et al. 2008). Direct Hela-cell line imaging was reported using GSH-capped CdTe QD (Chen et al. 2018). Poor resolutions of radiolabelled ligand and organic dyes were compromised by QDs. CdSe/ZnS QDs functionalised with phospholipid micelles was applied for labelling Rab 7 protein in cells (shown in Fig. 4) (Lu 2005). Phospholipid encapsulated QDs are stable, do not aggregate and are used in the cell labelling of embryos (Barroso 2011). At low concentrations, QDs-Micelles were not toxic to the cell but higher concentration (5×10^9 per cell) of QDs-Micelles produced abnormalities. The effect of starvation on *Dictyostelium discoideum* development was studied in the analysis of different population by means of different coloured QDs. These cells are tracked for long term without any loss of fluorescence.

Live cell labelling and tracking was achieved using multicoloured QDs and the stability of the cells was retained for more than a week (Jaiswal et al. 2002). Quantum dots play a vital role in labelling, imaging and tracking of viruses (Liu et al. 2016). The application of QDs in cancer imaging and cancer detection are summarised in Table 2 (Pericleous et al. 2012).

Table 2 Detailed report on wide spread application of quantum dots for cancer cell imaging and labelling in early stage (Pericleous et al. 2012)

References	Conjugates	Target	Potential
Imaging			
51	Transferrin-QD conjugates	Mouse heart and femur up to 0.8 mm deep beneath the skin	QD conjugates in the near-IR allow for greater visualisation depth
52	DHLA-QD conjugates	Interstitial fluid; in rats, where the QD conjugates exited the blood vessels	Potential to interrogate the delivery mechanism of QDs to tumour cells
Vasculature imaging			
53	Tri-peptide-QD conjugates	Human glioblastoma and human breast cells	Help differentiate cancer based on integrin expression levels
54	Biotinylated fibrinogen QD conjugates	Robust biocompatibility, longer stay in circulation without toxicity	Non-invasive visualisation of blood vessel development over time
Tracking			
55	Anti-HER2 monoclonal antibodies-QDs conjugaies	Visualisation of the nanoparticles in blood vessels serving tumour cells in mice	Track biomolecules and understand sub-cellular movements: drug delivery
56	PEG-coated QDs	Right dorsal flank of mice, monitor biodlstrlbutlon by ICP-MS	Determination of non-specific accumulation of the nanoparticles into the organs involved with immune response
57	Carboxyl groups-QDs conjugates	Study of the individual and composite drainage patterns towards primary lymph nodes	Non-invasive prediction of metastasis routes for cancer; prediction of cancer metastasis into the lymph nodes
58	Carboxyl-PEG and methoxyl-PEG-QD conjugates	Injected into the rumours themselves; Carboxyl-PEG QDs visualised in numerous lymph nodes while methoxyl-PEG QDs only tracked in lymph nodes close to the tumour site	Monitoring of cancer spread

(continued)

Table 2 (continued)

References	Conjugates	Target	Potential
59	EGF-QDs conjugates	Continuous observation of protein diffusion on the cellular membrane, even after the proteins where internalised	Specific recognition and tracking of plasma membrane antigens
60	Antibody-QDs conjugates	Antibody fragment specific for glycine receptors on the membranes of living neurones	Tracking of single receptors
61	Human mesenchymal stem cells loaded with QDs	Implanted into an extracellular matrix patch for use as a regenerative implant for canine hearts with a surgically-induced defect	Simultaneous monitoring of multiple types of cells in living organisms and identification using distinct optical codes
Circulation/distribution			
62	QDs with paramagnetic particles in a silica sphere	Simultaneous trace of the spheres in blood circulation, providing distribution information and anatomical reference	Tracing brain-vasculature and visualising neuron, astrocyte and blood vessel structures
63	CdSeS silica-hydroxyl shell particles	Detect cadmium in urine, faces and other organs in mice	Determination of QD clearance in vivo
64	QDs with a negatively-charged carboxyl group coating	Rapid uptake by the lymph vessels and retention in the lymph nodes	Determination of QD clearance in vivo
65	CdSe/ZnCdS QDs with a DL-cysteine coating	Lack of aggregation or protein binding in serum due to neutral charge	Bio-distribution and pharmacokinetics
Labelling/detection			
66	Antibodies-QDs conjugates	Five different antibodies against five cancer biomarkers (HER2, EGFR, PR. ER, mTOR)	Multiplexed cell labelling and analysis

(continued)

Table 2 (continued)

References	Conjugates	Target	Potential
Cellular imaging			
67	QDs with heterofunctional, biocompatible, charge-tunable surface coatings	Utility of the alloyed shell and surface coating	Live cell labelling
66	Phospholipid micelle encapsulated QDs	Internalisation in human pancreatic cancer calls	Functionalising-dependent cell uptake
69	QD-coated substrate	Non-specific endocytosis	Cell motility and metastatic potential studies
70	Organelle-targeting peptides-QDs conjugates	Specific stain of either cellular mitochondria or nuclei, following microinjection into fibroblast cytoplasms	Intracellular delivery of QDs
71	Tat-peptide-QDs conjugates	Cellular uptake and intracellular transport of nanoparticles in live cells	Development of nanoparticle probes for intracellular targeting and imaging
Targeting			
72	Folate-QD conjugates	Folate receptors in mouse lymphoma cells	Folate is critical for cell growth, may be important lo cancer diagnosis
73	Antibody-QD conjugates	Imaging of the retinal vasculature, spatial resolution down to the level of single cells	Cancer growth and metastasis

3.4 In Vitro and In Vivo Application of QDs

QDs have excellent photophysical properties suitable for use in in vitro and in vivo molecular imaging. In general, QDs can brighten biological imaging of biomolecules (Byers and Hitchman 2011). Imaging techniques such as fluorescence microscopy can be used for tracking purposes. For instance, Three-Dimensional Fluorescence Analysis was effective in probing QDs labelled Human Mesenchymal Stem Cells (hMSCs) (Rosen et al. 2007). Primary cause of heart attack and stroke in human is due to calcification. The combination of fluorescence lifetime analysis and scanning acoustic microscopy (SAM) was successfully utilised to image the CdTe/CdS QDs painted human carotid atherosclerotic plaques (Bilen et al. 2018). These dual-modal studies differentiated the calcified areas from the collogen-rich areas within

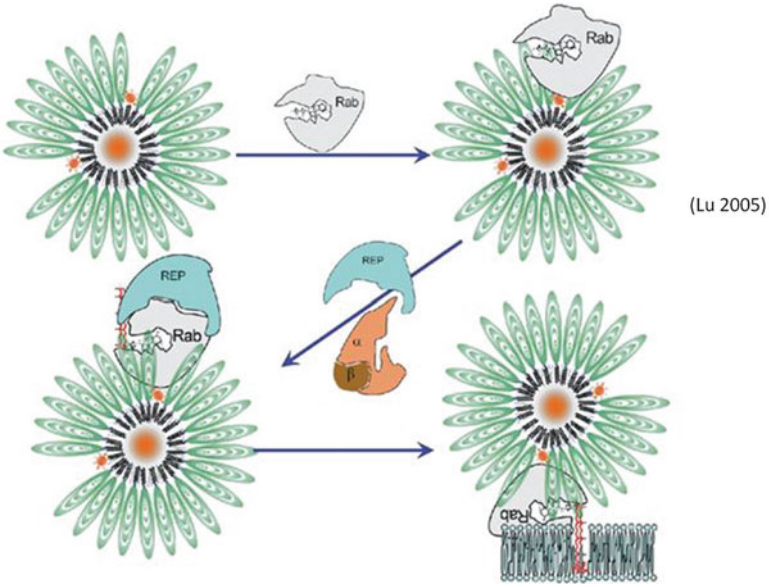


Fig. 4 Shows the prenylation technique for a PEG2000-QD/Rab7 bioconjugate. The Rab7 protein was attached to a functionalized QD and the prenylation reaction was done in the presence of Rab escort protein (Rep1), Rab geranylgeranyl transferase (RGGT) and Geranylgeranyl pyrophosphate (GGpp). At the final stage, the prenylated Rab7/QD conjugate was delivered to the membrane of organelles by the help of Rep1 (Stein et al. 2003)

the plaque. QD-receptor-mediated endocytic trafficking in live cells is done utilising fluorescence microscopy.

Enhanced binding affinity and the multivalency effect of QDs render their analysis of cancer biomarkers even at low concentrations (Li and Zhu 2013). QD bioconjugates have been used in a number of in situ tissue applications. Quantum Dots was widely used for studying the blood brain barrier for treatment of HIV-related encephalopathy (Xu et al. 2013). Bio-conjugates of QD/dopamine can function as redox coupled assemblies for in vitro and intracellular pH sensors (Medintz et al. 2010).

In vivo *or* cellular targeting and imaging is helpful for cancer diagnosis to yield more intuitive information (Michalet et al. 2005; Walling et al. 2009). For this, first QDs are encapsulated with an ABC triblock copolymer and it is linked to amphiphilic polymer tumour-targeting ligands. Subsequently, it is used as a probe for passive tumour imaging and active tumour imaging with high sensitivity and multicolour capabilities. From in vivo targeting studies on mouse it is clear that QDs can be delivered to tumour cells by both enhanced permeation and by antibody binding to cancer-specific cell surface biomarkers like prostate-specific membrane antigen (Bruchez et al. 1998; Rizvi et al. 2010; Zrazhevskiy et al. 2010).

Visualisation of cancer cell was possible with the help of luminescent and stable QDs. QDs coupled with fluorescence microscopy enabled the tracking of cells

at high resolution in living animals (Oliveira et al. 2014). Simultaneous targeting and imaging of prostate tumours was reported via QDs bioconjugated to prostate cells. This new type of QDs-Conjugate with amphiphilic triblock polymer and multiple PEG molecules for biocompatibility and circulation were employed for in vivo protection due to their better stability and bright detection signals. Different wavelength emission of QDs helped to realise the imaging of multiple tumour markers simultaneously thus enhancing the sensitivity and selectivity of cancer detection.

Recently, QDs with Near Infra Red Fluorescence (NIRF) signals were reported. It is to be noted that NIRF signalling predominates over the visible fluorescence signals due to the preferred penetration of the former into tissues. Further, this NIRF method possesses high signal-to-background ratio which is a favourable aspect required for better sensitivity and detection. Potentially dual modal (fluorescence/magnetic resonance) in vivo imaging was obtained using Gd^{3+}-functionalized near-infrared quantum dots (Jin et al. 2008). Despite their excellent optical imaging capability for in vitro and in vivo application in tumour cells and also their sensitive real time detection using NIRF, QDs contain Cd which are toxic to cells. Thus the viability of employing these QDs for future clinical applications is yet to be explored in order to minimise toxicity.

3.5 QDs as Photosensitiser (PS) in Cancer Treatment

QDs are very useful in photodynamic therapy (PDT) of cancer cells due to their ability to promote FRET (Fig. 5b). This is attributable to the close proximity of CdSe QDs with photosensitiser normally employed such as porphyrin, phthalocyanines, chlorin e6 (Charron et al. 2012; Shen et al. 2017). However CdSe promoted PDT activity led to lower yield of O_2^1 (single oxygen) compared to classical photosensitisers. But this is offset by the minimal photobleaching of QDs versus the rapid photobleaching encountered with the classical photosensitisers. Thus it was established that prolonged and repetitive exposure of QDs to light (as photosensitisers in PDT) may lead to a higher steady level of O_2^1 which will be enough to ensure apoptic and necrotic cell death in the target tissue (Samia et al. 2003) (Hong et al. 2016). When the QD conjugates are treated with coelenterazine, a substrate of RLuc8, energy is transferred from the substrate to the QD conjugates via bioluminescence resonance energy transfer (BRET), thereby emitting 655 nm photons and activating meta-tetra-hydroxyphenyl-chlorin (m-THPC, Foscan®) PS for ROS generation (Fig. 5a).

3.6 QDs and Neuroscience

The unique properties of QDs offered a promising future for neuroscience research. QDs serve as not only alternative to traditional immune cytochemistry but also they

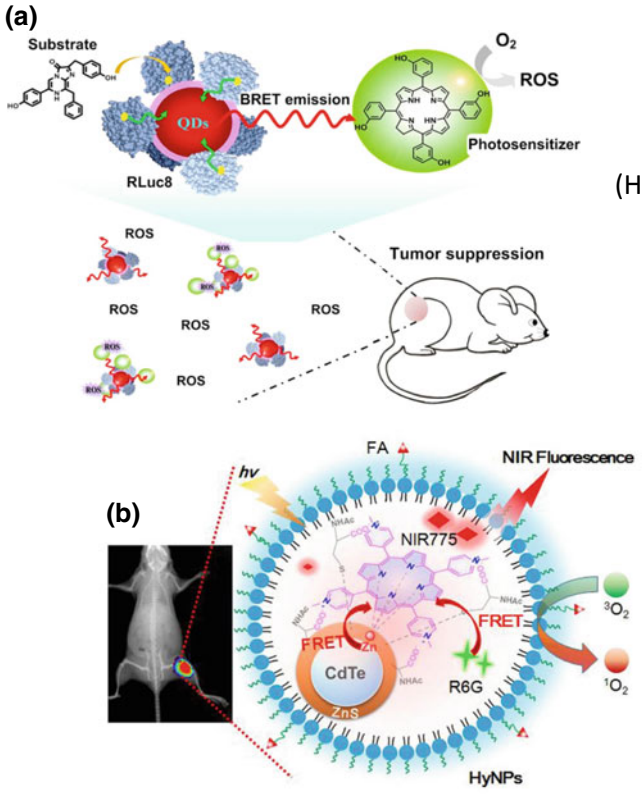
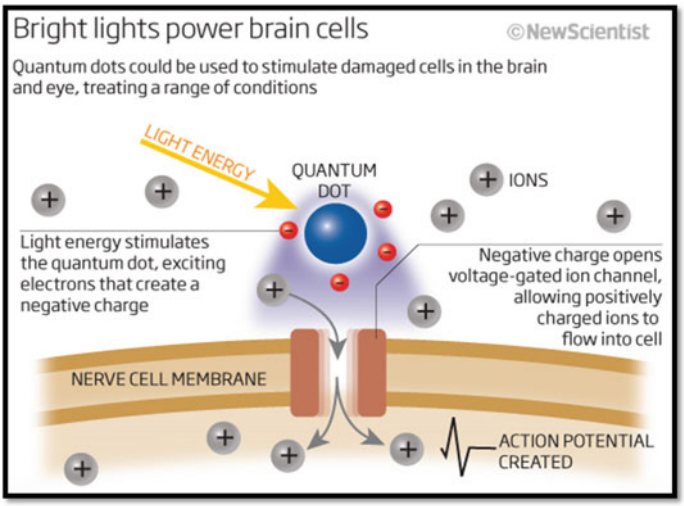


Fig. 5 **a** QD conjugate incorporating light-emitting Renilla luciferase 8 (RLuc8) was utilised to produce bioluminescence as an external light source for activating photosensitiser drugs in PDT. **b** Targeted and effective photodynamic therapy for cancer using functionalized nanomaterials

are valuable in the study of neurons (Silva 2012). Owing to their extremely small size and better optical resolution properties, QDs were used to visualise and track dynamic processes in neurons over a span of time resolution despite the restricted anatomy of neuron. Antibody conjugated QDs can able to track the spinal cord neuron, and control the brain cells (Lugo et al. 2012). Photo excited QDs cause the nerve cell membrane to open, and allow them to enter inside a cell shown in Fig. 6.

The utility of QDs in neuron science is well addressed. Neuronal-like differentiation in cultured pheochromocytoma 12 cells by Nerve growth factor (β-NGF) tagged QDs was reported by vu.et al. However this method suffered due to reduced activity when compared with β-NGF (Vu et al. 2005). Subsequent attempts by researchers reported on the remediation by using biocompatible water soluble QDs-Micelles which retained the optical properties of individual quantum dots (Howarth et al. 2005; Pathak et al. 2006; Silva 2009). In fact, these QD-micelles indicated the intracellular dispersion in cultured hippocampal neurons. Yet another method involving



(Lugo et al. 2012)

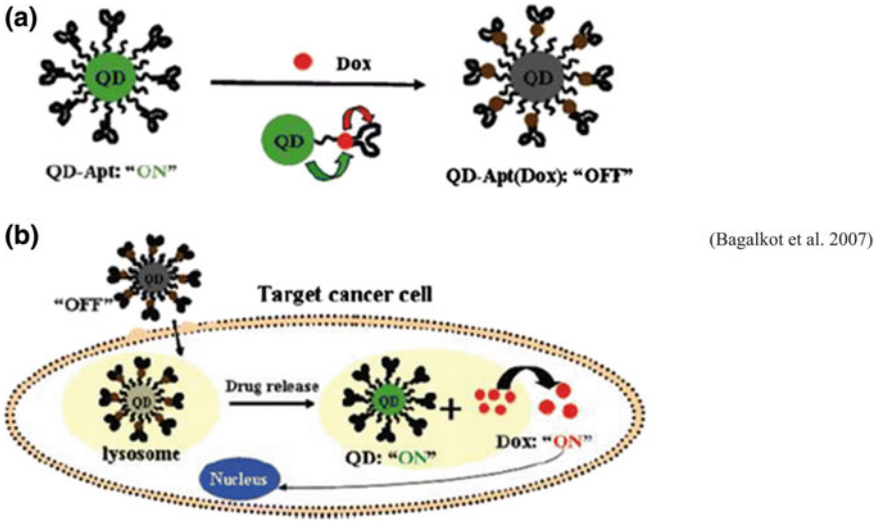
Fig. 6 QDs entering the nerve cell membrane (Courtesy to newscientist.com)

QD labelling was found to be useful in specific tracking of AMPA receptors in cultured hippocampal neurons. Glycine receptors and their diffusion dynamics inside the neuron were tracked effectively using single QDs (Dahan et al. 2003). Thus it is understandable that QDs represent a special class of biological probes which are easily modifiable, tunable, tagged to be used for specific tracking receptors even in otherwise difficult situation.

As an alternative material for toxic cadmium chalcogenide QDs, graphene based quantum dots can be used to treat Alzheimer's and Parkinson diseases. These graphene QDs slow down fibrillation of α-synucleinopathy and interact directly with mature fibrils, prevent the aggregation processes (Kim et al. 2018). Luminescent Si coated QDs can also be potentially applied in diagnostic and bioimaging applications due to less toxicity (Han et al. 2009). Thus it is clear that QD nanotechnology will need an easy-to-use approach that can be directly replicated in a characteristic neurobiology laboratory.

3.7 QDs Drug Delivery Systems

QDs serve as efficient vehicular or transport systems for optimal drug delivery. Such a phenomenon is attributed to the ability of QDs to form complexes with nanomedicines due to certain water soluble capping agents like mercapto acetic acid, mercapto ethylamine and polyethylene glycol when attached to QDs. It is interesting to recall the fluorescence emission of QDs which play a key role in specific targeting, measurement of delivery efficiency and drug release rate which will



Scheme 3 Represent the QD-aptamer (Dox) Bi-FRET system for drug delivery application

help to ensure the diagnostic recognition and establish the mechanism of drug delivery. Further enhancement of therapeutic effects and diminution of side effect for pharmaceuticals can be ensured by designing a single system consisting of QDs with therapeutic modality (Baba and Nishida 2012; Oliveira et al. 2014). Scheme 3, shows the labelling of traditional drugs to QDs and this type of studies can be established in a new nanocarrier for drug delivery application (Bagalkot et al. 2007). Photoexcited QDs can kill multidrug-resistant pathogenic bacteria (Courtney et al. 2016).

It is hoped that these points will lead to more prosperous and futuristic utility aspects of QDs for further applications.

3.8 Cytotoxicity of QDs

Prolonged use of CdSe QDs under UV light induces toxicity. This is due to UV light having sufficient energy close to a chemical bond that breaks down the QDs during photolysis leading to release of Cd ions into culture medium which harms the cells (Zhang et al. 2007). The formation of reactive oxygen species (ROS) like free radicals (for example, hydroxyl radical), superoxide and singlet oxygen ($^1O_2$), by QDs can cause irreversible damage to biological systems (cellular components) (Mazumder et al. 2009; Wang and Chen 2011). Physicochemical properties and cellular toxicity of QDs depends on their surface alteration (Hoshino et al. 2004). Henceforth, a direct way of avoiding the QD toxicity lies in making the QDs coated with low or non-toxic organic molecules/polymers (e.g., PEG) or inorganic layers (e.g., ZnS and silica), acetoacetic acid, mercaptopropanic acid, 11-mercaptoundecanoic acid and 2-aminoethanethiol are more toxic than QDs coated with silica. However non

toxic effects were noticed when further PEG molecules were attached onto the silica external surface. QDs can enter the cells through endocytosis and cell death was related to the uptake and quantity immaterial of nature of surface coating. Cancer cells labelled with QDs survived in circulation and extravagated into tissues just like the unlabelled ones and there was no significant difference in their capacity to form tumours in mice after a month and QDs had no adverse effects on the physiology of host animals or labelled cells. In another report, it was described that QDs coated with PEG had very little toxicity to cells if the measure was lower than 100 nM.

4 Conclusion

Despite recent research, much work still has to be done in order to achieve more worthy developments in nanomedicine. A better perspective of the distribution and fate of delivered cells may allow for assessment of protection and efficacy for any novel stem cell therapy before reaching human trials phase. Furthermore, this chapter emphasizes the potential applications of QDs in life sciences and biomedicine. Additional developments of advanced QDs-based nanotools are to be tackled in future.

Videos

- A Demonstration of Quantum Dots by Samsung, 2016. Watch quantum dots in action in this short lab demo (video link https://www.youtube.com/watch?v=96MJOJlApD4&t=48s).

Articles

A1 Your Guide to Television's Quantum-Dot Future by Zhongsheng Luo, Jesse Manders and Jeff Yurek. IEEE Spectrum, February 22, 2018.
A2 How a Mysterious Bacteria Almost Gave You a Better TV by Joanna Klein. The New York Times, May 5, 2016.
A3 The Cancer Surgeon's Latest Tool: Quantum Dots by Imad Naasani. IEEE Spectrum, September 21, 2016.

Patents

These few examples give a taste of how inventors are turning their thoughts to commercial applications of quantum dot technology:

P1 US Patent 20080172197A1: Method of manufacturing a quantum dot optical component and backlight unit having the quantum dot optical component by Mun-Ki Sim et al., Samsung, May 16, 2017. A detailed description of a quantum-dot display backlit by LEDs.

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Molecular Dynamics Simulations in Drug Discovery and Drug Delivery

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Abstract Molecular dynamics (MD) simulation acts as an important supporting tool to experimental methods in the process of drug discovery. With the recent growth in computational power and development of efficient and fast computational techniques, the role of MD simulations has become even more prominent. In this chapter, we discuss the role played by MD simulations at different stages of the drug discovery process. We also discuss the contribution of MD simulations in developing drug-delivery strategies and highlight how the molecular resolution offered by the MD simulations aids in better understanding of the systems involved.

Keywords Molecular dynamics · Free energy · Docking · Carbon nanotube · Dendrimer · Liposome

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1 Introduction

Among the most important motives of science is that of using scientific knowledge to create a better environment to live for the human race. In the past, many deadly outbreaks of dangerous diseases have resulted in the loss of a large number of human lives. As time passed and scientific knowledge on the working and behaviour of the human body started to increase, scientists, mostly biologists began investigating means to develop cures. The starting point was to get an insight into the cause of the disease, and once the cause is known, the aim was to devise means to cure them. This led to the rise of the field of drug discovery. Starting from the accidental discovery the first antibiotic, penicillin, till today, the field of physiology and medicine has seen a total of 108 Nobel prizes and 214 Nobel laureates among its peer. Starting from the initial stages of drug discovery, to the current times, the methods involved have changed drastically. Earlier, the field was mainly dominated by biologists and chemists, who, respectively, had the mechanistic knowledge of the working of the human body and of the nature of chemical compounds and how they can react with various biomolecules. Recently, the field has seen a significant intervention from physics and her glorious instruments. The knowledge of X-ray diffraction and the physical interpretation of the diffraction patterns led to the possibility of deciphering the structures of various biomolecules involved in diseases. With the knowledge of their structures the scientists had a physical picture of what their target was, making the choice of a molecule that can act as a drug more informed and quick.

Today, the process of drug discovery has become strongly multi-disciplinary. After a therapeutically important biomolecule is identified, potential molecules are to be identified that can interact with the target and block the disease-causing pathway. After a set of proper molecules is arrived at, they are modified for bio-compatibility. The drug molecule then undergoes multiple stages of trials before it can be commercially available. The process is usually decades long and extremely expensive. There are various factors that need to be taken care of. For example, during the tweaking of a potential drug molecule to make it biocompatible, one needs to take care of how its properties may change. The drug-drug interaction, the permeability of the drug molecule through the cell membrane, its diffusion properties are also a few considerations that result in prolonging the process. In addition, to find a molecule that can act as an efficient drug, one needs to have information on parameters like its energy of binding with the host molecule and the molecular mechanism underlying its action as a drug. Thus, to speed up the drug discovery process and to make it cost-effective, the process of screening a large swarm of molecules needs to be made efficient. Also, efficient methods are required for deciphering the molecular modes of action of the drug and measuring the strength of interaction between drug molecules, drug and non-target proteins and also drug and cell membrane. One would prefer a molecule that is effective in small amounts. The efficiency of a drug depends on many factors. If all the drug that is injected into the human body directly reaches the target area without interacting with other molecules that it can potentially bind, its efficiency would be high. Also, there can be situations where a molecule might show strong and

specific interaction to a target protein but is slow to diffuse across the cell membrane. So, to increase the efficiency and efficacy of a drug it is desirable to deliver it into the human body in such a way that a large portion of the drug reaches the target area and also does not face hindrance from the cell membrane and other "molecular sinks" on the way. To this end, one uses carriers that have favorable interaction both with the drug molecule and the target site. To decide on a molecule that can act as a carrier for a particular drug, one needs to know the favorability and strength of their interaction and also the molecular details of interaction in order to be able to tune better, the properties of the carrier *vis a vis* the drug molecule. In addition to these properties, one needs to have an idea of the rate of release of the drug from its carrier and the quantitative details of the energy barriers involved in the process. To this end, the introduction of computers and efficient tools of computational sciences has played a very important role in speeding up the drug discovery process and in making it less cumbersome and cheaper. Development of efficient methods and computational tools that can aid in speeding up quantitative in silico calculations of various drug and target properties are an active area of research. Many quantum mechanical and classical simulation tools are now available which aid in calculating the properties of molecules that can help one decide on its potency as a drug.

In this chapter, we discuss those aspects of drug discovery and drug delivery in which molecular dynamics (MD) simulations play an important role. We begin with a short introduction to the basics of MD simulations and various computational methods that have been developed with an aim to calculate various properties of intermolecular interaction between the drug and the target molecule. We discuss and describe important results from the literature that highlight the productivity and importance of MD simulations in the field of drug discovery and drug delivery. We conclude with a prediction of what is to come.

2 Brief Introduction to MD

An MD simulation starts with the details of the initial coordinates of all the atoms constituting the biomolecule. The forces are then calculated on each atom based on empirical potential functions and parameters. With the information of the forces acting on the particles in the initial configuration, the system (all the atoms) is evolved for a small time (Δt) using Newton's equations of motion, that are integrated to obtain the new positions and velocities. This process is repeated to generate a trajectory in time consisting of a sequence of conformations. The trajectories are used to calculate physical properties of the system. To have an accurate measure of a property, the system size needs to approach the thermodynamic limit. However, the size of the simulated systems are generally far smaller than the thermodynamic limit, which would give rise to large time dependent fluctuations in the measured values of the physical properties. A large percentage of molecules in a finite system lie on its surface, giving rise to surface effects. To take care of the above issues, the *periodic boundary conditions* (PBC) are utilized. With PBC, the system is repeated infinitely

in all three directions to mimic the bulk. Any given particle in the infinite system interacts with all other particles in all the infinitely repeated copies as well as the particles in its own cell. The use of PBC helps to mimic the bulk system sufficiently effectively. But there are some drawbacks. One of the major drawbacks is that the allowed wavelength for fluctuations in the system depends on the size of the periodic box. The longest allowed wavelength in the periodic system has to be equal to the size of the periodic box. Thus, while studying phenomena like the 2^{nd} order phase transition, PBC may lead to artifacts (Frenkel and Smit 2001).

The force calculation constitutes the most expensive part of any MD simulation. The evaluation of forces requires the calculation of distances between all the particles in a system, which is an N^2 process, N being the system size. To optimize the process, short range interactions like the van der Waals (vdW), often modeled by the Lennard-Jones potential, are evaluated only up to a cut-off distance, and tail corrections are added to adjust for the error induced due to the use of the cut-off. For long range interactions like the coulomb potential, Ewald method and its variants are used: the interactions up to a cut-off distance are evaluated in the real space, whereas, beyond the cut-off reciprocal space calculations are used for efficient evaluation and convergence (Frenkel and Smit 2001).

A good algorithm for integrating the equations of motion possesses properties like speed, long time energy conservation and time reversibility. Most of the MD packages use some flavour of the verlet algorithm (Frenkel and Smit 2001) to integrate the equations of motion. The integration scheme is mentioned below:

$$r(t+\Delta t) = 2r(t) - r(t-\Delta t) + \frac{f(t)}{m}\Delta t^2 \qquad v(t) = \frac{r(t+\Delta t) - r(t-\Delta t)}{2\Delta t} \quad (1)$$

Here, r, f, m *and* v are displacement, force, particle mass and velocity respectively.The verlet algorithm is fast, has exceptional long time energy conservation, is time reversible and produces a sufficiently accurate MD trajectory. There are various integration schemes that are equivalent to the verlet algorithm. One of the most common schemes is the velocity verlet algorithm (Frenkel and Smit 2001). It uses a simple Euler scheme (Taylor expansion) for the positions. The equations corresponding to this scheme are:

$$r(t+\Delta t) = r(t) + v(t)\Delta t + \frac{f(t)}{2m}\Delta t^2 \qquad v(t+\Delta t) = v(t) + \frac{f(t+\Delta t) + f(t)}{2m}\Delta t \quad (2)$$

It is important to point out that the trajectories generated by an MD simulation diverge significantly from the true trajectory of the system after sufficiently large times. No integration algorithm can produce an exactly accurate trajectory. In fact, the trajectories depend strongly on initial conditions and infinitisimally different initial

conditions eventually give rise to very different trajectories that are exponentially divergent and demonstrate Lyapunov instability (Frenkel and Smit 2001).

The trajectories generated from MD simulations are used to calculate the physical properties of the simulated systems. An accurate calculation of the properties depends on how well the MD trajectory describes the dynamics of an experimental system. This, in turn, depends largely on how well, the empirical interatomic interactions, that are used to evolve the system during an MD simulation, represent the actual inter-atomic interactions.

$$\begin{aligned} U(\mathbf{R}) = & \sum_{bonds} k_i^{bond}(r_i - r_0)^2 + \sum_{angles} k_i^{angle}(\theta_i - \theta_0)^2 \\ & + \sum_{dihedrals} k_i^{dihed}[1 + \cos(n_i\Phi_i + \delta_i)] \\ & + \sum_i \sum_{j \neq i} 4\epsilon_{ij}[(\frac{\sigma_{ij}}{r_{ij}})^{12} - (\frac{\sigma_{ij}}{r_{ij}})^6] + \sum_i \sum_{j \neq i} \frac{q_i q_j}{D r_{ij}} \end{aligned} \tag{3}$$

Equation 3 is a typical potential function representing interatomic interactions. The first three terms represent the bonded interaction (bonds, angles and dihedrals), while the last two terms represent the nonbonded interaction (Lennard-Jones and electrostatics). r_0 and θ_0 are the equilibrium bond length and angle respectively. ϵ and σ are the energy and size parameters for the vdW interaction, while q and D are the particle charge and the dielectric constant of the medium respectively. Normally an additional term corresponding to improper dihedrals is added to the potential function to maintain the planarity of rings. The specific potential function along with the parameters of the function (eg. bond length r_0, equilibrium angle θ_0 etc.) constitute what is known as the force field. The force field is a critical factor in deciding how well the values of physical properties derived from an MD trajectory match with experiments. There are different ways to obtain the parameters of the force field. Different force fields meant to be used for the same molecules use different methods to parametrize the potential function and hence different force fields are suited to describe different sets of properties. While performing a simulation it is thus important to decide on what properties one wants to derive from the simulation and to choose a force field appropriate to reproduce that property. AMBER ff99SB (Hornak et al. 2006) for proteins, parmbsc0 (Perez et al. 2007) for nucleic acids, CHARMM27 (Foloppe and MacKerell 2000; MacKerell et al. 1998) for proteins and nucleic acids and GAFF (Wang et al. 2004) for organic molecules are examples of some commonly used force fields.

One of the major issues with atomistic MD simulations is of sampling times. For a large system the sampling times are typically restricted to a few 100 ns. The accuracy of the values of physical properties derived from an MD simulation improves with sampling time. In addition, many biological phenomena take place at the time scales of milliseconds to seconds. Such timescales are rarely achievable with atomistic MD and hence the method is restricted to the study of phenomena that occur at short-enough time scales. To be able to study the phenomena occuring at long time

scales, MD simulations need to be longer and faster. To achieve this, the concept of coarse-graining is used, in which a group of atoms are represented by a single bead. The number of force calculations thus reduces and longer simulation times can be achieved. To represent the motion of the beads, an effective force field is used, and the parameters of the force field are calculated by matching certain physical properties of the coarse-grained (CG) system with the atomistic results. MARTINI model, developed by Marrink et al. originally for lipids (Marrink et al. 2007), is a popularly used CG force field. The model is designed such that it can be used for a very broad range of applications without the need of reparametrization. In this model, the non-bonded interaction terms have the same functional forms as the atomistic force fields, but they are truncated and shifted (Monticelli et al. 2008). Also, non-bonded interactions between nearest neighbours are not considered. The bonded interactions comprise of the bond, angle, dihedral and improper dihedral terms.

$$V_b = \frac{1}{2}K_b(r_i - r_0)^2 \qquad V_a = \frac{1}{2}K_a[\cos\theta_i - \cos\theta_0]^2 \tag{4}$$

$$V_d = K_d[1 + \cos(n\Phi_i - \Phi_0)]^2 \qquad V_{imp} = K_{imp}(\Phi_i - \Phi_{impro})^2 \tag{5}$$

To mimic the collective motions observed at the atomistic level, the force constants ($K's$) are set to low values. For proteins (Monticelli et al. 2008), the experimental results on the oil-water partitioning free energies of the amino acids is used to parametrize the non-bonded terms, while the bonded parameters are fixed by fitting the bond-length, angle and dihedral distributions obtained from simulations to the distribution obtained from structures in the Protein Data Bank (Berman et al. 2000). On an average, one bead is used to represent four heavy atoms. This number reduces to one bead per two atoms for rings. Four different kinds of interaction sites, namely, polar, non-polar, apolar and charged are used. An appropriate combination of the four is used to represent each amino acid. For DNA, each nucleotide is mapped to 6 or 7 beads (Uusitalo et al. 2015). The backbone is represented by three beads, one for phosphate and two for sugar. C and T are modeled as 3-bead rings, whereas, A and G are modeled as 4-bead rings.

The quality of a CG model is determined by its parametrization procedure and its accuracy. The model must lead to a significant acceleration in sampling, but the compromise on its ability to reproduce the atomistic results should be minimal.

Water has an important role to play in determining the nature and strength of interaction between receptors and ligands. Increase in system entropy due to water release from receptor cavities also aids in determining the strength and specificity of receptor-ligand binding. Simulating receptor-ligand systems in explicit water is thus very important to accurately understand the nature of their interaction. A set of parameters (bond length, angle, partial charges and bonded and non-bonded interaction parameters) meant to describe the structure and the inter-molecular interactions of water are referred to as water models. Different water models are good at capturing different properties of water. Thus an intelligent choice of the water model is

important, keeping in mind the problem at hand. There are different water models in use these days ranking differently in terms of their complexity. Some are rigid (TIP3P, SPC, SPC/E etc.), some are flexible (F3C, Ferguson etc.) and some others are polarizable (SWM-4DP, AMOEBA03 etc.). The complexity also goes up with the number of interaction sites. There exist water models with number of interaction sites varying from 3 upto 6. More the number of interaction sites, more interactions need to be evaluated every time step, resulting in more computationally demanding simulations.

TIP3P (Jorgensen and Madura 1983; Mark and Nilsson 2001) is a 3-point interaction site model of water. The vdW centre lies on the oxygen atom, while all the three atoms are involved in electrostatic interactions. The potential energy function of the model is given by: $U_{AB} = \sum_{i(A)} \sum_{j(B)} \frac{q_i q_j}{r_{ij}} + \frac{a}{r_{oo}^{12}} - \frac{b}{r_{oo}^{6}}$, where, r_{oo} is the distance between oxygen atoms of water molecules A and B. One notes that the vdW centres lie only on the oxygen atoms. TIP3P is a computationally efficient model and reproduces the experimental specific heat of water very well, but cannot reproduce certain important water-specific properties like its anomalous density behavior. The improvements over the TIP3P model are the TIP4P (Jorgensen and Madura 1983; Jorgensen et al. 1983) and TIP5P (Mahoney and Jorgensen 2000) water models. These are 4-point and 5-point models respectively. The TIP5P model captures the density anomaly of water really well. Figure 1 shows the difference between the three models.

The SPC/E (Mark and Nilsson 2001; Berendsen et al. 1987) model of water is also a 3-point model like the TIP3P model. The model, to capture bond-polarizability, adds a polarization correction to the potential energy function. The correction term is given by, $U_{pol} = \frac{1}{2} \sum_i \frac{(\mu - \mu^0)^2}{\alpha_i}$. Here, μ^0 is the dipole moment of polarized water molecule, μ is the dipole moment of an isolated water molecule and α is the polarizability constant. The correction adds 1.2 kcal/mol of intermolecular interaction energy. Also, while the H–O–H bond angle for the TIP3P model is $\sim$104.5°, the angle for the SPC/E model is fixed at $\sim$109.5°. The SPC (Mark and Nilsson 2001) model of water is similar to the TIP3P model with a bond angle similar to SPC/E. With the addition of the polarization correction, the SPC/E model can reproduce the density and diffusion constant of water better than the SPC model. Constant improvements

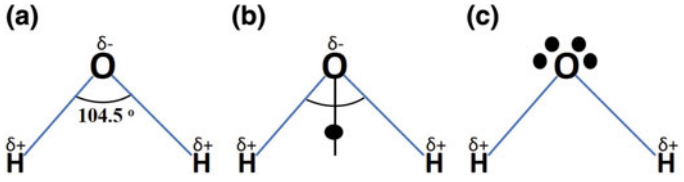

Fig. 1 **a** The TIP3P water model. There are three interaction centers. The vdW center lies on the O atom, while all the three atoms are charge centers and participate in electrostatic interaction. **b** TIP4P model with three charge centers on the three atoms and the vdW center lying on the bisector of the H–O–H angle. **c** TIP5P model. vdW center lies on the O atom, while the charge on the O atom is held at two off-atom points around it

on the current models are carried out to develop models to better suit special cases. For example the water model TIP4P-D, developed by Piana et al. (2015) uses a modified dispersion term with the original TIP4P water model. This water model reproduces better the conformational sampling of unstructured proteins.

In the MARTINI model, 4 water molecules are represented by one unified bead, significantly reducing the system size. The beads are neutral and interact via LJ potential (Monticelli et al. 2008).

3 MD in Computer-Aided Drug Design

The process of discovering a drug molecule is a billion dollar affair, and a prime contributor to this is the selection of lead compounds that can be tuned further to be used as drugs (Basak 2012), through an automated experimental process called *high throughput screening* (HTS). The traditional HTS involves the screening of a large number of molecules to single out those that show desired biological response. This method is basically hit and trial and needs minimal prior knowledge or compound design. The search space is typically of the order of 10^6 molecules. *Computer-Aided Drug Design* (CADD) helps in reducing the size of the sample space for a similar number of favourable hits, thus increasing the hit percentage. The process involves screening of virtual compound libraries to reduce the sample space for HTS and is also referred to as virtual HTS or vHTS. CADD also guides the optimization of the lead compounds. Figure 2 shows the sequence of methods in the drug discovery process and the position of CADD. CADD can be classified into (i) structure-based approach and (ii) ligand-based approach. The ligand-based approach does not need the structural details of the receptor and the drug. It uses prior information on active and inactive molecules and performs virtual screening based on similarity searches and prediction of quantitative structure-activity relations (QSAR) (Kalyaanamoorthy and Chen 2011). It involves processes like the pharmacophore modelling (Wolber and Langer 2005; Chen and Lai 2006) where the activity data of a ligand for a particular target is used to construct a model predicting the positioning of interaction sites participating in hydrogen-bonding and hydrophobic interaction. Molecules in ligand libraries can be compared with this model to select promising candidates. The structure-based approach, on the other hand, requires information about the target and ligand structure. Experimentally determined X-ray crystallography and NMR structures are used for the target. When a target structure is not present, the computational tool of homology modeling (Vyas et al. 2012) helps in predicting the structure. The method involves the prediction of the protein structure by searching the database for template structures with regions of similar sequences. The accuracy of the model building methods like homology modeling is important in order to have a protein model that can be used to predict lead molecules. The modeling needs to be especially accurate on an around the possible binding pockets on the protein surface. The generated model is then refined by fixing the bond lengths and geometries and then minimizing the structure to remove unfavourable contacts (Raval et al. 2012;

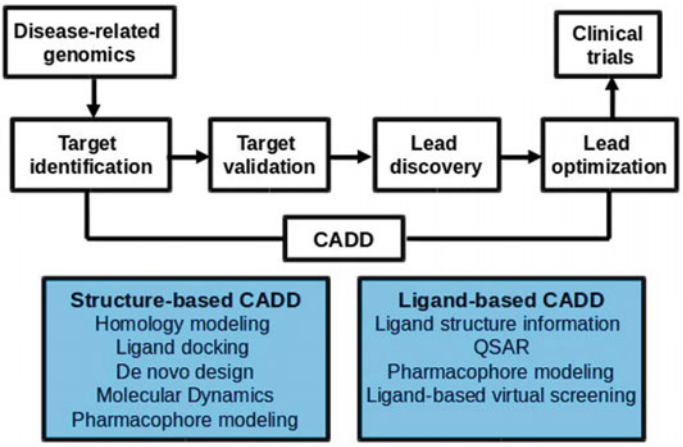

Fig. 2 The position of CADD in the drug discovery process. Various methods within the category of structure-based and ligand-based CADD are listed. De novo design involves building inhibitor molecules from scratch based on the structure of the binding site on the target

Misura and Baker 2005). The refined model is then evaluated by comparing it with experimentally determined protein structures (Melo and Sali 2007; Cozzetto et al. 2009). Ligand structures are taken from virtual ligand libraries (Fink and Reymond 2007; Blum and Reymond 2009).

Molecular docking is the process of predicting the binding site of a ligand on the surface of a receptor. Docking methodology employs a search algorithm which examines various relative conformations of the ligand and the receptor for minimum binding energy, and then using a scoring function, ranks different binding conformations. The process helps in predicting sites where ligands can potentially bind to a receptor, and their capability of binding to the target can be compared, resulting in a reduced sample space of potential lead molecules that can be further screened by experimental HTS. Docking methodologies are categorized as (i) rigid docking and (ii) flexible docking. In rigid body docking, the receptor and ligand are considered rigid. Various conformations are generated based on surface complementarity and then ranked based on electrostatic and van der Waals interaction. ZDOCK (Pierce et al. 2014) is an example of a popular rigid docking software. On the other hand, flexible docking methodology uses a scoring function that recognizes that for protein-ligand complexes, side-chain flexibility plays an important role. Autodock Vina (Trott and Olson 2010) is a popular docking software that is based on flexible docking methodology. Although rigid docking can efficiently explore the shape complementarity between binding partners, the method may not work when one wants to dock two proteins that have been crystallized separately. Using even a very complicated and fine-tuned scoring function may not remove the false structures that have high scores. Thus, although rigid docking softwares like ZDOCK have high accuracy, the resulting complexes are not very useful in designing inhibitors, especially for the protein

interfaces. The flexible docking algorithms have an advantage in this regard. It was found that glucose adsorption decreased on the inhibition of α-glucosidase resulting in a decrease in blood glucose level which hints towards α-glucosidase being a target for curing diabetes. Park et al. (2008) screened a library of 85,000 compounds using autodock and arrived at four novel inhibitors.

Usually, the target structure is not fixed but undergoes fluctuations at a finite temperature. Thus, using a single static target structure leads to an inaccurate prediction of binding sites. MD simulations are used to generate an ensemble of target conformations. At room temperature, an MD trajectory will consist of successive target conformations that lie very close to the local minimum corresponding to the starting conformation. To get rid of this, various advanced simulation methods like replica exchange MD (Zhou 2007), accelerated MD (Hamelberg et al. 2004), metadynamics (Laio and Gervasio 2008) etc. are used. Replica exchange MD involves performing parallel MD simulations at different temperatures. Frequent conformation exchange is performed between replicas at different temperatures with a certain probability that is dependent on the energies of the replicas. A replica corresponding to a higher temperature, when simulated at a lower temperature may thus relax to a minimum on the energy surface that is different from the minimum the system started from. Metadynamics is an enhanced sampling technique in which a system prepared in a local minimum is time evolved and at appropriate intervals, a gaussian hill, centred at the current location of the system along the reaction co-ordinate, is added. With repeated addition of such hills, the local potential well gets filled up with gaussians, and the system falls into another nearby minimum where the process of addition of the gaussian hills continues. The system can thus jump barriers and explore the complete potential energy surface. The depth of a particular well is measured by adding all the gaussian hills needed to fill up the potential energy well corresponding to that minimum. Metadynamics is also used in flexible docking algorithms to generate an accurate free energy surface. Accelerated MD is yet another enhanced sampling method in which the potential energy landscape is modified by raising energy wells for which the minimum lies below a certain energy threshold. This reduces barriers separating adjacent energy basins and allows the system to sample conformational space out of the local minimum it is prepared in. The conformational sampling obtained on the modified potential energy surface is reweighed to trace back the sampling on the true potential energy surface. The conformations generated using these enhanced sampling MD methods are then used to perform docking. The discovery of raltegravir, which is the first clinically approved inhibitor of HIV integrase, is attributed to this approach (Summa et al. 2008).

Accurate binding free energy calculations are crucial in comparing the binding specificity and affinity of different ligands to a target. An accurate calculation of free energy is a challenging task and developing techniques in this regard is an active area of research. Many free energy techniques are in use currently. Umbrella sampling (Torrie and Valleau 1977) is the most commonly used equilibrium free energy technique. If a system is in a free energy well, the fluctuations of the reaction coordinate (RC) (the coordinate as a function of which the PMF profile has to be calculated) during an MD simulation, about the minimum can be used to calculate

the free energy profile in the neighbourhood of the minimum. Umbrella sampling involves performing many such simulations with the system restrained using harmonic bias potentials at different points along the RC. A combination of the sampling at different values of the RC can be used to construct a free energy profile as a function of the RC (Kumar et al. 1992). Methods like metadynamics and accelerated MD can also be used to perform long enhanced sampling simulations leading to accurate free energy surfaces. A large set of steered MD simulations (Li and Mai 2012), in which the system is pulled along the reaction co-ordinate at a finite constant velocity, by the application of an external force, can be used to calculate the free energy profile using the Jarzynski equality, $e^{-\beta\Delta F} =< e^{-\beta W} >$ (Jarzynski 1997). The relation connects the non-equilibrium work done, W to the equilibrium free energy difference, ΔF between the starting and ending conformations of a non-equilibrium process (Park et al. 2003; Fox 2003). The ensemble average suggests that a large number of steered MD simulations are required to obtain a reliable result (Gore et al. 2003). Various hybrid simulation methodologies are being developed to obtain accurate results within the boundary of the limited simulation wall time. A notable example is the use of steered MD with accelerated MD (Mcksch and Urbassek 2016) to achieve lower pulling speeds in silico, which would lead to more accurate non-equilibrium calculation of free energy profiles from MD simulations.

With an increase in computational power and the development of faster and efficient supercomputers, μs to ms long MD simulations have been possible. Such long simulations can be used to accurately predict the binding site and the corresponding binding energy of a drug molecule. As a notable example, Shan et al. (2011) performed multiple 100 μs long simulation of systems consisting of Src kinase, with dasatinib (a cancer drug) or PPI (kinase inhibitor), randomly placed inside the simulation box. The unguided MD simulations not only succeeded in recognizing the crystallographic binding site of the drugs, but also resolved the location of waters in the X-ray structure of Src-PPI complex.

4 MD Simulations in the Development of Drug Delivery Strategies

To ascertain high drug efficacy, it is important to make sure that the drug is delivered at the intended site. Biomolecules are multivalent and show favourable interaction to a lot of different kinds of molecules. Drug molecules, thus, can get consumed before they reach the intended site. Another aspect of drug interactions inside the human body is its ability to permeate the cell membrane. Some drugs might be strongly hydrophilic and may refuse to interact with the cell membrane to enter the cell. These issues need to be addressed to increase the efficiency of the drug. Effective drug delivery strategies need to be developed to achieve this aim. The drug molecule is carried by another molecule which either has a favourable interaction with the target site or can permeate the cell membrane and carry the drug molecule inside

the cell. The birth of the field of drug delivery dates back to the time when it was observed that some molecules showed enhanced permeability and retention (EPR) effect in tumour regions (Golombek et al. 2018). The accumulation resulted from a change in the interaction properties of the affected tissues. It was realized that the drug molecules can be sent directly to the affected area using, as carriers, different macromolecules that show favourable interaction with the drug molecules and the affected tissues.

MD simulations play the role of supporting experimental studies in determining the appropriate carrier for a particular drug. Free energy calculations help in determining which macromolecules the drug molecule can strongly hold on to. Many MD simulations have been performed to determine the capability of a macromolecule to puncture the cell membrane and facilitate the entry of the drug molecules into the cell. MD simulations also help in determining how the structure and nature of drug molecules and other biomolecules change when they interact with their carriers.

4.1 Dendrimers as Drug-Carriers

Dendrimers are highly branched polymers. The dendrimer structure includes a central core, branching units and surface groups (Fig. 3). The structure of the dendrimer is highly controllable, and the synthesis methodologies are well established. The dendrimer can be used to encapsulate drugs in its voids. A knowledge of the change in the structure of the dendrimers on drug encapsulation is important. Also the release

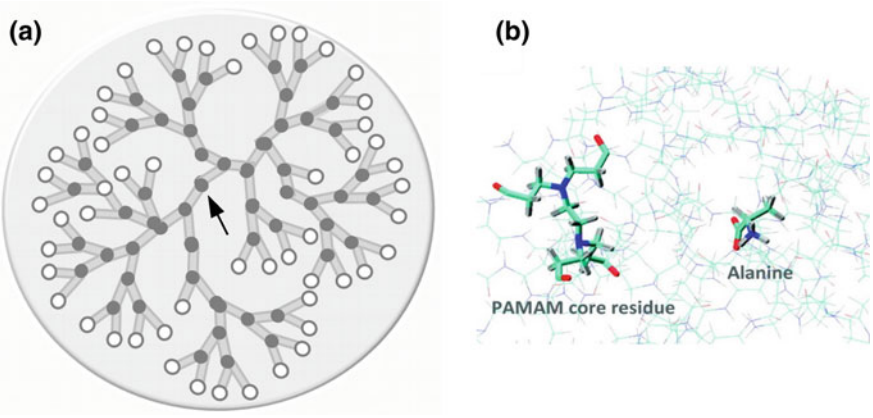

Fig. 3 **a** The structure of dendrimer showing the core (arrow), branching units (grey circles) and surface groups (white circles). The cavities that can be used to house the drug molecules are also visible. The number of layers of branching units around the core defines the generation of the dendrimer. **b** An l-alanine molecule caged in a PAMAM dendrimer void. Adapted with permission from Maingi et al. (2012). Copyright 2012 American Chemical Society

rates of the drug molecules from the dendrimer structure need to be determined. In addition, one needs to design strategies to increase the ability of the dendrimer for drug uptake and retention.

Maingi et al. (2012) performed MD simulations of various drug molecules (soluble drugs salicylic acid and l-alanine, and insoluble drugs phenylbutazone and primidine) contained inside the PAMAM dendrimer. They used the docking software AutoDock4 to dock the drug molecules in (G5)-PAMAM dendrimer. They performed PMF calculations using the umbrella sampling method and calculated the free energy as a function of the center of mass distance between the dendrimer and the drug. They found that the stabilization on encapsulation for the soluble drugs was lower as compared to the insoluble ones. From the energy barriers observed in the PMF profiles, they could rank the drugs based on their ease of release from the dendrimer interior. The soluble drug l-alanine faces the lowest energy barrier while phenylbutazone faces the largest barrier to release. Comparing there results to the experimental results of Zhao et al. (2010) they concluded that soluble drugs like the salicylic acid and l-alanine cannot be encapsulated inside the dendrimer and if the molecules do enter the dendrimer, they cannot be retained due to low energy barriers. In other words the encapsulation is diffusive rather than free energy driven. The insoluble drugs show large stabilization in the encapsulated state and the stabilization increases as the size of the insoluble drug increases. They also studied the pH dependence of drug-dendrimer interaction. For non-protonated G5 PAMAM dendrimer (corresponding to pH = 10) they found that the dendrimer-drug system achieved a stabilization of 9 kcal/mol, while for the protonated dendrimer (corresponding to pH = 7), the stabilization energy was 42 kcal/mol. Thus at low pH, the dendrimer retains the drug strongly and a controlled release of the drug is possible. The pH inside the human body is around 7.4 and thus the release of the drug into the blood stream will be in a controlled manner. The PMF profiles can be combined with theoretical tools of statistical mechanics like the Smoluchowski equation to churn out numbers for the release rates. There are some aspects which the simulations could not capture. For example, it is known that at low pH it is harder to encapsulate the hydrophobic drugs in the dendrimer. The PMF calculations in this work show that at pH of 7, the energy gap between the encapsulated and free state of the drug is 42 kcal/mol. This suggests that the drug should be gulped in with a larger force by the dendrimer at low pH as compared to that at high pH. The simulations cannot capture the polarisation of the otherwise nonpolar cavities of the dendrimer at low pH which should give rise to an energy barrier to encapsulation at the dendrimer surface. Constant pH MD simulations would be able to catch this effect better.

Earlier, Teobaldi and Zerbetto (2003) performed MD simulations on a G4 propylene amine dendrimer dissolved in CH_2Cl_2. They performed the simulations in the presence and absence of encapsulated eosin Y dyes. They showed that in presence of the dye molecules the cavities in the dendrimer that enclose the molecules shrink. The simulations were carried out in presence of a maximum of 12 dye molecules encapsulated in the dendrimer. MD simulations showed that after multiple entry-exit events, the number of encapsulated molecules equilibrate at a value of 6 which sec-

onds the experimental findings. The study demonstrates that MD simulations can be used to measure the drug-carrying capacity of potential drug-carriers.

Liu et al. (2009) performed explicit water MD simulations of PAMAM dendrimer to study the pH dependence of dendrimer structure. They observed that the size of the dendrimers were similar at different pH values but the internal structure showed dependence on pH. At high values of pH the dendrimers had dense cores but at low pH the density was higher at the periphery of the dendrimer. The study was important because the dendrimers have to carry the drug molecules in physiological pH conditions that range from 5 to 7.4. An understanding of the conformation of the dendrimers in these conditions is important to have an insight into the drug-release and retention properties of the dendrimer.

In order to increase the drug-carrying capacity of the dendrimers, various modifications may be made to their structure. Dendrimers are open to different modifications and a multivalent surface with multiple termini allow for external groups to be added. It is important to know what kind of structural change is imparted to the dendrimer after it is chemically modified. And also, how the modifications affect the drug-carrying capacity of the dendrimer. Li et al. (2010) performed MD simulation of protonated PPI dendrimer covered with 3,4,5-tris(tetrathyleneoxy) benzoyl units attached with PEG polymers. They showed that PEG modification increased the biocompatibility of the dendrimer. They performed the simulation of protonated and PEGylated PPI-5 dendrimers and obtained crucial information regarding the shape of the dendrimer and distribution of the PEG tails. They further studied the interaction of Bengal rose molecules with the modified dendrimer and found that the radius of gyration of the dendrimer decreases with increase in the number of encapsulated molecules, a result that is consistent with the experimental findings. To understand the effect of the PEG tails, they performed MD simulation of the unmodified and the PEG-modified PPI-5 with 32 Bengal rose molecules docked in. They found that the modified dendrimer could retain all the 32 dye molecules.

While trying to use dendrimers as drug carriers, it is important to understand their interaction with the cell membrane. Experimental studies in the past have revealed a dendrimer type, generation, surface chemistry and solution pH dependent interaction with the cell membrane (Mecke et al. 2005; Hong et al. 2004; Majoros et al. 2009; Nyitrai et al. 2013; Parimi et al. 2008; Ruggeri et al. 2013; Lombardo et al. 2016; Ainalem et al. 2010; Berenyi et al. 2014; Evans et al. 2014; Zeng et al. 2016). For example, ellipsometry and neutron reflection studies revealed that amine terminated G2 and G4 dendrimers penetrate the cell membrane while keeping it in tact, while the G6 dendrimers lead to disruption of the membrane structure. MD simulations have also contributed to understanding the nature of interaction between the dendrimer and the lipid bilayer and also the effect of the presence of the dendrimer on the structure and properties of the lipid bilayer. The early MD simulations to study the dendrimer-bilayer interaction were performed using the MARTINI coarse-grained model. Lee and Larson performed MARTINI level CG simulations of many aspects of dendrimer-bilayer interaction (Lee and Larson 2006, 2008a, b, 2011). From the MARTINI CG simulation of acetylated and unacetylated G5 and G7 PAMAM dendrimers with the DMPC bilayer (Lee and Larson 2008a), they found

that the acetylated dendrimers aggregated while the unacetylated dendrimers did not. The acetylated dendrimers did not show any insertion into the bilayer, even at high concentrations. When the simulation system contained only 1 unacetylated dendrimer, no insertion of the dendrimer was observed. while, when the dendrimers were present at higher concentrations, there was insertion, thus indicating that there was co-operativity. In systems with high unacetylated dendrimer concentration, the bilayer demonstrated a curved structure. It was observed that the dendrimers that were inserted into the bilayer resided in the region of positive bilayer curvature. Thus, it was shown that the dendrimer insertion is concentration dependent but is independent of dendrimer aggregation. The acetylated systems showed zero bilayer curvature and no dendrimer insertion even at high dendrimer concentration. In another study, MARTINI CG simulation (Lee and Larson 2006) of G3 and G5 PAMAM dendrimers (acetylated and unacetylated) at different salt concentrations in presence of the DPPC bilayer demonstrated that completely acetylated dendrimers do not show insertion into the bilayer while unacetylated G3 and unacetylated and partially acetylated G5 PAMAM shows insertion. Only unacetylated G5 PAMAM showed significant pore formation while the pores healed once the dendrimer was removed. It was also shown that as the salt concentration increases the unacytelated G5 PAMAM does not insert into the bilayer resulting from a reduction in the electrostatic interaction between the dendrimer and the bilayer. MARTINI simulations have also been used to demonstrate the molecular shape dependence on membrane disruption (Lee and Larson 2008b). Simulation of MARTINI DMPC bilayers with poly l-lysine and charged PAMAM dendrimers showed that the spheroidal shape is more effective in disrupting the structure of the bilayer. In yet another CG simulation study (Lee and Larson 2011) it was demonstrated that acetylated and PEGylated dendrimers form lesser pores than the charged ones. 50 % PEGylation resulted in no pore formation. The pore formation also reduced with increase in the size of PEG. The CG scheme thus allows long simulations of the bilayer-dendrimer systems, up to time scales not currently accessible with the atomistic scheme, but suffers from the demerit of not being able to account for the hydrogen bonding and hydration effects. There have been quite a few atomistic studies in this field. Kelly et al. performed implicit solvent atomistic MD simulations of the PAMAM dendrimers with DMPC bilayer. Calculation of free energy of interaction using umbrella sampling showed that the interaction depends on the chemical nature of the surface group termination. Carboxylic acid-terminated dendrimers showed a stronger binding (47 kcal/mol) as compared to the charged amine-terminated ones (36 kcal/mol). Whereas, the neutral acetamide-terminated dendrimers had a free energy of binding of 26 kcal/mol. Kim et al. (2014) performed explicit solvent atomistic MD simulation of G3 PAMAM dendrimer with DPPC and POPG bilayers and obtained the free energy of binding of 50 kcal/mol and 120 kcal/mol respectively. Bhattacharya et al., through explicit solvent atomistic MD simulation of 5 G4 PETIM dendrimers with the DMPC bilayer demonstrated dendrimer insertion, while the G3 dendrimers were bound to the surface and showed no insertion. In a recent study Subbarao et al. (2018) performed 200 ns long MD simulation of the interaction of G3 PAMAM dendrimer with the DMPC bilayer and compared the performance of different force fields.

In addition to being used as a drug-carrier, the dendrimer has also been used as a drug. The gp120 protein of HIV-1 interacts with the CD4 receptors of the T-cells and induces viral entry. The drugs that prevent the gp120-CD4 binding are known as entry-inhibitors. The G4 SPL7013 dendrimer is known to act as an entry-inhibitor against HIV-1 and is an active component of the microbicide Vivagel. To tune its properties as an entry-inhibitor, its mechanism of action needed to be determined. Nandy et al. (2015) performed MD simulation of the gp120-CD4 complex in explicit solvent. They performed similar simulations with the SPL7013 dendrimer bound to gp120 in the gp120-CD4 complex. Then they performed steered MD simulations to break the gp120-CD4 complex. They found that the rupture force in the presence of the dendrimer was lower than that in its absence (Fig. 4). On an analysis of the gp120-CD4 contacts, they found that some important gp120-CD4 contacts did not exist in the presence of the dendrimer. They further found that in the presence of the dendrimer, the gp120 in the gp120-CD4 complex acquires a tilt relative to CD4, that leads to an increase in the distance between residues that constitute inter-protein interaction pairs, resulting in the breaking of the contacts. They demonstrated that the dendrimer can bind the gp120 protein on multiple locations, leading to multiple possible modes of entry-inhibitory action, which, in turn, would increase the genetic barrier of the dendrimer *vis a vis* HIV entry. Prior to this, Nandy et al. (2013), in another work had demonstrated the entry-inhibitory properties of the PAMAM dendrimer using a similar methodology.

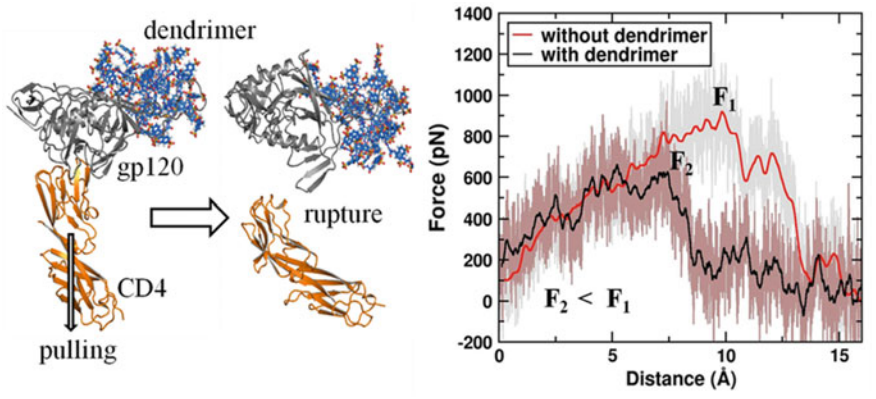

Fig. 4 Snapshots from a steered MD simulation with gp120 (gray) fixed and the CD4 (orange) being pulled along the direction of the vector joining the centers of mass of gp120 and CD4, with the SPL7013 dendrimer (blue) bound to gp120. The force v/s pulling distance plots in the presence and absence of the dendrimer are shown on the right. One notes that the presence of the dendrimer leads to a reduction in the rupture force of the gp120-CD4 complex. Adapted from Nandy et al. (2015) with permission from The Royal Society of Chemistry

4.2 Carbon Nanotubes for Translocating Drugs and Drug-Carriers

Single-walled carbon nanotubes (CNTs) have a cylindrical structure and are hollow. It has been found that functionalized CNTs can rupture cell membranes (Kostarelos et al. 2007; Lacerda et al. 2013; Pogodin et al. 2011) through an endocytotic pathway (Shi Kam et al. 2004; Shikam and Dai 2005), thus creating a path for drug molecules, carriers and genes to enter the cell, which would rather interact unfavourably with the cell membrane. Functionalization of the CNTs is needed to make them biocompatible and the resulting biodegradability makes them relevant as biomaterials. Both the outer surface of the CNT and region within can be used as interaction regions for various drug molecules and can also be used to house DNA and RNA for gene delivery. There have been many MD studies to understand how various molecules interact with the CNT and what kind of structural changes are induced in them as a result of this interaction.

Chen et al. (2009), used equilibrium and non-equilibrium MD simulations to study the encapsulation of peptide drug Zadaxin inside CNT in an aqueous environment. They found that Zadaxin was spontaneously encapsulated by the CNT. In their simulation, the peptide spontaneously entered the tube and stabilized near its centre. They found that the encapsulation is driven by the vdW interaction between the peptide and the CNT. They also investigated the effect of the tube diameter and tube length on the encapsulation process using steered MD simulations. They pulled the peptide through CNTs with lengths of 4.90, 7.53 and 9.80 nm and observed higher peaks in the force-displacement plots as a function of increasing length. They performed similar simulations with CNTs of different diameters, (14, 14), (16, 16), (18, 18) and (20, 20). The force-displacement plots for (14, 14) CNTs showed higher peaks and the peaks decreased as a function of increasing diameter. The CNT-peptide vdW interaction showed shallower potential wells with increasing CNT diameter.

Sahoo et al. (2018) performed extensive MD simulation of the encapsulation process of different biological and synthetic molecules into CNT. They started by performing MD simulations of CNTs embedded in lipid bilayers and found that the CNTs with length similar to the width of the lipid bilayer are stable and stay oriented with their axes perpendicular to the plane of the bilayer. While, longer CNTs tended to align with their axes parallel to the bilayer plane, an effect that can be attributed to the hydrophobicity of the nanotube. They further demonstrated spontaneous encapsulation of four different kinds of molecules namely dendrimer (PAMAM and PETIM), ubiquitin, asiRNA and ssDNA inside the membrane-embedded CNT (Fig. 5a). While studying the spontaneous encapsulation they made the important observation that while the PAMAM and PETIM dendrimers could spontaneously enter a (20, 20) CNT, the ubiquitin could not do so. It only showed spontaneous encapsulation for CNTs with a larger diameter of cross-section like the (28, 28) CNT. They attributed this effect to the higher structural rigidity of ubiquitin. Similar difference was observed for the ssDNA which could spontaneously enter a (16, 16) nanotube with the asiRNA which could enter only a (20, 20) CNT owing to its

larger diameter and rigidity. A calculation of the structural parameters like the radius of gyration and asphericity revealed that the more hydrophobic PETIM dendrimer showed a larger structural deviation on encapsulation as compared to the PAMAM dendrimer. Ubiquitin showed minimal structural change on encapsulation. For the asiRNA, the terminal bases stacked with the CNT wall while for the ssDNA all the bases showed stacking with the CNT wall. The structural changes induced in the molecules thus depend strongly on their nature and mode of interaction with the CNT, while the structural changes seem to be reversible. They used umbrella sampling to calculate the PMF profiles as a function of the distance between the molecules and the center of the CNT. The PMF profiles were again a reflection of the nature of the molecules and depended on how they interact with the CNT. For example, the stabilization on encapsulation was higher for the more hydrophobic PETIM dendrimer as compared to the PAMAM. The asiRNA showed larger stabilization as compared to the ssDNA because of the larger number of vdW contacts that the asiRNA can form with the CNT wall. Ubiquitin showed the least stabilization owing to the fact that the folded protein has few hydrophobic amino acid residues on its surface. But nevertheless, the PMF profiles showed that the molecules can be spontaneously encapsulated. Theoretical calculation of the encapsulation and translocation times using backward Smoluchowski equation revealed an infinitely long translocation time for the molecules across the CNT, suggesting that the molecules can enter the CNT but can never come out of it on their own. To overcome this and to make the molecules translocate through the CNT, the authors suggest making use of the fact that a CNT of lower diameter can spontaneously enter another CNT with a larger diameter. They demonstrate that a CNT of lower diameter than the encapsulating CNT can push the molecules out, thus, mechanically forcing them out of the CNT and into the cell (Fig. 5b). The same concept of release of a drug from the inside of a CNT through competitive replacement was proposed earlier by Cheng et al. (2009) and demonstrated using explicit solvent MD simulations. In addition to suggesting that a CNT of smaller diameter can be used to eject an encapsulated molecule from the inside of a larger CNT, Cheng et al. suggested that the opposite process can also be used to remove a molecule attached on the outer surface of a smaller CNT. They also demonstrated that a peptide stuck inside a CNT can be ejected by another larger peptide.

4.3 Drugs in Liposomes

Liposomes are nanoparticles, spherical in shape and consist of one or more concentric lipid bilayers about 20–200 nm in size. Encapsulation of drug molecules in the interior of the liposome leads to a significant reduction in drug accessibility to normal tissues. Using Liposomes as drug-delivery vehicles is an attractive aspect for drug delivery to solid tumours and inflammation sites. The liposome shows EPR effect for tumour tissues. The study of drug interaction with the liposome was initially carried out indirectly by studying the drug interaction with flat lipid bilayers. Bassolino et al.

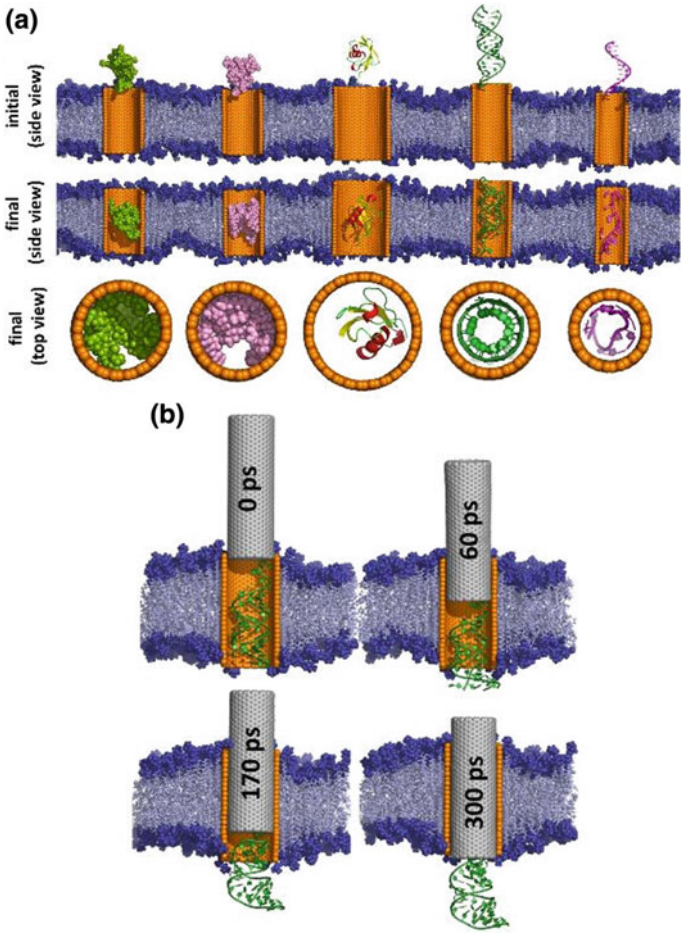

Fig. 5 **a** Snapshots from the simulation of various different biomolecules initially placed at the entrance of bilayer-embedded CNT. **b** Snaphots from a simulation, showing a CNT competitively replacing a biomolecule from the interior of a wider CNT. Adapted with permission from Sahoo et al. (2018). Copyright 2018 American Chemical Society

(1993, 1995) performed MD simulation of benzene molecules in DMPC bilayer. The simulations revealed that the benzene molecules perform a caged motion in the voids of the bilayer followed by jumps between voids. Pohorille et al. (Pohorille and Wilson 1996; Pohorille et al. 1996) performed MD simulations to evaluate the partition coefficients for polar and non-polar molecules from water into a lipid bilayer. Dipolar molecules were seen to accumulate at the lipid-water interface while the non-polar molecules showed a decrease in free energy when they move from water to the interior of the bilayer. Xiang et al. (2002) performed MD simulation of an anti-cancer drug

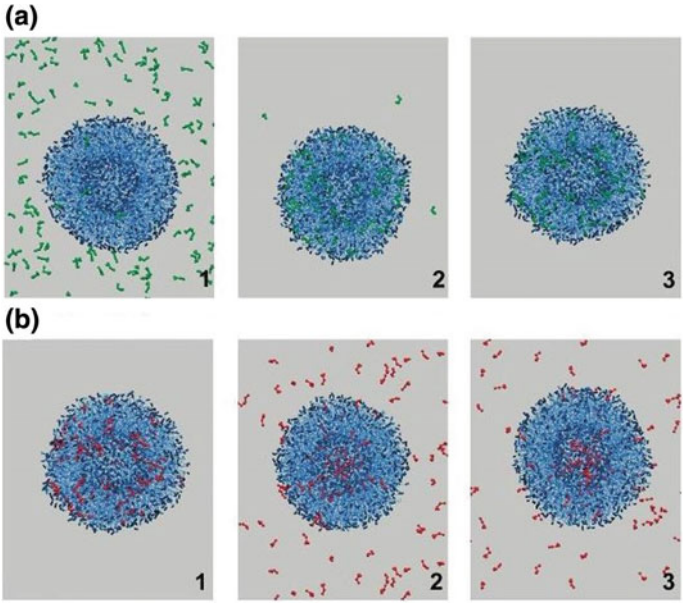

Fig. 6 **a** Simulation of neutral prilocaine and liposome with initial configuration such that all the drug molecules are outside the liposome. **b** Simulation of protonated prilocaine and liposome with initial configuration such that all the drug molecules are embedded inside the liposome. The snapshots correspond to 0 ns (1), 250 ns (2) and 1.25 μs (3). Adapted with permission from Pickholz and Giupponi (2010). Copyright 2010 American Chemical Society

DB-67 and a protonated prodrug DB67-AB in a DMPC bilayer. They found that the molecules oriented such that both the polar and non-polar interactions are satisfied.

As the liposomes are bulky structures, the atomistic simulations of liposomes over a long time scale are not feasible. The CG simulations play an important role in allowing for long simulations to study the drug-liposome interaction and associated phenomena in more detail. Using MARTINI model, Pickholz and Guipponi (2010) studied the interaction between liposome and a local anaesthetic called prilocaine. Under physiological conditions, prilocaine is found in both neutral and protonated states. It was seen that most of the neutral drug molecules get attached to the liposome within 200 ns (Fig. 6a). On the other hand, the protonated drug molecules do not enter the vesicle and do not get attached to it (Fig. 6b), while some of them interact with the membrane and get attached and detached intermittently. While, the interaction of the protonated drug with the liposome was more structured, with the charged tail of the drug molecules oriented towards the membrane, the neutral drug molecules did not display any preferred orientation. The simulation reveals that the driving force responsible for encapsulation is mostly hydrophobic. In yet another study, Jambeck et al. (2014) used the MARTINI model to study the interaction between hypericin, a neutral compound used in photodynamic therapy, and the liposome. They encapsulated 21–84 hypericin molecules in the liposome. They found that an

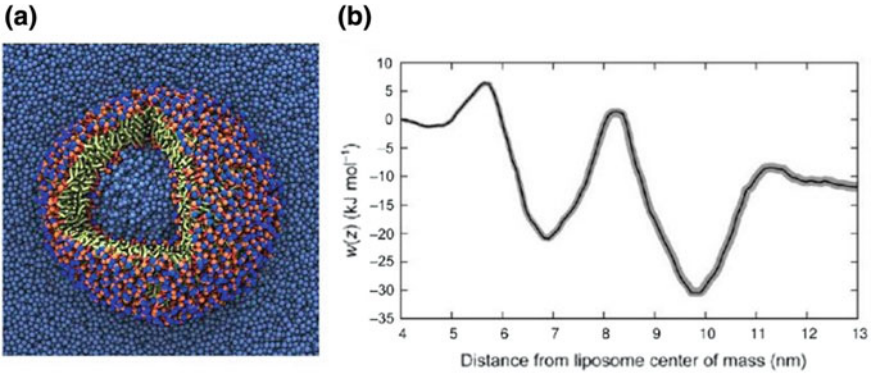

Fig. 7 **a** The MARTINI liposome in a water box. **b** The PMF profile to transfer a drug molecule from the centre of the liposome to its exterior, the difference in the interaction in the inner and outer layer of the liposome can be easily seen. Adapted with permission from Jambeck et al. (2014). Copyright 2013 American Chemical Society

increase in the amount of hypericin leads to expansion of the liposome. Most of the hypericin molecules reside near the polar headgroup of the outer lipid bilayer and the orientation is such that the hydrophobic and hydrophilic interactions are maximized. At high drug loading, the drug molecules aggregate through π-stacking, with only a few drug molecules penetrating inside the liposome. They calculated the PMF profile for the drug-liposome interaction with the distance between their centers of mass as the reaction co-ordinate. They found that the inner layer had a higher barrier to penetration and a shallower well-depth of penetration as compared to the outer layer (Fig. 7b), something that originates from the difference in lipid density and curvature of the two layers.

4.4 DNA Nanostructures for Drug Delivery

Recently, DNA origami techniques have been used to build a variety of structures. DNA icosahedra, which are cage-like structures, are one among them. Drug molecules and various imaging agents can be encapsulated inside these cages and then can be delivered to the target site. MD simulations have been performed to check whether these structures are stable in aqueous environment. Joshi et al. (2017) performed extensive explicit solvent MD simulations and measured structural properties of a DNA icosahedron (Fig. 8a). A knowledge of the structural properties like radius of gyration and average volume are important to estimate how much of cargo the icosahedron can carry. In addition to performing MD simulations on an empty icosahedron, they performed simulations of the structure with gold nanoparticle filled in its void (Fig. 8b). They notice reduced fluctuations in the structural parameters in the presence of the nanoparticle, suggesting that the cargo-loaded icosahedra are more

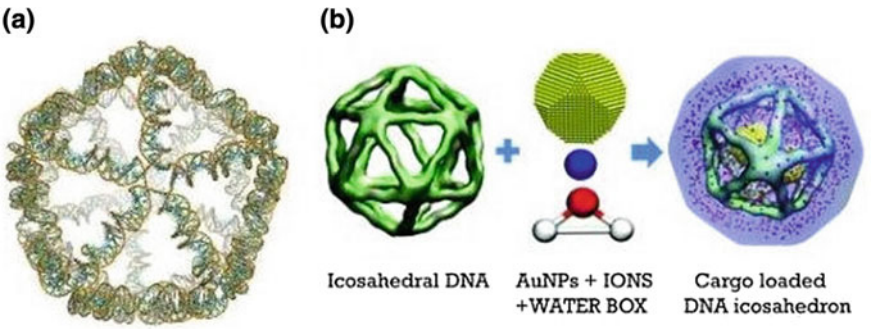

Fig. 8 **a** An empty icosahedron. **b** Schematic of simulation methodology for gold-nanoparticle-filled icosahedron. Adapted from Joshi et al. (2017) with permission from The Royal Society of Chemistry

stable as compared to the empty ones and thus the structures are good candidates for the delivery of drugs and other entities like the imaging agents. Joshi et al. (2018) also performed MD simulation of nanotube structures made up of 6 dsDNA molecules embedded in a lipid bilayer and demonstrated their stability. They found that the stability of the structure embedded in the lipid bilayer increases with an increase in salt concentration. Such structures can also be used in future in drug translocation into cells. Although, more information on the membrane rupture energetics of these structures is desired and awaits more experimental and computational studies.

5 Summary and Conclusion

The chapter presents details of the MD procedure and discusses how computational techniques have been used to predict potential drug candidates and also in developing various drug-delivery techniques. The field of molecular simulations is developing very fast. With the development of more and more powerful machines like the Anton developed by the D. E. Shaw group, larger and larger systems can be subjected to molecular simulations and the systems can be simulated for reasonably long times. With time, ms-long simulations would be routine and long time scale phenomena can be observed in MD simulations. Molecular simulations, in addition to being long, also need to be accurate. A considerable amount of work is being done in developing more and more accurate force fields, that would aid in improving the quantitative resolution of the MD simulations. Forcefields like ff99SB-ILDN and water models like TIP4P-D are some such examples. Developments of methods like the constant pH MD are helping in simulating systems that are closer in behaviour to their experimental counterparts. Hybrid simulation methodologies, like the accelerated steered MD, are being developed which combine the benefits of multiple methods to give rise to more efficient techniques. With these and many other developments taking place at a swift

rate, MD simulations can play a more effective role in the process of drug discovery and hopefully some day a computer will be able to develop a drug, considerably speeding up the process of drug discovery.

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Nanomedicine for Treating Specific Disorders

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Abstract Nanomedicine utilizes the molecular nanotechnology in the form of nanomaterial, and nanobiosensors to modify the properties of the drug for the treatment of human illness. The nanomedicine improves the pharmacokinetics, pharmacodynamics, stability properties of existing drugs. In addition, the nanomedicine serves as a diagnostic tool to monitor the physiological functions of the human body. The nanomedicine formulates the existing drug without using dose-limiting toxic excipients, and therefore nanomedicines reduce the toxicity of the drug. The sustained and controlled release of drug from nanomedicine also enhances the safety and efficacy. Overall, the therapeutic index of a drug is enhanced when the drug is administered in the form of nanomedicine. At present, a numerous number of nanomedicines have been developed to treat a wide range of human illness like cancer, HIV, kidney diseases, angiogenesis, etc. Recently, nanotechnology has been viewed as a revolutionary discipline in pharmaceutical and medical sciences. The advancements in nanomedicines are continuously growing to treat life-threatening diseases such as cancer, HIV, etc. Despite, there is a significant progress in the development of nanomedicines, the clinical translation of nanomedicine remains challenge in drug development. The present review describes the challenges, recent progress in development, therapeutic properties, clinical role and potential outcome of nanomedicine in treating specific human disorders. It will be useful to simplify the monitoring, diagnosis, and curing of diseases in personalized health care.

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1 Introduction

1.1 Nanomedicine

Nanomedicine is the emerging inter-disciplinary field which is associated with nanoscience, nanoengineering, chemical science, and biological science (Whitesides 2005). Nanomedicine is the application of nanotechnology to the field of medicine where the nanoparticle is the key component. Nanomedicine refers to either the size of the drug particle or therapeutic drug delivery system. Nanomedicines are developed as biologically active products or drug delivery systems. The therapeutic system of nanomedicine consists of active ingredient as one of the two components. Nanomedicine is usually referred to as a nanoparticle loaded therapeutic agent to improve the pharmacokinetics and pharmacodynamics properties at the therapeutic target (Matsumura and Maeda 1986). The nanomedicine may show an improvement over the freely administered drug in bioavailability, half-life, enzymatic degradation, drug accumulation, drug release. etc. The nanomedicines enhance the specific target recognition in the vascular and endovascular system. Due to small in size and surface modification in nanomedicines, it interacts efficiently with the binding site of the target and makes a better therapeutic response. Nanomedicines are broadly employed in two different areas of medical sciences, (i) diagnosis and imaging techniques (ii) treatment of human diseases (Fig. 1). Nanomedicines are expected to be the future of medicines (Sahoo 2005).

The size of biologically important molecules are categorized in the following nanoscale- glucose (1 nm), antibodies (10 nm), viruses (100 nm), bacteria (1000 nm) and human cells (10,000 nm) (Fig. 2). The development and implementation of

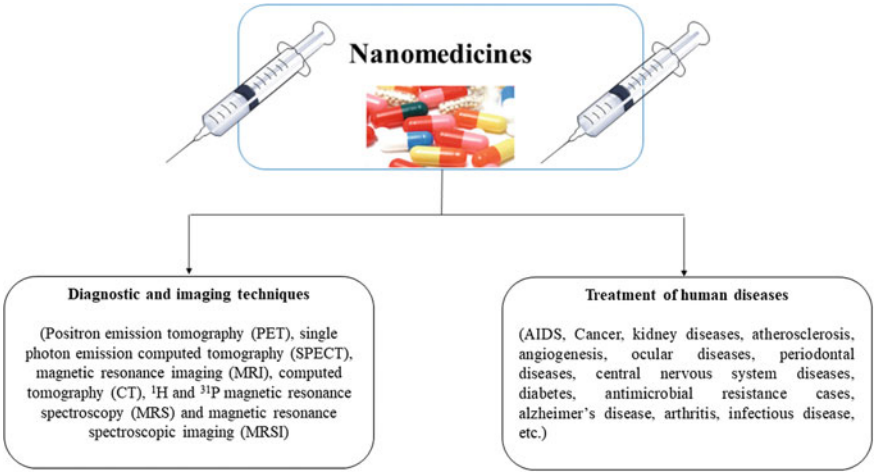

Fig. 1 Application of nanomedicines in medical sciences

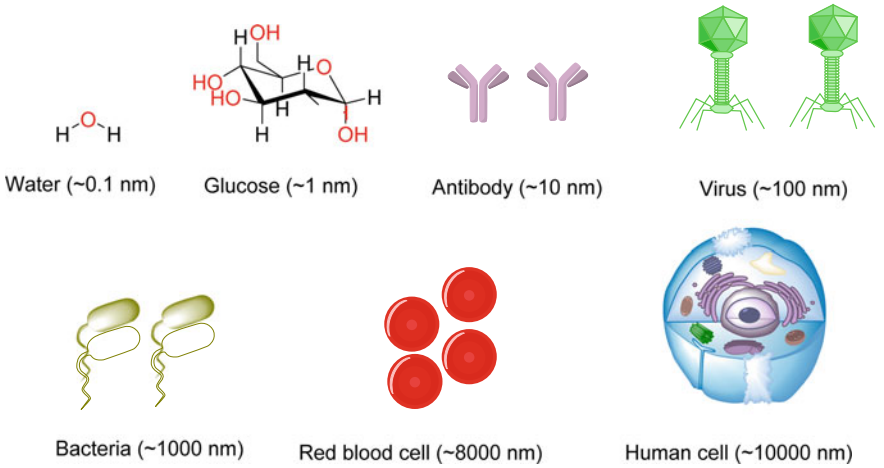

Fig. 2 The nanoscale dimension of the biologically important structure

nanoscale technologies with the aid of drug to prevent, diagnose or treat the diseases are achieved by the use of a nanomedicine. Molecular nanotechnology is greatly employed in the development of nanomedicines for various diseases. Nanotechnology is the design and fabrication of material in nanoscale. Nanomaterials are engineered macromolecules like lipids, polymers, metals, etc. The size of nanomaterials ranges from 1 to 1000 nm (Kreuter 2014). The components of nanoscale provides advantages on physical, chemical and biological interaction over macroscale components. The nanomaterials incorporate the small molecule of drug and small molecules of protein, nucleic acids, etc. The nanomaterials incorporating the drug molecules are usually achieved to control the release of drugs. After the incorporation of therapeutic molecules, the therapeutic molecule is solubilized or the solubility may be increased to higher fold leading to an improved clinical therapy (Singh and Lillard 2009).

1.2 Scope of Nanomedicines

Recently, nanomedicines have gained a special attraction from the researchers. A large number of research articles were reported on the development of nanomedicines. Polymeric nanoparticles, micelles, dendrimers, inorganic nanoparticles, metal-organic complexes, protein nanoparticles, protein cages, polyplexes, liposomes, etc. are widely employed as nano-construct to develop nanomedicines (Fig. 3) (Puri et al. 2009). The active moieties either therapeutic drug or imaging agent is encapsulated with nanoconstruct. The active moiety may be adsorbed or chemically binds with the nanoscale vector. The modified form of the drug is an improvement over drug solubility and drug release profile. Further, the delivery of

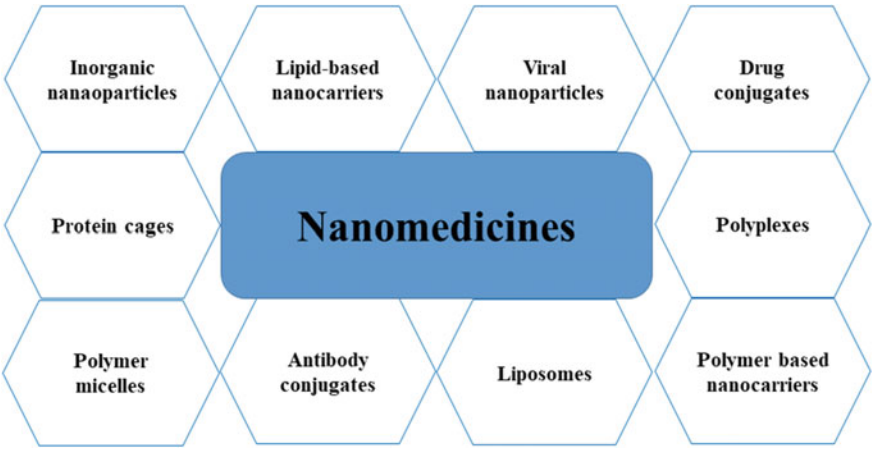

Fig. 3 Different nano-constructs used in the development of nanomedicines

drugs to a cellular or sub-cellular level is also enabled. The therapeutic response is enhanced to a higher fold. Therefore, the nanomedicines have shown a significant impact on the economy. In 2015, the cost of $16 billion nanomedicines was sold in the market. An approximately, $3.8 billion is invested every year in the research sector of nanotechnology (US National Science Foundation 2014). In 2013, 241 research organizations including the industries and institutes were found to be working on the development of nanomedicines (Venkatraman 2014). Nowadays, nanomedicines seem to offer new perspectives in the treatment of several diseases like AIDS, cancer, kidney diseases, atherosclerosis, angiogenesis, ocular disease, etc. More than 200 nanomedicines are under the trials (Etheridge et al. 2013; Min et al. 2015).

Inorganic nanoparticles *include elemental metals, metal salts, and metal oxides. As the size of the inorganic particle approaches nanoscale, novel physical properties are achieved. Silver nanoparticles are used as bactericide* (Asta et al. 2007; Bose and Wong 2015; Gannimani et al. 2016).

Lipid-based nanocarriers *Lipid-based nanocarriers composed of physiological lipids. These carriers are well tolerated and are nontoxic. Lipid-based nanocarriers like niosomes, ethosomes, transfersomes, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were employed to deliver anti-inflammatory drugs* (Chuang et al. 2018).

Viral nanoparticles *Viruses has been found as useful tools for numerous applications. Viruses are considered as nanocontainers to encapsulate synthetic nanomaterials. Drugs, reagents, and imaging molecules are attached to the surface of viral nanoparticles to develop devices for medical applications* (Pokorski and Steinmetz 2010).

Antibody-drug conjugates *In which, the antibody is conjugated with the nanoparticles of the drug. The antibody like drug conjugates are used for the treatment of cancer* (Arruebo et al. 2009).

Protein cages *are formed by the self-assembly of protein subunits. The caged structure has three surfaces units- (i) the interior (ii) the exterior (iii) the inter-subunit. Therapeutic and diagnostic molecules are loaded in the interior of nanocages and the external surfaces are engineered to enhance their biocompatibility and targeting abilities* (Bhaskar and Lim 2017).

Polyplexes *It is a complex of a polymer and DNA and it is designed to protect the DNA in gene therapy. It is an alternative method to use viral vectors in gene therapy* (de Ilarduya et al. 2010).

Polymer micelles *Micelles form when the hydrophobic core of the molecular structure is driven to an interior while the hydrophilic core of the structure is driven outward in water. In a polymeric micelle, the hydrophobic core of the molecular structure is used to hold the drug.*

Nanoemulsion *is an oil in water type emulsion in which the particles are reduced to less than 10,000 nm in the dispersed phase. A nanoemulsion of soyabean oil forms droplets in the range of 400–600 nm and kills viruses and bacteria* (Sonneville-Aubrun et al. 2004).

Dendrimers *are the polymers obtained from synthetic processes. The surface properties are modified for the better interaction with the therapeutic drug. Dendrimers are smaller than cells and can be synthesized in pre-determined size* (Buhleier et al. 1978).

Liposomes *are a spherically shaped vesicle composed of a bilayer membrane. It is used to administer the therapeutic drug. The membrane composed of a phospholipid and cholesterol bilayer. The size, hydrophobic and hydrophilic character of liposomes makes it as promising drug delivery system* (Akbarzadeh et al. 2013).

1.3 Design of Nanomedicine

The particle size is decreased drastically from micro scale to nanoscale in the preparation of nanomedicine. As the size of the particle is decreased, hardness, surface area, chemical reactivity, and biological response are changed (Allaker 2010). Nanomedicines were documented with varying size, composition, and surface properties. The organic materials like lipids, polymers and non-organic materials like iron oxide, silver, gold were employed in formulating nanomedicines. Further, the polymeric coating is applied to modify the surface properties. To maintain or improve the enhanced permeability and retention (EPR) effect and circulation time, the nanomedicine for the treatment of cancer is designed with a spherical shape. The diameter of the sphere in nanomedicine is around 100 nm approximately. The surface of the sphere may be coated with poly ethylene glycol (Klibanov et al. 1990).

1.4 Advantages of Nanomedicines

Nanomedicines are widely investigated for the site-specific delivery of the therapeutic drug in order to treat the multiples diseases. However, the approval of nanomedicines is primarily based on therapeutic efficacy and safety. The drugs available in the form of nanomedicines improve the (i) therapeutic index (ii) pharmacokinetics properties (iii) tissue distribution (iv) tolerability (v) solubility (vi) stability of existing drugs (Sahoo 2005; Ventola 2012). Nanomedicine enables the administration of a larger quantity of drugs to the patients. In the marketed formulation, dose-limiting toxic excipients are used and these excipients are eliminated in nanomedicines. Therefore, the toxicity is reduced when the drug is formulated as nanomedicine. The size, charge, shape, type of surface modification, and biocompatibility of the drug molecules have a greater influence in the delivery of nanomedicines. Administration of the drug in the form of nanomedicine would be a newer therapeutic approach for the existing diseases. The nanomedicines may show the significant outcome in therapy. The therapeutic approaches based on the use of nanomedicines decrease the degree of invasiveness and surgical methods. It also reduces the rate of mortality and morbidity (Sahoo 2005; Ventola 2012).

1.5 Disadvantages of Nanomedicines

The cost of nanomedicines is high. The success rate in the translation of nanomedicines from clinical trial to the market is also low. It is due to the lower therapeutic efficacy of nanomedicine in a clinical trial (e.g. Opaxio™ against anti-cancer), unknown toxicity or poor understanding of disease biology. The translation of nanomedicine into the clinic is a time-taking process (Hare et al. 2017). The approach involves sophisticated bio-analytical techniques like magnetic resonance, gamma scintigraphy, SPECT imaging, etc. The toxicity and an environmental impact of nanomedicine is a major concern. Therefore, the ethical issues related to the safety of nanomedicines is a serious concern (Gebel et al. 2014). Recognition and uptake of micelle delivery system of anticancer drugs by tumor cells remain a considerable challenge.

2 Analytical Techniques in the Characterization of Nanomedicines

2.1 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive diagnostic technique and is used to observe the anatomy and physiological process of the human body. It is based on

the principle of nuclear magnetic resonance. It involves the interaction of atomic nuclei with each other and the surrounding molecules in a biological tissue. MRI uses magnetism, radio waves, and computer to produce images of human anatomy. The technique involves an absence of radiation and high resolution. Tumors, sports injuries, spinal injuries, vascular abnormalities, pathological conditions of soft and bone tissues, etc. were studied using MRI technique (Pan et al. 2010). Miller et al. studied the bio-distribution of magnetic nanoparticles and fluorescent version of therapeutic nanoparticles using imaging technique by injecting the nanoparticles in mice. The study identified the EPR effect of nanoparticles in the development of nanomedicines (Miller et al. 2015).

2.2 Immuno-Fluorescence Techniques

Fluorescence is the property of molecules to absorb the light at one wavelength and to emit the light at longer wavelength when it is illuminated at different wavelength by light. Fluorescent techniques are used to visualize the antigen and antibody binding. Fluorescein isothiocyanate and tetramethyl rhodamine isothiocyanate are used as a dye in fluorescent technique. In this technique, the antibody is linked with the dye to react with its corresponding antigen. Immuno-fluorescence technique is mainly used in the characterization of nanomedicine delivery (Kawamura and Aoyama 1983). Zhang et al. studied the effect of captopril in the delivery of nanomedicine against cancer using immuno-fluorescence staining. Immuno-fluorescence staining indicated the distribution of nanoparticle in tumor slices after the treatment by captopril. The strategy has shown an improvement in tumor perfusion, and an enhanced vessel permeability in the delivery of nanomedicine against cancer (Zhang et al. 2017).

2.3 Electron Microscopy

Electron microscopy is used as a diagnostic tool in the discovery of nanomedicine. It provides a high resolution image by magnifying the sample of nanometer scale. The beam of electron is used as a source to obtain the image of the sample. The wavelength of an electron beam is 100,000 times lower than visible light. Therefore, the resolution is very high in comparison to the light microscope. Transmission electron microscopy, scanning electron microscopy, analytical electron microscopy, scanning electron microscopy is the type of electron microscopy, mostly employed to gain in-sights on molecular structure, morphology, topology, and composition of a material (Hall 1953). Yao et al. synthesized ginsenoside Re-based carbon dots for the effective inhibition of cancer cells. The diameter of Re-carbon dot is 4.6 $\pm$ 0.6 nm with an excellent luminescent properties and is facilitated by cellular uptake. The study used transmission electron microscopy to characterize the carbon

dots. Re-based carbon dots have shown bioimaging and anticancer effect (Yao et al. 2018).

2.4 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a preferred method to characterize the functional groups present in a given sample. During FTIR analysis, the infrared radiation is passed into the sample; some of the radiation is absorbed and some of the radiation is transmitted. The resulting spectrum creates finger print of the sample and is useful in the analysis of a given sample (Stuart 2005). Boussoufi et al. employed FTIR in the synthesis and study of cell-penetrating peptide-modified gold nanoparticles. In many cases, gold nanoparticles have been found to be useful for the treatment of cancer. FTIR was used to confirm the presence of peptide in gold nanoparticles. The study has been proposed to replace cancerous tissue with healthy soft tissue (Boussoufi et al. 2018).

3 Nanomedicines for Treating Specific Disorders

Nanomedicines research area mainly covers the development of a nanoscale delivery system for the controlled and targeted delivery of drugs and diagnostic agents against various diseases. Recently nanomedicines for AIDS, cancer, kidney diseases, angiogenesis, atherosclerosis, ocular diseases, periodontal diseases, etc. were reported. However, most of the research work was devoted to cancer therapeutics.

3.1 Nanomedicines for Treating AIDS

Acquired immune deficiency syndrome (AIDS) is a condition where opportunistic infections arise due to declining of immunity. AIDS is caused by the human immunodeficiency virus (HIV) and around 35 million deaths were reported due to AIDS all over the world. Highly active antiretroviral therapy (HAART) is recommended to treat AIDS. HAART reduces the progression of AIDS over a time. However, there is no cure for AIDS. In addition, the removal of HIV from the central and peripheral nervous system is also studious. Therefore, the development of nanomedicine to treat AIDS would be an interesting strategy.

Carson et al. (2016) developed a method for the controlled release of tenofovir using poly-caprolactone/poly(lactic-co-glycolic) acid (PCL/PLGA) electrospun nanofibers for the treatment of AIDS. The study established the newer platform for the sustained release of the antiretroviral drug. The drug was loaded on PCL/PLGA based on uniaxial electrospinning. The morphology of drug loaded

PCL/PLGA was characterized by scanning electron microscopy. The method has shown biocompatibility and inhibition of HIV in in-vitro studies. The tenability of release profile of drug loaded PCL/PLGA was achieved by varying the concentration of PCL:PLGA ratio (Carson et al. 2016).

3.2 Nanomedicines for Treating Cancer

The formulation of nanomedicine for the treatment of anticancer includes polymeric nanoparticles, polymeric conjugates, liposomes, polymeric micelles, microemulsion, nanoparticles, and polymer-drug conjugates, etc. The nanomedicines of anticancer are used to enhance the permeability and retention effect. Among the different types of nanocarriers, polymeric micelles greatly enhance the water solubility, blood circulation time of anticancer drugs. In 1995, Doxil™/Caelyx™ was developed as a first nanomedicine for the treatment of cancer. Doxil™ showed 300-fold increased response in a patient in comparison to doxorubicin. Myocet™, DaunoXome™, Depocyt™, Abraxane™, Genexol-PM™, and Onivyde™ are the other nanomedicines approved for the treatment of cancer. Jędrzak et al. synthesized multifunctional nanostructure based on PAMAM (polyamidoamine) dendrimer. The nanostructures were applied to a combined chemo- and photothermal therapy for the treatment of liver cancer. The desired cell death was observed from in-vitro studies of dual therapy (Jędrzak et al. 2018).

3.3 Nanomedicines for Treating Kidney Disease

The therapeutic molecules mostly are excreted from kidneys and those molecules exhibit poor pharmacokinetics profile at the kidneys. The drugs cleared from hepatobiliary process still have a less pharmacokinetics profile at the kidneys. Therefore, administering drugs to treat kidney diseases is challenging. No drugs were approved as nanomedicines to treat kidney diseases. However, the nanomedicines to target the glomerular and tubular part of the kidney have been reported. Bennett et al. (2008), developed a non-invasive method to detect the structural integrity of the basement membrane in kidneys by delivering cationic iron-oxide nanoparticles. The cationic iron-oxide nanoparticles accumulated at the basement membrane due to the higher negative charge of proteoglycans. The accumulation of cationic iron-oxide was characterized from the intensity of the signal using MRI in in-vivo and ex-vivo models of rats. Further, immuno-fluorescence and electron microscopy techniques described the selective accumulation of cationic nanoparticle at the glomerular basement membrane. The study proposed cationic iron-oxide nanoparticles as the contrasting agent to target the basement membrane. The basement membrane is integrated with the cell to form the tissues in organs. Therefore, cationic iron-oxide nanoparticles were suggested to be useful in the detection of the breakdown of basement membrane

in kidney diseases (Bennett et al. 2008). The nanoparticles to cross the glomerular filtration barrier were also developed. The study characterized the localization of nanoparticles at the epithelial cells of the lumen of the nephron (Zuckerman and Davis 2013).

3.4 Nanomedicines for the Treatment of Atherosclerosis

Atherosclerosis is a narrowing of arteries due to the build-up of plaques on the walls of arteries. The plaques are made up of cholesterol, macrophages, calcium, and others. This may lead to stroke and heart attack. Therefore, treating atherosclerosis is important to avoid the complications. The localization of monocyte depends upon chemokine receptor-2 (CCR2).

Leuschner et al. administered the lipid nanoparticle and SiRNA systemically in mice to treat atherosclerosis. The nanoparticles were localized at monocytes and decreased the accumulation of atherosclerotic plaques. The degradation of CCR2 mRNA in monocyte decreased the accumulation of inflammatory monocyte at the site. It also reduced the size of infarct in coronary artery occlusion (Leuschner et al. 2011).

3.5 Nanomedicines for the Treatment of Angiogenesis

Angiogenesis refers to the formation of blood vessels from the pre-existing vessels. Angiogenesis is also involved in the malignant transformation of tumors, tumor growth, and metastasis. Integrin, cadherin, and selectin are the key factors in angiogenesis. Integrin is overexpressed in the angiogenetic process and is one of the important biomarkers to monitor the physiological event of angiogenesis (Santulli 2013; Lampri and Elli 2013; Birbrair et al. 2014, 2015) Therefore, modulating angiogenesis by targeting integrin has been found as a striking strategy (Duro-Castano et al. 2017). Gao et al. developed a system to deliver anticancer drug at integrin $a_v\beta_3$-rich prostate cancers. Cyclic RGDyK (cRGDyK)-conjugated pH-sensitive polymeric micelles were employed to deliver paclitaxel using pH-sensitive copolymer poly(2-ethyl-2-oxazoline)-poly(D,L-lactide) (PEOz-PLA) and cRGDyK-PEOz-PLA. The micelle had a diameter of 28 nm and enhanced the cellular uptake leading to increase the cytotoxicity of paclitaxel. The antitumor activity was evaluated by in-vivo method using PC-3 xenograft-bearing nude mice. The micelle was found to be effective and safe in delivering anticancer drugs by targeting $a_v\beta_3$ integrin in cancer cells (Gao et al. 2015).

3.6 Nanomedicines for the Treatment of Ocular Diseases

Pink eye, dry eye, styes, chalazia, glaucoma, retina and choroid diseases are ocular diseases. These diseases directly affect the vision of the eye (Azar 2006; Pascolini and Mariotti 2012). The disease mainly affects the old age people of above 50 years. The anatomical nature that includes a biological barrier (blood-aqueous and bool retina) as well as tight junctions to protect the eye, physiological process and pathological mechanism of the eye complicates the delivery of drug against ocular diseases. The topically administered drugs are cleaned within 2–3 min of tear film restoration time. Therefore, the bioavalability of the ocular drug is poor (5%). However, bioadhesive, sustained release, etc. were developed to treat ocular diseases. These nanomedicines reduces the eye irritation and improves the bioavailability of ocular drugs (Weng et al. 2017).

To overcome the barrier associated with ocular drug delivery, a nanowafer was developed by Yuan et al. (2015) Nanowafer is a small, circular and transparent disc that was loaded with the drug in the form of nanoreservoir. The nanowafer releases the drug slowly and enhances the drug absorption at the ocular tissue. Yuan et al. evaluated the in-vivo efficacy of axitinib nanowafer to treat corneal neovascularization. The nanowafer was fabricated from poly(vinyl alcohol), polyvinylpyrrolidone, (hydroxypropyl)methyl cellulose, and carboxymethyl cellulose polymers with a diameter and depth of 500 nm wells. Then these wells were filled with drug/polymer solution. The delivery system was found effective twice in comparison to eye drop therapy with the reduced toxicity. The enhanced therapeutic efficacy was suggested due to increased drug residence time and drug distribution. The polymers and drugs employed in the nanowafer are already in use and the delivery system may be translated into the clinics (Yuan et al. 2015).

3.7 Nanomedicines for the Treatment of Periodontal Diseases

The infections around the teeth are called periodontal diseases. The infection may affect the gum, cementum covering the root, ligament, alveolar bone, etc. Bacteria around the dental plaque is the major cause of periodontal diseases. The bacteria adheres and forms a biofilm over the teeth. Nano particles play a key role in the controlling of these films (Rosan and Lamont 2000). Bactericidal effect of silver nanocoating on a plaque to control the biofilm formation was reported by Besinis et al. (2014) Bioassay study on bacterial growth and cell viability quantitatively showed the bactericidal effect of silver nanoparticle and silver nitrate coating on dentine. The study proposed silver nanoparticle as an alternative to silver nitrate and chlorhexidine in the protection against dental plaque.

3.8 Nanomedicines for the Central Nervous System Diseases

The central nervous system is composed of blood-brain barrier. Blood brain barrier limits the penetration of drugs into the central nervous system. Blood-brain barrier is associated with endothelial cells with the tight junction. The transport of more than 90% of small molecule of drugs and a large molecule of molecular weight greater than 500 Da were hindered (Pardridge 2005). It functions as a protective barrier. Therefore, treating the central nervous system disorder is challengeable. However, nanomedicine could be an interesting strategy to cross the blood-brain barriers. The drugs carrying the nanoparticles improves the distribution of drugs in the central nervous system. The distribution of drugs into the central nervous system was achieved by receptor-mediated endocytosis, transcytosis into the brain and drug was released within the endothelial cells. Zhou et al. fabricated nanofibrous polycaprolactone to load the Schwann cells and induced pluripotent stem cells-derived neural stem cells in cell transplantation strategy. The study has been proven to be an evidence for the treatment of spinal cord injury (Zhou et al. 2018).

3.9 Nanomedicines for the Treatment of Diabetes

Nanoceria is a cerium oxide nanoparticles which has shown the diverse application in biomedical science as an antioxidant, anticancer agent, antibacterial, etc. Cerium oxide is an inorganic compound of lanthanide series. It is present in two different oxidation states of +3 and +4. The polymeric coating of this nanomaterial has shown enhanced solubility, stability, and biocompatibility. Nanoceria has been synthesized by chemical and physical methods (Rajeshkumar and Naik 2018). Recently, the antidiabetic effect of nanoceria has been reported in streptozotocin-induced type 1 diabetes pf Swiss mice (Khurana et al. 2018). Nanoceria was characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and powder x-ray diffraction (pXRD). Nanoceria at the low dose level (0.2 mg/kg, ip,) reduced the glucose level and diabetogenesis in 50% of cases. Nanoceria exhibited the antidiabetic effect by modulation of Nrf2/NF-κB pathway. The study has been proposed as a novel strategy to attenuate the type-1 diabetes mellitus (Khurana et al. 2018).

3.10 Nanomedicines for the Treatment of Antibacterial Resistance Cases

A nanoscale liquid film-forming system containing Benzalkonium bromide was developed by Yang et al. based on Chitosan-polyvinyl alcohol against Methicillin-resistant *Staphylococcus aureus* (MRSA) (Yang et al. 2018). Nanoscale liquid film-forming system was prepared initially, by dissolving Chitosan in acetic acid. Then the dissolution of polyvinyl was prepared in hot water and Benzalkonium bromide was added. This resulting solution was added into the initially prepared Chitosan solution and mixed. The liquid film-forming system was characterized by SEM, transmission electron microscope, atomic force microscope and was evaluated against MRSA in in-vitro as well as in-vivo studies. MRSA is prevalent at wound infectious site. Due to its resistance and biofilm forming ability, MRSA is difficult to treat. The Nanoscale liquid film-forming system has shown potency against MRSA252 in comparison to benzalkonium bromide. The nanosystem (2 and 5 mg/mL) inhibited the biofilm formation and destroyed the membrane integrity formation. The nanoscale liquid film-forming system has been proposed as a promising drug delivery system for the treatment of MRSA cases (Yang et al. 2018) (Table 1).

4 Toxicity Concern

Nanomedicines used for the treatment of various human diseases must be free from toxicity. The nanomedicines should be biocompatible, biodegradable, etc. Nanotoxicology is the field that describes the toxicity of nanoparticles. The following three class of nanomaterials were proposed to cause health risks- (i) Rigid biopersistent respirable fibrous nanoparticles (ii) Respirable granular biodurable particles (iii) Nanoparticles with the specific chemical properties of its components (Gebel et al. 2014). Rigid biopersistent respirable fibrous nanoparticles may cause lung cancer and Respirable granular biodurable particles may cause inflammation upon inhalation. The toxicity of nanomedicines requires an evaluation in an appropriate animal models (Gebel et al. 2014).

5 Conclusions and Future Perspectives

Nanotherapeutics and nanodiagnostics are the important tools of current medicine. Nanomedicines have been a topic of great interest in the research of medical sciences. Over the past two-decades, a plethora of nanomedicines has been proposed for the treatment and diagnosis of diseases. Most of the nanomedicines were designed in a spherical shape with a diameter of few hundreds. The fundamental design of nanomedicines has not been changed. Many of the nanomedicines

Table 1 Development of nanomedicines for the various human diseases

No.	Therapeutic category	Drug/therapy	Therapeutic targets/site	Nanomedicines	References
1.	Anti-AIDS	Tenofovir	–	Tenofovir loaded poly-caprolactone/poly(lactic-co-glycolic) acid (PCL/PLGA) electrospun nanofibers	Carson et al. (2016)
2.	Anticancer	Chemo- and photothermal therapy	Liver	Dendrimer-based theranostic nanostructures	Jędrzak et al. (2018)
3.	Kidney diseases	Cationic iron-oxide nanoparticles	The basement membrane of kidneys	–	Bennett et al. (2008)
		–	Glomerular filtration barrier	–	Zuckerman and Davis (2013)
4.	Atherosclerosis	Lipid nanoparticle and SiRNA	CCR-2	Lipid nanoparticle and SiRNA	Leuschner et al. (2011)
5.	Angiogenesis	Paclitaxel	$A_v\beta_3$ integrin	Cyclic rgdyk-conjugated and paclitaxel-Loaded ph-responsive polymeric micelles Doxil™/Caelyx™	Gao et al. (2015)
6.	Ocular disease	Axitinib	Ocular tissue	Nanowafer	Yuan et al. (2015)
7.	Periodontal diseases	Silver and silver nitrate nanoparticles	Teeth	Nanoparticles	Besinis et al. (2014)
8.	Central nervous system diseases	Schwann cells and induced pluripotent stem cells -derived neural stem cells	Spinal cord	Nanofibrous polycaprolactone	Zhou et al. (2018)
9.	Diabetes mellitus	–	Nrf2/NF-κb pathway	Nanoparticles	Khurana et al. (2018)
10.	Antibacterial resistance cases	Benzalkonium bromide	Cell membrane	Anoscale liquid film-forming system	Yang et al. (2018)

were not reached yet. Therefore, newer approaches and techniques are still required for the nanomedicines to reach the clinic. The present work reviews the use of nanomedicines for the several diseases. This could help in deep understanding of the use of nanomedicines. The present review may provide an inspiration and impetus in the development of nanomedicines. The nanomedicines may deliver newer research tools, diagnostic agents, and clinical therapies in near future.

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Nanomedicines in Cancer Therapy

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Abstract Cancer is one of the most controversial diseases known for humanity and emerged as a global health problem all the time. The drug discovery scientists and clinicians have attempted to cure cancer since centuries. Conventional cancer treatments such as radiotherapy and chemotherapy have many limitations including low specificity, lack of stability, rapid drug clearance, biodegradation and limited targeting besides number of side effects associated with these treatments on the actual patients. Nanomedicine has evolved over the past few years and became a breakthrough technology for the diagnosis and the treatment of several cancer types. Specifically, the drug is being carried out through carriers called nanoparticles in which the properties of these carriers are very important for the successful treatment of deadly diseases like cancer. In this chapter, we describe the application of nanotechnology and nanomedicines in the diagnosis and treatment of cancer. Further, we discuss the targeted-nanodrug delivery to cancer cells in a broad context. Moreover, we provide a glimpse on marketed nanomedicines available for the management of cancer.

Keywords Nanodrug delivery · Nanomedicine · Brain cancer · Breast cancer · Lung cancer · Nanoparticles

List of Abbreviations

ABC	ATP-binding cassette
AuNPs	Gold nanoparticles
BBB	Blood brain barrier
Bcl-XL	B-cell lymphoma protein extra-large
BCNU	Bis-chloroethylnitrosourea
BTC	Brain tumor–cell barrier
BTSCs	Brain tumor stem cells

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CED	Convection-enhanced delivery
EPR	Enhanced permeability and retention effect
FDA	Food and drug administration
GI	Gastrointestinal
HER2+	Human epidermal receptor 2
HSV	Herpes simplex virus
IR	Infrared
IV	Intravenous
LH	Lutenizing hormone
mAbs	Monoclonal antibodies
MDR	Multi drug resistance
MMP	Matrix metalloproteinase
MMR	Mismatch repair
MPS	Macrophage phagocytic system
MM	Multiple myeloma
NP	Nanoparticle
PBAEs	Poly(β-amino esters)
PDT	Photo dynamic therapy
PEG	Poly ethylene glycol
PSM	Prostate specific membrane
P-gp	P-glycoprotein
PLGA	Poly-(lactic acid-coglycolic acid)
PSA	Prostate-specific antigen
PTT	Photothermal therapy
PTX	Paclitaxel
TAMS	Tumor-associated macrophages
TME	Tumor microenvironment
TNBC	Triple-negative breast cancer
TRAIL	TNF-related apoptosis inducing ligand
WHO	World health organization

1 Introduction

Cancer is a widespread disease which can affect any cells or tissues of the body. It is the disease caused by excessive growth of abnormal cells through the rapid and continuous divisions of these cells in the body. It can also referred as a tumor in which the swelling of specific part of the body that can be enlarged, without being infected. Tumor could be a solid or filled with water and it may develop into cancer in certain cases. It is important to know that not all tumors are cancerous. Generally, abnormal growth of tissue refers to one of these possibilities either benign, pre-malignant or malignant. Generally, tissue biopsy and respective analysis under the microscope can diagnose the cancer or any other abnormalities in the cell growth. According

to the results of biopsies, benign tumor is not a cancerous type and harmless to the health. Further, it will not be able to invade to the surrounding tissues and if removed, it will not relapse again thereby it just be centralized at certain point without any migration to nearby tissues. For instance, hemangiomas, benign tumor which are known as strawberry marks indicates that it has excess of blood. The premalignant is a type of tumor which needs a regular follow up because it has the potential ability to develop into cancer upon maturation. Further, malignant tumor is a type which grows and spreads rapidly and attacks the surrounding tissues by entering into the blood stream which is called as metastatic and considered as a serious disease that threatens the life of patients. Principally, tumor refers to the uncontrolled cell growth and the cancer denotes to growth in the body which can spread to other nearby tissues. Generally, metastasis refers to the spreading and migration of cancerous cells from a primary site 'the origin' to distant organs. All kind of solid tumors can metastasize except the liquid cancer such as leukemia as there is no primary tumor. Metastatic tumor is responsible for many of the cancer deaths. There are several factors such as use of tobacco, radiation, lack of physical activity, obesity and certain infections can increase the risk of cancer (Hassanpour and Dehghani 2017). To have the best therapeutic effect, an optimal combination of an appropriate drug, the dose, and the site-specific drug delivery should be accomplished. The nanomedicines are useful in cancer therapy as they exhibit optimal physico-chemical and pharmacokinetic properties. The challenges in chemotherapy of cancer include low specificity, rapid clearance of drug, biodegradation and limited targeting. Unlike the conventional cancer treatments, nanoparticles offer various advantages that make them superior in oncological applications.

2 Cancer Therapy

According to the statistics, annually about 500,000 people in US and more than 8 million people world over are dying due to the deadly disease 'cancer'. The incidence of cancer is rising along with aging and scientific predictions expect that the disease will spread and affect about 22 million people by the year 2030. This increasing number of cancer patients by every year is dreadful. In 2012 itself, 14.1 million new cancer cases were diagnosed worldwide, and 8.2 million cancer-related deaths were recorded (Siegel et al. 2018). There are various types of cancer and they are named according to the tissue or organs where it attacks. The most common type of cancer is skin cancer which includes various types such as squamous cell carcinoma, basal cell carcinoma and melanoma (dangerous cancer). Ultraviolet (UV) radiation from either sunshine or tanning bed can trigger the damaged cells of skin to multiply rapidly and produce a malignant tumor. Each year there are 132,000 new melanoma recorded and presented by World health organization (WHO). Six types of cancers such as prostate, colon, lung, stomach, liver and breast cancers are registered for more number of deaths globally. Among all the cancer types, lung cancer tops with a mortality of 1.6 million followed by liver and stomach cancers accounting for 1.4

million deaths together. It is advised to the people that to diagnose the metastatic cancer at an early stage before the tumor become a metastatic. Lymphoma is another type of cancer which begins in lymph cells and is divided into two main groups such as Hodgkin and non-Hodgkin lymphoma. It is mainly affecting the lymphocytes, a type of white blood cells which play an important role in the immunity. Plasma cell myeloma also known as multiple myeloma (MM) which is a type of cancer that occurs due to the presence of malignant plasma cells within the bone and can spread easily to rest of the tissues in the body. Most of the cancer types can be treated by surgery or radiation therapy. Sometimes, cancer can be recurrent and becomes metastatic which threatens the patient life. A lot of cancer therapies have been discovered till date and each one of them depend on the type of cancer and severity of the disease. These therapies include immunotherapy, hormone therapy, surgery, stem cell transplantation, targeted therapy and chemotherapy (Arruebo et al. 2011).

Cancer chemotherapy is a systemic treatment involving medicinal compounds that assaults abnormal cells. Once the medicinal compound enters the blood stream and reaches the site of cancer cells, it will destroy and kill cancer cells. This kind of approach is effective to treat the early stages of the disease. Further, it is used immediately after surgery to reduce the possibility of relapse of cancer. On the other hand, chemotherapy may be employed before surgery to reduce the size of the cancer. Generally, chemotherapy targets are the fast-dividing cells and to certain extend normally dividing cells also affected. Hence, chemotherapy may cause some undesirable side effects such as nausea, vomiting, hair loss, susceptibility to infections and decrease in blood cell count (Falzone et al. 2018). Similarly, immunotherapy is a type of cancer treatment which enhances the body's defense mechanism to combat the disease. Monoclonal antibodies, T cell therapy, oncolytic virus therapy and vaccines are the different types of immunotherapy. The immunotherapy had a clear mechanism to prevent the progression of the disease, decrease the growth of cancer cells and prevent its spreading capacity to other parts of the body. Nowadays, interferons and some cytokines are used to trigger the immune response in patients suffering from renal cell carcinoma, melanoma and bladder cancer (Oiseth and Aziz 2017).

Hormone therapy is a treatment used to stop the growth of cancer by altering the hormone levels. This type of therapy is used to treat people with breast and prostate cancer based on hormone consumption for growth. This therapy relieves the symptoms of prostate cancer patient who have not yet undergone surgery or radiation therapy. Furthermore, this therapy can be used to treat other types of cancer because of its ability to shrink the tumor before surgery and is also known as neoadjuvant therapy. Moreover, it reduces the possible risk of cancer recurrence after completion of the treatment which is called as adjuvant therapy. Common side effects of hormonal therapy depend on the type of hormone administered to the patient and its effect on the body (Abdulkareem and Zurmi 2012). Similarly, photodynamic therapy (PDT), a therapy used to treat and alleviate the symptoms of esophageal and lung cancers. This therapy uses a drug which is called as photosensitizing compounds and a suitable light source, it could be either infrared (IR) or visible light with specific

wavelength for each photosensitizer. This light will trigger the drug to create a proper form of oxygen that can kill the nearby tumor cells. The principle of this therapy is very simple. Once the drug is injected into blood stream of the patient, it will be absorbed by the cells throughout the body. After 1 to 3 days of time, most of the drug particles will be removed from the normal cells and remain inside the cancer cells. Once these particles exposed to the light, they produce an active form of oxygen that devastate cancer cells. PDT can destroy the tumor under the skin because the light can't reach the distant cells. Therefore, it is less effective to treat large tumors. PDT nanoparticles is preferable due to multiple reasons. First of all, the specific targeting enhances the concentration of the photosensitizer at the desired target-site and reduces the cytotoxicity on the surrounding healthy tissues. Secondly, the photosensitizer can be maintained at a stable rate as there is no kinetic release or zero order release. Finally, the hydrophobicity of the hydrophobic photosensitizer can be improved using suitable nanoparticles (Zhang et al. 2018).

Another cancer treatment is photothermal therapy (PTT) which is based on the translation of the photon's energy to heat to destroy cancer cells. The most effective particles for this therapy are the gold-nano particles in size range from 20 to 300 nm. They have an ability to absorb the light in IR region and convert it into heat that is adequate to destroy the cancer cells. Scientists have been advised to attach specific antibodies to the surface of the gold particles to become more site-specific for certain cancer (Zou et al. 2016). Targeted drug delivery includes the use of small organic drug molecules that have an ability to enter into the cancer cell and target a specific gene or an enzyme which are mutated or over-expressed so that it will help in preventing the distribution and multiplication of cancer. It is often used with other therapies like chemotherapy. Another strategy of this type of therapy is the use of monoclonal antibodies which can attack a specific antigen on the cancer cell. The most common type of monoclonal antibody is the naked monoclonal antibody which mainly blocks the antigen and preventing the cancer from invading to other tissues. Another type is the conjugated monoclonal antibody that can be employed along with other types of therapies like chemotherapy. Also, it carries a toxic substance to transmit it directly to the target cell. Similarly, bispecific monoclonal antibody also exists which consists of two parts of different proteins. The possible side effect of this therapy is allergic reaction, apart from other common side effects such as fever, nausea, vomiting, weakness, chills and headache. This therapy will help other treatment to work exactly at the target site and found to be effective in many types of cancer like melanoma and leukemia (Weiner 2015).

3 Drug Resistance in Cancer Chemotherapy

In general, chemotherapy can be given in combination with gene therapy to destroy both the metastatic and recurrent cancer. Apart from the myelosuppression (reduced bone marrow activity which further reduces the blood cells), alopecia (partial or complete loss of hair) and mucositis (GI inflammation), chemotherapy may

aid to develop secondary cancer also. Several drugs can be used as an effective chemotherapeutic agents and are categorized into five major classes namely, alkylating agents, antimetabolites, anthracycline antibiotics, vinca alkaloids and topoisomerase inhibitors. The choice of a suitable chemotherapeutic agent depends on the type and the nature of the cancer and the cancer therapist (Oncologist) can prescribe a suitable combination of drugs to the patient considering the dose, duration of therapy which entirely depends on the type and size of the tumor and the cytotoxicity profile of drugs toward normal healthy cells. Upon long-term administration of the chemotherapeutic agents inhibiting the cell mitotic processes, cancer cells may develop the resistance against the administered drugs which may happen anytime during the therapy (Housman et al. 2014).

There are two types of resistance namely, intrinsic resistance and acquired resistance. There are multiple reasons for drug-resistance such as drug-inactivation, epigenetics, DNA damage repair, drug-target alterations, drug efflux mechanism, cell death inhibition, and epithelial-mesenchymal transition. The difficulty in the absorption of cytotoxic agents is mainly due to the alterations in the expression of transport proteins thereby resulting in the development of drug-resistance. Tumor cells possess membrane efflux pumps pertaining to the ATP-binding cassette (ABC) family protein members which are overexpressed and pumping out the drugs finally decreasing the intracellular concentration of anticancer drugs. Any modification in drug metabolism may lead to the drug-resistance. The tumor cells overexpressing the metabolic enzymes can deactivate the administered drugs. Specifically, the drug Paclitaxel was deactivated inside the colorectal cancer cells due to the enhanced activity of CYP_{3A4}, an enzyme of cytochrome P_{450} family. Similarly, the drug Irinotecan is reduced in the cancerous cells due to the drug-resistance caused by the altered level of the intracellular enzymes such as carboxylesterases. The anticancer drug anthracycline binds with DNA topoisomerase and exhibits anticancer activity. Mutations in the TOP1 gene, encoding topoisomerase 1 in cancer cells generated the drug-resistance. In case of DNA repair, a repair of excised nucleotides was mediated by alkylating agents and the mismatch repair (MMR) corrects the wrongly matched nucleotides. Any loss of MMR generates a genetic instability thereby developing the drug-resistance to various anticancer drugs. The protein p53 has an important role in chemo-resistance. In parallel, any mutation in the gene of the BAX protein, enhanced the growth of cancer cells that are resistant to the drug, Oxaliplatin. Similarly, upregulation and abnormal expression of other proteins like Bcl-2 or Bcl-XL (B-cell lymphoma protein extra-large) are linked to the development of drug-resistance (Luqmani 2005).

4 Limitations of Conventional Therapy

Each type of cancer therapy had its own risk factors and certain limitations associated with it. Although the chemotherapy is one of the most popular treatments that is used in many cases of cancer, it has a lot of characteristics and distinctive ability to slow

down the growth of the tumor. Further, it has different effects and varied efficiency from one person to the other person. In case of hormone therapy involving GnRH agonist may suppresses the testosterone and inhibits the lutenizing hormone (LH) production thereby eliciting the anticancer activity against the prostate cancer. On the other hand, it may cause some side effects such as hot flashes as well as pain and redness at the site of injection. In surgical removal of cancer tissues, it takes longer time to recover from the operation which interferes with the individual's daily life. In radiation therapy, patients will face several problems like skin rashes and sensitivity at the site where exposed to the radiation waves. Further, it can cause stretching marks in tissues and damaging a part of the organ. Thus, the above mentioned limitations of the present therapy paved a way to the identification of novel approaches such as gene therapy (Mross and Kratz 2011).

5 Gene Therapy

Recent epidemiological and cytogenetic research showed that cancer is a genetic disease involving genes and it could be a better way to fight against the cancer using the gene therapy which mainly focusing on the use of nucleic acid as a therapeutic agent. The principle of gene therapy involves the insertion of a correct and healthy gene in order to correct a gene which has some kind of defect. If a specific mutated gene produces an incorrect protein, gene therapy may be used to insert a correct gene to generate the correct protein, successfully. Gene therapy can be considered as an alternative therapy that could be used in the treatment of cancer and include three different methods namely immunotherapy, oncolytic virotherapy and gene transfer (Wirth and Ylä-Herttuala 2014).

The immunotherapy utilizes cells that are genetically modified and some viral particles are incorporated in order to activate the immune system to kill the cancer cells, effectively. Of 1200 clinical studies, only few trial reports showed promising results on many types of cancer such as lung cancer, prostate cancer and pancreatic cancer. The immunotherapy enhances the immunogenicity of the tumor to stimulate the immune system and the cultured lymphocytes from the blood can be designed to provide T-cell receptors specifically for antigens of certain types of tumor. The immunotherapy suffers with several drawbacks such as limited expression, the low avidity between the gene and tumor cells, and the escape of tumor cells from immune system (Brown et al. 2018).

Oncolytic virotherapy employs viral particles that replicate inside the cancer cells and destroy them. This therapy exhibited a positive outcome in many metastatic cancers and oncolytic viruses are employed which replicate and destroy tumor cells. HSV virus, adenovirus, poliovirus and newcastle disease viruses are mostly used due to their ability to target cancer cells. When a patient had already been exposed to a specific virus, it will become familiar to the body and the immune system will produce antibodies against the virus. Further, the virotherapy is very expensive as

the administered virus must possess adequate safety which is established through clinical trials (Singh et al. 2012).

The gene transfer is a method of introducing new genes into cancer cells or the tissue that surrounded cancer cells to slow down the process of growth of the cancerous cells. This therapy was proved to be successful in case of head and neck cancer using p53 and MDA-7 as transferred genes, respectively. The gene transfer approach can also be employed to convert the anticancer pro-drug to active drug using a gene as an enzyme activator and the common example is herpes simplex virus (HSV)—thymidine kinase. Vectors can be used to achieve the gene transfer process successfully by delivering a desirable gene into the patient's body. Variety of vectors available for this purpose including viruses like retrovirus, adenovirus, and non-viral vectors such as plasmids and liposomes. Each one of them has a special criteria and selection of suitable vector is not a random process but depends on different types of cancer and the gene causing the disease (Das et al. 2015).

There are several strategies to achieve a successful gene transfer and few of them are mentioned below:

1. Correction of the mutated gene that causes malignancy.
2. The activation of T-cell-mediated immune response against the tumor (immunotherapy).
3. The application of oncolytic viruses that kills the tumor cells (virotherapy).
4. The application of enzyme that convert the non-toxic medicines to cytotoxic metabolites.
5. The insertion of tumor suppressor genes into tumor cells.
6. The administration of suicide gene into tumor cell.
7. The administration of specific genes into stem cells that can deactivate the chemicals and minimize the side effects.
8. The administration of antisense gene to counteract the expression of oncogenes in tumor cells.

In gene therapy, a gene can be administered to stimulate the apoptosis or increase the sensitivity of tumor towards drugs and radiation. The apoptosis can be induced by introducing gene encoding ligand as an inducer. TNF-related apoptosis inducing ligand (TRAIL) is an example of apoptosis-inducer employed in the cancer therapy which showed higher efficiency on the variety of cancer types with low toxicity on normal cells. Similarly, MDA-7 is apoptosis-inducer showed the selectivity towards cancer cell without exhibiting any toxicity on normal cells. The tumor suppressor gene such as p53 (regulator for cell cycle and apoptosis), PTEN (which regulates cell survival) and retinoblastoma gene Rb (regulator) are identified and delivered to the cancer cells for an effective treatment of cancer (Kumar et al. 2016).

Future of gene therapy depends on two factors namely, bioengineering and traditional clinical development. The major issue in the gene therapy is the selectivity towards cancer cells and various strategies to overcome this problem are still in preclinical or early clinical stages. The successful application of the gene therapy depends on the selectivity to cancer cells, selection of appropriate vectors, and avoiding possible neutralization by the immune system. Presently, the gene therapy

is developing rapidly and playing crucial role in the treatment of cancer. Similarly, many cancer vaccines are in the advanced stages of clinical trials and the gene transfer technology increase the effectiveness of cancer chemotherapy as well. Though these alterative cancer therapies available, a fastest growing nanotechnology-based medicines are now available for an effective management of cancer starting from diagnosis to therapy of various cancer types (Amer 2014).

6 Nanomedicine

Researchers from the literature described 'Nanomedicine' as the science derived from nanotechnology and nanoscience that is specialized in the diagnosis and treatment of diseases using small nano-sized molecules to cure the disease at the cellular and molecular level which are called as nanoparticles. Nanomedicine science is a breakthrough technology which carried many advantages over the conventional methods. It provides an effective approach by focusing on the vascular and cellular characteristics of tumorous cells. By using nanoparticles, the treatment can passively accumulate the drug in cancerous cells through the leaky vasculature via the enhanced permeability and retention effect (EPR) (Yang et al. 2017). Nanomedicine also gives the option of decorating nanoparticles with active targeting ligands which specifically binds to the needed targets on tumor cells and this enhances the accumulation inside tumorous cells. It helps to achieve a good balance between the efficacy and the toxicity of the drug by enhancing the bio distribution and the target accumulation of chemotherapeutic drugs with a lower damage effect on the surrounding tissues (Arranja et al. 2017). Nanomedicine overcame the problems of the conventional treatments such as the lack of selectivity, lack of aqueous solubility of drugs and the multidrug resistance (MDR) that is developed from the repetitive administration of the same drug. It basically satisfies the need of the drug delivery methods suffering with poor drug solubility, poor bioavailability, nonspecific cytotoxicity, suboptimal pharmacokinetics and pharmacodynamics towards specific targeting to increase the safety, efficacy and patient compliance to the chemotherapy (Xu et al. 2015).

A particle is described as a nanoparticle when it has a dimension range in between 1 and 100 nm. Nanoparticles are the materials precisely engineered to have special physiochemical properties. They are designed in such a way that they specifically targets the injuries and diseases without affecting the surrounding healthy tissues. They are approved for the treatment and diagnosis of certain diseases and nanotherapies are approved for the treatment of cancer and without being toxic to normal cells (Shi et al. 2017). There are two method such as bottom-up technique (nanoparticles are being built atom by atom) and the bottom-down technique (nanoparticles are being built by eliminating existing atom from the main particle) were available in nanomedicines (Pal et al. 2011). The emergence of nanomedicine for the past 5 decades is presented in Fig. 1.

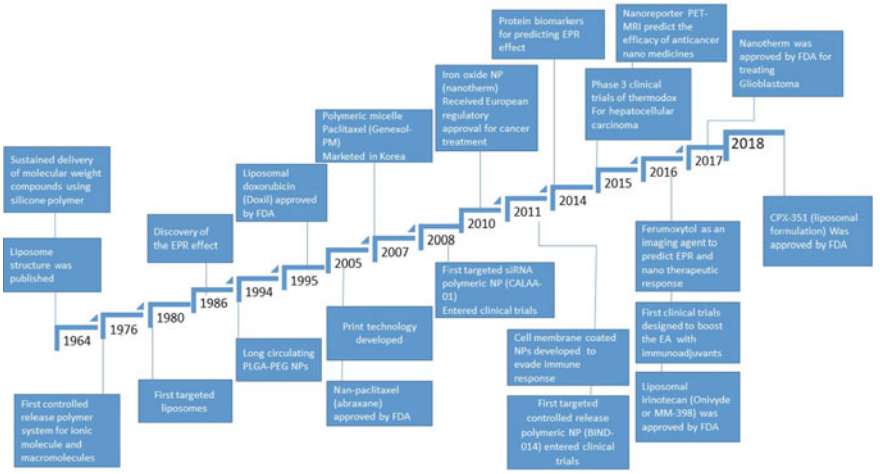

Fig. 1 The emergence of nanomedicine for the past 5 decades

7 Significance of Nanomedicine in Cancer

One of the most important features is the targeted drug delivery so as to minimize the unwanted side effects and tissue toxicity. The diameter of small capillaries is in between 5 and 6 micron. Since drugs that are used to treat cancer is larger than the capillaries diameter, they cannot penetrate efficiently unless they are reduced in their particle size. Since the size of nanoparticle is smaller than the microparticle they can efficiently penetrate the capillaries and deliver the drug for an effective treatment. Furthermore, the drug effectiveness increases when it is carried by nanoparticles as they will be consumed efficiently through the cells. Since the nanoparticle has a high surface to volume ratio, the dissolution rate can be increased. The administration of poorly soluble drugs also would be more effective when they are integrated through a nanocarrier as the uptake of these drugs would be improved at the site of action (Sengupta and Kulkarni 2013).

An important advantage of carrying the drug through a nanoparticle is maintaining drug molecules safe and protecting them as it would be encapsulated by the nanocarrier. This in return will decrease the degradation by the body before reaching the target and improves the therapeutic index. This also will increase the bioavailability and the absorption of the drug in the tumor cell. Important characteristics of nanomedicines are prolonged circulation, adequate half-life, delivering high concentration of drug into the tumor cells, increased bioavailability, and enhanced tumor disposition (Peer et al. 2007). Nanoparticles face many barriers such as blood, lungs, liver, spleen, kidneys, and brain during their transportation to the tumor sites. The blood as a barrier is characterized by the opsonization process. The hydrophobicity and the charge of the nanoparticle are important factors affecting the protein opsonization process. It results in the activation of the complement immune system in which it

triggers the rapid uptake by phagocytes (Owens and Peppas 2006). Nanoparticles had a large surface to volume ratio which allows the increased solubility of the drug in the blood. Their outer surfaces can be modified to target specific cells which gives them the advantage of having optimized size and shape. They are convenient for the design, preparation, biodegradable and biocompatible nanomedicines. They have better stability compared to conventional treatments and they can be designed to be sensitive to certain stimuli which makes the treatment easier and target-specific manner (Gelperina et al. 2005).

8 The Design of the Nanocarriers for Tumor Targeting

It is important to consider the physiochemical characteristics while designing a nanocarrier for tumor targeting. Surface properties such as hydrophobicity and charge affect the opsonization process. If the nanoparticles are neutral and hydrophilic, they would be less prone to opsonization. Moreover, cationic nanoparticles extravasate more rapidly than neutral or anionic particles in the tumor cells as the cationic NP would have an attractive electrostatic forces with the anionic endothelial glycocalyx. The size plays an important role in designing an effective nanoparticle. It determines the accumulation and penetration. As the particle gets smaller, more penetration and accumulation will be achieved. A nanoparticle less than 100 nm would be optimal for an effective EPR. If the particle size is smaller than 5 nm, it would be efficiently and quickly eliminated by renal filtration. Large particles more than 200 nm quickly accumulate in healthy organs such as liver, lungs or spleen. They also would be rapidly taken up by macrophages. The optimal size would be in between 5 and 250 nm as particles can extravasate from the leaky vessels of tumor and this allows for efficient accumulation (Perrault et al. 2009).

When the nanoparticle enters the blood stream, it forms what is known as a Corona. The corona formulated by binding proteins already available in the bloodstream with the surface of the nanoparticle and accumulates on it. This accumulation causes alteration of the nanoparticle characteristics such as size, stability and surface properties. This binding can also play a role in the triggering a physiological response. Most famous example is the osponization process by the plasma protein called 'opsonin' which facilitates the uptake of foreign particles that enter the blood and coats them in order to initiate the process of phagocytosis (Bourzac 2016). The binding of opsonin can trigger the recognition and clearance by the mononuclear phagocyte system (MPS) which eventually cause the reduction of the availability of the drug at the sites needed (Mahmoudi et al. 2011). In order to suppress the protein absorption to the nanoparticle, PEGylation can be performed, which involves coating the surface of the nanoparticle with poly ethylene glycol (PEG). PEG is a considered as hydrophilic, therefore, it avoids phagocytosis in the reticuloendothelial system. The affinity of the proteins responsible of opsonization will be reduced, thus, the uptake will be delayed by macrophages along with the clearance rate of the NP and prolonging circulation time (Gustafson et al. 2015).

9 Types of Nanocarriers

There are many types of nanocarriers used in cancer nanomedicines such as dendrimers, polymeric micelles, silica nanoparticles, metal nanoparticles and liposomes. These nanocarriers are briefly discussed in the following subsections.

9.1 Dendrimers

Dendrimers have tree-like structure with an inner core made up of one molecule and generated layer by layer of repeated units. The core molecule controls the size and degree of branching. Different functional groups can be added to hold certain hydrophobic drugs. Temperature or any other stimuli on these dendrimers can break and release the drug as required (Sun et al. 2014).

9.2 Polymeric Micelles

One of the most popular nanoparticles used for drug delivery applications is polymeric micelles. They comprised of dense matrices and have degradation curves. Basically, they are the structures of synthetic polymers which allows them to be easily customized into diversely important nanoparticle properties (biodegradability, molecular weight and hydrophobicity). They are amphiphilic copolymers that has a hydrophilic shell with a hydrophobic core (Otsuka et al. 2012).

9.3 Silica Nanoparticles

They were firstly introduced in 2000 by Vallet-Rego and Co-workers. An example is the mesoporous silica-based nanoparticle. The pore size can be adjusted from 2 to 50 nm which allows a controlled accommodation of guest molecules with varying sizes. They have ample of reactive 'silanol groups' on their surfaces which has the capability to conjugate with many diversified functional groups (Jaganathan and Godin 2012).

9.4 Liposomes

They are self-assembled structures consisting of lipid bilayers. As lipids are used to construct cells, they are considered as biocompatible and suitable to carry the drugs into the body. Many studies demonstrated that the encapsulation of drugs by

liposomes improved the pharmacokinetics and bio-distribution. Liposomes consists of lamellar structures encapsulating a hydrophilic payload. They are small structures spherical in shape generated when an amphiphilic lipid is added to water or other hydrophilic liquids to result in a nanosize (50–500 nm) (Elizondo et al. 2011).

9.5 Metal Nanoparticles

Metal nanoparticles are flexible to be modified with diverse functional groups which permit them to associate with drugs of interest, ligands, antibodies which opens different potential applications in biotechnology. The pore size can be ranged from 1 to 500 nm. The nanoparticles generally consists of magnetic nanoparticles, nanocages, nanoshells, silver nanoparticles, gold nanoparticles, and iron oxide. Hence, these nanoparticles are useful in targeted drug delivery, pre-concentration of target analytes, gene therapy, magnetic separation and in case of imaging technologies (ultrasound, MRI, CT, PET and optical imaging) as well (Mody et al. 2010).

9.5.1 Albumin-Based Particles

Albumin, as protein act as a carrier and demonstrate non-immunogenic, non-toxic, biodegradability and biocompatibility properties. They also possess higher binding capacity to various drugs without any serious side effects and can be well tolerated. Due to the presence of different binding sites in albumin, appreciable quantity of drugs can be loaded. Similarly, a high concentration of charged amino acids in albumin facilitates electrostatic adsorption of ionized molecules. In comparison with liposomes, they display a smaller size 50–300 nm for the better controlled release properties. Some examples of albumin employed as nanocarrier include bovine serum albumin, ovalbumin and human serum albumin (Elzoghby et al. 2012). Different types of nanocarriers with their shape and size range are presented in Fig. 2.

10 Mechanism of Targeting to Tumor Tissues

There are mainly three types of targeting the nanoparticle delivery to tumor tissues namely, passive-targeting, active-targeting, and triggered release. A brief discussion on these mechanisms is provided in the following subsections.

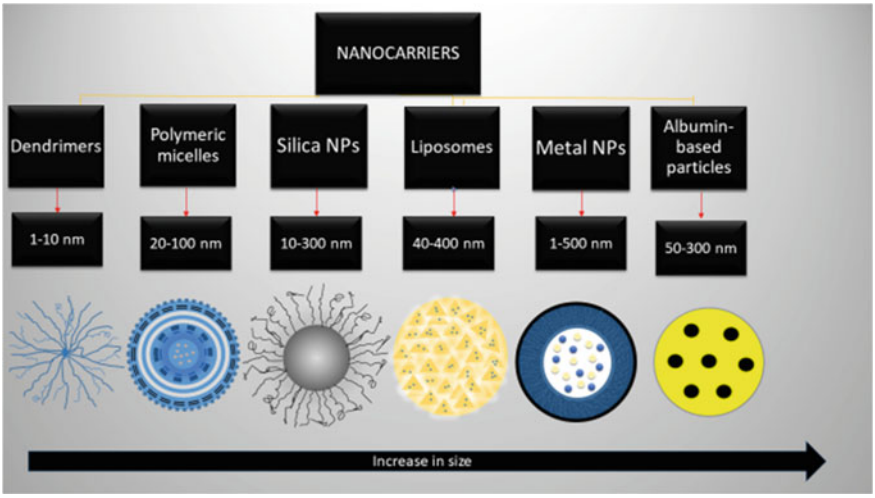

Fig. 2 Different types of nanocarriers with their shape and size ranges

10.1 Passive Targeting

As vascular walls of the tumorous tissues are malformed, cell to cell junctions are weakened and lymphatic drainage becomes dysfunctional, therefore leaky vasculature and hyper-permeability are possible in tumor cells along with large intracellular pores and interrupted endothelial cells (Yang et al. 2017). These characteristic properties in tumorous cells allow the nanoparticles to be accumulated or retained into the tumorous cells. This process is called the enhanced permeability and retention effect (EPR) and is responsible for the passive targeting. This process tolerates the cytotoxic drugs carried by the nanoparticles to be accumulated where they are needed, therefore protecting the healthy surrounding tissues from being affected. This reduces the side effects associated with the chemotherapy. Studies showed that the nanoparticles in the range of 10–100 nm are suitable for the accumulation of such drugs into tumor tissues (Danhier et al. 2010). There are several other factors affecting the EPR mechanism such as different tumor types, tumor size and tumor heterogeneity. This leads to differences in the biodistribution of the nanoparticles and the lack of uniformity (Shi et al. 2017).

10.2 Active Targeting

This approach is based on the use of specific ligand conjugation to perform the modification on the surface of the nanoparticle. These modifications specifically binds to receptors available at the target site. Cancerous cells have specific molecules

expressed on their surfaces and this is how the system differentiates between cancerous cells and normal cells. When the modified ligands on the nanoparticle surface binds to these specific receptors, cells are induced to absorb the nanocarrier and triggers cell apoptosis. This process decreases the uptake by the macrophage phagocytic system (MPS) which in return increases the circulation time. Moreover, active targeting helps in the delivery of particles that cannot cross the cellular membrane by themselves such as proteins and nucleic acid (Sykes et al. 2014). Moreover, it decreases the MDR because proteins that induce the resistance such as P-glycoprotein (P-gp) cannot pump out the nanoparticles associated with drug or drug polymer conjugates that entered the cell via endocytosis. Normally, cancerous cells have one or more molecular targets expressed on their surfaces as they deliver sites for active targeting (Blagosklonny 2003).

10.2.1 Target Ligands

Small molecules such as folic acid, antibodies, transferrin, peptides and aptamers are considered as target ligands. Folic acid is a very selective marker that is highly expressed on tumor cells (100–300 timer more than normal tissue) and plays an important part in the biosynthesis of nucleotide bases and DNA. It is being carried out across the plasma by either folate receptors or folate carrier. Due to the higher affinity receptors and limited interaction with normal tissue, they help in the delivery and the selectivity of the chemotherapeutic agents. Transferrin and folate receptors are overexpressed in cancerous cells. In order to make a successful active targeting, the nanoparticle can be modified suitably using these ligands to increase the effectiveness and absorption of the drug. Nanocarriers expressed with transferrin ligands had a better tumor targeting than non-targeted ones. Antibodies, aptamers and peptides also have overexpressed on cancer cells (van der Meel et al. 2013).

Aptamers are considered as a form of macromolecules that consist of a single stranded oligonucleotides, DNA or RNA and they are selected according to their binding affinity to antigens. Their length is about 25–100 nucleotides (8–25 kDa) and they can bind with molecular targets. Aptamers are also called “chemical antibodies” and they became very popular and used widely for the decoration of nanoparticles. They have many characteristics to make them favorable as ligands, specifically over monoclonal antibodies (Yang et al. 2011).

(1) Ease of isolation chemically
(2) Selectivity and the binding affinity
(3) Small size
(4) Non-immunogenicity which makes good homing ligands for the cancer targeting
(5) Broad range of potential targeting.

Aptamers targeting can be done by modifying the surface of their molecules with different functional groups in order to make it easier for the conjugation with the nanomaterial so that they can be used for selective targeting. An example of this is

the combination of the A10 aptamers with the PLGA-block-PEG co-polymer. It has been used for targeting the prostate cancer specific membrane (Gardikis et al. 2010).

Monoclonal antibodies (MAbs) are the antibodies derived from a single clone of the parent immune cell. They consist of complex protein-based immunoglobulins that has two parts, Fab which is the antigen binding fragment and the Fc which is the complement fixing fragment accountable for targeting specific antigen. They act as an optimal platform for conjugated drug targeting if they were specific for certain antigen. However, the Fc region can be considered as detrimental for the application in targeting, as it could be accessible by the Fc receptors on the macrophages and can lead to the increased accumulation in the spleen and liver, respectively. Their size is approximately 150 KD, 15 nm long and 5 nm in diameter as they are considered as the largest among other targeting ligands. Certain antigens can be overexpressed on the surface of tumors either primary or metastatic. Examples of these antigens are the HER2 which is overexpressed on breast cancer cells. Chimeric and fully humanized derivatives are made to target the MAbs on tumor cells. Examples of FDA approved mAbs to treat cancer are Rituximab, Certuximab and Alemtuzumab (Chames et al. 2009).

Peptides are also useful targeting ligands and specifically, vasoactive intestinal peptide is considered as a neuro modulator peptide. It consists of 28 amino acids from the glucagon secretin class which is widely distributed at the central and peripheral nervous system. Their application mainly is in the breast cancer treatment as they are found to be expressed 5 times on tumorous breast cell than the normal cell. It is also being naturally biosynthesized in the eyes and plays an important role in the ocular immunological functions (David 2017).

10.3 Stimuli Responsive Release

Modification in nanocarriers can be triggered by internal and external stimuli. Various internal stimuli are possible such as ionic strength, pH, redox, and stress in target tissues. Similarly, different external stimuli triggering the drug release are electric fields, light, ultrasound, magnetic force and temperature. A representative for each of internal and external stimuli is discussed briefly in the following subsection (Cai et al. 2008).

10.3.1 pH Sensitivity as an Internal Stimuli

These nanoparticles are stable at physiological pH and release the drugs exclusively under acidic conditions. Usually tumor cells had lower pH which is due the fact that the angiogenesis cannot meet the demand of oxygen for the new tumor cell growth and tumor cells are low in blood vessels, all of which generate the regions with low oxygen supply. This low oxygen/hypoxic conditions changes the metabolism of the tumor cells to become anaerobic instead of aerobic respiration. This produces high

levels of CO_2 and carbonic acid (H_2CO_3) which in turn decreases the pH of the cell. In this case, a combination of pH responsive and targeting ability of nanoparticle would be very useful. Similarly, magnetic field and enzyme-sensitive nanoparticles are the other stimuli responsible for the drug release (Arranja et al. 2017).

10.3.2 Temperature as an External Stimuli for Drug Release

This concept is based on the process of cell hyperthermia. Nanoshells are designed in such a way that it can absorb different frequencies of light, allowing the heat to be accumulated inside the tumor and destroying it from the inside. When the nanoshells enter into the tumor cell via an active targeting, a near-IR is applied and absorbed to generate an extreme heat inside the cancerous tissue which finally leading to the destruction of the cancer cells, selectively (Liu et al. 2016). Poly(*N*-isopropyl acrylamide) is an example of thermosensitive material commonly used in nanomedicine which can change the hydrophobicity and hydrophilicity rapidly at a temperature of 32 °C (lower critical solution temperature). In a normal situation, nanoparticles that are responsive to keep their payload at a temperature between 36 and 37 °C (Li et al. 2015). Liposomes also are used as thermosensitive material to release the encapsulated drugs at certain temperatures and this can be successfully applied as a triggered release method when drugs needed to be delivered to sites that are treated with light or high-intensity ultrasound and others. Figure 3 represents the types of targeting to the tumour cells (Frenkel 2008).

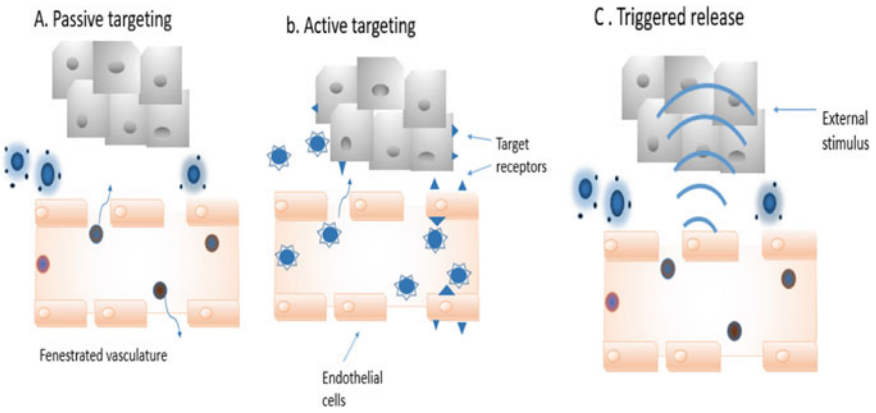

Fig. 3 The types of targeting to the tumor cells

11 Nanomedicines in the Therapy of Cancers

Nanomedicines are very useful in the treatment of cancers such as brain cancer, breast cancer, prostate cancer, and lung cancer. The application of nanomedicines in the treatment of different cancer types is discussed in the following subsections.

11.1 Brain Cancer

Brain cancer is one of the life-threatening cancers of humankind which affecting low- and middle-income countries and observed 9–17% of all cancers. A metastatic primary brain tumor is known as glioblastoma multiforme contributing 12–15% of all brain tumors recorded in adults. In 2016 alone, 23,770 new brain cancer cases and 16,050 deaths are reported (Leece et al. 2017). Due to the intracranial location of the brain, the brain cancer is challenging to treat. The principal hurdle and the poor survival rates of brain cancer patients is due to inability of drugs to cross the blood–brain barrier (BBB). BBB protects the brain by acting as a physical or metabolic barrier and regulates the entry of molecule to the brain. Therefore, lipophilic small molecules can passively diffuse through the BBB, while the large molecules with high molecular weight or hydrophilic type cannot pass this barrier. As most of the tumors are associated with an intact BBB, it is very hard to notice the accumulation of the nanoparticles within the tumor cells. To overcome such difficulty, researchers discovered that P-gp substrate-efflux pump which can circumvent the brain tumor-cell barrier (BTC) (Tiwary et al. 2018). Apart from BBB, the BTC generated by the efflux action of the tumor cells also limits the accessibility of systemically administered drugs to reach the site of action in cancerous cells (Agarwal et al. 2011).

In case of targeted-drug delivery, there are three possible mechanism involved such as passive and active targeting using nanoparticles and theranostics. The passive targeting is an ideal way of delivering the nanoparticles which passively targeting drugs towards the intracranial tumors for an efficient delivery across the BBB as the nanoparticles favorably gather the drug at tumor sites. Intravenously (iv) administered nanoparticles exploit the improved permeability and retention and attain a vicinity to the tumor cells, accordingly. This has been illustrated with an example of P-gp substrate, doxorubicin, when iv administered in poly(butylcyanoacrylate) nanoparticles, yielded enhanced concentration of drug in the tumor tissue. Similarly, other polymers like cyanoacrylates and poloxamer 188-coated poly-(lactic acid-coglycolic acid) (PLGA) nanoparticles also resulted in the higher tumoricidal effect when incorporated with the drug Doxorubicin (Zhang et al. 2016). Sometimes, nanomedicines can be formulated with more than one drug for the therapy of brain tumors. The iv administration of chitosan surface-modified PLGA nanoparticles loaded with carmustine with O^6-benzylguanine formulation yielded synergistic effect than the administration of these two drugs individually or the nanoparticle consisting of the drug carmustine alone (Qian et al. 2013).

The active targeting utilizes nanocarrier having different surface ligands to attain the drug transport across an intact BBB. For the purpose of delivering an anticancer drug taxol derivative, Tf– c[RGDfK] paclitaxel micelles were synthesized and administered through an iv-route thereby achieved higher concentration of the drug in the brain, wherein Tf facilitated the drug transportation across the BBB and c[RGDfK] mediated the uptake of drug by tumor cells (Zhang et al. 2012). Similarly, the use of GLUT to transport the drug across the BBB was attained by employing 2-deoxy-D-glucose-modified poly(ethylene glycol)-co-poly (trimethylene carbonate) paclitaxel nanoparticles (Jiang et al. 2014). Few clinical trial reports showed the passively targeted nanoparticles NCT02340156, NCT02820454, NCT01266096, NCT03020017, and NCT00734682 in brain cancer, however, their efficacy profiles have not yet established (Anselmo and Mitragotri 2016).

Theranostics are imaging agents and drugs which are transported by a single nanoparticle for the brain cancer therapy (Meyers et al. 2013). In PDT, photofrin encapsulation was employed for the accumulation of surface decorated tumor vasculature targeting F3 peptide within the intracranial tumor and visualized using MRI involving the polymeric nanoparticles loaded with iron oxide nanoparticles. This theranostic enhanced the survival rate in 9L glioma rat model following PDT when compared with PDT or photofrin alone (Reddy et al. 2006). Iron oxide act as an MRI imaging agent for several theranostics. Similarly, elongated iron oxide nanoparticles (nanoworms) are used in combination of a tumor-homing peptide (CGKRK), to target the mitochondria of tumor along with a proapoptotic peptide D[KLAKLAK]2 as a drug. The results of the study demonstrated that the targeted nanoworms were highly efficient than the nontargeted nanoworms (Agemy et al. 2011). Thus, the image-competent nanotherapeutics can be an alternative choice for the drug delivery to the brain tumor.

Currently, in USA, Gliadel wafer i.e. carmustine wafer or bis-chloroethylnitrosourea (BCNU) wafer is the only drug approved for the localized treatment for brain tumours (Lin and Kleinberg 2008). Gulati et al., synthesized titanium wires to work as nanotube holders or drug nanocarriers for the delivery of doxorubicin which had limited drug loading and unable to control the drug release profile. A self-ordering electrochemical anodization method was employed on the surface of titanium wires (thickness-0.75-mm; length-30 mm) and producing titania nanotube (170 nm in diameter) arrays for loading of doxorubicin (up to 1200 μg). The generated nanoengineered wires were found to be biocompatible, mechanically stable for doxorubicin loading and displayed a sustained drug release of the drug over 8 days (Gulati et al. 2012). In another study by Wang et al., formulated different formulations involving the use of Paclitaxel (PTX) including nanofiber discs made up of PLGA nanofibers (100 nm in diameter) and microparticles embedded in hydrogels. The authors demonstrated that the PLGA nanofiber discs exhibited rapid, prolonged release of the drug and exposure to more surface area of biological fluids, thereby effectively treating glioma cells (Ranganath et al. 2010). Another nanotech approach is magnetic nanoparticles which respond to an external magnetic field and impacting the delivery of drugs. For the treatment of brain cancer, magnetized nanoparticles loaded with carmustine can be administered.

The BCNU-loaded magnetic nanoparticles blocked the growth of glioblastoma cells with the cell viability ranged from 49.8 to 7.3%. Nanoparticles displayed no significant cytotoxicity individually, demonstrating that the observed cytotoxicity against brain tumor is mainly due to the drug carmustine and the drug release rate can be optimized by their composition, ranging from 78.8 to 30.2% after the duration of 72 h. In presence of an external magnetic field, the magnetic nanoparticles can induce the heat that can be used as intratumoral thermotherapy (Akilo et al. 2016).

The non-viral gene therapy for glioblastoma involves the use of non-viral vectors by encapsulating the nanoparticles. In this technique, poly(β-amino esters) (PBAEs) were employed to generate the nanoparticle carriers and integrated with DNA into a distinct nano-objects which can encapsulate about 100 plasmids each and analysed for their influence on brain tumor stem cells (BTSCs). An outcome of the study reported the higher efficiency in the transfection of glioblastoma astrocytes and BTSCs than the normal cells. Further, an in vivo study of the most effective PBAE–DNA nanoparticles was conducted by lyophilization and subsequent injection into tumor-bearing mice. The results of the study indicated that the naked DNA exhibited lower expression when compared with nanoparticle-encapsulated DNA which transfected comparatively better when administered into tumor-bearing mice (Tzeng et al. 2011).

The nanoparticle carriers are used overcome the drawback in crossing the brain parenchyma, premature degradation of the drugs and the drug clearance from the capillaries. The drug PTX was encapsulated within 70-nm PEG-coated PLGA nanoparticles to evaluate their in vivo activity on brain tumor. The outcome of the study indicated the 92% tumour growth inhibition was observed with PLGA–PEG nanoparticles to deliver PTX, when compare with 55% inhibition observed using PLGA nanoparticle encapsulating the PTX alone. Thus, PEG-coated nanoparticles exhibit good diffusion through the brain TME to suppress the tumor growth when loaded with the drug PTX (Nance et al. 2014).

Convection-enhanced delivery (CED) technology can be employed for the delivery of the multifunctional nanoparticles to attain a synergistic activity. Zhang et al., employed magnetic nanoparticles as carriers for O^6-benzylguanine, an MGMT inhibitor. When these nanocarriers coated with a crosslinked, biocompatible, redox-responsive chitosan–PEG copolymer, the stability of the nanomedicine was enhanced substantially in the course of the drug transportation and subsequent release. The nanomedicine distribution can be imaged through T2-weighted MRI, immediately after delivery using CED technology (Stephen et al. 2014). The CED-based nanomedicine delivery had a greater impact in producing synergistic effect while integrated with chemotherapy and radiotherapy. The optimized nanoparticle-based drug delivery systems are needed to overcome the challenges of localized brain cancer treatment, sustained release of drug for longer period of time, drug resistance issues, profound tumor penetration, and targeting cancer stem cells (Tzeng and Green 2013).

11.2 Breast Cancer

Breast cancer is the main cancer and second leading cause of mortality among women population world over. In the past decade, the incidence of breast cancer was raised up to 20% and 1.5 million new breast cancer cases were reported in each year (Torre et al. 2017). Lipoplatin® is a type of liposomal nanoparticle (110 nm diameter) carries the drug, cisplatin and evaluated clinically for the breast cancer therapy. In a phase-II clinical trials involving lipoplatin/vinorelbine combination in HER2/neu-negative metastatic breast cancer, high effectiveness was observed without neuro- and nephron-toxicities (Mylonakis et al. 2010). Similarly, Onivyde® is another nano-liposomal formulation containing the drug, irinotecan (Zhang 2016). Though, it is not employed in the breast cancer chemotherapy, a phase-I clinical evaluation with advanced refractory solid tumors including breast cancer cells indicated the cancer control rate 45.5% (Chang et al. 2015). In the Phase I and II clinical trials on metastatic or recurrent breast cancer, another nano-formulation Genexol-PM containing paclitaxel with polymeric micelles demonstrated complete (12.2%) and partial (46.3%) responses, respectively with higher maximum tolerable dose (Werner et al. 2013). Phase-III clinical evaluation of this product is currently taken up by Samyang Biopharm in Korea.

About one quarter of breast cancers are human epidermal receptor 2 (HER2+) which had a tendency of faster progress than breast cancers, except triple-negative breast cancer (TNBC). For an instance, HER2 monoclonal antibodies like trastuzumab is currently employed for the breast cancer therapy and for nano-delivery, the antibody was decorated by the nanocarrier surface (Gianni et al. 2016). Similarly, trastuzumab-mimetic peptide was also investigated for the nano-delivery to decrease the immunogenicity, manufacturing costs and difficulties associated with the antibody, trastuzumab. Around 15–20% of breast cancer cases are triple negative (estrogen receptor negative, progesterone receptor negative and HER2 negative) type and are difficult to treat them. The TNBC had high chances of mutation of BRCA-1 and can only be diagnosed at the advanced stages. Different therapeutics such as siRNA, Doxorubicin, siRNA silencing oncoprotein lipocalin-2 are delivered to the protein targets such as EGFR, folate receptor and CXCR4 all of which demonstrated appreciable cellular uptake through the TNBC cells with high efficacy in pre-clinical experimentations. For the nanodelivery of drugs, folate receptor is frequently targeted due to its higher expression (50–86%) in metastatic TNBC. In order to prevent the relapse and metastasis of cancer, nanotherapy targets the breast cancer stem cells (BCSCs) which are having the self-renewal capacity and differentiation to cancer cells. Further, these cells have high metastatic potential, more aggressive and are drug resistant too. As the CD44 receptors highly expressed in BCSCs, they could probably act as a biomarker that associate the growth factors and cytokines from the tumor microenvironment (TME). Anti-CD44 monoclonal antibodies and hyaluronic acid are the common ligands for the delivery of anticancer drugs targeting CD44 (Muntimadugu et al. 2016). Similarly, CD133 is also a potential target which receives the drug Paclitaxel integrated with PLGA nanoparticles decorated

with anti-CD133 antibody as a nanocarrier (Swaminathan et al. 2013). This study indicated the potential advantage of targeting BCSCs in the prevention of the relapse of the breast cancer which is very crucial in the therapy.

TME-based nanotherapy is another potential approach in the nanomedicine delivery for the breast cancer treatment. In general, TME is acidic (pH ~ 5) and hypoxic than the normal cells and these properties were explored for the nanodelivery of drugs. In case of pH-dependent nanodelivery, nanocarriers are designed in such a way that they can release the loaded drug faster through a hydrolysis at lower pH. This approach not only enhances the concentration of drugs at the site of action, but also the release rate (van Sluis et al. 1999). The pH-sensitive nanocarriers such as chitosan-based glycolipid, triblock copolymeric micelles with cell-penetrating peptides, amphiphilic copolymer integrated with β-thiopropionate linkage are employed by the researchers for the delivery of the drug, Doxorubicin. Due to due the poor vascularization, breast cancer cells are under hypoxic environment which substantially enhance the cancer spreading and consequently developing the 'pan-chemoresistance' to most of the anticancer drugs (Velaei et al. 2016). The active and passive targeting mechanisms of nanomedicines are highly valuable in this approach. The liposome of the drug Disulfiram was formulated to inverse the 'pan-chemoresistance' due to hypoxia-induced nuclear factor (NF)-κB. The results of this investigation suggested that the liposomal disulfiram was found to be effective in disrupting NF-κB in breast cancer cell model and reversed the pan-chemoresistance which translated the higher in vitro and in vivo efficiency (Liu et al. 2014). Similarly, the nanoformulation of CRLX101 (an investigational nanoparticle)—camptothecin conjugate was assessed separately or in combination with bevacizumab (antiangiogenic drug) in murine breast cancer model. This CRLX101 nanoformulation was effective in blocking the hypoxia-inducible factor-1α, enhancing the cancer sensitivity towards bevacizumab, increasing the tumor perfusion and decreasing the hypoxia, accordingly (Pham et al. 2016).

Tumor-associated macrophages (TAMs) are also significant targets for the nanodelivery of anticancer drugs. TAMs have significant functions in neoangiogenesis, matrix modeling, and immune suppression which is involved in the growth, invasion and spreading of cancer (Noy and Pollard 2014). PLGA nanoparticles with mannose and abraxane target the TAM and EPR, respectively for the successful delivery of drugs, Doxorubicin and Paclitaxel (Cullis et al. 2017). The macrophage-based nanotherapy remains in initial stages of investigation and more studies are needed to warrant their therapeutic potential. The matrix metalloproteinase (MMP), FSH receptor and stromal cells are responsible for the mammary gland development and breast cancer progression, thereby exhibit as alternative targets in TME. Anticancer drugs such as Docetaxel and Doxorubicin are delivered to those target cells employing diversified nanocarriers like liposome with degradable lipopeptides, Cellax® (nanoparticles of acetylated carboxymethylcellulose linked with PEG) and nano-graphene oxide with FSH antibody (Ernsting et al. 2012).

Drug resistance is the principal hurdle in the breast cancer treatment and the resistance process is highly complex involving overexpression of P-glycoprotein, mutations of genes, mutations in crucial amino acids, and overexpression of HER2

(Marquette and Nabell 2012). Apart from the combination therapy, nanomedicines contribute to overcome the anticancer drug resistance issues. The breast cancer is one of the deadly diseases and difficult to treat if it becomes metastasis stage. It can spread to the important organs like lung, brain, liver and bones. The design and delivery of the nanoformulation that sufficiently penetrate the site of cancer without initiating any adverse effects is of primary importance in breast cancer therapy.

11.3 Prostate Cancer

Prostate cancer is the major cancer affecting men population for about 1.3 million new prostate cancer cases were reported in the current year (2018). Age-related incidence (more than 65 years old men) rates of the prostate cancer have raised exponentially which is due to the accessibility of screening for prostate-specific antigen (PSA) in men without symptoms of the prostate cancer. This PSA test guides convenient diagnosis of the prostate cancer which is small or unrecognized, and may develop in future to the advanced stage of cancer. For the prostate cancer, various options such as surgery, endocrine therapy, and hormonal therapy are available in the current medical practice. Nanomedicines play a crucial role in the prostate cancer treatment in which the drug has been delivered through both active and passive targeting methods. In the passive method, enhanced permeability and retention (EPR) effect has been employed and the prostate cancer can be visualized by fluorescent nanoprobe and intravital microscopy techniques (Sandanaraj et al. 2010).

Myocet®, a non-PEGylated liposomal doxorubicin formulation is studied under Phase-II clinical trial against the prostate cancer cells and exhibited superior effect than the other formulation of Caelyx® (Montanari et al. 2012). Similarly, a liposomal formulations comprising of curcumin that targeted to prostate cancer cells specifically by coating with an antibody to prostate membrane specific antigen and demonstrated 10-fold higher inhibition than the drug alone. For the targeted drug delivery of drugs to prostate cancer, polymer-based devices were developed by different researchers (Thangapazham et al. 2008). Farokhzad et al., developed functionalized targeted aptamer-combined polymeric nanoparticles involving co-precipitation of docetaxel with the co-polymer (PLGA-PEG) followed by the surface functionalization with A10 aptamer and displayed 77-fold higher efficiency than the non-nanoparticles (Farokhzad et al. 2006). Further, a mixture of docetaxel-PLGA-PEG and PLA-PEG encompassing cisplatin was evaluated to overcome the drug resistance problem. A synergistic effect was observed between these two drugs against LNCaP cell line (in vitro), demonstrating 5.5-fold higher cytotoxicity than single drug formulation (Kolishetti et al. 2010).

The silver nanoparticles (100 nm) using an aqueous extract of *Alternanthera sessilis* were synthesized by researchers and the resulting NPs demonstrated substantial activity against PC3 prostate cells (Firdhouse and Lalitha 2013). Similarly, glucose-bound gold nanoparticles (AuNPs) were synthesized which displayed higher toxicity and sensitivity towards DU-145 prostate cancer cell line. Another study indicated

that the nanoparticles of 50 nm without a PEGylated surface exhibited good cellular uptake of AuNPs in PC3 prostate cell line. It was also proved that the AuNPs encapsulated antineoplastic drug docetaxel demonstrated good results (Arnida and Ghandehari 2010). Similarly, liposomal delivery of gemcitabine demonstrated promising approach and it shows the dose reduction while providing 45-fold higher antitumor effect than the drug alone (Jantscheff et al. 2009). While, most of the anticancer drugs evaluated for the delivery to prostate cancer exhibited passive targeting, the only one drug, doxorubicin showed the active targeting. Further research studies are warranted for the development of highly efficient and specific liposomal drug carriers for the therapy of advanced prostate cancer (Kroon et al. 2014).

12 FDA Approved Nanomedicines

Doxil was the first FDA approved nanomedicine that came out in the year 1995. It is a PEGylated liposomal doxorubicin. It was initially approved for the therapy of ovarian cancer, refractory breast cancer and Kaposi's sarcoma. It is designed to have a good balance between the toxicity and efficacy of doxorubicin. This drug is known for its cardiotoxicity when it is given alone in higher doses (accumulative 550 mg/m^2) for cancer treatment. But the drug administered in an encapsulated formulation, the side effects are reduced and demonstrated longer drug circulation in the blood while formulating the drug by encapsulating with 80–90 nm unilamellar liposome coated with PEG. The nanomedicine formulation exhibit DNA intercalation and Topoisomerase II-mediated DNA repair disruption mechanisms to treat cancer (Bobo et al. 2016). Table 1 lists all the approved nanomedicines by the FDA with specifications.

13 Future of Nanomedicines

Multiple nano products such as nanorobots, respirocytes, microbivores, and clottocytes are currently emerged in the nanomedicines (Sosnik and Carcaboso 2014). Nanorobots are the devices as small as microbe but lack the ability of self-replication and their main function is to repair the damage caused by metabolism by preforming specific procedures (nanorobotics). It assists in repairing almost every known disease that generates physical injury (Venkatesan and Jolad 2010). Respirocytes are synthetic red blood cells smaller than the natural one, almost the same size of a bacterium. Despite of its small size, it has an ability to carry hundred times more capacity of both oxygen carrying and carbon dioxide removing processes (Surendiran et al. 2009). Microbivores are synthetic white blood cells, bacteria with drug resistance has no chance to go against it. Although the lethal infectious pathogens can be easily removed within few minutes to few hours, conventional methods may take years. It may also be used for early detection and killing of cancer cells and

Table 1 FDA-approved nanomedicines

Therapy modality	Generic name and/or proprietary name	Nanotech. platform	Active pharmaceutical ingredients	Cancer type	Status	Adverse labeling	The year of approval/study completion date	Manufacturer	Country of manufacture
Chemotherapy: non-targeted delivery	Liposomal doxorubicin (Doxil)	PEGylated liposome	Doxorubicin	HIV-related Kaposi sarcoma, ovarian cancer multiple myeloma	Approved by FDA	Cardio-toxicity Infusion reaction Myelosuppression	1995	Janseen	Belgium
	Liposomal daunorubicin (DaunoXome	Liposome	Daunorubicin	HIV-related Kaposi sarcoma	Approved by FDA	Hemorrhage Cardio-toxicity Tissue Necrosis	1996	Galen limited	UK
	Liposomal vincristine (Marqibo)	Liposome	Vincristine sulfate	Acute lymphoblastic leukaemia	Approved by FDA	Neurologic Toxicity Fatigue Hepatic Toxicity	2012	Talon Therapeutics	Canada
	Liposomal irinotecan (Onivyde or MM-398)	PEGylated liposome	Irinotecan	Post-gemcitabine meta-static pancreatic cancer	Approved by FDA	Interstitial lung disease Severe diarrhea neutropenia	2015	Merrimack	USA
	Liposomal doxorubicin (Myocet	Liposome	Doxorubicin	Metastatic breast cancer	Approved by FDA	Hand foot syndrome Cardiac toxicity Fetal mortality	2000	Teva pharmaceuticals	Israel
	Mifamurtide (Mepact	Liposome	Muramyl tripeptide phosphatidyl-ethanolamine	Nonmetastatic, resectable osteosarcoma	Approved by Europe and Canada	Tachycardia Tachypnea Vomiting	2003	Takeda	Japan
	Nab-paclitaxel (Abraxane	Albumin NP	Paclitaxel	Breast, lung and pancreatic cancer	Approved by FDA	Hypersensitivity Hepatic Impairment Sepsis	2005	Abraxis Celgene	USA
	SMANCS	Polymer conjugate	Neocarzino-statin	Liver and renal cancer	Approved in Japan	Mild fever Dull pain	1989	NA	NA
	Polymeric micelle paclitaxel (Genexol-PM)	Polymeric micelle	Paclitaxel	Breast cancer and NSCLC	Approved in South Korea	Neutropenia Neuropathy Myalgia	2006	Samyang	South Korea
	Liposomal cisplatin (Lipoplatin	PEGylated liposome	Cisplatin	NSCLC	Phase III	Neurotoxicity Nephrotoxicity Anaphylactic-like reaction	2019	Regulon	USA

(continued)

Table 1 (continued)

Therapy modality	Generic name and/or proprietary name	Nanotech. platform	Active pharmaceutical ingredients	Cancer type	Status	Adverse labeling	The year of approval/study completion date	Manufacturer	Country of manufacture
	NK-105	Polymeric micelle	Paclitaxel	Metastatic or recurrent breast cancer	Phase III	peripheral sensory neuropathy anaphylaxis hypersensitivity	2017	Nektar Therapeutics	USA
	Liposomal paclitaxel (EndoTAG-1)	Liposome	Paclitaxel	Pancreatic cancer, liver metastases and HER2-negative and triple-negative breast cancer	Phase II	Abdominal pain Pyrexia Nausea	2012	Medigene/SynCore Biotechnology	Taiwan
	Nab-rapamycin (ABI-009	Albumin NP	Rapamycin	Advanced malignant PEComa and advanced cancer with mTOR mutations	Phase II	Mucositis Fatigue Rashes	2021 (proposed)	Abraxas-Celgene	USA
	CRLX-101	Polymeric NP	Camptothecin	NSCLC, metastatic renal cell carcinoma and recurrent ovarian, tubal or peritoneal cancer	Phase II	Fatigue Cystitis anemia	2017	Cerulean pharma	USA
Chemotherapy: targeted delivery	MM-302	HER2-targeting liposome	Doxorubicin	HER2-positive breast cancer	Phase II	Neutropenia, Diarrhea, Vomiting.	2016	Merrimack	USA
	BIND-014	PSMA-targeting polymeric NP	Docetaxel	NSCLC and mCRPC	Phase II	Lymphopenia Nausea Dyspnea	2016	Bind therapeutics	USA
	MBP-426	TfR-targeting liposome	Oxaliplatin	Gastric, oesophageal and gastro-oesophageal adenocarcinoma	Phase Ib/II	Nausea Anemia Neuropathy	2012	Mebio pharm	Japan
	Anti-EGFR immunoliposomes loaded with doxorubicin	EGFR-targeting liposome	Doxorubicin	Solid tumors	Phase II	Neutropenia Septicaemia Fetal massive oral bleed	2016	University of Basel	Switzerland
Chemotherapy: stimuli-responsive delivery	ThermoDox	Liposome	Doxorubicin	Solid tumors	Phase III	Heart problems. Alopecia Anemia	NA	Celsion Corporation	USA

(continued)

Table 1 (continued)

Therapy modality	Generic name and/or proprietary name	Nanotech. platform	Active pharmaceutical ingredients	Cancer type	Status	Adverse labeling	The year of approval/study completion date	Manufacturer	Country of manufacture
Chemotherapy: combinatorial delivery	Liposomal cytarabine–daunorubicin (CPX-351 or Vyxeos)	Liposome	Cytarabine and daunorubicin	High-risk acute myeloid leukaemia	Approved by FDA	Neutropenia Bacteremia epistaxis	2017	Celator Pharmaceuticals	USA
	CPX-1	Liposome	Irinotecan and floxuridine (1:1)	Advanced colorectal cancer	Phase II	Diarrhea Vomiting Neutropenia	NA	Celator Pharmaceuticals	USA
Hyperthermia	NanoTherm	Iron oxide NP	NA	Glioblastoma	Approved by FDA	sweating, tachycardia hypotension	2018	Magforce nanotechnologies	Germany
	AuroLase	Silica core with a gold nanoshell	NA	Head and neck cancer, primary and metastatic lung tumours	Not applicable	Diarrhoea Vomiting Alopecia	2014	Nanospectra Bioscience	USA
Radiotherapy	NBTXR3	Hafnium oxide NP	NA	Adult soft tissue sarcoma	Phase II/III	Tumor hemorrhage Pain Hematologic reactions	2018	Nanobiotix	France
Gene or RNAi therapy	SGT53	TfR-targeting liposome	Plasmid encoding normal human wild-type p53 DNA	Recurrent glioblastoma and metastatic pancreatic cancer	Phase II	Anemia Chills fevers	2019	SynerGene therapeutics	USA
	PNT2258	Liposome	DNA oligonucleotide against BCL-2	Relapsed or refractory non-Hodgkin lymphoma and diffuse large B-cell lymphoma	Terminated	Fatigue Nausea Vomiting	2016	ProNAI theraputics	Canada
	SNS01-T	Polyethylenimine NP	siRNA against eIF5A and plasmid expressing eIF5A-K50R	Relapsed or refractory B cell malignancies	Phase II	Hypercalcemia Renal insufficiency Anemia	2014	NA	NA
	Atu027	Liposome	siRNA against protein kinase N3	Advanced or metastatic pancreatic cancer	No longer recruiting	Fatigue Nausea	2017	Silence therapeutics	USA

(continued)

Table 1 (continued)

Therapy modality	Generic name and/or proprietary name	Nanotech. platform	Active pharmaceutical ingredients	Cancer type	Status	Adverse labeling	The year of approval/study completion date	Manufacturer	Country of manufacture
	TKM-080301	Lipid NP	siRNA against PLK1	Neuroendocrine tumours, adrenocortical carcinoma and advanced hepatocellular carcinoma	Phase II	Pyrexia Chills nausea	2016	Tekmira	Canada
	DCR-MYC	Lipid NP	Dicer-substrate siRNA against MYC	Hepatocellular carcinoma	Terminated	Fatigue Nausea Infusion reaction	2016	Dicerna pharmaceuticals	USA
	MRX34	Liposome	miR-34 mimic	Primary liver cancer, solid tumours and haematological malignancies	Withdrawn	Back pain Fever Fatigue	2017	Mirna therapuetics	USA
	CALAA-01	TfR-targeting polymeric NP	siRNA against ribonucleotide reductase M2	Solid tumours	Terminated	Nausea Vomiting	2012	Calando pharmaceutics	USA
	ALN-VSP02	Lipid NP	siRNAs against KSP and VEGFA	Solid tumours	Phase I	Fatigue Nausea	2012	Alnylam	USA
	siRNA-EPHA2-DOPC	Liposome	siRNA against EPHA2	Advanced cancers	Phase I	had mild neutrophilic moderate lymphohistiocytic inflammation in the esophagus	2021 (proposed)	MD anderson cancer center	USA
	pbi-shRNA STMN1 LP	Lipid NP	shRNA against stathmin 1	Advanced and/or metastatic cancer	Phase I	Nausea Fatigue	2017	Gradalis	USA
Immunotherapy	Tecemotide	Liposome	MUC1 antigen	NSCLC	Terminated	Arthralgia Myalgia Nausea	2015	NA	NA
	dHER2 + AS15	Liposome	Recombinant HER2 (dHER2) antigen and AS15 adjuvant	Metastatic breast cancer	Phase II	Myalgia Fatigue Musculoskeletal pain	2012	NA	NA

(continued)

Table 1 (continued)

Therapy modality	Generic name and/or proprietary name	Nanotech. platform	Active pharmaceutical ingredients	Cancer type	Status	Adverse labeling	The year of approval/study completion date	Manufacturer	Country of manufacture
	DPX-0907	Liposome	Multi-tumour associated antigens	HLA-A2-positive advanced stage ovarian, breast and prostate cancer	Discontinued—Phase-I for Breast cancer, Ovarian cancer and Prostate cancer in USA	Injection site reactions	2011	Depovax	NA
	Lipovaxin-MM	Liposome	Melanoma antigens	Malignant melanoma	Phase I	Anemia	2012	NA	NA
	JVRS-100	Lipid NP	Plasmid DNA	Relapsed or refractory leukaemia	Phase I	Fever Pain Fatigue	2017	Juvaris BioTherapeutics	USA
	CYT-6091	Colloidal gold NP	TNF	Advanced solid tumours	Phase I	Lymphopenia Hypoalbuminemia Hypokalemia	2009	Cytimmune Sciences	Italy

NA data not available in the literature

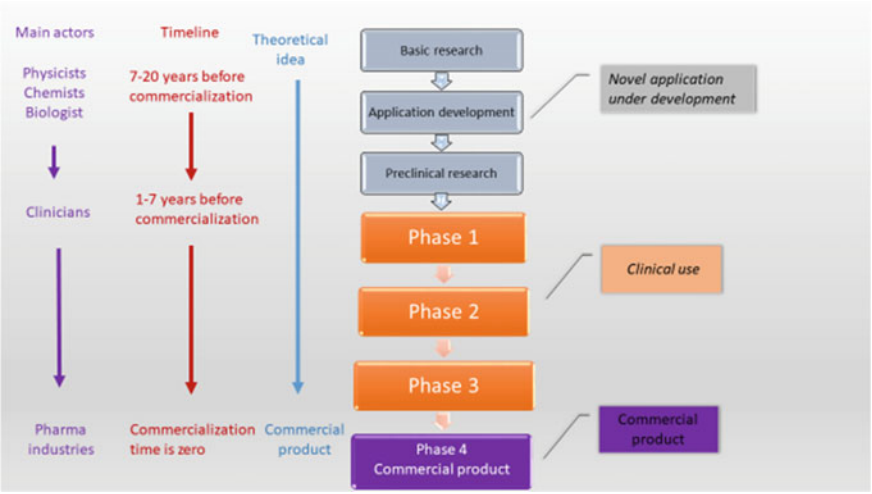

Fig. 4 General time-line in the development of nanomedicine from basic research to commercialization

removing any occlusions in blood stream to avoid ischemic damage in the condition of stroke (Li et al. 2018). Clottocytes are artificial mechanical platelets that can act 1000 times faster than the natural platelets that exist inside the body. They allow fast clotting of any wound despite of its size within seconds or less and stop the bleeding immediately (Nabipour and Assadi 2015).

14 Timeline for the Development of Nanomedicines

FDA is currently developing certain regulations for the products related with nanomedicines by generating "Nano Task Force" which provides adequate details about the FDA process in accepting nanomedicines (Venditto and Szoka 2013). General time-line in the development of nanomedicine is presented in Fig. 4.

15 Conclusion

Nanomedicine has the particle size less than 100 nm and demonstrate enormous future impact on many aspects such as quality of life of the patient, reduce cost of health care, early detection of fetal diseases and target-specificity. Literature evidences demonstrated the useful applications of nanomedicines in the therapy of different diseases. Amongst, cancer nanomedicines are advancing in the clinical trials and emerging as novel therapeutics for the management of the dreadful cancer. This

chapter provided a highlight on the applications of different nanomedicines involved in the therapy of cancer. Still, much more research outcomes are anticipated that will turn on the new horizons in fighting the deadliest disease cancer.

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Nanomedicines in Tuberculosis: Diagnosis, Therapy and Nanodrug Delivery

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Abstract Nanoparticle-based delivery systems represent a promising nano medications to deliver a therapeutic agent, selectively and effectively, to a specific tissue or organ in the body; thus treating chronic diseases such as tuberculosis. The delivery of first-line and second-line antituberculosis drugs, using synthetic or natural polymeric carriers, has been extensively reported as a potential intermittent chemotherapy. In addition to the prolonged drug release, this delivery system can enhance the therapeutic efficacy, reduce dosing frequency and side effects, and increase the possibility of selecting different routes of chemotherapy and targeting the site of infection. The choice of carrier, system stability, toxicity and production capacity are the main considerations during the development of such system. Regardless of the obstacles, the nano drug delivery have systems shown a promising effectiveness in treating TB.

Keywords Nanomedicines · Nanodrug delivery · Chemotherapy · Tuberculosis

1 Introduction

Millions years ago, the presence of tuberculosis in creatures was distinguished and distributed the exploration in 2018. Various recorded reports and medicinal research materials vouch for the omnipresent spread of tuberculosis in the inaccessible past. Prior to the most antiquated find inconter that is related to the appearance of tuberculosis in people, had a place with Paul Bartels. In 1907, they depicted that the tuberculous thrashing of the thoracic vertebrae with the arrangement of a mound in the skeleton, which was found close Heidelberg and had a place with body that belong to the B.C era.

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Mycobacterium tuberculosis (MTB) has been a major worldwide medical issue. Approximately, 9 million new active cases had been expected and almost million once had passed in 2011 (WHO 2011). 40% of these new cases occurred in the south-eastern countries of Asia. In 2010, only 65% of the assessed cases were reported, which indicates the requirement for a better conclusion (Van Rie et al. 2010). This exhibits the need for a new methodologies for early finding, treatment observing, and sickness monitoring. TB diagnosis (dynamic or inert) is fundamental in treating an affected persons as well as resisting its spread. In areas with high persistence of TB, the main goal of TB care programs is to treat patients affected by dynamic TB rather than inert TB (LTBI), which is just a proved by the immunological reactions to MTB proteins (Barry et al. 2009). In spite of the fact that LTBI patients are not irresistible, their distinguishing proof has been similarly critical since 10% of these people, especially the immune suppressed cases, can form dynamic tuberculosis. The normal conclusion of dynamic TB contamination utilizes different methodologies such as smear microscopy, MTB bacillus culture, identification of MTB nucleic acids (NAATs, nucleic corrosive enhancement tests), and clinical side effects. Tuberculin skin test (TST) and interferon gamma (IFNγ) discharge (IGRAs) tests are usually used to recognize LTBI. The low specificity of clinical conclusion, the inaccessibility of symptomatic strategies to create world research centres, and inadequacy to screen quiet consistence to the six-multi month long treatment are the major drawbacks of the current TB discovering strategies. Significantly, further propelled specialists of the medieval East, specified the centered of tuberculosis (Avicenna, 980–1037 gg.). In the Group of Restorative Science, Avicenna (Abu Ali Ibn-Sina) talks about utilization as a malady passing on others and transmitted by legacy, demonstrating disease with tuberculosis with "ruined air," that is, irresistible air or airborne beads. Avicenna perceived the impact of nature on the course of the sickness; prescribed different techniques for recuperating, specifically, appropriate sustenance. In spite of the fact that the current diagnostics strategies of TB are promising (e.g., a POC manual NAAT unit utilizing circle intervened isothermal intensification from Eiken/Discover Establishment for Development New Diagnostics) and a handheld NAAT gadget from Epistem/Xcelris (Pai and Pai 2012; WHO 2011), nonappearance of a sans instrument test and surprising expense keep on being a bottleneck. In this manner, there is a neglected need to build up a basic, economical, touchy and convenient measure for the location of dynamic MTB contamination, and for the separation of dynamic TB from LTBI for the purpose of-mind (POC), where reasonable budgetary help, research facility foundation, and a well prepared administrators are constrained (Wang et al. 2010). In this audit, we (I) centred around the present TB analytic advances as far as their potential for distinguishing dynamic TB disease at the POC sooner rather than later, (ii) feature the group between present tests and clinical need to oversee TB patients, and (iii) assess the capability of nanotechnology and microfluidics to create POC diagnostics for TB.

2 Present Measures for TB Determination

The diagnostic methods of TB have promoted from sputum smear microscopy to the most recent WHO-supported GeneXpert TB test. This involves the recognition of complete MTB bacillus, MTB-particular invulnerable reactions or nucleic acid amplification. These diagnostics methods are categorized according to their potential (low, direct and high) for POC testing considering affectability, cost and celerity as well as precision and specificity. Even though urine antigen and sputum tests are basic and generally utilized for asset in compelled situations, they are restricted to recognize dynamic TB in highly anticipated areas. These two assessments are considered together as "tests with direct potential for POC testing". The nucleic acid intensification tests are considered as "tests with high potential for POC testing", which account a high affectability and specificity. Furthermore, these examination (such as GeneXpert) can be modest, fast and computerized for POC testing in deconcentrated situations.

3 Recognizing High-Hazard Gatherings for *M. tuberculosis* Testing

As a feature of their normal assessment, human services suppliers should recognize and test people who are at high hazard for getting TB contamination or at high danger of advancing to TB sickness if tainted. In some select settings, dynamic case finding might be more suitable than testing for M. tuberculosis contamination, adaptability is required in characterizing high-chance gatherings for testing. The changing study of the disease transmission of TB demonstrates that the hazard for TB sickness or LTBI among bunches as of now thought to be high hazard may diminish after some time, and gatherings right now not distinguished as being in danger may along these lines be viewed as high hazard.

Tuberculin skin test (TST) 100 years prior, TST was distinguished as the cleansed protein subsidiary (PPD) test. It depends on the utilization of the PPD tuberculin, an encourage of non-animal categories particular atoms separated from sanitized and focused societies of MTB, to distinguish resistant reactivity in subjects (Andersen et al. 2000). This test is straightforward and broadly utilized to recognize MTB presence. On the off chance that the subject is presented to MTB, intradermal infusion of PPD tuberculin prompts induration of the skin following 48–72 h, because of an invulnerable reaction. In spite of the fact that the method is generally basic, the measure requires very well prepared administrators to perform and break down outcomes. Incorrect outcomes may emerge from the nearness of nontuberculous mycobacterial contamination or Bacillus Calmette-Guérin (BCG) immunization (Farhat et al. 2006). As detailed, BCG inoculation in earliest stages causes a TST false positive rate of 8.5% and 1% when tried previously or following 10 years old, individually. These discoveries demonstrate the consequences of TST in high-commonness nations, for

example, India and China ought to be deciphered with the alert, as BCG immunization is given to new-born children after birth (Zwerling et al. 2011). It ought to likewise be noticed that the affectability of TST is low in HIV patients due to trade off invulnerable reactions (Cobelens et al. 2006).

4 Interferon-Gamma (IFN-γ) Discharge Tests

IFN-γ discharge tests were produced and executed 17 years ago with fluctuating stages of progress (Mazurek et al. 2005). They measure Lymphocyte arrival of IFN-γ (have cell safe) endless supply of entire blood with MTB-particular antigens, including early-discharged antigenic target 6 (ESAT6), culture filtrate protein 10 (CFP10) and TB7.7 (an extra antigen utilized in altered IGRA units). IGRA examines are accessible in two arrangements, ELISA (e.g., QuantiFERON®-TB, GFT) and catalyst connected immune spot test (ELISPOT, e.g., T-SPOT® TB test). QuantiFERON®-TB (counting QFT-Gold and QFT-Gold In-Tube) evaluates the centralization of IFN-γ, while T-SPOT® TB test checks the quantity of IFN-γ-delivering antigen-particular T lymphocytes. Despite the fact that these examines are not influenced by earlier BCG immunization as the antigens are missing in all BCG strains, they cannot recognize between LTBI and dynamic infection (Dheda et al. 2009). Furthermore, efficient audit and meta-examinations demonstrated that IGRA tests have no preferable affectability in recognizing dynamic or inert TB contamination in HIV-tainted people compared to TST (Cattamanchi et al. 2011; Metcalfe et al. 2011). This means that they cannot be utilized to discount in or administer dynamic TB cases, particularly in HIV-contaminated people (Chen et al. 2011; Ling et al. 2011). This constraint has driven a WHO master gathering to debilitate the utilization of IGRA tests for dynamic pneumonic TB conclusion in low-and center wage nations (Andersen et al. 2000). Moreover, the examine takes 24 h to create results and requires a huge instrumentation and all around prepared staff.

5 Indicative Holes Between Present Advances and Neglected Clinical Need

Despite the fact that *Mycobacterium tuberculosis* was recognized as the main cause of TB years ago, the discovery of TB in the creating scene stays a critical social insurance problem attributable to various difficulties. MTB is a moderate developing bacterium, and in this way culture, in spite of high affectability, can't give direction to nearby patient care. For instance, MTB takes 2–4 months to develop with conventional strong cultures, 10–14 days with quick fluid cultures. Respiratory TB can lead to moderately low clinical side effects right off the bat in the course of ailment,

which prompts delays in looking for understanding, consideration. Dynamic respiratory TB can initially display low bacillary weight, which regularly prompts low affectability for sputum tests. Also, using sputum tests for the determination of TB with existing system is more perplexing compared with that using urine and blood tests. Especially, institutionalized sputum accumulation, transportation as well as capacity techniques are required to guarantee a predictable analytic results. Some difficulties are presented by the moderate developing kind of TB, the absence of dependable and approved natural indicators (used for the identification) of dynamic TB and as recognizable proof of LTBI. Inaccessibility of solid bioindicators is due to the lack of understanding the complex communication between MTB and its host and defensive insusceptible reactions amid contamination. Moreover, heterogeneous insusceptible reactions from people with various infection statuses for example, idle disease/re-contamination or with various inoculations can frustrate the translation of immunoassay outcomes. A perfect POC measure would (1) recognize initial contamination/sickness specifically with high affectability, (2) yield the results rapidly and easily, (3) request an isolated visitation, (4) result in practically zero patient inconvenience, (5) avoids using sputum, (6) distinguish the spread of negative cases and evaluate tranquilize defencelessness, (7) be able to detect bioindicators specifically and effectively and can lead to a better treatment and (8) be accessible to all people with suspicious TB, where there is little access to a reference research center. Efficient early diagnosis would offer extra preferences as those patients are less infectious (Behr et al. 1999); thus decreasing in general horribleness and mortality (Siddiqi et al. 2003). In spite of the current innovations of tuberculosis diagnosis, the improvement of a basic POC test sooner rather than later is still difficult (Pai and Pai 2012; WHO 2011). The need for novel diagnostic method and solid bio marks are necessary to improve the TB diagnosis in highly affected communities (for example, those with HIV as well as additional pneumonic diseases, kids from whom sputum tests are hard to gather). In spite of the fact that the GeneXpert presenting a method of choice, but the high cost restricts its use. An elective system to create reasonable TB indicative tests for asset compelled settings is to scale down TB finding. Microfluidic and Nano-based technologies have shown a promising signs in developing a better TB diagnostic methods with no/little limitations.

6 TB Current Treatment and Confinements

The current Tb treatment involves the start with a four drugs regimen using the first-line drugs; isoniazid (INH), pyrazinamide (PZA), rifampicin (RIF) and ethambutol (EMB) for two months. This is followed by a two drugs regimen using INH and RIF for four months. The failure of treatment using the first line drugs demands the use of second line drugs such as streptomycin, kanamycin, amikacin, capreomycin, ofloxacin, levofloxacin, gatifloxacin, ethionamide, prothionamide, cycloserine, terizidone and paraamino salicylic acid (Douglas and McLeod 1999; Ahmad and Mokaddas 2014; Zumla et al. 2015). These drugs are less viable, more poisonous,

and inaccessible in numerous nations because of high expenses (WHO 2015a, b). Until the December of 2012, the latest medications gone back 50 years (Grosset et al. 2012). Sarkar and Suresh (2011) accentuated how essential additionally look into the new medication target, is required in mind the end goal to battle sedate safe TB (Sarkar and Suresh 2011). There are a few new drug competitors presently looking into furthermore, in the clinical preliminaries such as bedaquiline and delamanid, which have been affirmed for the treatment of MDR-TB, at the point where different options are not accessible (WHO 2015a, b). Bedaquiline was affirmed by the Nourishment and Medication Organization (FDA) in December 2012 and has finished stage II preliminaries. Keeping in the mind the end goal to enhance treatment regimens, stage III preliminaries and stage IV think (WHO 2015a, b). Delamanid was endorsed by the European Solution Office (EMA) in April 2014 furthermore, is at present being tried in a stage III clinical preliminary for the treatment of MDR-TB in grown-ups and in youngsters (Grosset et al. 2012). Likewise, a few existing medications are in a condition of re-assessment (Siddiqi et al. 2003). The current TB remedy is generally connected with extreme adverse effects, bringing about poor consistence, leading to the appearance of multidrug safe strains and treatment's disappointment (Pinheiro et al. 2011; Dartois 2014; Das et al. 2015). Additionally, the present treatments have a constrained capacity to infiltrate granulomas and effect sly affect torpid bacilli (Wallis and Hafner 2015). In this unique situation, enhanced medicines are expected to abbreviate TB treatment span, anticipate obstruction and lessen lung damage.

Other than the previously mentioned confinements, the organization courses have likewise basic difficulties. The oral course is the most helpful and minimum costly; in any case, a delayed organization of high dosages is required and sub-helpful levels of hostile to TB drugs achieve the site of disease, due to the slower beginning of activity, the hepatic first-pass digestion and also the brutal gastro-intestinal retention (Pham et al. 2015; Turner et al. 2011). The oral course is additionally associated with extreme reactions because of high foundational introduction (Mehanna et al. 2014; du Toit et al. 2006). In comparison with the oral course, a higher bioavailability is achieved using the parenteral and pneumonic delivery systems, which avoid the first-pass effect (Pham et al. 2015). In any case, parenteral organization is an excruciating course of organization and requires the nearness of human services laborers (Prabakaran et al. 2004). In this unique circumstance, coordinate lung conveyance of hostile to TB drugs utilizing pneumonic conveyance frameworks could be invaluable and will be talked about in additional detail in the following area.

7 The Respiratory Administration Course: Favorable Circumstances and Difficulties

Lung is the most imperative course of access on account of disease by MTB (Misra et al. 2011; Sethi and Agrawal 2011), being the inhalatory course for medicate conveyance an energizing theory to be investigated keeping in mind the end goal to battle TB malady (Hokey and Misra 2011; Amani et al. 2011; Muttil et al. 2009). Undoubtedly, the lungs are the perfect target site of hostile to TB medicate conveyance and could give a non-invasive conveyance entry, requiring lower organization dosages for accomplishing a superior viability and poisonous quality decrease in examination with oral course (Willis et al. 2012). Lung mucosa has a vast surface of ingestion, a thin alveolar epithelium and a broad vascularization from which medications might be foundationally introduced into the circulation system; thus avoiding the first-pass effect (Misra et al. 2011). Respiratory organization likewise gives an enhanced bioavailability of medications in the focused on area, because of the constrained medication utilizing compound movement contrasted with different organs, for example, the gastrointestinal tract and liver (Lee et al. 2015). In comparison with the parenteral administration, the respiratory conveyance framework is self-administrated and non-invasive "free of needles" and can be self-administrated, which makes it considered as an intriguing methodology to treat respiratory diseases (Mehanna et al. 2014). Additionally, pathogenic TB bacilli set up disease principally in AMs (Hokey and Misra 2011). In this way, pneumonic conveyance of hostile to TB drugs establishes an intriguing methodology for the treatment of respiratory and furthermore extra pulmonary TB. In such manner, it would bear some significance with convey sedates straightforwardly to the lungs, accomplishing a superior focusing toward the contaminated AMs on account of pneumonic TB. On the other hand, the pneumonic conveyance of hostile to TB drugs related to their foundational ingestion could be utilized to treat extra pulmonary TB (Das et al. 2015; Pandey et al. 2005).

8 Lung Affidavit

The sciences and the defensive covering of the lungs are just parts of the challenge when an inhalatory system is being outlined. The affidavit of breathing in remedial operators in the respiratory aviation routes are exceedingly reliant on a few factors, which incorporates the characteristics of inhaled medications such as molecule number, shape, thickness, electrostatic charge and streamlined molecule measure dissemination (Lee et al. 2015; Ferron 1994; Ferron et al. 2013; Dunlap et al. 2000). Molecule measure is the most essential trademark to consider so as to accomplish a profound lung statement (Sung et al. 2007). Figure 2 shows the molecule measure impact in lung testimony. Particles, with diameter more than 5 μm, tend to settle in the mouth and upper airways by impaction, particles with breadths extending from 1 to 5 μm are the most proficient to achieve the profound lung by inertial impaction

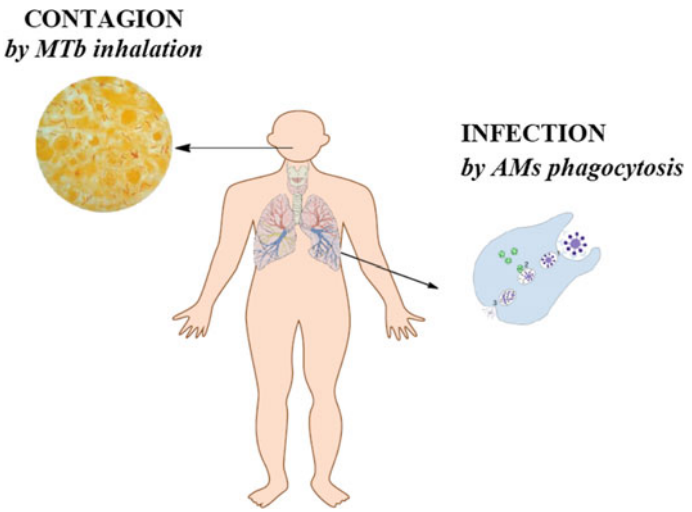

Fig. 1 Schematic portrayal of the contamination by MTB

and sedimentation (Mitchison and Fourie 2010; Ranjita et al. 2011). With particles howl 1 μm, components, for example, dispersion and sedimentation wind up imperative for achieving the pneumonic alveoli and such could be misused to advance respiratory conveyance methodologies (Chow et al. 2007). The molecule estimates between 50 and 200 nm is wanted for augmented medication limitation upon organization by inward breath (Ranjita et al. 2011). Molecule measure is likewise a critical trademark in the latent focusing of macrophages as it influences the accomplishment of disguise inside these cells. In such manner, particles with measurements of around 500 nm have been accounted for as perfect to experience phagocytosis by AMs (Fig. 1) (Muttil et al. 2009).

As depicted previously, adjusting the measure of the bearer framework, the phagocytosis of NPs by AMs can be upgraded. Related with latent focusing on system, there are dynamic focusing on methodologies that can be used to enhance the disguise of the NPs in the AMs. In dynamic focusing on systems, the structure and constitution of the nanosystems are changed. The presence of various receptors associated with macrophages make them susceptible to be used by nanostructures with suitable ligand. For example, the receptors of sugars (such as mannose and lactose) are profoundly associated with macrophages (Nimje et al. 2009; Jain et al. 2010). Different ligands regularly with a similar reason incorporate maleylated ox-like serum egg whites, O-steroyl amylopectin and anionic lipids. Recently, a few nanoparticle-based frameworks have been examined with the objective of tending to the previously mentioned angles. Pneumonic administration of medications should finish utilizing an appropriate gadget that creates a suitable airborne. As of now, there are three primary conveyance gadgets utilized for this reason, to be specific nebulizers, pressurized metered-measurement inhalers (pMDIs) and dry powder inhalers (DPIs).

These are used widely with various types of particles (Bosquillon et al. 2001). They are ordinarily utilized in the treatment of respiratory constant sicknesses. DPIs are fuel free, versatile, simple to work and ease gadgets (Amani et al. 2011).

9 Nanosystems for the Pneumonic Conveyance of Hostile to TB Drugs

Nanotechnology is concerned with the plan and study of nano-structures, called nanoparticles (NPs), which have a diameter in the range of (1–1000 nm). These particles are characterized by special properties, which can be altered through changing the particle size (Pinheiro et al. 2011). Some particles, with a diameter of more than 1 μm, are viewed as nanoparticles as they share a few, or even most, of these physical concoction qualities. NPs can be utilized for therapeutic purposes, to be specific as nanocarriers for restorative and demonstrative specialists by implies of exemplification, covalent connection, or surface adsorption of these operators (Moghimi et al. 2005).

Nanotechnology has shown the ability to develop a promising pneumonic drug delivery system. This can be explained by the ability of these carriers to deliver the drugs to different regions in the lungs, the possibility of developing drug delivery systems via modifying their surface and using special ligands as well as a high bioavailability can be achieved using nanosuspensions (Ranjita et al. 2011; Gill et al. 2007; Gao et al. 2012). All these reasons can enhance the therapeutic efficacy; reduce dosing frequency and side effects (Ranjita et al. 2011). Inhalable nanocarriers offer a potential incentive in the neighborhood the and uninvolved conveyance of hostile to TB treatment, as inspected beforehand (Mehanna et al. 2014; Andrade et al. 2013). Neutral NPs, polymeric and lipid based are the most used ones for pneumonic drug delivery. Different details right now looks into incorporating the creation of medication nanocrystals, pressurized canned products with attractive nanoparticles carriers, with foaming movement, and gold based carriers.

9.1 Liposomes

Liposomes were found in 1965 (Pinheiro et al. 2011). They are vesicular nanoparticles, established by phospholipid bilayers encasing a fluid medium (Bangham 1993; Mouritsen 2011). Their structure can be modified to adapt with lipophilic, hydrophilic or amphiphilic substances (Pinheiro et al. 2011; Chimote and Banerjee 2005). The possibility of modifying their size and structure paved the road to deliver the drugs, effectively and specifically, to different regions of the lung. Surface mannose alteration is a standout amongst the best models of this procedure, as it has been appeared to expand the take-up of NPs by AMs (Chono et al. 2010; Andrade et al. 2013; Wijagkanalan et al. 2008; Kong et al. 2012). Liposomes appear to be especially

proper for respiratory conveyance, as they may be detailed from endogenous mixes, for example, the segments of respiratory surfactant (PS) (Justo and Moraes 2003). Notwithstanding, numerous aerosolization systems can bargain liposome structure respectability. In generally aerosolized liposome details for focused aspiratory conveyance, liposomes are shaped before bundling. This more often than not brings about bursting of vesicle structure amid organization, in this manner losing the capacity for managed discharge (Anabousi et al. 2006). This makes them able to locate countless including pneumonic conveyance of medications with liposomal details, a large number of them concentrating on anti-microbial furthermore, especially hostile to TB drugs (Table 1). A research group investigated the theory of framing liposomes in locally, since the lung has a wet surface, which could give a watery stage to unconstrained development (Gaur et al. 2010). The plan embodying RIF with a stacking productivity of 29–38% was framed by egg phosphatidylcholines (EPCs), Chol and DCP. They have detailed that no vesicle break was seen with in situ framed liposomes and delayed medication discharge was accomplished.

Ciprofloxacin was one of the principal hostile to TB medications to be typified in liposomes. Finlay and Wong (1998) investigated liposomes made of phosphatidylcholine (PC) and Chol epitomized with ciprofloxacin with a high stacking proficiency of 90% preceding nebulization, of which 2–30% remained embodied after nebulization. They contemplated liposome disturbance amid aerosolization, utilizing 25 nebulizers (Finlay and Wong 1998). Afterward, the same creators distributed outcomes on unconstrained development of liposomes on scattering of phospholipid-based powder plans (Desai et al. 2002a, b). The plans comprised by PCs (dipalmitoylphosphatidylcholine (DPPC) or on the other hand EPC) and Chol showed a stacking productivity of 97% preceding lyophilization, of which up to 90% were held by the lyophilized cake and up to 40% after stream processing. In the other examination, among all the tried definitions: dimyristoylphosphatidylcholine (DMPC), DPPC, dimyristoylphosphatidylglycerol (DMPG), EPC:DMPG and DMPC:DMPG; the best outcomes as far as the stacking efficiency was gotten to the details with the adversely charged phospholipid DMPG, being right around 100%. Also, the nebulization of the particles causes a general diminishing of the medication's stacking effectiveness. Cysteine was utilized keeping in mind the end goal to encourage post-organization tweak of the medication discharge rate since it frames cross-interfaces between nanosized liposomes to frame the agglomerates. It could permit the treatment regimens where the organization of one single measurements would be adequate for an expanded timeframe, since medicate discharge could be intermittently quickened. They additionally found that the dynamic arrival of the medication does not cause critical irritation, dissimilar to the organization of free ciprofloxacin. Other enemies of TB drugs have just been epitomized into liposomes. Liposomes made of DPPC for INH conveyance were produced (stacking proficiency of around 37%) by Chimote and Banerjee (2009). The creators watched a supported arrival of INH epitomized in liposomes held more than 24 h, after a burst discharge in the initial 5 h of half of the medication. They have likewise directed biocompatibility and soundness studies, and found that these plans were hemocompatible, cytocompatible, and stable for the length of no less than multi month. Justo and Moraes (2003) contemplated

Table 1 Liposomes for the pulmonary delivery of anti-TB drugs

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
Liposomes (PC:Chol)	5–7 μm	N/A	Ciprofloxacin	90% (before nebulization), of which 2–30% remained	Nebulizer	N/A
Liposomes (DPPC:Chol and EPC:Chol)	Lyophilized cake: 1–7 μm After jet milling: 1–2 μm	N/A	Ciprofloxacin	Before lyophilization: 97% After lyophilization: 90%	Dry powder	N/A
Liposomes (DMPC, DPPC, DMPG)	2–3 μm	N/A	Ciprofloxacin	DMPC: 50% DPPC: 30% DMPG: 95%	Nebulizer	N/A
Liposomes (DPPC:Chol:mPEG-DSPE and DPPC:Chol:DSPE-PEG-NH2)	Liposomes: 140–460 nm Agglomerates: 1–140 μm	N/A	Ciprofloxacin	N/A	Nebulizer	The plan did not cause critical aggravation, dissimilar to the organization of free medication
Liposomes (DPPC)	750 nm	N/A	INH	~37%	Nebulizer	The plan is hemocompatible and cytocompatible
Liposomes (DSPC:Chol)	286–329 nm	N/A	INH, PZA, RIF, ethionamide, and streptomycin	INH: 3% PZA: 2% Streptomycin Ethionamide and RIF: 0% Streptomycin: 42%		N/A

(continued)

Table 1 (continued)

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
Liposomes (Lecithin:Chol) 6	6.373 μm	N/A	RIF	79.25%	Dry powder	The created NPs were inside respirable size range upgrade of medication pervasion in alveolar epithelium
Pre formed and in situ formed liposomes (EPC:Chol:DCP)	2 μm for preformed and in situ formed, respectively	N/A	RIF	29–38%		Drawn out medication discharge was accomplished to the in situ definitions
Liposomes (PC:Chol)	2–4 μm	Negatively charged liposomes (DCP)	RIF	47–49%	Nebulizer	Lung maintenance of medication was higher with liposomes than with free medication
Liposomes (EPC:Chol)	DSPE-PEG liposomes: b200 nm O-SAP coated liposomes: ≥200 nm	O-SAP	INH and RIF	INH: 8–10%	INH: 8–10% RIF: 44–49%	Exemplified drugs were seen to be less destructive than free sedates in cell lines O-SAP covering enhanced lung total

(continued)

Table 1 (continued)

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
Liposomes (EPC)	25–65 nm	Tuftsin	RIF	28–32%		Covered liposomes given twice week after week for about fourteen days were extensively more viable than uncoated ones in controlling TB
Proliposomes (HSPC, Chol, stearylamine or stearic acid)	442–803 nm		Rifapentine		Dry powder	Supported medication discharge with longer maintenance of medication in lungs and most astounding focusing on potential In vivo considers: The immediate conveyance of rifapentine in the type of RLDPI to the lungs, accomplishes higher medication fixation in lungs and diminishes fundamental harmfulness

the likelihood of co-embodiment of a few against TB drugs (i.e., INH, PZA, RIF, ethionamide, and streptomycin) in liposomes made of distearoylphosphatidylcholine (DSPC) and Chol (Justo and Moraes 2003). Be that as it may, under the tried conditions, RIF and ethionamide were not effectively typified. Low epitome effectiveness was accomplished to both INH and PZA, with stacking efficiencies individually of 3% and 2%, being the epitome of streptomycin the most astounding (42%). Gaur et al. (2010) distributed an attainability think about where they utilized RIF as the model medication (Gaur et al. 2010). In this investigation, in situ framed liposomes made of PC and Chol demonstrated preferred managed discharge profile over the preformed liposomes, however both liposomal vaporizers demonstrated an enhanced conveyance of RIF over plain medication aerosols, with exemplification efficiencies around 30%. As of late, Patil et al. (2015) created RiF-stacked stop dried liposomes also; their outcomes affirmed that as the convergence of Chol expanded, the medication discharge diminished (Patil et al. 2015). Their advanced plan has 79.25% of medication ensnarement effectiveness and has demonstrated moderate and managed arrival of the medication. In vitro results uncovered an upgraded dissolvability of the medication and higher enemy of TB action, when contrasted with the unadulterated medication alone. Streamlined portrayal information recommended that the created NPs were inside the respirable size range and in vivo contemplates upheld the job of liposomes in upgrade of medication saturation in alveolar epithelium (Patil et al. 2015). The likelihood of surface covering to accomplish dynamic focusing with liposomes shaped by PC and Chol has likewise been a subject of intrigue. Vyas et al. (2004) epitomized RIF (stacking productivity of about half) when considering liposomes covered with macrophage specific ligands (i.e., DCP, MBSA or O-SAP) and announced a particular aggregation of ligand-covered plans in the lung macrophages (Vyas et al. 2004). O-SAP surface adjustment was additionally the focal point of Deol et al. (1997), who was created to cover liposomes shaped by EPC and Chol for the exemplification of both RIF (stacking effectiveness 44–49%) and INH (stacking proficiency 8–10%) (Deol et al. 1997). They looked at the results with the uncoated ones and revealed that epitomizing drugs inside liposomes lessened poisonous quality and that O-SAP covering has prevailing with regards to improving lung collection. Agarwal et al. (1994) contemplated the tufts in functionalization of EPC liposomes embodied with RIF (stacking effectiveness around 30%) (Agarwal et al. 1994). They announced that thinking about one single organization, tufts in functionalization improved outcomes than uncoated.

Definitions, however with general organization more than about fourteen days, tufts in liposomes were more productive in controlling TB (Agarwal et al. 1994). Patil-Gadhe and Pokharkar (2014) effectively connected the standards of value by configuration to create rifapentine-stacked proliposomes for inward breath by shower drying in a single step. Their outcomes showed a supported medication discharge and the pulmokinetic parameters were enhanced, uncovering longer maintenance of sedate in the lungs and most elevated focusing on potential (Patil-Gadhe and Pokharkar 2014). After one year, it was assessed the counter TB action, in vitro cytotoxicity also, in vivo poisonous quality of rifapentine-stacked proliposomal dry

powder for inward breath (RLDPI). Their outcomes affirmed the counter TB potential of rifapentine in splash dried RLDPI. Additionally, the immediate conveyance of rifapentine as RLDPI to the lungs, accomplishes higher medication focus in the lungs and diminishes foundational poisonous quality (Patil et al. 2015). These investigations obviously show that enemy of TB-epitomized liposomes have an exceptional potential as immediate medication conveyance frameworks to the lungs.

9.2 Lipid Nanoparticles

Lipid NPs indicate higher medication stacking limit, higher steadiness, and may not require the utilization of natural solvents amid generation in difference to liposomes and polymeric NPs (Sosnik et al. 2010). The good biocompatibility and the ability to deliver medicines, specifically and effectively, to different areas in the lung make these carriers good candidates for respiratory drug delivery systems (Videira et al. 2002; Pandey et al. 2005; Yu et al. 2010). Solid lipid nanocarriers (SLNs) and nanostructured lipid bearers (NLCs) are the most well-known lipid NPs utilized. Table 2 recorded the precedents established in the writing of lipid NP definitions for the conveyance of against TB drugs. The distributed outcomes by Jain and Banerjee (2008), who analyzed four unique nanocarriers for the joining of ciprofloxacin, demonstrated that SLNs could advance a drawn out medication discharge (Jain and Banerjee 2008). This work is one of the four reports that were discovered with respect to the respiratory conveyance of SLNs stacked with INH, RIF and PZA. Nimje et al. (2009) arranged rifabutin stacked SLNs made of tristearin, and contrasted uncoated definitions and details covered with mannose (Nimje et al. 2009). The outcomes demonstrated that the detailing is reasonable for maintained conveyance and due to mannose covering the cell take-up by AMs was about six times upgraded. In vivo results affirmed the nearness of higher medication sum in the lungs and less immunogenicity for covered SLNs with respect to uncoated definition. Pandey and Kuller (2005a, b) have arranged SLNs comprised by stearic corrosive for pneumonic conveyance through nebulization (Pandey et al. 2005). They consolidated INH, RIF and PZA with stacking efficiencies of about half for each tranquilizes. Every one of the definitions were equipped for managed medicate discharge with a burst sedate discharge lower than 20% in the initial 6 h, and 11–15% amid 6–72 h on account of INH and PZA; for RIF, 9% were the discharged in the initial 6 h, what's more, 11% amid 6–72 h. The nebulized SLNs were effectively saved in the lungs and were recognized in different organs up to 7 days after the organization. Administrated free medication was cleared within 24–48 h. Jain and Banerjee (2008) included SLNs made of stearic corrosive in their rundown of nanosystems to convey ciprofloxacin (with a stacking effectiveness of 39%) and reasoned that these NPs were fit for supported medication discharge up to 80 h (Jain and Banerjee 2008). As of late, Chuan et al. (2013) created RIF-stacked SLNs as AM-focusing on the sedate conveyance framework. RIF-stacked SLNs had a normal size of around 800 nm and demonstrated moderately low cytotoxicity when fixation was higher than 20 μg/mL. Also, the creators illustrated that the RIF-stacked SLNs were disguised all the more specifically in

Table 2 Lipid NPs for the pneumonic conveyance of hostile to TB drugs

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
SLN (tristearin)	Uncoated: 251 nm Coated: 389 nm	Mannose	Rifabutin	Uncoated: 82% Coated: 87%		Maintained medication discharge; macrophage take-up was higher for covered SLNs. Higher medication nearness in the lungs for covered SLNs; less immunogenic
SLN (stearic acid)	1–2 μm	N/A	INH, RIF, PZA	INH: 45% RIF: 51% PZA: 41%	Nebulizer	Medication discharged was b20% in the initial 6 h, and 11–15% amid 6–72 h for INH/pyrazinamid; 9% in the initial 6 h and 11% amid 6–72 h for RIF The three medications were identified in the lungs, liver and spleen of the creatures up to day 7 following the nebulization
SLN (stearic acid)	74–99 nm	N/A	Ciprofloxacin	39%	–	SLNs were equipped for a drawn out medication discharge up to 80 h
SLN	800 nm	N/A	RIF	86.5%	–	RIF-stacked SLNs were disguised more specifically in AMs than in alveolar epithelial compose II cells
NLC	160 nm	Mannose	RIF	N90%	–	RIF-stacked NLCs demonstrated tissue selectivity and altogether enhanced the lung amassing of RIF, when contrasted with RIF arrangement

AMs than in alveolar epithelial compose II cells (Chuan et al. 2013). As a novel sort of lipid NPs, NLCs are made of blend out of strong and fluid lipids, which makes a flawed gem lattice that upgrades tranquilize stacking limit and limits sedate ejection amid long haul stockpiling (Radtke et al. 2005). From our insight, there is just a single report about the improvement of against TB medicate stacked NLCs frameworks for AMs focusing on. Song et al. (2015) created RIF-stacked cationic mannosylated NLCs with a normal size of 160 nm, embodiment proficiency higher than 90%, least cytotoxicity and no incendiary reaction (Song et al. 2015). Besides, RIF-stacked NLCs indicated tissue selectivity and altogether enhanced the lung amassing of RIF, when contrasted with RIF arrangement (Yu et al. 2010). Because of their attributes, lipid NPs offers an efficient methodology for the respiratory organization of hostile to TB drugs.

9.3 Polymeric Nanoparticles

Natural and synthetic polymers have been involved in the development of nanocarriers for controlled drug delivery (Moghimi et al. 2005). Basic precedents of regular polymers for pneumonic conveyance are alginate, chitosan and gelatin. Manufactured polymers incorporate poly (lactide-co-glycolide) corrosive (PLGA), poly lactic corrosive, poly anhydride and poly acrylate (Pandey and Ahmad 2011). Polymeric NPs are among the most generally examined frameworks for medicating conveyance by and large, and many reports center around the pneumonic conveyance specifically. These conveyance frameworks satisfy most prerequisites set for pneumonic conveyance, for example, adequate relationship of the helpful agent with the bearer particles, focusing of particular locales or cell populaces in the lung, and empower the security of medications against corruption what's more, the medication discharge at remedial levels for accomplishing the attractive impact. They additionally can be moved into an airborne, present low poisonous quality and security against powers produced amid aerosolization (Beck-Broichsitter et al. 2012), making them fascinating materials for the designing of biodegradable nanocarriers (Rytting et al. 2008). Medication conveyance plans with hostile to TB drugs have just been utilized with these nanocarriers (Table 3). Jain and Banerjee (2008) thought about four distinctive NP details for ciprofloxacin conveyance, the three of them being polymeric NPs (Jain and Banerjee 2008). The creators fused the medication into egg whites, gelatin and chitosan NPs and concentrated their medication discharge profiles. Out of the three polymers, the egg whites and chitosan NPs turned out to be more equipped for medicate consolidation (48 and 35% stacking proficiency, separately) what's more, the supported discharge (up to 120 and 96 h, individually). Different examinations more often than not center around one sort of nanosystem, despite the fact that with different medications co-exemplified. Alginate NPs have been contemplated by Ahmad et al. (2005) for the joining of RIF, INH and PZA (Ahmad et al. 2005).

The said particles had streamlined distances across in the respirable run, and introduced high medication exemplification efficiencies for each of the three medications

Table 3 Polymeric NPs for the pneumonic conveyance of against TB drugs

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
Albumin, gelatin and chitosan	Albumin: 140–175 nm Gelatin: 143–184 nm Chitosan: 247–322 nm	N/A	Ciprofloxacin	Albumin: 48% Gelatin: drug unstable and prone to flocculation	–	Albumin and chitosan NPs were fit for delayed tranquilize discharge up to 120 h and 96 h, separately
Alginate	236 nm	N/A	RIF, INH, PZA	RIF: 80–90% INH and PZA: 70–90%	Nebulizer	Streamlined widths in the respirable range Expanded bioavailability contrasted with oral free medications
Gelatin	234 nm (uncoated) and 343 nm (coated)	Mannose	INH	55% (uncoated) and 43% (coated)	Nebulizer	An underlying burst, trailed by a slower managed discharge over a time of 120 h Macrophage take-up higher with covered NPs than with uncoated ones Higher medication content in the lung for mannosylated NPs

(continued)

Table 3 (continued)

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
mPEG2000 and mPEG5000 DSPE	mPEG2000: 226–396 nm mPEG5000: 163–233 nm	N/A	RIF	84–104%	Nebulizer	Drawn out medication discharge more than 3 days Streamlined distances across in the respirable range
Chitosan/tri-poly phosphate (TPP)	Chitosan/TPP (3:1): 241 nm Chitosan/TPP (6:1): 449 nm	N/A	INH	Chitosan/TPP (3:1): 13% Chitosan/TPP (6:1): 17%	Dry-powder	An underlying burst arrival of medication up to 4 h, trailed by a maintained discharge amid 6 days
Chitosan	230 nm	N/A	RIF, INH	RIF: 70.8% INH: 68.8%	Nebulizer	Lower cytotoxicity and critical decrease in the number of bacilli in the lungs, contrasted with free medication

(continued)

Table 3 (continued)

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
PLGA PNAPS	NPs: ~195 nm PNAP: ~4 μm	N/A	RIF	N/A	Dry-powder	An underlying burst of the medication happens, trailed by a managed discharge past 8 h Medication stayed noticeable in lung up to and past 8 h
PLGA	121–184 nm	Lactose	RIF	38–42%	–	In correlation with unconjugated NPs, lactose conjugated NPs indicated more prominent normal size and medication payload, slower medicate discharge, and upgraded take-up in lung tissue

(continued)

Table 3 (continued)

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
PLGA	186–290 nm	N/A	RIF, INH, PZA	RIF: 57% INH: 66% PZA: 68%	Nebulizer	RIF stays in plasma up to 6 days, while INH and PZA stay up to 8 days. RIF, INH, PZA were available at restorative focuses in the lungs till day 11
PLGA	Uncoated NPs: 180–290 nm Coated NPs: 350–400 nm	Wheat germ agglutinin	RIF, INH, PZA	RIF: 54% INH: 64% PZA: 67%	Nebulizer	RIF stays in plasma amid 6–7 days, while INH/PZA stays plasma amid 13–14 days
PLGA	110–700 nm	N/A	Levofloxacin	4–23%	Dry-powder	Epitome productivity was expanded utilizing changed techniques for PLGA arrangement, and medication discharge was kept up or improved. Antibacterial action was kept up after splash drying

(continued)

Table 3 (continued)

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
PLGA (lipid-polymer hybrid)			Levofloxacin, ciprofloxacin and ofloxacin	Levofloxacin: 10–19% Ciprofloxacin: 4–6% Ofloxacin: 10–25%	–	Mixture levofloxacin-NPs demonstrated a burst discharge in the initial 5 h, and afterward a moderate discharge in the accompanying 20 h. With non-mixture NPs, nearly the whole medication is discharged in the initial 5 h
Copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester	45.51–300.4 nm		PZA	80.09%	–	The streamlined definition was steady for 2 months furthermore, has been productively up taken by AMs

(somewhere in the range of 70 and 90%). Bioavailability everything being equal was examined and these details indicated preferable outcomes over the oral organization of free medications. Researchers developed gelatin-based nanoparticles, linked with mannose, as a promising targeted pneumonic drug delivery of INH. Abdulla et al. (2010) utilized two diverse sub-atomic weights of poly-(ethylene oxide)–square distearoylphosphatidyl-ethanolamine (mPEG2000–DSPE and mPEG5000–DSPE) polymers to create nano carriers for pneumonic conveyance of RIF (Abdulla et al. 2010). They announced high tranquilize stacking and ensnarement efficiencies (84–104%) and took note that these qualities were impacted by drug: polymer proportion, yet not by mPEG–DSPE atomic weight. Molecule measure and streamlined portrayal demonstrated that readied definitions were appropriate for lung statement through inward breath.

Chitosan has been used widely in the development of nano systems due to its attractive properties such as the good biocompatibility, biodegradability, low toxicity, being mucoadhesive and advancing macromolecule saturation through efficient epithelia (Grenha et al. 2005; Sharma et al. 2012). This examination additionally demonstrated that this methodology could be utilized for neighborhood treatment of lung maladies, for example, TB. Pourshahab et al. (2011) utilized chitosan/tripoly phosphate NPs as nanocarriers for INH with 449 nm in measure and a stacking proficiency of 17%. The detailing displayed a discharge profile with an underlying medication discharge burst, trailed by moderate and supported discharge in the accompanying 6 days (Pourshahab et al. 2011). Garg et al. (2015) arranged and described a shower dried inhalable chitosan NPs for supported conveyance of INH and RIF (Garg et al. 2015). Their outcomes incorporate NPs with a normal size of 230 nm and a medication exemplification proficiency of 69% for INH and 71% for RIF. Moreover, these details appeared bring down cytotoxicity and critical decrease in the quantity of bacilli in the lungs, contrasted with free medication (Garg et al. 2015). PLGA NPs are to a great degree regular in nano systems, and have been used to epitomize some enemy of TB drugs. Sung et al. (2009) illustrated that PLGA NPs stacked with RIF could be detailed, bringing about "permeable NP-total molecule" (PNAP), particles with airborne properties reasonable for lung conveyance (Sung et al. 2009). In vitro and in vivo thinks about were performed, what's more, found a postponed arrival of the medication up to 8 h. They displayed a more prominent normal size and medication payload, slower tranquilize discharge, also, and demonstrated to have a superior take-up by lung tissue. Pandey et al. (2003) also, Sharma et al. (2004) developed PLGA NPs as a potential co-delivery system of RIF, INH and PZA. They announced the nearness of RIF in plasma for 4–6 days, and of INH and PZA for 8–9 days. Surprisingly a further work demonstrated that five dosages of nebulized hostile to TB PLGA NPs accomplished the identical restorative advantages of 46 every day dosages of orally regulated free medication (Pandey et al. 2003). In 2004, they covered comparable NPs with wheat germ agglutinin and announced an expanded period amid to which all medications were recognizable in plasma, in particular 6–7 days for RIF and 13–14 days for INH and PZA (Sharma et al. 2004). Cheow and Hadinoto (2010) modified PLGA arrangement strategies to accomplish higher embodiment efficiencies of water soluble anti-infection agents,

utilizing levofloxacin as the model medication (Cheow and Hadinoto 2010). The detailing displayed a stacking productivity somewhere in the range of 4 and 23%, and displayed antibacterial action even after the splash drying. In other example, they created lipid–polymer half and half NPs to consolidate levofloxacin, ciprofloxacin, and ofloxacin (Cheow and Hadinoto 2011). These details showed a productivity epitome of 10–19% (levofloxacin), 4–6% (ciprofloxacin), and 10–25% (ofloxacin). After introductory burst discharge, cross breed NPs demonstrated a slower or slower tranquilize discharge than its non-hybrid partners. With a specific end goal to manage the discharge profile and the diminish dosing recurrence of PZA, Varma et al. (2015) figured PZA-stacked polymeric NPs, utilizing a copolymer of ethyl acrylate, methylmethacrylate and a low content of methacrylic corrosive ester with quaternary ammonium bunches (Varma et al. 2015). The outcomes uncovered that the NPs demonstrated a size range from 45 to 300 nm and a maximum drug entanglement effectiveness of 80.9%. This upgraded detailing was steady for 2 months and has been effectively uptaked by AMs. The discharge rate of the medication diminished with an expansion of polymer's fixation, and a proportion of the drug: polymer (1:2) empowered the PZN discharge for over 1 day (Varma et al. 2015). These investigations bolster the thought that polymeric NP-based for sedate conveyance frameworks are appropriate for focusing on the cell stores of MTB.

9.4 Different Nanocarriers

The utilization of remedial operates as a nanocarrier has been presented as a framework for tranquilizes conveyance. The medicated nanocarriers break down quickly in the pulmonary fluid prompting a nearby high focus, which is useful for a confined treatment of pulmonary sicknesses such as pneumonic TB. The outcomes demonstrated that these frameworks could be utilized in tranquilize conveyance details to enhance both the pharmacokinetic and the pharmacodynamic properties of ineffectively solvent medications. Table 4 recorded the models of NPs diverse from the most widely utilized for the conveyance of hostile to TB drugs. Gao et al. (2012) detailed two various types of pneumonic definition containing sedate nanocrystals (Gao et al. 2012). Spore like medication particles for profound lung statement have been also proposed as an inventive framework (Shen et al. 2012). Empty and spore like nanoagglomerates were acquired by blending the medication arrangement with an antisolvent in a high gravity condition. The manufacture of medications like spores can enhance the pneumonic medication conveyance.

Productivity in DPIs, out of context, financially savvy and simple large production capacity technique compared to other conventional techniques such as milling, homogenization, and shower solidifying into fluid and supercritical antisolvent precipitation to get a ready nanosuspensions. Monodisperse particles with controlled morphology can be obtained using this system (Shen et al. 2012). Right now, just a single report was announced in regards to the creation of nanocarriers carriers for the delivery of TB drugs. El-Gendy et al. (2010) arranged ciprofloxacin nanosuspensions

Table 4 Medication nanocrystals and NPs with fizzing action for the respiratory conveyance of hostile to TB drugs

Nanoparticle	Size	Ligand	Drug(s)	Loading efficiency	Inhaled form In.	In vitro/in vivo results
Drug nanoparticle agglomerates	NPs: 8–722 nm NP agglomerates: 2–4 μm	N/A	Ciprofloxacin	81–96%	Dry powder	Disintegration rate was progressed in correlation with the free medication
Effervescent NPs (PBCA)	Bubbling arrangements: 244 and 252 nm when shower drying, individually Bubbling arrangements containing l-leucine what's more, PEG 6000: 150 and 177 nm, prior and then afterward splash drying, separately Bearer particles: 2 μm	N/A	Ciprofloxacin	N/A	Dry powder	Fizzing bearer particles discharged 56% of ciprofloxacin into arrangement in correlation with 32% at the point when lactose particles were utilized

with a higher stacking proficiency (i.e., 81–96%) (El-Gendy et al. 2010). Disintegration tests were performed. The results demonstrated that the disintegration rate was enhanced, exhibiting that these strategies may survive a portion of the solvency issues exhibited by the new enemy of TB drugs, extraordinarily by atoms that, in spite of the fact that it did not pass the clinical preliminaries because of dissolvability issues, indicated higher potential as hostile to TB drugs. NPs with bubbling action have been recommended for respiratory conveyance. Oral medication conveyance related to bubbling pharmaceutical definitions is utilized for quite a while, in stomach trouble solutions, analgesics and vitamins. The fizzing action of the transporter particles happens when the bearer particles are presented to moistness, adding a functioning discharge instrument to the pneumonic course of the organization. Moreover, bubbling nanostructures with a suitable size can be orchestrated for a better lung settlement, and the innovation seems, by all accounts, to be alright for respiratory conveyance (Azarmi et al. 2008). Albeit fizzing NPs have been generally examined

as a promising respiratory conveyance technique for the conveyance of various medications (Azarmi et al. 2008; Al-Hallak et al. 2012; Roa et al. 2011), just a single report has been announced with respect to their utilization for the conveyance of ciprofloxacin (Ely et al. 2007). Ely et al. (2007) have created and considered diverse powder syntheses with fizzing movement, and announced two plans reasonable for pneumonic conveyance (Ely et al. 2007). Inhaled gold nanoparticles, with a mean diameter of 16 nm, were recently studied as potential targeting delivery systems (Tom et al. 2004), yet the referred to consider do not center around the pneumonic conveyance, and no different reports have been discovered with respect to the utilization of these particles for pneumonic conveyance, notwithstanding the model medication. The utilization of attractive mist concentrates utilizing superparamagnetic press oxide NPs has likewise been recommended as an approach to enhance pneumonic conveyance (Dames et al. 2007). Ciprofloxacin has been utilized as a model medication in the advancement of superparamagnetic nanocomposites with attractively intervened arrival of the stacked enemy of TB sedate (Bajpai and Gupta 2011). Be that as it may, no examination has been discovered joining these two procedures to accomplish attractively interceded respiratory conveyance of hostile to TB drugs.

10 The Potential Application of Nanotechnology/Microfluidics in TB Diagnosis

Nanotechnology and microfluidics have been used to develop biosensitive sensors for MTB diagnosis (Table 1). This system consists of an expository gadget combined with an organic sensor reacts to physicochemical changes in the detecting region. Contingent upon the utilized flag creating instrument, TB biosensors can be put into one of the accompanying classes: mass/piezoelectric, biochemical, electrical, also, optical sensors as talked about underneath. These detecting stages are in light of distinguishing antibody–antigen connections, entire mycobacteria or on the other hand nucleic corrosive hybridization.

10.1 Biosensors in View of Identifying Antigen/Neutralizer/Entire Mycobacteria

10.1.1 Mass and Piezoelectric Sensors

These sensors use quartz precious stones, which are highly sensitive to mass and surface changes. They can identify sub-atomic cooperation among target and ligand, what's more, screen biochemical responses happening on the surface of a detecting stage. Two methods have been developed to recognize MTB, including (1) quartz

gem microbalance (QCM) innovation (2) arrangement piezoelectric precious stone based sensors. In QCM, changes in gravitational load on the sensor also, viscoelastic properties of the example cause a recurrence move of a quartz precious stone resonator (Höök et al. 2001; Peh et al. 2007). In an immuno-piezo sensor, a QCM detecting surface was first covered by a styrene–butadiene–styrene copolymer as a film emulating layer to immobilize hostile to MTB antibodies on the sensor (He et al. 2003). Amid the hatching with MTB on the stage, the catch of MTB cells was checked continuously by watching the recurrence move because of the change in mass stacking on the sensor. The point of confinement of-recognition of this framework was seen to be 105 CFU/mL (He et al. 2003). Despite the fact that this innovation is fast, basic, and mark free, the exactness is influenced by various factors, for example, thickness, consistency, dielectric steady and the sample electrical conductivity (Ren et al. 2008). Another system was developed using a multi-channel arrangement piezoelectric quartz precious stone (MSPQC) sensor framework (Ren et al. 2008). The MSPQC framework comprised of a different example discovery stage, a microchip framework, and an information yield framework. The silver-covered generator delicate to changes in the recurrence. The framework intended to distinguish unpredictable metabolites (such as CO_2 and NH_3) that are created therefore of MTB development. After that, they were consumed due to the presence of KOH in the medium, also created a recurrence move. With a cutoff of 100 Hz change in the recurrence move, this test had a wide linearity running from 102 to 107 CFU/mL and a location point of confinement of 10 CFU/mL. Contrasted with the regular measures, for example, "BACTECTM MGITTM 960 and Lowenstein–Jensen (L–J)" inclines, MSPQC examine is more economical (under $1000 for the setup and $4.2 for the measure) furthermore, delicate (Ren et al. 2008). In any case, the examine needs 2–4 days to culture MTB, and necessities test pretreatment to dispense with possible tainting by other microbes that might be less appropriate for POC testing.

10.1.2 Light Identification Advancements

Raman spectroscopy has been used to recognize refined MTB and have turned out to be quick and profoundly particular in separating MTB from other strains (Buijtels et al. 2008). In this method, a Raman spectra system is coupled to a custom-manufactured transformed magnifying instrument with computerized XY-arrange. To energize the examples, a laser light utilized at an estimated wavelength of 750–1000 nm to produce spectrographic fingerprints of a numerous mycobacterial strains. These fingerprints spoke to the sub-atomic piece of the practical organism at both the strain and species levels. The affectability of the depicted technique for different strains was seen to be 95.2%, contrasted with 16S rRNA distinguishing proof (sequencing), which has disadvantages, for example, staggering expense, unpredictability, and unambiguous translations (Buijtels et al. 2008). The spectra of warmth inactivated tests demonstrated negligible contrast from that of practical mycobacteria tests and this can enable this stage to be utilized outside Biosafety Level 3 research facilities, with a warm inactivated tests. At any rate, this approach is restricted by a

few difficulties for POC testing, which include the economy (utilization of laser and a fluorescence magnifying instrument), long system (roughly 3 h) and prerequisite to set up fingerprints for non-tuberculosis bacteria and other pathogens. Furthermore, the affectability for identification of MTB in the sputum should be additionally assessed. Another optical location innovation for MTB conclusion is RBS breathalyzer fluorometry (Rapid Biosensor Systems, Cambridge, UK). This framework comprises of a convenient gadget dispensable plastic gathering tube (3.5 × 10 cm) into which the patient hacks. The accumulation tube was intended to gather pressurized canned products and particles hacked out by the patient and embedded into a little battery-worked device, which contains a diode laser and a photomultiplier for detecting.

In the accumulation tube, the hack test is pushed around a plunger and disseminated onto the surface of a crystal at the base. Since the crystal was covered with fluorescence marked simple, the nearness of native MTB antigen (e.g., Ag85B) dislodged the simple and prompted a fluorescence flag change. The computerized readout of the fluorescence change was acquired within 10 min. In the field preliminary, this RBS breathalyzer fluorometry identified 23 tests gathered from 31 tuberculosis patients, with an affectability of 74% (95% CI 55–87). Of the 29 negative examples, 6 were false positive, bringing about a specificity of 79% (95% CI 60–91). Despite the fact that this test cannot supplant the utilization of sputum microscopy in asset obliged settings, it can positively help the analysis of some patients such as kids who cannot give sputum tests. The high cost, the low specificity and affectability are the main limitations of this test. Nagel et al. (2008) created three distinctive names free optical immunosensors for fast recognition of tuberculosis-particular antibodies (Fig. 2). The grinding coupler and the interferometric biosensor, both working in refract metric mode, observed the progressions in the compelling refractive list at the sensor

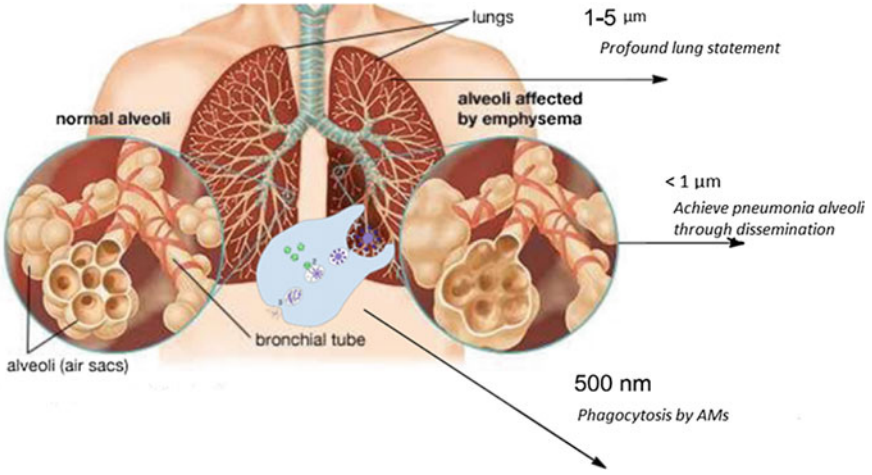

Fig. 2 Depiction of the effect of particle gauge in lung sworn statement and phagocytosis by the alveolar macrophages

surface (Ta_2O_5 and SiO_2). The grinding coupler biosensor decided the move of the coupling point, and the interferometric biosensor distinguished stage change of two waves going through a detecting branch and a reference branch within a waveguide. The RIFS in light of the reflecting metric approach decided the optical thickness of a thin sensor layer by distinguishing light reflection at interfaces. In spite of the fact that these three sensors varied in identification components, they used a similar surface science, where 1,4-phenylenediisothiocyanate, a cross linker, is utilized to immobilize a 38-kDa MTB lipoprotein antigen. These unique advancements were utilized to identify MTB-particular antibodies that may be available in the serum. As revealed, the grinding coupler has 100% specificity and 75% affectability contrasted with ELISA. The other two sensors appeared comparable sensor grams. Since these three optical sensors were sans mark advances, they required stringent particular conditions. In this examination, the utilization of 1,4-phenylenediisothiocyanate accomplished the impact of finish surface inclusion to evacuate non-particular official. In spite of close to nothing non-particular authoritative, high chip-to-chip variety was watched. Including to this test, the prerequisite of costly couplers, lasers, and a pump framework restrains the utility of this framework at the POC. Surface plasm on reverberation (SPR) is an optical strategy in light of the ongoing checking of changes in the surface refractive record caused by affiliation or separation of atoms onto/from the sensor (Homola 2008). This optical innovation had likewise been produced to recognize MTB particular antigens (e.g., CFP-10 antigen) in tissue liquid (Hong et al. 2011). In this immuno-based discovery technique, monoclonal enemy of CFP10 antibodies were first immobilized on a business immunosensor chip. The chip was coordinated with a SPR-based optical immunosensor framework (Bia Core 3000, Sweden) and used the CFP10 antigen as a delicate TB marker. The outcomes showed that the change in SPR point expanded directly with CFP10 focuses, i.e., in the range from 0.1 to 1 μg/mL (Hong et al. 2011). SPR-based immunosensors can likewise be adjusted to detect different antigens and pathogens by modifying the discovery particles (e.g., antibodies), along these lines offering exceptionally flexible stages. Despite the fact that this SPR immunosensor offers the points of interest of effortlessness, little test utilization, mark free, super specificity, affect the ability and reusability, it requires a very much prepared research center foundation. In this way, convenient what's more, modest SPR-based MTB biodetection frameworks are expected to limit research center prerequisite for POC testing. On the other hand, a micro tip-based framework has been appeared to focus what's more, catch MTB (Yeo et al. 2009). The framework was made out of microelectrodes, a micro tip, and a curl. MTB cells were concentrated to the finish of a micro tip due to electro-osmosis, coming about because of the use of a rotating current (AC) field. The concentrated cells were suctioned into a micro tip by a slim power. Expansion of fluorophore-named, MTB-particular, polyclonal antibodies encouraged fluorescence identification. By means of this approach, MTB cells were distinguished inside 10 min at a focus as low as 8000 CFU/mL, which is practically identical to sputum spread microscopy. Despite the fact that the electro-osmotic focus approach is promising to recognize

without culture TB, its application to move MTB in sputum tests should be additionally assessed. The prerequisite of fluorescence-marked antibodies additionally limits this framework from being utilized in for remote settings.

10.1.3 Atomic Attractive Reverberation (NMR)

As of late, the advancement of another, scaled down the analytic attractive reverberation (DMR) framework that has the ability to recognize MTB as few as 20 CFU/mL in the natural sputum test during 30 min (Chun 2009; Lee et al. 2008). The DMR framework was basically a closeness examine that can recognize the mass change in spin–turn unwinding time of encompassing water particles, when attractive nanoparticles collected because of the nearness of target biomarkers. The framework comprised of 3 parts; a micro coil exhibit, a microfluidic system and on-board NMR gadgets. Attractive particles were covered with antibodies particular for target biomarkers, which can be mammalian cells, microbes, or protein. Since the measure works on the guideline of NMR, turbid examples, for example, blood, sputum or pee can be utilized. Contrasted with the benchtop relax meter, this framework shown 80-crease increment in mass affectability in distinguishing avid in Lee et al. (2008). The prevalent affectability and diminished measure time makes this framework appropriate for POC testing, this gadget has potential to be converted into a MTB POC test (Chun 2009). Other striking highlights incorporate taking care of little example volumes (5–10 μL) and short turnaround time, flexibility and multiplexing capacity, too. The NMR part costs under $200 and the expendable microchip costs under $1, making the innovation conceivably appropriate for the creating scene.

10.1.4 Enzymatic-Based Immunosensor

TB diagnosis based on enzymatic immunosensors is considered the most well-known diagnostic method up to date. Diaz-Gonzalez et al. (2005) utilized an ELISA gadget to recognize the immuno-complex, which was caught by a streptavidin changed screen-printed carbon cathode (SPCE). In this investigation, two antigens (Ag360 and Ag231) of MTB were utilized in mix with their monoclonal antibodies to shape a particular immuno-complex. The immuno-complex was caught by biotinylated against MTB antibodies immobilized on the sensor surface by means of streptavidin– biotin association. At that point, the immuno-complex was distinguished utilizing a nonexclusive location immune response that was conjugated to a basic phosphatase (AP). The compound processed an electrochemical substrate 3-indoxyl phosphate (3-IP), to accomplish voltammetric discovery. The utmost of discovery of this immunosensor was appeared to be (1.0 ng/mL) for the match of Ag231 and its monoclonal neutralizer. This method use entrenched screen-printing microfabrication, which takes into consideration large scale manufacturing of economical cathodes (Diaz-Gonzalez et al. 2005). What's more, the scaled down identification is incorporated into an expendable convenient gadget, which is promising for recognition of TB at the POC.

Nonetheless, the measure time is more than 4 h. In this way, this examine could profit by shorting the turnaround time for quick outcomes at the POC. As of late, another enzymatic TB sensor that used a characteristic BlaC enzyme from tubercular bacilli was created (Xie et al. 2012). BlaC, a catalyst from class a β-lactamase family, hydrolyzes all classes of β-lactam substrates, including cephalosporins. Attributable to the exceptional adaptability of BlaC, chemically modified BlaC-particular fluorogenic substrates (i.e., cephalosporins) were utilized as fluorescence tests, which separated MTB from other microbes, for example, Pseudomonas, Staphylococcus and natural Mycobacterium like *M. smegmat* is likewise, the utilization of these fluorescence tests likewise enhanced the affectability of recognizing BlaC MTB other than homologue TEM-1 BlaC and β-lactamases from other microscopic organisms. As detailed, the altered tests upgraded the fluorescence force from 100–200 overlap, and enhanced the selectivity by 1000 folds, contrasted with TEM-1 β-lactamase. As low as 100 bacilli spiked in natural patient sputum were distinguished in under 10 min. To dispose of the requirement for fluorescence identification, a LED-based, modest, and open door for quick MTB recognition in asset obliged setting. Be that as it may, this framework should be additionally assessed in the field utilizing natural sputum tests contrasted with the culture, spread microscopically or then again PCR.

10.1.5 Mirror-Based Immunosensor

A liking based immunosensor identifying antibodies against mycolic acids (MA) of MTB in TB quiet examples has been depicted (Thanyani et al. 2008). This thunderous mirror biosensor, IAsys (Affinity Sensors, Cambridge, UK), estimated the authoritative and disassociation of antibody–antigen on the sensor. Quickly, the surface of a twin-celled biosensor cuvette was actuated with cetyl-pyridinium chloride (CPC). Liposomes containing MA were then immobilized at first glance taken after by mechanically straightforward and fast for POC testing, this biosensor could advantage from lessened cost and enhanced consistency between cuvettes.

10.1.6 Acoustic Wave-Based Sensor

An acoustic wave-based impedance biosensor was created to quickly recognize the development of MTB in culture (He et al. 2003). MTB takes 1–3 weeks to achieve confluency relying upon the sort of media utilized. Notwithstanding, by utilizing this mass acoustic sensor, the location time was abbreviated to multi day. The gadget chipped away at the rule of conductivity changes in the way of life media coming about because of MTB development. The development of MTB yielded the creation of protein, greasy acids and nucleic acids, and additionally metabolic side-effects; thus affecting the reference flag's quality, which declines and recurrence to increment. This was distinguished by the mass acoustic wave impedance sensor. Further, the sensor evaluated the underlying convergence of MTB, with beginning adjustment, by watching the time at which the distinguished recurrence changed. As illustrated,

the sensor can reasonably distinguish and measure MTB focuses going from 2×10^3 to 3×10^7 cells/mL in fundamentally diminished test time. What's more, it has been demonstrated that the normal reaction bend related to MTB is unique in relation to that of other microorganisms such as *E. coli*, *St. aureus* and *P. mirabilis*. The impediment is that this sensor needs a difficult culture procedure that is not reasonable for POC testing. The feasibility of this examine should be assessed via extra testing.

10.2 Biosensors in Light of Nucleic Acid Hybridization

Notwithstanding the identification of insusceptible reaction created against MTB, nanotechnology has additionally been used to encourage identification of MTB-particular nucleic corrosive. For example, gold nanoparticle-based test, tests have been created to distinguish MTB PCR intensification items by breaking down examples of shading change because of nanoparticle conglomeration (Costa et al. 2010; Soo et al. 2009). Despite the fact that these methods disentangled the discovery of MTB PCR items, despite everything they required enhancement of MTB nucleic corrosive by PCR, which stays testing at the POC. To defeat this test, a without pcr electrochemical biosensor was created for recognition of MTB genomic DNA in light of the double marking of gold nanoparticles with basic phosphatase and particular DNA oligonucleotides (Thiruppathiraja et al. 2011). Quickly, MTB genomic DNA was first separated and broken into little pieces utilizing ultrasound sonication. The produced MTB DNA pieces were hybridized onto anodes having immobilized with particular catch tests. Expansion of double marked gold nanoparticles permits age of an electroactive types of para-nitrophenol, which fills in as a substrate in the accompanying electrical detecting. As illustrated, this technique recognized MTB DNA down to 1.25 ng/mL. Additionally, this strategy indicated equivalent affectability also, specificity of PCR in distinguishing MTB from clinical sputum tests. Be that as it may, the necessities for stringent hybridization conditions (counting various wash and temperature controls) and long hatching time make it less perfect for POC testing. SPR has likewise been utilized to detect MTB genomic DNA by means of hybridization with cysteine adjusted NH_2-end peptide nucleic corrosive (PNA, 24-mer) test and 5′-thiol end marked DNA tests (Prabhakar et al. 2008). For nucleic corrosive hybridization, the DNA tests were intended to identify the succession of MTB either with or without mutations. In this examination, the change in SPR point was observed amid the hybridization of DNA tests with PNA and DNA immobilized on gold (Au) cathodes. The results demonstrated that there was no non-particular official of noncomplementary arrangements to the DNA/Au and PNA/Au cathodes. Further, the PNA/Au anodes were more effective for location of arrangements with single-base crisscrosses, having a lower breaking point of location (1 ng/mL) than DNA-Au terminal (3 ng/mL) (Prabhakar et al. 2008). Despite the fact that the SPR innovation indicates delicate discovery of MTB successions, promote endeavors can add to scale down the location system to a versatile gadget, which can be executed

at the POCO wing to the difficulties related to spread microscopically (e.g., the requirement for a massive light microscopy and microbiology abilities for pathogen identification), fluorescence recognition of MTB has been created to illustrate the plausibility of an incorporated and versatile cell phone microscopy framework (Breslauer et al. 2009). The stage had a double magnifying instrument mode, which can screen tests under brightfield and fluorescence. To take a picture, a mobile phone having a 3.2 M.

10.3 Phone-Based Fluorescence Microscopy

Attributable to the difficulties related with spread microscopy (e.g., the requirement for a massive light microscopy and microbiology aptitudes for pathogen recognition), fluorescence discovery of MTB has been produced to illustrate the possibility of a coordinated and convenient cell phone microscopy framework (Breslauer et al. 2009). The stage had a double magnifying instrument mode, which can screen tests under brightfield and fluorescence. To take a picture, a mobile phone having a 3.2 Megapixel camera was utilized. The phone was mounted on an optical rail stage. For fluorescence imaging, the framework was furnished with a cheap Driven excitation source, which emits within the excitation scope of fluorescent Auramine O-recolor regularly utilized for recognition of TB bacilli in sputum smears. To picture the example, the light created from the LED source initially went through the authority focal point, and after that the excitation filter with a wavelength range to see TB bacilli. The pictures are then exchanged to a PC for advance examination utilizing the ImageJ program (http://rsb.info.nih.gov/ij/). Contrasted with the non-fluorescent Ziehl–Neelsen recolor, this strategy utilizes a lower control (20×) objective with a bigger field-of-see, hence lessening the quantity of field pictures to cover the whole screening territory. Further, the utilization of a 20 × 0.4 NA objective permits adequate light-social occasion for fluorescence location without utilizing a conventional fluorescence magnifying instrument. In any case, this framework requires costly channels and focal points, and the example pictures should be transported to a PC for promoting examination.

11 Elective Non-intrusive Courses of Organization of Nanomedicines Against TB

TB is caused by the inward breath of air tainted by microscopic organisms MTB. Due to the lungs being the principle organ where the contamination starts, new pharmaceutical frameworks with the point of pneumonic organization have been created. Be that as it may, in the late phase of the advancement of this irresistible illness, TB can spread and reaches the lymphatic and blood course, influencing different organs

(pleura, lymph hubs, digestive system, meninges, kidney, skin, bones), so new frameworks with various pharmacokinetics properties that permit a superior adequacy are required (Lee 2015). The prescribed treatment for extrapulmonary-TB treatment is the same prescribed for respiratory TB (Lee 2015). The primary of the current against TB drugs showcased is controlled by oral course, what's more its symptoms, oral enemies of TB drugs are simple organization and have higher patient consistence when contrasted and parenteral course. To permit a higher biocompatibility and a decrease of symptoms, against TB drugs have been detailed in new pharmaceutical frameworks, some of them with a particular ligand on its surface that permit the AMs focusing on (Costa et al. 2015). Respiratory course is one of principle investigated course as depicted in the past segment, anyway the advancement of new oral conveyance frameworks has been investigated with a specific end goal to enhance against TB sedate bioavailability, viability and to decrease tranquilize harmfulness. These frameworks have been utilized for treating respiratory TB, yet now and again can be likewise appropriate for treating additional respiratory TB. Then again, frameworks for against TB drugs conveyance through topical course stay for being investigated.

11.1 Oral Nanosystems Delivery for TB Treatment

Amid the advancement of pharmaceutical frameworks for oral organization, a few issues must be considered. They ought to secure the medication from the low pH in the stomach as well to enzymatic corruption by intestinal/pancreatic compounds that can lessen the counter TB tranquilize bioavailability. This is specifically compelling for RIF conveyance as it degrades at low pH and the procedure of debasement is upgraded at the point when INH is co-directed (Singh et al. 2013). The oral conveyance frameworks are defenseless to first pass metabolism, which affects their bioavailability, imperiling the effectivity of hostile to TB drugs. Medication conveyance frameworks for hostile to TB conveyance ought to likewise be successful in giving a long maintenance of medications at lung tissues, the neighborhood of primocontamination, what's more, chiefly focused on the AMs. The supported discharge is additionally vital for TB treatment that will permit the decrease of the quantity of oral organizations and the day by day measurement of medications, bringing about less reactions. Through sub-atomic unique recreation, (Bellini et al. 2015) made an investigation to assess the relationship of RIF with a 4th generation poly(amidoamine) (G4-PAMAM) dendrimer. In this investigation, it was demonstrated that the complex was more steady at recreated impartial pH than recreated corrosive pH. In this way, because of the quick arrival of the RIF at corrosive pH, this complex could be a reasonable approach for focusing on AMs, once the medication is conceivable to be discharged at acidic compartment of macrophages. Anyway since of the RIF introduces low security at corrosive pH (Bellini et al. 2015), this complex must be consolidated into an enteric covering or low-pH-safe cases that would evade the arrival of RIF at the stomach. Distinctive nanosystems have been quality, temperature, weight) their present distinctive swelling properties that permit to control the

medication discharge at diverse conditions (Vashist et al. 2014; Ahmed 2015). (Kajjari et al. 2012) played out an examination to create hydrogel microspheres which are touchy to warm and pH boosts, all together to maintain the controlled arrival of INH at various pH, and subsequently to limit the dangerous impacts. Hydrogels were comprised by various rates of poly(N-isopropylacrylamide)-g-guar gum and of sodium alginate and agreeing with the microsphere mixing, it was conceivable to accomplish distinctive sorts of detailing that displayed a solid pH depend swelling, and thusly an alternate INH discharge design. Besides, microspheres comprised just by sodium alginate were ready to cause an in vitro INH discharge amid 12 h at physiologic pH, in a temperature-autonomous way, while other hydrogel plans introduced a higher arrival of INH at 25 °C than 37 °C, since in the first case hydrogel was in a swollen state, and once it is hydrated ensnared medication will diffuse from network polymer effortlessly (Kajjari et al. 2012). Notwithstanding, in vivo concentrates to survey the INH discharge design after oral organization were not performed. In option in contrast to hydrogel that exhibits a high size, nanogels have been produced for being utilized as a transporter for medicate conveyance arrangement of low atomic medications too macromolecules (proteins, DNA, oligonucleotides). They are formed by polymer systems with high water solvent properties and due to nanoscale estimate they can saturate natural films. Like hydrogels, then present a decent stacking limit also, swelling properties in watery condition when in contact with diverse condition upgrades (Kabanov and Vinogradov 2009; Kennedy 2013). Distinctive polymers can be utilized to get a ready nanogels, to be specific PCL, poly(ethylene glycol)-cl-polyethyleneimine, PLGA, alginate, chitosan what's more, dextrins (Kennedy 2013; Schütz et al. 2011). A portion of these polymers were at that point utilized to create nanosystems for hostile to TB tranquilize conveyance (Pandey and Khuller 2004; Malathi and Balasubramanian 2011; Feng et al. 2013; Choonara et al. 2011), however the potential utilization of nanogel for TB treatment is ineffectively considered. Chen et al. (2016) joined INH and RIF in poly(methacrylic acid) (PMAA)-based nanogel with the expect to advance a controlled discharge after oral organization with less lethality. After the generation of PMAA nanogel, the counter TB drugs were consistently scattered on the nanogel through ultrasonication, framing the PMAA/INH/RIF nanogel. The swelling property of this nanogel was pH-subordinate. In vitro discharge contemplate demonstrated that there was under 10% of medication discharges in recreated gastric liquid (pH 1.2), demonstrating its appropriateness for being controlled by oral course, since it can go through the stomach without corruption, what's more, just permitted the medication discharge at the small digestive system (26.7% and 32.3% of INH and RIF amid 6 h individually, at pH 6.8) and colon (48.7% of INH and 55.9% of RIF within 44 h at pH 7.2). Moreover, in vitro considers demonstrated that PMAA/INH/RIF nanogel displayed less cytotoxicity than INH and RIF alone after 24 h of brooding, and nanogel drenching displayed 72 h of antibacterial movement. The long-term antibacterial action was given due to nanogel balance out the counter TB drugs, with the goal that the antibacterial property was just appeared after disintegration and dissemination of nanogel. With respect to properties the creators theorized that this definition could be

helpful for multidrug-safe intestinal MTB better than the current ordinary medicines in light of INH and RIF (Chen et al. 2016).

11.2 Topical Nanosystems Delivery for TB Treatment

Cutaneous tuberculosis (CTB) is caused by atypical mycobacterium species, specifically *M. leprae*, *M. haemophilum*, and *M. ulcerans*, among different species, however when it is caused by MTB or even through BCG immunization it is outlined as evident CTB. Skin can be viewed as a great course for the treatment of CTB. It just speaks to 1–1.5% of extrapulmonary cases, influencing principally the face, yet middle and neck regions can be additionally influenced (van Zyl et al. 2015). CTB can happen with exogenous instruments, to be specific by coordinating passage of microbes trough sore skins or mucosa (contact with tainted needles, task, or wounds). It happens in people, which were not past sharpened with microbes, and emerge to be specific in the endemic zone with poor immunization. After contamination, an underlying sore called TB chancre is for the most part shaped, however this injury can likewise be available in sharpened people that have resistance against MTB; frequently it shows up in a wellbeing proficient because of direct immunization of TB through the skin (van Zyl et al. 2015; Semaan et al. 2008; Santos et al. 2014). Endogenous components like optional disease got from an essential center injury (tissues consistent with skin, for example, lymph hubs or bones) are likewise in charge of CTB. Besides, it very well may be likewise due to hematogenous spreading from contaminated tissues, similar to the lungs (van Zyl et al. 2015; Santos et al. 2014). Tuberculids are additionally skin issue portrayed by the extreme touchiness response to TB in people's immunocompetent, being considered as not a genuine CTB, but rather it can likewise happen because of BCG inoculation, in spite of the fact that immunization confusions being infrequently (van Zyl et al. 2015; Keijsers et al. 2011). Analysis of CTB is extremely unpredictable on the grounds that skin injuries are fundamentally the same as with other skin injuries, so that, for a right demonstrative of CTB past clinical highlights microbiological and histological exams are necessary (Asadi Gharabaghi 2012). CTB can be treated with tradition hostile to TB drugs. WHO suggests an administration of a 2-month concentrated stage with INH, PZA RIF, and EMB, trailed by an upkeep stage with RIF and INH for a period of 4 months. Other than that, treatments with 2% of corrosive lactic, topical anesthesia or on the other hand careful intercession can be additionally performed (van Zyl et al. 2015; Sethuraman and Ramesh 2013). INH, one the primary line hostile to TB sedate, is all around perceived for causing hepatotoxicity. One approach to take care of this issue is growing new pharmaceutical items from being directed by different courses, in particular topical course, which is extremely satisfactory by the patients, permit a drawn out managed discharge and stay away from the hepatic first pass digestion. This course is the unique enthusiasm for instances of CTB because of vicinity with irresistible tissue, which can give a nearby treatment. Caon et al. (2015) exhibited that INH can be combined with distinctive synthetic enhancers and according with

log P properties it is conceivable to accomplish a topical or transdermal conveyance of INH. The consolidation of INH on transcutol (log P −0.42) permitted a higher skin maintenance when looked at with limonene. Transcutol advances a store impact of medication and this impact was most likely caused by swelling of the intercellular lipids of the stratum corneum, empowering the medication maintenance and thus the development of the terminal, without influencing the structure of lipid bilayer. By other hand limonene, a high lipophilic (log P 4.58) terpene, empowered a higher INH skin penetrability and a decrease of slack stage, by expanding the medication dispersion into the skin This compound can associate with lipids of stratum corneum and thus permit lipid extraction, being considered by the creators a decent excipient for transdermal details, while transcutol would be more appropriate for topical organization or for INH maintained discharge (Caon et al. 2015). To allow a supported arrival of against TB drugs, hydrogel can be a decent approach, permitting the decrease of various organization and thus an expansion of patient consistence. Hydrogels present a few properties that make it appropriate as a framework for medicate conveyance (Vashist et al. 2014). The utilization of hydrogel dressing may be of enthusiasm for the treatment of CTB, permitting a neighborhood activity on skin sores, and thusly could be an adjuvant of current treatments. In any case, topical or transdermal medicines for CTB were not depicted in the writing, and there is no enemy of-TB framework on the advertised for being regulated through topical or transdermal course. Other than the high number investigations of against TB drug loaded into various sorts of frameworks for respiratory TB, there is a still an absence of options for treatment CTB.

12 Difficulties and Future Points of View

TB is as yet an ongoing worldwide general wellbeing concern, bringing about a substantial monetary, social and human weight (Ginsberg 2010). There have been built up endeavors to battle this infection, and the scan for new enemy of TB drugs assumes a significant job (Sosnik et al. 2010; Onozaki and Raviglione 2010). Regardless of these endeavors, the latest tranquilizes in the market goes back 50 years, thus new conveyance systems that enhance the adequacy of existing medications may progresses toward becoming imperative in this battle. In addition, and since the lung is the essential disease site of in TB, the respiratory course for against TB organization is by all accounts a promising methodology to battle this infection (Pitt et al. 2013).

Albeit promising and immensely examined, the pneumonic conveyance systems confront snags hard to survive. With the specific case of hostile to TB drugs, the vast majority of the primary line drugs have been considered in vitro for use in powder definitions for inward breath (Das et al. 2015; Son and McConville 2011; Hanif and Garcia-Contreras 2012; Manion et al. 2012; Chan et al. 2013). In any case, so far no breathable definition for the treatment of TB has come to the market (Misra et al. 2011). These challenges have been accounted for all through the logical writing, including the utilization of protected and acknowledged excipients,

creating adaptable procedures, creating beads with legitimate molecule size and morphology for lung statement, and accomplishing agreeable medicate stacking (Misra et al. 2011). Additionally, usable methodologies must have the capacity to represent unique lung structures, breathing examples, and changes in the aviation route morphology caused by the pathogenic operator (Sethi and Agrawal 2011). They must accomplish get to to ineffectively circulated air through zones of the lung and extracellular microorganisms in all around circulated air through lung tissue, beating acceptance of obstruction due to low intracellular medication focuses, and outperforming confinements due to the conceivable intrinsic reactions of the host (Yadav et al. 2011). Once breathed in, the particles ought to have the capacity to movement to the pneumonic alveoli and come to the fundamental cell focus of MTB (i.e., AMs). Delivered particles in this way should have a fitting size, generally they will be caught in the upper aviation routes or then again leave the lung amid exhalation. Additionally, it is well realized that AMs have particular receptors that quandary to sugars. In this way, surface adjustment might be performed to exploit these receptors and move forward cell take-up by AMs (Martin 2005). Oral course is typically one of non-intrusive courses particular picked, since medicine allow by this course is simple, effortless, permitting to move forward the patient consistence when contrasted and parenteral course. A few thinks about with respect to various oral conveyance frameworks (NPs, microspheres, polymeric micelles, nanogel) were specified for the treatment of TB. In a powerful endeavors, distinctive innovative parameters, like mean molecule estimate, Pdi, zeta potential, tranquilize stacking, in vitro discharge properties in distinctive conditions were portrayed so as to build up a framework that isn't degradable at corrosive pH of the stomach and achieve the duodenum, where medications can be discharged for being ingested or even the counter TB tranquilize stacked nanosystems can simply be ingested, achieving the blood stream. As of not long ago, a few in vivo pharmacokinetic contemplates demonstrated an awesome change of oral bioavailability when contrasted and current hostile to TB drugs. However, there are still few animal thinks about that demonstrate the against bacterial adequacy of these oral frameworks, also of sub-intense and sub-endless poisonous quality investigations. Organization of medications by topical course with the plan to treat CTB could be favorable, since it would advance an expansion of medications on tainted skin tissue, with less reactions. In any case, since CTB is an uncommon condition, it tends to be a conceivable clarification why this non-obtrusive course is still inadequately investigated. After in vitro, the in vivo studies ought to be performed keeping in mind the end goal to get it in the event that the definition has the alluring attributes to come to the advertise. Also, the investigations ought to be intended to answer a few appropriate and basic inquiries. Will the conveyance system permit the medication to achieve the circulatory framework, consequently be utilized to battle extrapulmonary TB? Will the counter TB medication's fixation inside the AMs be sufficient to murder all the MTB populace, coming to likewise the granulomas furthermore, not the circulatory framework, and therefore be utilized in pneumonic TB without the foundational reactions? Will oral course approach be appropriate for the treatment of pneumonic tuberculosis or additional respiratory TB, achieving focuses enough to eliminate microbes, with less day by day measurement, less number of

organization and with less side effects? Will oral approaches empower to focus on the AMs without being right off the bat phagocytized by various macrophages? In spite of every one of these difficulties, the quest for a NP-based plan to battle TB ought not be dropped. In actuality, a portion of the introduced results are exceptionally encouraging, thus they ought to urge us to go further.

13 Conclusion

The present treatment of TB is compelling yet it is related to the extreme unfavorable impacts and rebelliousness to endorsed regimens. Albeit current enemy of TB pharmaceuticals are chiefly managed through oral highway, an exertion has been made to grow new frameworks that permit the change of the bioavailability of current enemy of TB drugs, as well diminishing its harmfulness. In any case, none of miniaturized scale and nano-based frameworks investigated has come to the showcased yet. With the approach of creative NP-based details, a more up to date trust has developed out. The achievement of these nanodelivery frameworks will most likely rely upon the outline of savvy plans that location distinctive impediments of against TB pharmacotherapy, making the treatment more functional what's more, moderate to all patients.

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Nanomaterials in Nutraceuticals Applications

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Abstract Diverse strategies are adopted to fight against various diseases and probable health risks. Besides the pharmaceutical approach, diet-based strategies are also deemed apt to avert various disorders. "Nutraceuticals" considered as bioactive components found in natural products. Bioactive components are additional nutritional ingredients that typically present in small quantities of foods that are used in day-to-day life and strongly believed to play a crucial role in the maintenance of our health. The food products used as nutraceuticals can be categorized as dietary fiber, prebiotics, probiotics, polyunsaturated fatty acids, antioxidants and other different types of herbal/natural foods. These nutraceuticals facilitate in combating a number of the major health problems including microbial infections. In recent years, nanotechnology-based formulations like micro- and nanoencapsulation have been a rising interest for nutraceutical, food and pharmaceutical applications. To enhance nutritional quality and stability of the nutraceuticals, one option is to encapsulate the functional ingredients using food-grade or "generally recognized as safe" (GRAS) materials that can exhibit controlled-release behavior. These diversity of building blocks and formulation methods led to nanocarriers like nanoemulsion, nanodispersion, nanoparticles, liposomes etc. with diverse physicochemical properties and functional characteristics. Based on the above-mentioned facts, this chapter provides an insight of some of the emerging nanomaterial-based applications being commercialized in nutraceuticals. A glimpse on various research work undertaken for the nanomaterials in the field of nutraceuticals is also discussed in this chapter.

Keywords Nanomaterials · Nutraceuticals · Nanoformulations · Physicochemical parameters

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1 Introduction

Nanotechnology is one of the recently emerging practices which enabled the potential technology to transform the agriculture, food, herbals or nutraceuticals materials into nanomaterial delivery systems. Therefore, the purpose of nanoscience is emphasized on the developing or manufacturing, and evaluation of inorganic, organic, herbal and nutraceutical materials to shape into innovative components, materials and delivery systems at the nanoscale level which have at least one dimension 1–100 nm in size (Duncan 2011). In recent years, Nanotechnology-based formulations like micro- and nanoencapsulation has been a rising interest for nutraceuticals, food and pharmaceutical applications. The fast rising nano-based technologies have offered brawny support for developing inventive novel nutraceuticals formulations. The nutraceuticals are foods or food elements that endow with health gains away from basic nutrition however numerous nutraceuticals demonstrate poor solubility, stability and bioavailability.

Hence, to develop the nanotechnology-based nanomaterials has allowed to surmounting confronts and technological obstructions such as solubility, stability, bioavailability and delivery of bioactives or nutraceuticals. The fast developments of nutraceutical nanotechnology carry great assurance to make available effective and new efficient foods as a tool for averting and possibility to cure some non-communicable diseases (Gopi et al. 2016). Nutraceuticals or nutraceuticals-based bioactive constituents are claimed to have various physiological benefits or improve immunity of ailments/disorders for instance cardiovascular disorders (Choudhary and Tomer 2013), obesity and diabetes (Annunziata et al. 2018), cancer (Prasad et al. 2017), stress, Alzheimer's and Parkinson's diseases (Klyachko et al. 2012).

The polyunsaturated fatty acids, antioxidants, probiotic and proteins are prevalently used bioactive constituents (nutraceuticals). The nanomaterials-based encapsulation nanotechnology will aid appreciably in terms of improving or retaining their nutraceutical values as well as stability. Since, many of these nutraceuticals are effortlessly subjected to quick degradation or inactivation, encapsulating them into nanomaterials will help to avert or slow down the degradation progressions until their delivery.

Various nanomaterials-based encapsulation methods have been used although not any of them can be deemed as a perfect method for encapsulating a broad series of bioactives/nutraceuticals. This is mainly because of the fact that each bioactive show unlike physico-chemical attributes i.e. solubility, polarity, molecular weight (Augustin and Hemar 2009). The rising number of innovative formulations are being formulated and the oral bioavailability of encapsulated bioactive/nutraceuticals is being considerably augmented. The potential application of nanotechnology in the area of functional foods bestow promising possibilities for the future prospective of the food industry. Functional foods are attracting interest in the food commerce as well as in food research science. The advancements in molecular biology, biochemistry and other analysis techniques, a number of chemical constituents i.e. natural

bioactive compounds that found in traditional foods are possessed good health promoting benefits. By means of this information, the food industry has incorporated these compounds now usually called nutraceuticals or natural bioactives into foods and these products are called functional foods.

A number of literature assessed the application of nanomaterials-based nanotechnology in nutraceuticals particularly in encapsulating and delivery technologies of nutraceuticals or food bioactives. The most common nanomaterials based delivery systems for instances microemulsion, self nano- and micro-emulsifying delivery system, nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), nanoparticles, liposomes and nanoemulsion have been comprehensively discussed (Singh et al. 2018; Singh et al. 2016a, b; Tripathi et al. 2018; Shah et al. 2018; Kanoujia et al. 2016; McClements and Rao 2011; da Silva Malheiros et al. 2010; Spernath and Aserin 2006). In this chapter, we describe the nanonutraceuticals, protein-based nanomaterials, cellulosic nanomaterials and carbohydrate-based nanomaterials as nano-sized nutraceuticals for their potential applications in therapy and general health.

2 Nano-nutraceuticals

The nutraceutical was basically originated from nutrition and pharmaceutical and term coined by Stephen L. De Felice in 1989 (Cencic and Chingwaru 2010) as it has illustrated in Fig. 1. Basically, we can say nutraceuticals are hybrid of pharmaceutical technology and nutrition. It was formerly explained as "a food or part of the food that gives medical or health benefits, together with the mitigation or cure of illness" (Cencic and Chingwaru 2010). Nutraceuticals is in broad expression used to demonstrate any artifact resulting from food resources that offers supplementary health promotions besides the fundamental nutritional value. Nutraceuticals are classified as non-toxic constituents. They are also called as nutritional supplements, designer foods, medical foods, dietary supplements and comprise a number of matters varied from herbal products, natural diets, biofortified crops, genetically modified and

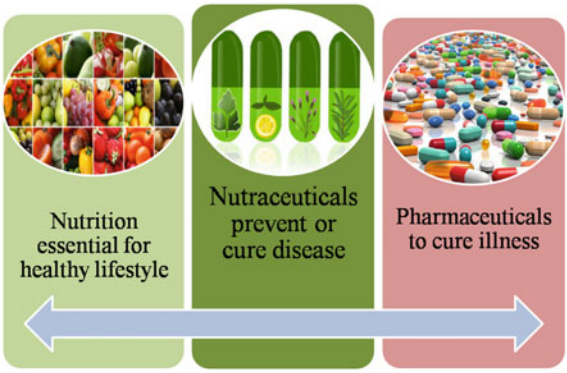

Fig. 1 Nutraceuticals originated from nutrition and pharmaceuticals

processed food products (De Felice 1995; Pandey et al. 2010). These nutraceuticals usually have the essential sum of proteins, vitamins, lipids, minerals, carbohydrates, and other necessary nutrients. When food assists in prevention or cure of illness other than fulfilling deficiency conditions then these are called as nutraceuticals. The compounds such as vitamin C and E, vitamin D, flavanoids, omega-3 fatty acid, minerals such as chromium, magnesium, phytoestrogens and dietary fibres are used for treating metabolic syndrome and diabetes (Davi et al. 2010; Choudhary and Tomer 2013; Shinde et al. 2014).

Herbal nutraceuticals are valuable in retaining health and they also helpful for nutritionally provoked acute or chronic ailments, promote longevity, quality of life, and health. Nutraceuticals have established significant curiosity since of their supposed safety, prospective nutritional value and therapeutic outcomes. People may perk up their healthiness by taking foods that have been fortified or formulated with bioactives. One more reason for the increasing trend in nutraceuticals are public education, cultivation and processing, renewable source, environmental friendliness, and local availability. Germany, Canada and UK defined nutraceuticals as 'a product manufactured from foods but sold in powders, pills, and other therapeutic forms not usually correlated with food.' In India, nutraceuticals are the food segment prepared from botanical or herbal raw matters, which are used for averting or mitigating various types of acute and chronic illness.

The decreased permeability, stability and bioavailability of the bioactive constituents/or nutraceuticals in the gastrointestinal (GI) fluids shows to their limited absorption from the GI tract. This leads to their diminished or nearly no biological activity, which is a most important drawback for the research scientist nowadays. Through the conventional approach of deliverance of nutraceuticals, a fraction of the given nutraceutical quantity is absorbed to the pharmacological active-site of proteins while the rest of the fraction may be excreted or set off non-specific toxicity and adverse effects due to unwanted bio-distribution. For instance, milk thistle, which has benefits in liver detoxification and the majority of its active constituent silymarin is obliterate after ingestion, therefore it is not bioavailable (Calani et al. 2012). In addition, a range of nutraceuticals that encompass antioxidant components, for example ascorbic acid and α-tocopherol are usually unstable, low water-soluble and poorly distributed in vivo. So, there is a requirement to deliver nutraceutical or bioactive products more proficiently by manufacturing them to become more bioavailable through oral administration. To surmount these difficulties, the theories of nanotechnology have been employed for the proficient deliverance of nutraceuticals or bioactives. The perception of nano-delivery system has materialized to acquire numerous approaches of administration to understand the difficulties related with nutraceutical absorption. There has been a considerable advancement in the improvement of nano-nutraceuticals and deliverance of hydrophobic nutraceuticals by the use of nanotechnology. The efficient solubilization, stabilization, encapsulation and delivery of nutraceuticals/bioactives-based on nanotechnology leads to properties such as enhanced absorption in low doses, reduction in the frequency of dose administration and improved therapeutic index. One significant application of nanotechnology in nutrition and food is to prepare new functional food components i.e. nutraceuticals

with enhanced aqueous solubility, sensory attributes, oral bioavailability, thermal stability and physiological performance. The nano-formulations can also be used as a targeted delivery of the loaded bioactives/nutraceuticals. For this reason, there is an anticipation for improved development of the current nutraceutical products. By the use of nanotechnology, numerous acquired functional features are observed. The resulting features can be helpful in transforming the problematical biological procedure into advantageous procedure. While the size is decreased, the surface area of particles enhances and this aids to augment the needed biological process. For instance, when silymarin was re-formulated into nano-liposomes, a noteworthy augment in absorption was found (Javed et al. 2011).

An important step in the progression of delivery systems is the selection of the loading materials, since it affects fundamentally the encapsulation efficiency and delivery systems stability. This is a key confront in designing food grade-based deliverance systems intended for bioactives/nutraceuticals because of the restricted option of materials that can be utilized in the formulation.

3 Classification of Nano-nutraceuticals

The use of nanomaterials perk up the health-promoting features of nutrients and bioactive constituents in foods using encapsulation technologies. The encapsulating techniques such as liposomes, nanoparticles, and nanoemulsions improved the dispersion and suspension of water insoluble components thereby enhancing the bioavailability and stability. Nanomaterials are being employed in the food industry as food additives, food contact materials, food ingredients, novel food, enzymes, flavoring agents and supplements. Nanotechnology is able to increase the solubility, augment bioavailability and guard the strength of bioactive compounds and micronutrients throughout the manufacturing process, storage and distribution.

In contemporary years, micro- and nanoencapsulation technologies have been raising vigorously for pharmaceutical, nutraceutical and food industries. For food applications, these micro- and nanoencapsulation processes grabbed special attention for any one of the following rationales: (a) To guard the constituent of concern from its early degradation during processing or storage or unwanted interactions with the atmosphere, (b) To camouflage astringent tastes, (c) To improve the water solubility and dispersability, (d) To prolong and/or control its release and (e) To improve its oral bioavailability of the functional compounds. Different stages of nutra to nanonutraceuticals are shown in Fig. 2.

The carbohydrate, fat molecules and natural proteins have been tailored with nanotechnology and the tailored varieties are being used in food ingredients and food packaging, food additives and nutraceuticals. There has been a roar of nanotechnology in the majority of the segments that is food and health industry. Loading of active food or bioactive ingredients in nano-based delivery systems is an imperative purpose of this technology in the field of nutrition and food. The nanotechnology acquires massive possibilities for commercialization of the bioactives by overcoming

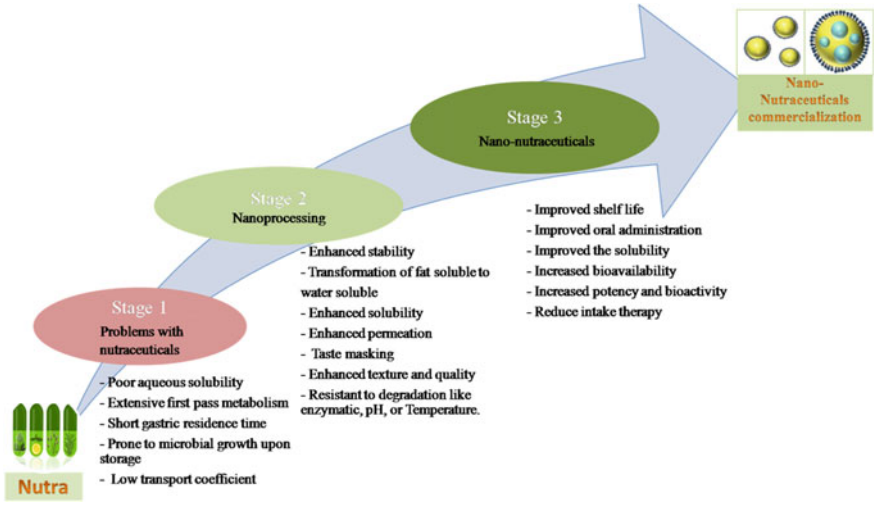

Fig. 2 Diagrammatic representation of stages of development from nutra to nanonutraceuticals

the boundaries related with them (Augustin and Hemar 2009). There are numerous desirable characteristics of nanotechnology-based delivery systems which must be taken into consideration, as given below:

- Should be physically and chemically stable to environmental harsh conditions while conserving its functional character.
- Should be capable to enhance gastric stability of labile bioactives.
- Should be proficient to uphold constant dosage level within the systemic circulation.
- For highly hydrophobic bioactive constituents, it should be able to facilitate the lymphatic transport.
- Should be capable to broaden the gastric retention time.

The enhancements in uniformity, flavor, taste and texture characteristics may be attained via the preparation of nanostructures.

Various nanomaterials used for encapsulations of bioactives/nutraceuticals are classified as:

- Lipid-based nanomaterials
- Polymer-based nanomaterials
- Cellulose-based nanomaterials
- Protein-based nanomaterials
- Polysaccharide-based nanomaterials.

4 Lipid-Based Nanomaterials for Nutraceuticals

Lipid-based nanomaterials like solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanoemulsions, liposomes, and nanoliposomes systems have an immense prospective for encapsulation and deliverance of susceptible bioactives/nutraceuticals. They are useful for the majority of the natural bioactives. Furthermore, in these systems, high dose of diverse molecules can be encapsulated and used as a target delivery system on unambiguous sites through passive or active mechanisms.

4.1 Solid Lipid Nanoparticles (SLNs)

SLNs are colloidal dispersions of solid lipid in aqueous emulsifier solution (Weber et al. 2014). They encompass several benefits, for example enhanced stability of the loaded constituents, capability to carry both hydrophilic as well as lipophilic constituents, controlled or sustained release and simple to scale up. In addition, these deliverance systems comprise the ingredients generally recognized as safe (Severino et al. 2012). SLN offers distinctive properties like small size, large surface area, good encapsulation capacity, easy and simple for large scale production, biodegradable, comprise excellent biocompatibility, capability to be control-released and eye-catching for their possibilities to enhance performance of nutraceuticals, pharmaceuticals and other materials (Mukherjee et al. 2009; Vakilinezhad et al. 2018). Besides the acidity and toxicity, issues are not viewed in SLNs as the resource of lipids used to prepare SLNs is more biodegradable and biocompatible than polymeric materials. The lipids used for the preparation of SLNs may be triglycerides e.g. tri-stearin, partial glycerides e.g. Imwitor, fatty acids such as stearic acid, palmitic acid, steroids (cholesterol) and waxes (cetyl palmitate). A variety of emulsifiers and/or their amalgamation such as Pluronic F 68, Pluronic F 127 and polysorbate, lecithin, sodium cholate, tyloxapol, taurodeoxychocolic acid sodium, sodium glycocholate, butyric acid and butanol are used to stabilize the lipid-based dispersion (Campos et al. 2014; Severino et al. 2012). The mixture of emulsifiers may avert particle agglomeration more proficiently (Mukherjee et al. 2009). SLNs formulations for different routes like oral, parenteral, dermal, pulmonary, ocular and rectal have been developed and characterized for in vitro and in vivo properties.

Various techniques are used to formulate SLN-like high shear homogenization, hot homogenization, cold homogenization, ultrasonication such as probe ultrasonication and bath ultrasonication, solvent emulsification/evaporation, microemulsion-based SLN preparations, SLN preparation by using supercritical fluid, spray drying method

and double emulsion method (Mukherjee et al. 2009). Amongst, the methods used to prepare SLNs, a universal approach is utilized i.e. the hot homogenization process which engrosses homogenizing a constituent encapsulated lipid and an aqueous phase with a hydrophilic surfactant at a temperature above the melting point of the lipid phase.

The curiosity in the preparation of novel nutraceutical and food products having components with antioxidant activity is rising in recent days. Because of the incomplete assimilation of antioxidant constituents and poor bioavailability resulted in sub-therapeutic concentrations at the target-site. To surmount the bioavailability problems, nano-particulate carriers are proposed to offer targeted or localized deliverance of such agents, and could provide a more feasible therapeutic option. The polyphenols found in rosmarinic acid (RA) interact easily with matrices in which incorporated and hence it is not stable (Campos et al. 2014). To surmount such drawbacks, preparation of polyphenols encapsulated SLNs may be an effective method to guard such compounds. SLNs encapsulated with RA were prepared by using 3^2 fractional factorial design and hot melt ultrasonication process employing Witepsol H15 as lipid and Polysorbate 80 as a surfactant (Campos et al. 2014). By applying this approach, nanoparticulate containing antioxidant constituents can be used as therapeutic and prophylactic agents in various ailments such as myocardial infarction (MI), atherosclerosis, hypertension, aging, diabetes, Parkinson's and Alzheimer's diseases (Klyachko et al. 2012), autoimmune disorders (Caccamo et al. 2012), cancer (Riemann et al. 2011), Huntington's disease (Tasset et al. 2011), inflammation (Lee et al. 2011) and hyperoxia (Gao et al. 2012).

In recent times, SLNs have been intended as an innovative ophthalmic/ocular delivery system, which may augment dwelling time on the ocular outer surface and hence enhance the bioavailability of drugs. Baicalin loaded SLNs (BA-SLNs) was evaluated for anti-cataract activity in sodium selenite-induced cataract rat model by Li et al. (2018). Baicalin is a chief flavonoid obtained from the root of *Scutellaria baicalensis* Georgi (*Scutellariae Radix*). Various studies have established that Baicalin is an efficient antiviral, antioxidant, antitumor, and anti-inflammatory (Li et al. 2018). Flavonoids have anti-cataract, anti-cancer, cardioprotective, anti-inflammatory and antioxidant activities and most potent nutraceuticals in a variety of food and phytopharmaceutical products. At the end of the study, it was found that nearly all lenses of model group developed partial nuclear opacity; while in the blank group, all lenses were clear and normal (Li et al. 2018).

β-Carotene is a carotenoids class of nutraceuticals and act as pro-vitamin A. It is found in green leafy vegetables, carrot and yellow or orange fruits (Souyoul et al. 2018). It protects cells from injury by restraining free radical and singlet oxygen-induced lipid peroxidation (Souyoul et al. 2018). It shows hydrophobicity and low chemical stability (Mehrad et al. 2018). The physico-chemical stability of β-carotene can be enhanced by loading whey protein isolate (WPI) stabilized SLNs encompassing palmitic acid and corn oil (Mehrad et al. 2018).

Formulation of a controlled release (CR) drug delivery system for inclusion of hydrophilic active components is a challenge for researchers. Various investigations

have been carried out for this purpose. SLNs have magnetized substantial consideration in current years because of their beneficial attributes. Nicotinamide (NA) is hydrophilic in nature and water solubility in the range of 691–1000 mg/ml. Nicotinamide is used as a nutraceutical and has antioxidant and anti-inflammatory properties, respectively (Takechi et al. 2013). Various in vivo and in vitro studies suggested that the pharmacological agents with anti-oxidative or anti-inflammatory activity can optimistically regulate blood brain barrier (BBB) integrity via the regulation of systemic inflammatory pathways. In recent years, based on anti-oxidative and anti-inflammatory features of NA has allured scientist's interest for impediment in the development of Alzheimer's disease under preclinical studies. By obtaining the high loaded system of this freely soluble constituent not only helped the controlled release of NA but also used to target and treat such neurodegenerative disorders. By considering its hydrophilic attributes SLNs with enhanced encapsulation efficiency and controlled release were prepared and optimized by using response surface method (RSM) (Vakilinezhad et al. 2018). SLNs were also prepared by using microemulsification method.

Quercetin is the flavonoid and has been revealed an array of biological and pharmacological functions like decreasing blood lipid, dilating coronary arteries, anticancer, antiplatelet aggregation, antioxidant, anti-inflammation, antianaphylaxis effects and anti-anemic (Wang et al. 2007). Quercetin-encapsulated SLN (QT-SLN) were formulated by using an emulsification and low-temperature solidification procedures. The QT-SLNs showed an average particle dimension of 155 nm, zeta potential greater than 30 mV and spherical shape. The loading capacity and encapsulation efficiency were found 13% and above 90%, respectively. The absorption of prepared QT-SLNs was studied in rats using an in situ perfusion method. A pharmacokinetic study was conducted which augmented the absorption of QT-SLNs compared with quercetin suspension. In an another research (Wang et al. 2007), authors prepared SLNs comprising total flavones of *Hippophae rhamnoides* (TFH-SLNs) by using cold and hot high-pressure homogenization methods. The effect of development parameters such as particle size, zeta potential, crystal form, encapsulation efficiency and in vitro release profile were examined. It was found that SLNs enhanced the oral bioavailability of TFH with prolonged drug release and maintenance time.

4.2 Nanostructured Lipid Carriers (NLCs)

The nanostructured lipid carriers (NLCs) consideration is rising in current years. NLCs are new 2nd generation of lipid nanoparticles that surmount the limitations of SLN (Muller et al. 2002). NLCs are more cost-effective to fabricate, not require the use of organic solvents, have high drug-loading capacity, and simple to scale-up (Doktorovova et al. 2014; Muller et al. 2002). NLCs consist of both solid as well as liquid lipids, hence resulting in low crystallinity, and a less dense lipid packaging over time (Muller and Keck 2004). The high encapsulation capacity and long-term storage stability are accomplished when compared with SLN (Muller et al. 2002; Muller and

Keck 2004). Three methods are generally used to formulate NLCs that are cold homogenization, hot homogenization and microemulsification. NLCs can be used to enhance the bioavailability and chemical stability of hydrophobic nutraceuticals through encapsulation.

Curcumin is a lipophilic polyphenol (rhizome of the herb *Curcuma longa*) and genistein (isoflavones found in soy) are phytotherapeutics which have health promoting and disease averting properties against various ailments including cancer (Aditya et al. 2012). Both curcumin and genistein are susceptible to oxidation, light and heat, and also show poor bioavailability because of low permeability, poor aqueous solubility (<1 μg/mL) and significant first pass metabolism (Aditya et al. 2012). NLCs were fabricated to enhance the oral bioavailability of curcumin and genistein (Aditya et al. 2012). The curcumin–genistein co encapsulated NLCs were prepared by nanoemulsion technique with high-speed homogenizer and ultrasonic probe. The entrapment efficiency was found more than 75% for curcumin and genistein-loaded NLCs.

Vitamin D (cholecalciferol) is a hydrophobic vitamin and act like a steroid hormone which plays an important role in the formation of bones and cartilages. Due to the lipophilic nature it has low water solubility hence low absorption in gastrointestinal tract. To enhance the absorption of Vitamin D, Compritol and Precirol (solid lipids), Octyloctanoate and Miglyol (liquid lipids), Tween 20, Tween 80 and Poloxamer 407 (surfactants) were used for preparation of vitamin D3-loaded NLCs by using hot homogenization method (Mohammadi et al. 2017). The in vivo results after oral administration of vitamin D3-NLCs, and vitamin D3-diluted in Miglyol oil in rats were showed that the inclusion of vitamin D3 into NLCs enhanced the absorption of vitamin D3 by oral administration. Various nutraceuticals/natural bioactive compound(s) encapsulated in NLCs are shown in Table 1.

4.3 Liposomes

Liposomes are utilized as an effective drug delivery system from several years to till date. Liposomes are spherical bilayer vesicular system composed of amphipathic molecules, like phospholipids which has aqueous compartment inside. Classically, they are formulated either from lipids or lipid mixtures with different ratios. These are produced by the self-association property of amphiphilic phospholipids with cholesterol molecules. Benefits of these carrier systems are capability to interact with cells, can be formulated with a broad choice of compositional and structural designs and also an ability to encapsulate biopharmaceutical drugs. Liposomes encompass a small size hence large surface area to contact with biological tissues, and thus having an improved bioavailability of loaded constituents.

There are many antioxidants were formulated in liposomes such as quercetin (Ghosh et al. 2011), lycopene (Tian et al. 2007), curcumin (Takahashi et al. 2009), vitamin E (Nacka et al. 2001), silymarin (Wagoner et al. 2011) and resveratrol (Wang et al. 2011). Wang et al. (2011) were scrutinized the protecting effect of resveratrol

Table 1 Encapsulation of nutraceuticals/natural bioactive compounds within NLCs

S. No.	Nutraceuticals/bioactive compound(s)	Preparation method	Purpose
1.	Turmeric extract	High shear homogenization	Improvement of antimicrobial and antioxidant activity
2.	α-tocopherol	Miniemulsions methodology	Improve antioxidant activity with scavenging activity
3.	β sitosterol	Hot melt emulsification method	Application in functional foods
4.	Cardamom essential oil	Low energy nano-emulsification method coupled with high shear homogenization	Used as food supplements
5.	Astaxanthin	High-pressure homogenization	Enhancement of antioxidant activity
6.	Astaxanthin	High-pressure homogenization	Improve the chemical and physical stability
7.	6-Gingerol	High-pressure homogenization	Oral bioavailability enhancement
8.	Quercetin	Phase inversion-based process method	Potential for chemo-preventive use in breast cancer
9.	Quercetin and linseed oil	High pressure homogenization	To improve antioxidant activity
10.	β-Carotene	Phase inversion temperature	To study the degradation prevention of β-carotene
11.	Vitamin A palmitate	High pressure homogenization	NLCs stabilized with 6% Poloxamer showed significantly lower particle size and particle size distribution
12.	Hesperetin	Melt emulsification	To entrap hesperetin as the functional ingredients in food sectors
13.	Omega-3 fatty acid	Hot homogenization method	Encapsulation of acids decreased their oxidation significantly
14.	Ascorbyl palmitate	High mechanical shear method	Enhancement of the chemical stability of ascorbylpalmitat

obtained from *Polygonum cuspidatum*. Resveratrol and resveratrol loaded liposomal formulations were administered orally on nigral cells of Parkinson's disease-bearing rats. After oral administration of resveratrol alone and resveratrol-loaded liposome, it was found that the loss and apoptosis of nigral cells, the abnormal rotational behavior and the levels of total reactive oxygen species (ROS) were noticeably reduced. Resveratrol-loaded liposome was showed good effects than free resveratrol. An improved bioavailability indicated that resveratrol loaded liposomes were more effective and can be an alternative choice to cure the Parkinson's disease.

Docosahexaenoic acid (DHA) is dietary ω-3-polyunsaturated fatty acids (PUFAs), useful in the treatment of cardiovascular diseases and cancer. These diseases have common pathophysiological pathways such as an inflammatory microenvironment, aberrant cell proliferation and unbalanced oxidative stress, which are potential therapeutic targets for PUFAs (Alaarg et al. 2016). A nanomedicine-based development will be good to deliver efficient amount of PUFAs to inflammatory cells. The ω-3 fatty acid i.e. docosahexaenoic acid was loaded into liposomes and evaluated for anti-inflammatory outcomes in various types of immune cells (Alaarg et al. 2016). This class of nanonutraceutical symbolizes a nanomedicine approach to deliver the practically safe nutraceutical constituents that may be useful for management or treatment of chronic inflammatory ailments and cancer. Similarly, carotenoids have abundant health advantages like reducing the risk of prostate cancer, anti-inflammatory action and they are furthermore helpful to fight against the age-related cataract and muscular deterioration. Carotenoids are prone to oxygen, light, auto-oxidation and instability inside food matrices (Xianquan et al. 2005). Hence, it is essential to load them in a suitable delivery carrier system before inclusion in the food system.

In one study, constituents of green tea (catechins and epigallocatechin gallate) were incorporated in liposomes (Rashidinejad et al. 2014). The liposomes were prepared of soy lecithin and size was 133 nm and encapsulation efficiency found >70%. Liposomes containing antioxidant constituents were efficiently maintained in a low-fat hard cheese and an easy and efficient deliverance vesicle for antioxidants (Rashidinejad et al. 2014). Authors were investigated the in vitro release performance and storage stability in the simulated gastrointestinal media of different carotenoids loaded in lipid vesicles (Tan et al. 2014). They were found that carotenoids exhibited unlike encapsulation efficiencies into the lipid vesicles. Inclusion of phenolic constituents into phospholipids, like soy lecithin, is called 'phenolipids formulation' and offers potential novel use for loading phenolic constituent(s) in food as well as in pharmaceutical preparations. Furthermore, the antiviral and antimicrobial activities of phenolic components could be improved by using soy lecithin. Liposomes are the elegant loading system due to their smaller in size, biodegradability, biocompatibility, absence of toxicity and ability to carry a wide range of bioactive substances because of amphiphilic nature of phospholipids used for encapsulation.

4.4 Nanoliposomes

As the advancements in nanotechnology the term 'nanoliposome' has been commenced in place of liposomes. Nanoliposomes refer to liposomes with nanoscale lipid-based vesicles. Usually, liposomes and nanoliposomes acquire the identical physical, structural, and thermodynamic characteristics. Nanoliposomes offer additional surface area, enhanced solubility, increased bioavailability, controlled release and facilitated targeting of the loaded actives to a superior manner than liposomes. The route of administration for nanoliposomes may be oral, topical, parenteral, or nasal (Shoji and Nakashima 2004). Liposomes and nanoliposomes need energy for the preparation of phospholipid and lipid molecules dispersion in aqueous medium (Mozafari 2005). Furthermore, nanoliposomes may get deteriorated by assorted forces like van der Waals (vdW), electrostatic, and hydrophobic forces. Hence, to make them steric, stable inert polymers are used.

Nanoliposome and Liposome are dynamic structures that may be liable to fuse to form an aggregate which resulted in the enhancement of size; means vesicles prepared in nanoscale size are transformed to micrometric vesicles on storage. Though, nanoliposomes must comprise adequate stability profile to keep their sizes within nanoscales. Hence, a technically sound explanation for nanoliposomes, thus, may possibly be: "bilayer lipid vesicles possessing and maintaining nanometric size ranges during storage and application". Probable methods to enhance the shelf-life and stabilities of nanoliposomal formulations by adding sterols such as cholesterol, vitamin E as an antioxidant and polymers like chitosan during preparation. Cholesterol enhances the stability of the bilayer vesicles by altering the fluidity of the phospholipid bilayer. A fascinating characteristic of nanoliposome vesicles is the metastable form, which resist to the change in vesicle size upon dilution with water when compared with other micron size lipid vesicles. An additional benefit of nanoliposomes over other carrier systems is that they are capable to load lipid soluble, water-soluble and amphiphilic constituents. Further, two bioactives can be encapsulated simultaneously in nanoliposomes which have dissimilar solubility profiles. Vesicles having two bioactive agents are known as bifunctional vesicles.

Alexander et al. (2012) examined an outcome of hydrophilic and hydrophobic plant sterol on the encapsulation efficiency and stability of model hydrophilic nutraceutical i.e. ascorbic acid. Nanoliposomes were formulated by using high pressure homogenization methodology. All the nanoliposome formulations were showed particle size between 115 and 150 nm. The inclusion of plant sterols were enlarged the average size of nanoliposomes and showed a sustained release patterns. Bochicchio et al. (2016) were formulated nanostructures (<100 nm in size) loaded with special vitamins such as vitamin B_{12}, tocopherol and ergocalciferol. Nanoliposomes were prepared by sonication method. Bioactive nutrient properties of vitamins can be enhanced by loading high amount into nanoliposomes. Conversely, in another study, the hydrophilic constituents like green tea catechins were loaded in nanoliposomes (Rashidinejad et al. 2014). The nanoliposomes were prepared by using soy lecithin and size found to be 130 nm with an encapsulation efficiency of >70%. When the

prepared formulation was mixed to the milk during low-fat hard cheese production and found that almost no catechin was lost (Rashidinejad et al. 2014).

Antioxidant bioactives are a new class of nutraceuticals that will take a considerable benefit from the nanotechnology in terms of enhancing the nutritional values. One good example is the antioxidant ascorbic acid and α-tocopherol containing nanoliposomal formulation (Niki 2014). Vitamin E when reacts with peroxy radicals present in the food and form α-tocopheroxyl radicals which are less effective than peroxy radicals in the oxidation chain reaction initiation. The α-tocopheroxyl radical is reduced to α-tocopherol by adding ascorbic acid, thus extending the antioxidant outcome of the α-tocopherol molecule. However, α-tocopherol is hydrophobic and therefore cannot interact with the water-soluble ascorbic acid. The unique properties of nanoliposomes which comprised of both lipid and aqueous phase, may give a valuable solution to above mentioned issue.

Bioactive peptides are food derived constituents which have functional value and shows a physiological consequence in the body (Shahidi and Li 2014). In general, peptides encompass 2–20 amino acid units and their molecular masses are <6000 Dalton (Najafian and Babji 2012). Bioactive peptides obtained from fish have a range of biological roles including immunomodulator, inhibition of angiotensin converting enzyme (ACE), antimicrobial and antioxidant activities (Najafian and Babji 2012; Shahidi and Li 2014). Bioactive peptides encompass bitter tastes that decreases the user suitability. Besides, bioactive peptides can also be denatured in the harsh acidic condition of the stomach hence reduces the activities, simultaneously proteolytic enzymes present in the intestine and stomach also degrades small peptides. For protein and peptide delivery through the gastrointestinal tract, a transporter system is required to overwhelm acidic, diffusion and enzymatic barriers. The antioxidant peptide with a molecular mass less than 30 kDa (PF30) was separated from rainbow trout (*Oncorhynchus mykiss*) and encapsulated in chitosan-coated nanoliposomes (Ramezanzade et al. 2017). The chitosan-coated nanoliposomes preserved the antioxidant action of the PF30 and can be used as a possible candidate for proficient delivery of bioactive ingredients found in functional foods and nutraceuticals. Different nutraceuticals/ingredients encapsulated in liposomes/nanoliposomes are shown in Table 2.

4.5 Nanoemulsions

Emulsions are usually the amalgamation of different components such as a lipophilic phase (oil or lipid), a hydrophilic phase (water or aqueous buffer), surfactant and co-surfactant and classified microemulsion as water-in-oil (w/o), oil-in-water (o/w) type or bicontinuous that can be used as colloidal drug delivery systems to solubilize lipophilic drug(s) or bioactives to enhancement of absorption hence bioavailability. Nanoemulsion is a kinetically stable system of oil and water (two immiscible liquids), which is stabilized by an interfacial film of surfactant and co-surfactant. It is isotropically clear colloidal dispersion that has droplets size range from 50 to 200 nm

Table 2 Nanoencapsulation of bioactive ingredients/nutraceuticals in liposomes and nanoliposomes

S. No.	Encapsulated agents	Objective(s)
1.	Catechin and epigallocatechin gallate	To increase the encapsulation efficiency
2.	Thymoquinone	Liposomal system for topical delivery
3.	Resveratrol	To study the absorption of Res-nanoliposomes from varied intestinal segments and the absorptive mechanism in vivo
4.	Phenolic components	To improve activities of phenolic components
5.	Carotenoids (lutein, lycopene, β-carotene, canthaxanthin)	To study in vitro release behavior and storage stability in different GI media
6.	Ascorbic acid	To investigate the stability and encapsulation efficiency
7.	Eugenol	Physicochemical characterizations of EN were evaluated by combining the ethanol injection method with the dynamic high-pressure microfluidization method
8.	Catechin	Preventing decomposition of hydroperoxidase into free radicals
9.	Lysozyme	Increase efficacy and stability against Listeria monocytogenes by nisin and lysozyme encapsulated in liposomes
10.	Vitamin C	To examine the plant sterols on the encapsulation efficiency and stability
11.	Vitamin B_{12}, Tocopherol and Ergocalciferol	To enhance the loading amount
12.	Vitamin E	To investigate the loading efficiency and payload
13.	Vitamin E	To increase the physicochemical properties
14.	L-carnosine	To study the influence of lipid composition on physicochemical properties
15.	Ascorbic acid and α-tocopherol	To encapsulate and improve antioxidant properties
16.	Bioactive peptides	For preservation of antioxidant properties
17.	Curcumin	To improve stability and mucoadhesive properties
18.	Quercetin	To study the physicochemical and sensory properties in a whey drink
19.	Polyunsaturated fatty acids (docosahexaenoic acid and eicosapentaenoic acid)	To optimize conditions for preparation of nanoliposomes
20.	Medium-chain fatty acids	To investigate the encapsulation efficiency and stability

(Solans et al. 2005). Nanoemulsions have significant potential for protecting, encapsulating, and delivering lipophilic and hydrophilic bioactive constituents through the oral route and dermal route, similar to that of delivering pharmaceuticals and nutraceuticals. Oil-in-water (O/W) nanoemulsions are predominantly useful for the design of excipient foods because they enhance the bioavailability of amphiphilic, lipophilic, and hydrophilic food components. O/W nanoemulsions are chiefly appropriate for the incorporation of lipophilic nutraceuticals because of their capability to form stable delivery systems with high oral bioavailability.

The systemic bioavailability of bioactive constituents is significantly influenced by their poor solubility in oil and water since their characteristic features like partition coefficient, lipophilicity, and solubility which decides their route of administration, transport and target sites. The most important factors restraining the oral bioavailability of hydrophobic bioactive agents are absorption, bioaccessibility and transformation (Aboalnaja et al. 2016). The loading of such bioactive components into nanoemulsions could be useful since the small globule size of nanoemulsions will augment the surface area hence resulted in an improved digestion rate, quick diffusion across membrane and improved cell permeability (Sivakumar et al. 2014; Ting et al. 2014). Furthermore, nanoemulsions can possibly protect the chemically susceptible bioactives from oxidation, thus improved shelf life and decreased their degradation in the GIT (Frede et al. 2014). There are various research studies on loading of bioactives into nanoemulsions and latest drift have revealed the use of food-grade nanoemulsions. The carrier oil (liquid lipid) is a major constituent in formulation of food-grade nanoemulsions as it decides the bioavailability of loaded constituents. The carrier oil must be capable to form mixed micelles with a good solubilization capability for an active constituent and must be completely digestible (Li et al. 2012a, b).

Nanoemulsion is also useful for skin care due to its good sensorial properties and biophysical properties. The high solubility potential for actives of the nanoemulsion system may enhance the thermodynamic activity towards the skin and due to the presence of surfactant and mixture of surfactants act as permeation enhancers that augment the drug flux via the skin (Aparna et al. 2015). The coenzyme Q10 is poorly soluble and permeable antioxidant which acts as anti-wrinkle and anti-aging agent (El-Leithy et al. 2018). To enhance the topical permeability, oil/water (o/w) nanoemulsion was formulated and evaluated as a proficient carrier to deliver coenzyme Q10 through the skin barriers to enhance its anti-wrinkle efficiency (El-Leithy et al. 2018).

Curcumin is a well-known natural bioactive curcuminoid having several health-promoting benefits like anti-inflammatory, anticancer, and antioxidant activities (Yu and Huang 2012). Its poor bioavailability restricts the application in functional foods. Solubilization, metabolism and permeation are significant factors that affect the bioavailability of bioactives. Yu and Huang (2012) developed and evaluated organogel-based nanoemulsions for the oral delivery of curcumin. The in vitro lipolysis result showed the faster digestion of nanoemulsion than the organogel. The permeation study on Caco-2 cell line was revealed the diffusion type absorption mechanism for curcumin present in nanoemulsion. In addition, the oral bioavailability of

curcumin was augmented by 9-fold in the nanoemulsion compared with free curcumin. This type new formulation approach can also be utilized for oral deliverance of other poor or low-soluble nutraceuticals and it has major impact on dietary supplements, functional foods, and pharmaceutical industries. Various nanoemulsions containing bioactives/nutraceuticals are shown in Table 3.

5 Protein-Based Nanomaterials for Nutraceuticals

Proteins are polymers that comprise of amino acids linked by peptide bonds. Proteins can be the part of compositionally and structurally complex natural ingredients for example milk, eggs, or they might be isolated functional ingredients for instance gelatin, whey protein, and caseinate. From the last few decades, protein has been considered as the most striking and flexible natural bio-macromolecule amongst all of the existing biopolymers. It is mostly used as a nano-carrier system in the pharmaceutical industry due to its biocompatibility, low cytotoxicity, biodegradability, considerable attaching capability, clinically usefulness in targeting, and site-specific proficient uptake (Chakraborty and Dhar 2017). Protein is used as a nano-vehicle for abundance of therapeutics such as drugs, hormones, enzymes, genes, antibodies, nutraceuticals, and peptides (Chakraborty and Dhar 2017).

The whey and milk proteins are broadly utilized as functional and nutritional components in prepared foods as they are comparative economical, generally recognized as safe constituent and hold major physical, chemical and biological functionalities (Gunasekaran et al. 2006). Aggregation and denaturation property of these proteins are of meticulous significance toward assembling of novel nanostructures. Furthermore, these proteins encompass imperative biological properties such as anticarcinogenic activity, antiviral, antimicrobial, digestibility, immune system modulation and amino acid pattern, chemical and physical functionalities like gelation, foaming, water absorption and emulsifying agents which are essentials in food appliances (Dissanayake and Vasiljevic 2009). These pertinent properties make proteins as promising building blocks for an encapsulation of the bioactives/nutraceuticals. The chief constituents of whey proteins are immunoglobulin, β-lactoglobulin (β-Lg), bovine serum albumin (BSA) and α-lactalbumin (α-La). The main nanostructured carrier systems are prepared from whey proteins are nanotubes nanohydrogels, nanoparticals and nanofibrils etc. which protect sensitive compounds from deterioration and improve the bioaccessibility to digestive enzymes and consequently the bioavailability.

β-carotene (BC) is a hydrophobic carotenoid produced by photosynthetic microorganisms found in plants, cyanobacteria and algae and it is drawing interest more than the last two decades for its multiplicity of health-promoting advantages (Eggersdorfer and Wyss 2018). Their important functions are strong antioxidant activity, vitamin A precursor, and decrease the risk of several chronic ailments and cancers. The consumption of BC in food or food systems, particularly in nutraceutically fortified food is hindered due to its very poor aqueous solubility, chemical and metabolic instability and poor bioavailability. Yi et al. (2018) were prepared and evaluated

Table 3 Food bioactive/nutraceuticals constituents nanoencapsulated in nanoemulsions

S. No.	Constituents	Methods	Objective(s)
1.	Astaxanthin	High-pressure homogenizer	To formulate and stabilize O/W nanoemulsions
2.	Vitamin E acetate	Phase inversion	To produce food-grade nanoemulsions enriched with vitamin E acetate
3.	Vitamin E acetate	Low Energy Approach	For preparation of food grade nanoemulsion
4.	Vitamin E acetate	high pressure homogenization	To prepare whey protein isolate and gum Arabic stabilized nanoemulsion
5.	Resveratrol and curcumin	Spontaneous emulsification	For the transnasal treatment of neurodegenerative diseases
6.	Tocotrienol-rich red palm oil	High pressure homogenizer	To prepare nanoemulsion for cosmetic formulations
7.	ω-3 fatty acids	Microfluidization	To prepare food grade O/W nanoemulsions
8.	Flax seed oil	Ultrasound-assisted	To establish targeted delivery into the lower sections of GIT
9.	Vitamin D	High pressure homogenizer	To study nanoemulsion as carrier of vitamin-D
10.	β-carotene	High pressure homogenization	To formulate stable nanoemulsions
11.	β-carotene and α-tocopherol	High pressure homogenizer	To improve aqueous solubility, oxidative stability and bioavailability
12.	β-carotene	High pressure homogenization	By application of modified starch to improve stability of O/W nanoemulsions
13.	Curcumin	emulsion inversion point	To improve aqueous solubility
14.	ε-polylysine and d-limonene	High pressure homogenizer	To investigate the synergism between ε-polylysine and d-limonene
15.	Quercetin	High speed homogenization	To prepare quercetin-loaded stable nanoemulsions using RSM
16.	Quercetin	–	To improve oral bioavailability

(continued)

Table 3 (continued)

S. No.	Constituents	Methods	Objective(s)
17.	Quercetin	–	Compared the effects of a quercetin-loaded nanoemulsion with the free form of the drug in a collagenase-induced ICH rat model
18.	Resveratrol	High pressure homogenization	To enhance oral bioavailability
19.	Resveratrol	Spontaneous emulsification followed by high-pressure homogenization	To formulate a kinetically stable o/w nanoemulsion for better management of Parkinson's disease
20.	Resveratrol and linseed oil	High pressure homogenization	To improve solubility, bioavailability and stability
21.	Lycopene	Emulsification evaporation/High pressure homogenization	To prepare stable and high encapsulation efficiency nanoemulsion by using a Box-Behnken design for beverage applications
22.	Lycopene	High-pressure homogenization	The impact of lycopene on the formation and physical properties of o/w nanoemulsions
23.	Carotenoids and Phenolics from Mangoes	High speed mixer	To improve bioaccessibility
24.	Coenzyme Q10	High pressure homogenization	To improve the bioavailability
25.	Coenzyme Q10	Pseudoternary phase diagrams by using aqueous titration method	To improve permeability through topical application
26.	Capsaicin	High pressure homogenization and ultrasonication	To improve stability and antimicrobial activity
27.	Capsaicin	Ultrasonication	To prepare a food-grade nanoemulsion to improve dissolution profile and bioaccessibility
28.	Capsaicin	–	To examine the pharmacokinetic properties of the capsaicin-loaded nanoemulsions

the resveratrol (RES)-loaded whey protein isolate (WPI)-dextran nanocomplex to improve the physico-chemical stability of BC emulsions. WPI-dextran was produced by Maillard-based glycation, while WPI-RES and WPI-dextran-RES nanoparticles were formulated by nanocomplexation method. The chemical stability of BC with WPI-RES and WPI-dextran-RES was found amazingly improved when rendered to thermal treatment and UV light. These protein-polysaccharide conjugate can be employed as novel stabilizers and offer a superior substitute to efficiently guard and deliver other lipophilic nutraceuticals.

One research study reported the entrapment of the α-tocopherol in whey protein nanodispersions using high pressure encapsulation technique to enhance the antioxidant properties (Relkin and Shukat 2012). Authors prepared a stable nanohydrogel from a composite of protein polysaccharide i.e. β-Lg-pectin for loading and delivery of lipophilic nutraceuticals for example ω-3 fatty acids and developed nanohydrogel to protect DHA against oxidation, therefore this can provide health-improving characteristics to food products and beverages during storage (Zimet and Livney 2009).

Li et al. (2012a, b) prepared nanohydrogels of β-Lg of epigallocatechin-3-gallate which is a most important catechin found in green tea and has an effective antioxidant property. A clear and stable nanosystem was obtained at pH 6.4–7.0 and maximum protection of epigallocatechin-3-gallate antioxidant activity was obtained with β-Lg when heated at 85 °C. In a similar way, some more authors have formulated nano-entrapped epigallocatechin-3-gallate after cooling and vortexing pre-heated β-Lg solutions (Shpigelman et al. 2012). Thermally-induced protein-epigallocatechin-3-gallate co-assemblies were found <50 nm and loading efficiency of 60–70% of epigallocatechin-3-gallatein β-Lg nanocomplexes. The slow release of epigallocatechin-3-gallate was found in simulated gastric media from β-Lg–epigallocatechin-3-gallate nanoparticles which recommending that they can be utilized as nanomaterials for shielding of epigallocatechin-3-gallate in the stomach.

Some researchers were disclosed that both WPI and pure β-Lg produce nanofibrils on prolonged heating, at low ionic strength and pH below 2.5 (Nicolai and Durand 2013; Gosal et al. 2004). A small number of researches are available about the applications of β-Lg and WPI nanofibrils as nanomaterials. In recent times, potential applications have fascinated a wide attention from biomedical and food industries, for the microencapsulation of bioactive ingredients, enzyme immobilization, or even the development of biosensors.

Protein nanotubes are produced via a partial hydrolysis of α-La in the presence of serine endoprotease obtained from *Bacillus licheniformis* using divalent cationic ions ((Ipsen and Otte 2007). Protein nanotubes acquire numerous built-in advantages than other protein nanostructures such as probability of functionalizing properties of outer as well as inner layers of nanotubes, controlled release pattern of bioactive(s)

and high stability (Sadeghi et al. 2013). Despite the remarkable properties with their intrinsic potential of protein nanotubes as a carrier system, only one research work unfolded the application of BSA nanotubes to load curcumin (Sadeghi et al. 2013). Nanotubes prepared from α-La were used for health and food applications due to biocompatibility, absence of toxicity, ease of functionalization, biodegradability and low-cost. The promising applicability of α-La nanotubes as thickener agents and effective nanocarriers for bioactives because of gelation ability of α-La (Ipsen and Otte 2007). Because of the above mentioned properties, α-La nanotubes might be utilized to perk up an effectiveness and stability of some nutraceuticals in addition to a controlled release pattern of such constituents in particular sites of the gastrointestinal tract (GIT).

6 Cellulosic-Based Nanomaterials in Nutraceuticals

Nanomaterials can be obtained from non-renewable sources via unsafe/dangerous chemical processes and so there is an inclination of researcher to discover instinctive nanomaterials with energy efficient and green formulation methods (a substitute to synthetic nanomaterials). The cellulosic nanomaterials (CNMs) are organic and green nanomaterials that obtained from renewable resources and having light weight, good biocompatibility and outstanding mechanical strength. The distinctive chemical and physical characteristics have made these nanomaterials a fascinating idea in different applications such as nutraceutical and food industries. The immobilization of various enzymes, bioactive agents, pathogen, emulsion stabilizer, food additives and the production of intelligent packaging systems are the potential applications of CNMs. A range of CNMs can be obtained depends on the source as well as on extraction/processing conditions (Grishkewich et al. 2017; Lin et al. 2012). For an instance, the cellulose nanocrystals (CNCs) are fabricated by using cellulosic resources at controlled acid hydrolysis procedure which produces rod-shaped and highly crystalline cellulosic nanoparticles. The dimension of CNCs might differ depend on the resources while the processing conditions are same. CNCs developed from hard wood have 3–5 nm width and 100–300 nm length than those derived from tunicate (a marine animal) which have a 15–30 nm width and length of 1000–1500 nm (Grishkewich et al. 2017).

The cellulose nanofibrils (CNFs) are obtained from the cell wall of cellulose fibers via exhaustive mechanical or amalgamation of chemical and mechanical procedure like microfluidization, high pressure homogenization, high ultrasonic treatment and cryocrushing are used to take out CNFs from a variety of bacterial and cellulosic

resources (Khan et al. 2017). CNFs made up of mixtures of amorphous and crystalline cellulose chains having width range of 4–20 nm and length in several micrometers. The CNFs have various properties such as the capability to form strong entangled network, semi-crystalline structure, good flexibility and high intrinsic mechanical strength.

Bacterial cellulose (BC) is prepared through various processes like biosynthesis of a variety of microorganisms such as *Gluconacetobacter xylinus* and in vitro enzymatic pathways and chemosynthesis of glucose derivatives (Ullah et al. 2016). The BC nano/microfibrils are microns in size, encompass a high aspect ratio (>50) (Moon et al. 2011). Probiotics (bacterial cells) are live microorganisms (e.g. *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, bifidobacteria etc.) belong to natural biota with little or no pathogenicity, used as nutraceuticals in a diversity of food formulations with additional health benefits and well-being of the host (Huq et al. 2017; Khan et al. 2017; Cencic and Chingwaru 2010). A probiotic bacterium is needed at a lowest concentration of 106–107 cfu/g, if we would like to get noteworthy health benefit from the ingestion of these microbes. The main drawback of probiotic bacteria is shelf-life and probiotic bacterium having food products experience from the intrinsic vulnerability of these microbes viability from high water activity and low pH. A broad study was conducted on increasing the viability of probiotic bacteria by using the nanomaterial of cellulose nanocrystal (CNC) (Huq et al. 2017). It was found that CNC showed good filler for microencapsulation of probiotic bacteria *L. rhamnosus* (Huq et al. 2017). It was found that the viability of probiotic by 38% was increased by CNC incorporation in alginate microbeads compared to alginate micro-beads with no CNC in simulated gastric fluid (SGF) at pH 1.5. In another study, it was found that CNC-containing probiotic tablet formulation was enhanced the survivable of probiotic bacterium by 84% in SGF (Huq et al. 2016).

The cellulose acetate nanofibers (CAN) and CNC are the cellulosic nanomaterials that can be used for the immobilization of a range of bioactive components like antioxidants and vitamins. Various animal studies were indicated that the sufficient delivery of dietary antioxidants can delay or avert diabetes complications such as renal and neural dysfunction by offering defense against oxidative stress. Vitamin C is a good antioxidant and scavenges ROS directly. Akhlaghi et al. (2015) prepared CNF-chitosan oliogosaccharide matrix for encapsulation of vitamin C. The in vitro release study showed a higher stability and high antioxidant activity of vitamin C prepared matrix when compared with the control vitamin C solution. Criado et al. (2016) prepared cellulose nanocrystals (CNCs) containing an antioxidant agent gallic acid. The green functionalization's of CNCs were brought out using redox reaction with ascorbic acid and hydrogen peroxide. A γ-irradiated CNC suspension was reacted with ascorbic acid and hydrogen peroxide, followed by mixing with gallic acid. It was found that the functionalized CNC derivative showed a high antioxidant value compared to that of the native CNC.

7 Polysaccharide-Based Nanoparticles

Polysaccharides are the main class of biological polymers, which are efficiently bioactive, hydrophilic, biodegradable, biocompatible, inexpensive, non-toxic and offer a broad variety in structure and properties. These can be effortlessly modified and biochemically/chemically to augment the bioadhesion with biological tissues, enhance bioavailability and provide better stability to drugs or bioactive or nutraceuticals. Their biocompatibility, non-toxic, low-cost, stable structure, hydrophilic nature with accessibility of reactive sites for chemical modifications makes them the material of alternative (Sinha and Kumria 2001). Polysaccharide materials are classified as non-polyelectrolytes and polyelectrolytes. The polyelectrolytes can be divided into cationic such as chitosan, anionic like alginate, pectin, hyaluronic acid, heparin and neutral (pullulan, dextran subgroups), based on their intrinsic charge. Based on sources of origin, polysaccharides are mainly classified as: (a) algal origin (alginate), plant origin such as guar gum, pectin, (b) microbial origin e.g. xanthan gum, dextran and (c) animal origin e.g. chondroitin, chitosan (Zheng et al. 2015; Augustin and Hemar 2009).

The majority of the natural polysaccharides are believed to be used for nanoencapsulation of various bioactives. The choice of method and polysaccharides depend on various factors like economic, safety and environmental considerations, etc. Different methods can be utilized for nanoencapsulation of bioactive components depending upon the physical and chemical characteristics of bioactives and polysaccharides. Polysaccharides are capable to encapsulate both hydrophilic as well as hydrophobic constituents. Their structural flexibility and site-specific digestion features project them as appropriate carriers for the controlled and targeted delivery of nutraceuticals along the human GIT (Sinha and Kumria 2001). Some other works done on encapsulations of nutraceuticals are collected in Table 4. The nanomaterials used for encapsulations of nutraceuticals are shown in Fig. 3.

8 Conclusion

For quite a long-time, bioactives/neutraceuticals obtained from plants were utilized in various diseases as aversion and treatment. Nanotechnology-based products progressed to the development of nanocarriers (nanoparticles, nanoemulsion, liposomes, polymer micelles, nanodispersion) with diversified physico-chemical properties and useful traits will open up new potential outcomes for oral conveyance of nutraceuticals. Nanoparticles may enhance the solubility properties through an encapsulation of nutraceuticals under suitable environment so as to avoid the degradation/oxidation in GIT. A glimpse on nutraceuticals, types, nanocarriers framework, nanomaterials and their encapsulation efficiency of nano-sized nutraceuticals for their potential applications in therapy were discussed. Thus, an improvement of nanotechnology-based system for the manufacturing of nano-nutraceuticals can offer an extra wellspring to

Table 4 Some other works on nanoencapsulation of nutraceuticals

S.No.	Nutraceuticals	Nano-delivery system	Material(s) used	Inference
1.	Allicin	Nanoparticles	Locust bean gum	Providing protection and stability
2.	Glycyrrhizic acid	Polymeric nanoparticles	Chitosan, katira gum	Increased anti-inflammatory activity
		Polymeric nanoparticles	Chitosan	Enhanced anti-bacterial activity
3.	Green tea extract	Nanostructured lipid carriers	Cetyl palmitate, glyceryl stearate, grape seed oil, St. John's wort oil (*Hypericum perforatum* oil), sea buckthorn oil	Improved antioxidant activity
4.	Hesperetin	SLNs	Glycerol monostearate, stearic acid, glyceryl behenate, oleic acid, Tween 80	Could well mask the bitter taste, after taste, and obviate poor solubility of hesperitin
5.	Capsaicin	Polymeric micelles	Phospholipid, sodium cholate and PVP K30	Prolonged plasma circulation with enhanced oral bioavailability
6.	Curcumin	Hydrogel nanoparticles	Hydrolyzed tetramethyl orthosilicate, chitosan, polyethylene glycol 400	Increase antimicrobial and wound healing properties
		Nanoparticles	Bovine serum albumin (BSA)-dextran	Improve the cellular antioxidant activity
		Polysaccharide nanoparticles	*Enteromorpha prolifera* polysaccharide (EP) and chitosan	Improved storage, thermal and photo stability
		Nanoparticles	Zein, gelatin, alginate	Improved pH stability and antioxidant activity
		SLNs	Tween 80, curcumin, cholesterol	Effective oxygen scavenging activity
7.	Anthocyanins	SLNs	Palmitic acid, span 85 and egg lecithin	Improved the stability against high pH
8.	Bromelain	Nanoparticles	Katira gum	Enhanced anti-inflammatory activity

(continued)

Table 4 (continued)

S.No.	Nutraceuticals	Nano-delivery system	Material(s) used	Inference
9.	Caffeine	SLNs	Softisan, pluronic F68	Increased skin permeation through skin
		Nanohydrogels	Lactoferrin-glycomacropeptide	Increased antimicrobial
		Nanoparticles	PLGA	Pronounced increase in the endurance of dopaminergic neurons, fibre outgrowth and expression of tyrosine hydroxylase (TH)
10.	Lutein	Polymeric nanoparticles	Poly-γ-glutamic acid, chitosan	Improved solubility
		Polymeric nanoparticles	Chitosan and dextran sulphate	Improved chemical stability
11.	Melatonin	Polymeric nanoparticles	Poly(D,L-lactide-coglycolide), polyvinyl Alcohol	Sustained release
		Polymeric nanoparticles	Lecithin, chitosan	Improved wound epithelialization
12.	Quercetin	Polymeric nanoparticles	Polyhydroxybutyrate-cohydroxyvalerate, polyvinyl alcohol	enhance the bioavailability
		Nanoparticles	Chitosan hydrochloride and carboxymethyl chitosan	Enhance the bioavailability, chemical stability and solubility
		Nanoparticles	Bovine serum albumin (BSA), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)	Prolonged quercetin release and improved antioxidant activity
		Polymeric nanoparticles	Poly(lactic-co-glycolic acid)-D-α-tocopherylpolyethylene glycol 1000 succinate	Enhanced pharmacological effects of quercetin with increased liver targeting
13.	Resveratrol	SLNs	Cetyl palmitate, polysorbate 60, miglyol-812	Validated for trans-resveratrol protection, stabilization and intestinal permeability

(continued)

Table 4 (continued)

S.No.	Nutraceuticals	Nano-delivery system	Material(s) used	Inference
		SLNs	stearic acid, omega-3 PUFA	To increase the efficiency
		SLNs	Phosphatidylcholine from soybean, D-α-tocopheryl polyethylene glycol 1000 succinate	Significantly higher cytotoxicity than resveratrol against C6 glioma cells
		Polymeric Nanoparticles	Poly(lactic-co-glycolic acid)	Cytotoxic and mode of apoptotic cells death against prostate cancer cell line (LNCaP)
		Nanoparticles	β-lactoglobulin	Improvement in treatment of oxidative stress
14.	Thymoquinone	Nanoparticles	Poly(styrene-β-ethyleneoxide)	Enhanced antitumor activity

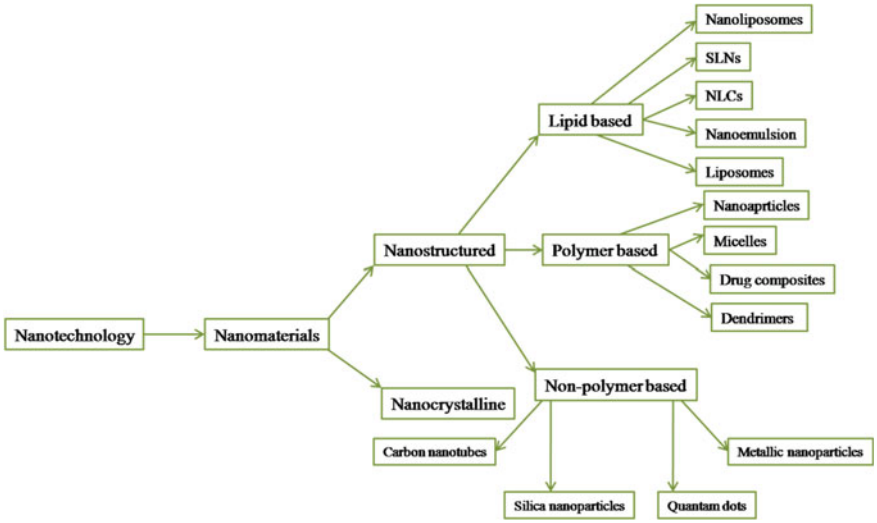

Fig. 3 Schematic diagram of various type pharmaceutical nanosystems for nutraceuticals/drug

the clinicians, dieticians and related health care professionals for the management of certain diseases and improvement in general health of people.

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Anand Krishnan and Anil Chuturgoon

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This book was inadvertently published with the incorrect Book Editor affiliation.

The "About the Editors" information has been updated. One of the co-authors "Bilal A AL Jaidi" name has been corrected as "Bilal A Al Jaidi" in the Chapter "Nanomedicines in Tuberculosis: Diagnosis, Therapy and Nanodrug Delivery".

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