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Application of Nanoparticles in Tissue Engineering

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Nanoparticles for Tissue Engineering: Type, Properties, and Characterization

1

Dikshita Deka, Alakesh Das, B. Priyadrshini, Surajit Pathak, and Antara Banerjee

Abstract

The multidisciplinary area of tissue engineering unifies various fields such as medical biology, engineering, and material science that focus on developing biological alternatives to increase the functions of organs or tissue or to replace or repair the damaged tissue and organs. Though tissue engineering technique has progressed in the scientific field, it still confronts with various challenges; for example, insufficient synthesis of growth factors required for cell-to-cell communication, the toxicity of the biomaterials used, and inability to regulate the cellular functions are the major drawbacks in the field of tissue engineering. Therefore, there is an elevating concern about the significance of the proper insight into the behavior and nature of the nanoparticles. Nanoparticles due to their specific size-dependent characteristics have exhibited their potential in solving several challenges encountered by tissue engineering. However, in spite of the immense advancement of nanoparticles for their application, the complete potentiality of nanoparticles in resolving tissue engineering challenges is still to be analyzed. This chapter represents an outline of various categories of nanoparticles and their potent applications in the challenges of tissue engineering which are necessary to prevail by nanotechnology to attain its complete potentiality.

Keywords

Nanoparticles · Tissue engineering · Drug delivery · Hydrophobicity · Regenerative medicine · Toxicity

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1.1 Introduction

Tissue engineering (TE) can be defined as the analysis of the growth and development of new organs and tissues, developing from a base of scaffolds and cells (Hasan et al. 2014a, b; Hasan et al. 2015). The three-dimensional (3D) structures of scaffolds are used for the growth of cells, proliferation, and differentiation into several types of cells. To intend the behavior of the cell concerning any desired process, the growth factors are introduced into the scaffolds where the final goal is to generate completely functional tissues or organs possessing the potential of regeneration and growth, appropriate for implantation (Hasan et al. 2014a, b; Paul et al. 2016). Regardless of such possibilities, TE also comes across various drawbacks, and translating these ideas into existence appears like an arduous task. The inefficiency to mimic the natural features of the tissue by the engineered materials is known to be another limitation of TE. Nanotechnology can aid to solve this challenge by customized engineering of the nanoparticle (Hasan et al. 2014a, b).

Nanoparticles, that are featured by their nanoscale measure, permit to develop the perilous chemical and physical features which improve the activities, thus makes nanoparticles promising for a wide field of applications. In the area of biomedical field, nanoparticles are utilized for imaging specific sites, drug delivery regulation (Wilson et al. 2010), biomolecular sensing, probing of DNA structures (Koo et al. 2005; Mironov et al. 2008), photothermal ablation of cells, gene delivery, and currently in TE. Moreover, various therapeutic approaches use nanoparticles to treat allergies, cancer, diabetes, inflammation, and infection (Hasan et al. 2018).

The potential of nanoparticles in TE arises due to their smaller size and their large associated ratio of the surface to volume, which is similar to small proteins along with peptides. Nanoparticles can diffuse efficiently through the membranes and can also aid in uptake by the cells. However, it is not restricted by a prearranged size of the nanoparticles, as it can be prepared in customized surface features and sizes to conform to any objective. Nanoparticles have the capacity to imitate the size scale of natural nanometer of extracellular matrix components of tissues themselves. The structure of the nanoscale is considered a necessary component of the body in which the elements of tissues and organs like cells and extracellular matrix consist of several molecules, atoms, microstructures, nanostructures, and macroscale structures. Simultaneously, there are several extrinsic nanoparticles that frequently penetrate and exit the body via oral or inhaled air and topical routes, which rely on the nature or toxicity of the nanoparticle that may or may not be toxic to the body (Hasan et al. 2015; Hasan et al. 2018; Rachel et al. 2020).

Therefore, understanding the particular intrinsic characteristics of specific material and its appropriate characterization may provide the answer to face the current challenges in TE, where the utilization of engineering materials has not been explored to its full potentiality.

1.2 History of Nanoparticles

Nanoparticles are objects of any structure that range from 1 to 100 nm in size (Radomska et al. 2016). The exceptional size accords these entities, the characteristics of both molecular structures and bulk materials. Therefore, nanoparticles are considered as an "arch" between the microscopic and macroscopic structures. The smaller size of the nanoparticles gives them their most appealing intrinsic characteristics: a higher surface-to-volume ratio. One of the notifying properties of nanoparticles is that in a free state they are immensely mobile, making the nanoparticles to have a slow rate of sedimentation. Additionally, nanoparticles are differentiated over a broad range of arrangements varying from soft materials to hard depending upon their applications and may contribute to the quantum effect. This effect permits in having immense conduct over the particles surface energy, which as a result can conduct the initial absorption of protein to dictate cellular associations (Khang et al. 2007; Subramaniam et al. 2019).

Based on the shape of the nanoparticles, it can be of 0 Dimensions, 1 Dimensions, 2 Dimensions, or 3 Dimensions structure. Further, depending on their type or source of materials used, they can be differentiated into distinct sub-groups such as metal-based, ceramic-based, polymeric-based, carbon-based, lipid-based nanoparticles and semiconductor-based (Khan et al. 2019). The nanoparticle synthesis is mainly done by two approaches: bottom-up and top-down methods (Ali et al. 2016) as depicted in Fig. 1.1.

The properties of nanoparticles provide exceptional applications of nanoparticles in a broad variety of research fields. Although compelling advancement has been done for several applications of nanoparticle in these fields, their applicability in TE is still in their initial stage.

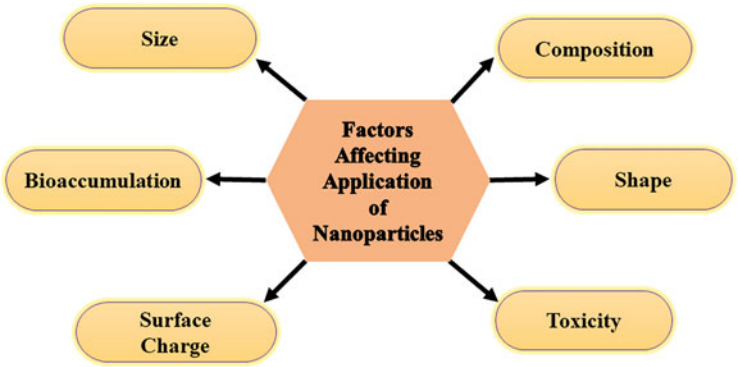

Fig. 1.1 Figure depicting the synthesis of nanoparticles

1.3 Types of Nanoparticles

Nanoparticles of specific nature are presently accessible in clinics and for research purposes, and the attempt to create superior ones are still in progress. Some of the most frequently utilized nanoparticles are reviewed below.

1.3.1 Organic Nanoparticles

1.3.1.1 Liposomes

Liposomes are vesicles made up of one or more phospholipid bilayer membranes. Each layer encloses a water compartment, where the hydrophobic component of the phospholipids is congested collectively, while the polar groups of the head region are in association with the aqueous compartment. However, the properties of liposomes are affected by various factors like the composition and arrangement of lipid, size, surface charge, and method of preparation (Immordino et al. 2006). The composition of lipid regulates the fluidity and charge of the bilayer and its responses toward an external stimulant.

Usually, liposomes are employed as vehicles in targeting or delivery systems. Due to that reason, it is significant to integrate the required molecules within the liposomal structure. A major limitation of this approach is that liposomes are fast recognized by the reticuloendothelial system, which accelerates the process of elimination of liposome from the bloodstream. This process can be slowed down by utilizing liposomes covered with a biocompatible polymer such as poly-ethylene glycol known as stealth liposomes (Torchilin 2005). As they are greatly biodegradable, biocompatible, and displayed a reduced level of toxicity, liposomes are further deliberated as an appropriate approach for clinical uses.

1.3.1.2 Polymeric Nanoparticles

Considering the extent of physical characteristics, biodegradability, and adaptable architecture polymers have been intriguing in current years. The method for the synthesis of polymers is pliable and the chains of polymer can generally be operationalized with a broad range of molecules. With this aspect, the final polymers can display specific properties and compositions, envisaging a broad extent of functions and approaches. Generally, the polymeric nanoparticles are spherical with a diameter of around 100 nm; however, this size can be changed, based on their definite functions.

The notable benefit of the polymeric nanoparticles is associated with their particular high drug loading potentiality. Inorganic and organic molecules can be physically dispersed, dissolved, or associated with covalent interplay with the polymeric component (Nicolas et al. 2013). Hence, currently, these carriers are utilized for the delivery and transport of molecules in various clinical fields, like tissue regeneration, cancer, vaccination, inflammation, and neurologic pathology (Nicolas et al. 2013; Hu et al. 2015).

1.3.2 Dendrimers

It has arisen as an adaptable and versatile element, with an arrangement that divaricate out from a principal core and further divided into units of a stratified branch, finally causing external capping units. Dendrimers are biocompatible, spherical, and biodegradable polymeric-associated nanoparticles that maintain a huge count of surface functional groups, resulting in a unique function for an appreciable count of applications (Menjoge et al. 2010). Generally, an amine exists in the core of the dendrimer. Although, other molecules such as sugar can be used for identical reaction binding two monomers (Tomalia 2005). Additionally, the physiochemical characteristics of dendrimers are increased by the polar surface functional groups which in turn can show increased potential in the application of targeted drug delivery, with an advancement on the solubility of the drug (Gillies and Frechet 2005).

1.3.3 Inorganic Nanoparticles

Inorganic nanomaterials are also being employed for application in the biomedical field. In this field, metallic nanoparticles, nanoparticles of silica, bio-ceramic nanoparticles, and carbon nanotubes are the most novel.

1.3.3.1 Silica Nanoparticles

Silica is noted to be biocompatible, with exceptional surface properties and chemical stability (Slowing et al. 2008). Therefore, silica has been utilized for various applications in the biomedical field, like drug delivery and imaging, either used as a coating material for other compounds or as itself (Aryaei et al. 2014). Silica nanoparticles can be generated by the method of Stőber, by hydrolysis of silane, and the size can be adjusted by shifting the ratios of ammonium hydroxide, tetraethyl orthosilicate (TEOS), and deionized water (Lipski et al. 2008). Based on their definite application, silica nanoparticles can be generated both as bulk silica particles as well as mesoporous silica nanoparticles, and core/shell silica nanoparticles. Core or shell silica nanoparticles are usually utilized in imaging as the delivery agents. The silica shell shields the core, made up of imaging agents such as fluorescent probes, reducing the intensity of photobleaching and permitting the monitoring of the labeled materials for a longer period (Lee et al. 2012; Wu et al. 2014).

1.3.3.2 Metallic Nanoparticles

Gold Nanoparticle

Metallic nanoparticles possess novel properties, varying from the gross material, which is a direct effect of the quantic consequences (Zhao et al. 2013). Among the metallic nanoparticles, gold nanoparticles have emerged as a privilege for various applications of biotechnology. Biosensing, drug delivery, photothermal therapy, and

bioimaging are some of the fields where these materials can be used (Vial et al. 2013; Zhang et al. 2013). Such flexibility is elucidated by the number of optical characteristics, like probable surface modifications, decreased toxicity, and biocompatibility, in comparison to other metallic materials (Yeh et al. 2012). The association of gold nanoparticles with other compounds like silica, leading to core-shell nanoparticles, is also used. As the property of gold nanoparticles, the surface plasmon resonance (SPR) is confirmed on all the indicated configurations, which means that it is likely to appraise a few of their characteristics, like the shape or diameter, via analysis of the UV–vis spectra. Gold nanoparticles are also alluringly used in the process of bioimaging; they can be adjusted to absorb the infrared in the near range, hence leading to increased visualization of the deep tissue by the imaging approach. Also, the higher atomic number can cause strong X-ray attenuation leading to an increased contrast of the computed tomography. Though the gold nanoparticles are treated safer in comparison to other metallic nanoparticles, it is still significant to evaluate their biodistribution, biocompatibility, bioelimination, and environmental effect before their synthesis on a large scale for in vivo applications (Vieira et al. 2017).

1.3.4 Magnetic Nanoparticle

With a size of nanometer the magnetic elements like nickel, iron, cobalt, and their oxides are being noted to have diverse applications in the biomedical field. The intricate information about the most frequently utilized magnetic nanoparticles (MNPs) like magnetic cationic liposomes, metallic, superparamagnetic iron oxide nanoparticles (SPIONs), and bimetallic were recently reviewed. Like other nanosystems, the surfaces of the magnetic nanoparticles can also be functionalized to identify the distinct targets. Therefore, these nanoparticles can be utilized for the cell tracking and isolation, imaging processes, guided drug, hyperthermia, 3D cell organization, biosensors, and gene delivery (Sun et al. 2008; Colombo et al. 2012).

1.3.4.1 Bio-ceramics and Bioactive Glass Nanoparticles

Glass-ceramics, bio-ceramics, and glasses are distinguished as an element with a nonmetallic or inorganic content that are biocompatible and suitable for their application in the biomedical field. Bio-ceramic nanoparticles such as hydroxyapatite (HAp), tricalcium phosphate, and calcium defective HAp (CDHA) have been mixed with synthetic or natural polymers leading to nanocomposite materials (Yan et al. 2013; Pina et al. 2015). This combined biomaterial hold exceptional mechanical characteristics that can be utilized to improve the strategies of tissue regeneration, usually the ones associated with bone disorders. Therefore, bio-ceramic nanoparticles can be endorsed to enhance the performance of the scaffolds, and also can be used for biologically active compounds as a regulated delivery system such as genetic materials or drugs (Verma et al. 2011; Madhumathi and Kumar 2014). On the other hand, bioactive glasses are usually amorphous silicate-associated elements that can evidently forge chemical interaction with the bone

tissue. When the bioactive glass is embedded, the silicon constituent is released instigating the development of a hydroxyapatite layer deficient in calcium on its surface. The layer formed can interact with the impaired bone via the collagen fibrils, permitting the bioactive glass to bind to the enclosing tissue. The mechanism involved in producing monodisperse bioactive glass is a complicated process, though it has drawn great concern from researchers in the field of biological material. Due to this, like bio-ceramics, the nanoparticles of bioactive glass have been associated with various groups of polymers, including natural as well as synthetic polymers to synthesize polymeric composites of bioactive nanostructure with advanced characteristics (Vieira et al. 2017).

1.3.4.2 Carbon Nanotubes

In the field of biomedical research, scientists are working to estimate the potentiality of carbon nanotubes (CNTs) for the delivery of drugs, microscopy, theranostics, biosensing, and reinforcement of composite materials. CNTs are graphitic hollow tubular structures, which is produced by a sheet of atoms of carbon, associated with sp^2 bonds, having very good electrical characteristics along with chemical and mechanical stability. Usually, CNTs have high chemical stability, with a limitation for the process of covalent functionalization, because they need impaired carbon atoms on the tubular structure. For clinical uses, it is essential to do the deeper and standardized evaluations to interpret their toxicity. Various factors like length, diameter, shape, surface, purity, charge, and agglomeration are thought to be significant players in CNTs toxicity (Balasubramanian and Burghard 2005; Liu et al. 2013).

1.4 Physiochemical Properties of Nanoparticles

1.4.1 Electronic and Optical Properties

The electrical conductivity of the material changes and decreases down to the nanometer size. In the case of ceramics nanoparticles, it has higher conductivity due to the small size scale, but in the case of metals, it has lower conductivity when the size is small. When light enters the material, it is either absorbed or scattered. Absorption and diffusion depend upon the particle size. When the dimension of the material is less than 2 nm, the absorption is high, whereas when the size of the material is 100 nm or more, the scattering is large. Therefore, optimal absorption or diffusion can be achieved by designing nanoparticles of different sizes. The optical property of nanomaterials is capable of restricting their electrical characteristics in order to generate a quantum effect with the feasibility of difference in shape and size, or color change. For example, a material that is not transparent at the volume level becomes transparent at the nanoscale (copper nanoparticles), gold nanoparticles at 25 nm appear green, and spherical gold nanomaterial with a diameter of 100 nm appears orange, whereas spherical silver nanomaterial with a diameter of 100 nm looks yellow. The optical properties are dependent on the electronic structure of the

nanoparticle. The color observed in nano-sized materials is a function of the surface plasmon resonance due to light wavelengths resonate on the outer electron band of nanomaterial. The link between particle size and color can be described quantitatively (Adewuyi and Lau 2021). For example, metal nanoparticles are interdependent on size and optical properties which shows strong UV absorption bands that are absent in the spectra of solid metal. This type of excitation band occurs due to the collective excitation of conduction electrons called local surface plasma resonance (LSPR), while the frequency of the incident photon remains unchanged. Studies show that the peak wavelength of the LSPR spectrum depends on its unique dielectric properties and the local environment, comprising the substrate, solvent, adsorbed water along with particle size, shape, and the distance of the nanomaterial (Eustis and El-Sayed 2006).

1.4.2 Mechanical Properties

The mechanical characteristics of nanomaterials increase in proportion to their size reduction. The hardness, yield strength, and modulus of a material increase with decreasing grain size. For example, copper nanoparticles (Cu NPs) with less than 50 nm are considered cemented carbide materials but lack the translucency and ductility of bulk copper. The mechanical properties have a significant role in lubrication systems and are material-dependent in nanoparticles (Akbulut 2012). The intrinsic mechanical property of nanoparticles is the key to new applications in various fields like physics, surface engineering, nanoprocessing, and nanofabrication. The mechanical characteristics of nanoparticles are affected by surface coatings, coagulation, and lubrication. Nanomaterials exhibit distinct mechanical properties compared to microscopic granular and large materials. Additionally, when the contacts are lubricated, the contrast in hardness between the nanoparticles and the outer surface controls whether the nanoparticle is flattened or deformed at high pressures. This important information can reveal how nanomaterials work with exposure to certain conditions. Proper control of the mechanical function of nanoparticles and the interaction with all types of surfaces is important to improve the surface quality and increase stock removal. Obtaining useful results in this field often requires thorough understanding properties of nanoparticles, like modulus and stiffness, the law of motion, the law of friction and surface adhesion, and their size-dependent properties (Guo et al. 2013).

1.4.3 Magnetic Properties

The magnetic property of nanoparticles is caused by the movement of particles that consists of both mass and charge. These particles are electrons, protons, cations, and anions. The rotating charged particles create a magnetic dipole, the magneton. In ferromagnets, magnets are grouped. A magnetic domain (known as a Wyeth domain) is a mass of ferromagnet due to an exchange force all magnets are aligned

in the same direction. The concept of this region distinguishes between ferromagnetism and paramagnetism. Nanoparticles exhibit excellent magnetic properties. Each magnetic moment of the nanomaterial is ferromagnetic oxides that are commonly used in ceramics, paints, and synthetic pigments in ceramics. Magnetic nanoparticles exhibit enormous properties such as highly irreversible and highly saturated fields, super magnetism, anisotropy contributions, and then displaced rings. These properties form narrow and finite-size effects and surface effects that control the individual nanoparticles. Magnetic nanoparticles react with null residue and high speed in an external magnetic field. These characteristics establish the groundwork for biological imaging and data storage applications. According to studies, the efficiency of nanoparticles is greatest when the particle size is less than a critical value, which is between 10 and 20 nm. At these low scales, these particles are valuable and can be utilized for various applications because the magnetic properties of nanoparticles are effectively governed. The magnetic characteristics are caused by an unequal distribution of electrons in nanoparticles. These features are regulated using synthetic processes such as solvent, coprecipitation, microemulsion, pyrolysis, and flame atomization synthesis (Lu et al. 2007; Faivre and Bennet 2016; Qi et al. 2016).

1.4.4 Thermal Properties

The thermal properties of nanoparticles depend on factors that are not normally critical for bulk materials. In specific, the surface property, interface structure, and classical-quantum effects primarily determine heat transfer in nanomaterials, resulting in non-obvious carrier diffusion and localization in materials in bulk. Studies show that metallic nanoparticles have high thermal conductivity compared to solid and liquid. Aluminum oxide is more thermally conductive than water. Therefore, liquids containing suspended solid particles have to significantly improve thermal conductivity compared to existing heat transfer fluids. Production of nanofluids is done by spreading nano-sized solid particles in a liquid medium. Nanofluids are likely to show superior characteristics by comparing local surface plasmon resonance (LSPR) localized to the outer surface of nanoparticles (Khan et al. 2019). A larger total surface area also improves suspension stability (Thomas and Sobhan 2011). Recently, nanofluids composed of copper oxide or aluminum oxide nanoparticles in water or ethylene have been shown to exhibit high thermal conductivity (Cao et al. 2002). Shanbedi et al. showed that the thermal conductivity of nanofluids could be improved in comparison to pure water with elevating concentration and temperature (Shanbedi et al. 2015). A study distinguishes the thermal conductivity of the pure process with the silicon oxide nanocomposite and found that the thermal conductivity of the nanocomposite was improved compared to the pure process with the least temperature-dependent change (Shin and Banerjee 2015; Nikkhah et al. 2015).

1.5 Characterization of Nanoparticles

1.5.1 Particle Size

The characterization of nanoparticles is principally appraised by the size of the particle morphology and distribution. With the help of electron microscopy, it is now attainable to determine the size along with the morphology of nanoparticles. By utilizing various tools, the applicability of nanoparticles in drug targeting and drug release can be determined efficiently. It has been documented previously that the particle size of nanoparticles has a subtle impact on the activity of drug release. The smaller the particle size of the nanoparticles, the greater the surface area, which leads to the immediate delivery of the drug. The loaded drug when unveiled to the surface area of the particle it causes a significant release of the drug. On the other hand, the diffusion of the drugs slows inside the nanoparticles due to the occurrence of the larger particles. Whereof, the smaller particles likely congregate during the transportation and storage of nanoparticle dispersion. Hence, there is a shared arrangement between the smaller size and maximum stability of the nanoparticles. Additionally, polymer degradation can also be influenced by the size of the particle (Redhead et al. 2001).

1.5.2 Surface Charge

The interplay of the nanoparticles with the biological milieu is generally governed by their surface charge and its intensity along with their electrostatic interplay with the bioactive components. The zeta potential of the nanoparticles determines the stability of the colloidal compounds, which indirectly determines the surface charge of the nanoparticles. The zeta potential can be attained by assessing the potential difference between the surface of shear, and the outer plane of Helmholtz. Therefore, the values for the zeta potential are acquired to avert the congregation of the nanoparticles and to assure stability. These properties can be used in determining the surface hydrophobicity and the quality of encapsulated material within the nanocapsules (Otsuka et al. 2003).

1.5.3 Hydrophobicity

For determining the surface hydrophobicity, techniques like adsorption of probes, biphasic partitioning, hydrophobic interaction chromatography, etc. can be used. Current development in the field of research provides various advanced analytical techniques for evaluating the nanoparticles surface property, for example, X-ray photon correlation spectroscopy. This technique evaluates two effects benefits such as determining the surface hydrophobicity and secondly enables the determination of distinct chemical groups present on the surface of the nanoparticles (Scholes et al. 1999).

1.5.4 Drug Release

It is very significant to evaluate the magnitude of drug release and to acquire the data; the majority of the release approaches need the delivery vehicle and drug to be parted. The ability of the nanoparticles in drug loading is described as the quantity of drug bound per mass of the polymer. Various approaches like high-performance liquid chromatography (HPLC) post ultracentrifugation, UV spectroscopy, ultra-filtration, centrifugal ultra-filtration, or gel filtration are utilized to evaluate these parameters. Techniques that are used for the analysis of drug release are also comparable with the assays of drug loading which are more likely determined for a period of time to analyze the mechanism of drug release (Bhatia 2016).

1.6 Application of Nanoparticles

Nanoparticles have been utilized to aid several activities in the field of tissue engineering, varying from an increase of the electrical, mechanical, and biological characteristics to DNA transfection, gene delivery, patterning of cells, viral transduction and to promote the development of different types of tissues to biosensing and molecular detection as depicted in Fig. 1.2. The utilization of the precise of nanoparticles in tissue engineering can substantially increase the mechanical, electrical, and biological characteristics of the scaffold and also aids several functions depending on its applications as discussed below.

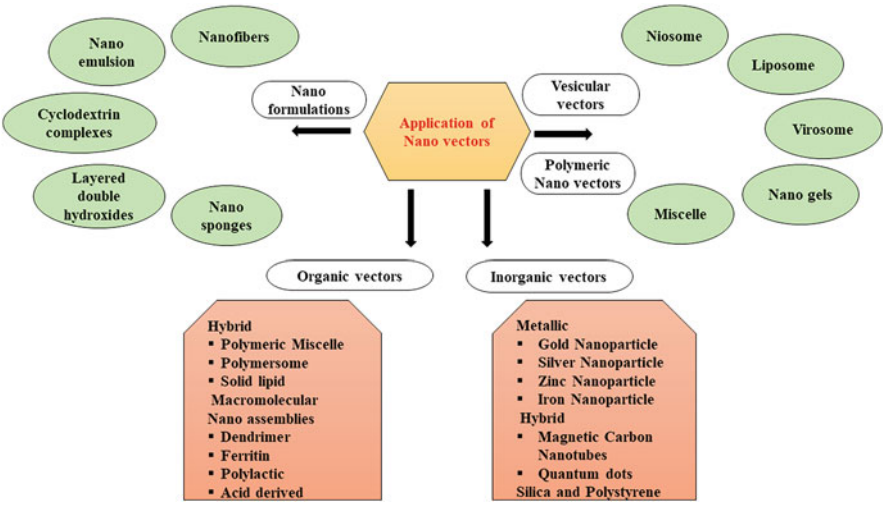

Fig. 1.2 Potent application of nanovectors in the field of tissue engineering

1.6.1 Biological Property Enhancement

Specifically, two kinds of nanoparticles, viz. titanium dioxide nanoparticles and gold nanoparticles (GNP), have been utilized to increase the proliferation rates of the cell for regeneration of the cardiac and bone tissue. GNPs have displayed remarkable capability for surface modification and biocompatibility, which has led to an interesting application in biomedical sciences (Giljohann et al. 2020). Reportedly, in bone TE, it was initially noticed that GNPs initiate the transformation of the osteogenic cells of MC3T3-E1 cells which is an osteoblast precursor cell line. Additionally, these nanoparticles also affect the formation of osteoclast from hematopoietic cells while serving a protective impact on the mitochondrial impairment in osteoblastic cells. Reports from another study revealed that GNPs can instigate varied lineage differentiation in mesenchymal stem cells (MSCs). This lineage differentiation has been reported to be towards the adipocytic cells and osteocyte cells in MSCs. This appears after the intracellular uptake by the GNPs during the establishment of the p38 mitogen-activated protein kinase (MAPK) cascade. However, later it was confirmed that this transformation of the osteogenic cells is reliant on the particle size (Hasan et al. 2018).

Further, in another analysis, titanium dioxide nanoparticles were reported to increase the cellular proliferation of the human embryonic stem cell-derived cardiomyocytes when added to the scaffold. Titanium dioxide nanoparticles have also been 3D printed with polymers, to accurately maintain the nanostructured roughness of bone itself and notably enhance the function of the bone cell. Alluringly, the titanium dioxide nanoparticles were reported to reduce the toxic impact of the acid byproducts freed by poly(lactic-co-glycolic-acid) during deterioration, known to destroy the cells (Liu et al. 2006). Hence, the inclusion of specific types of nanoparticles into the scaffolds can necessarily modify the biological characteristics of the scaffolds and so aid in TE.

1.6.2 Mechanical Property Enhancement

Nanoparticle entrenched in nanocomposite polymers both in the pattern of electro spun fibers and hydrogels has shown preferable mechanical characteristics for applications of TE in comparison to the scaffolds without reinforcements of the nanoparticle (Wanjale and Jog 2006). For example, a titanium dioxide entrenched biodegradable patch displayed a greater tensile intensity in emphasizing the scar after myocardial infarction. In tissue engineering for skin, 3D scaffolds of the nanocomposite were made utilizing a combination of polyvinylpyrrolidone (PVP) and type I collagen-coated titanium dioxide nanoparticles. In this combination, hydrogen bonds were developed within titanium dioxide, collagen, and PVP which enhanced the eventual tensile capacity of the scaffolds. Moreover, in other analyses, metallic nanoparticles, GNPs, and silver nanoparticles were entrenched in scaffolds to increase the mechanics of the scaffold, while in a few analyses, hydroxyapatite (HA) nanoparticles improved the mechanical characteristics of

electro spun silk fibroin scaffolds by forming bonds between silk fibroin fibers and HA (Kubota et al. 1994; Kim et al. 2014). Hence, nanoparticles can perform a pivotal function in improving the mechanical characteristics of scaffolds in tissue engineering.

1.6.3 3D Tissue Construction

In the field of tissue engineering, nanotechnology displays promising applications. The nanoscale structures of the nanoparticles are capable to regulate the cellular functions like adhesion, segregation, and most essentially, nanomaterials have novel magnetic and visual characteristics and therefore are potential agents for controlling in vivo cellular activities subsequent to transplantation. For bone, vasculature or skin tissue, nanomaterials were later developed as 3D tissue-engineered scaffolds. Furthermore, reports have been generated stating nanostructures potentiality in regulating the functions of primary stem cells like growth, differentiation, and adhesion (Xu et al. 2004; Lavenus et al. 2012).

1.6.4 Antibacterial Applications

Metal ions' antimicrobial effect is based on their capacity to inhibit or suspend enzymes and create reactive oxygen species, induce cell membrane damage, and prevent microorganisms from absorbing microelements; moreover, metals can exert the direct genotoxic activity. Nanosized particles act along two major lethal paths, which are interrelated and often occur simultaneously: firstly, the disruption of consistency with membrane potential, and secondly generation of reactive oxygen species (ROS) radicals, and nanomaterial, that acts as nano-catalysts. Membrane damage causes nanoparticle to be electrostatically bound to the cell wall of bacteria, resulting in changes in membrane potential, depolarization of the membrane and loss of integrity, resulting in transport imbalances, respiratory changes, transfer of energy discontinuation, and/or it occurs when it results in cytolysis, eventually cell death. ROS, which is the most efficient determinant of nanoparticle in vivo and in vitro cytotoxicity, is induced indirectly by disruption of the respiratory chain or directly by the nanomaterial itself. Generation of ROS by oxidative stress induces damage to all cellular macromolecules, causing lipid peroxidation, protein alterations, enzyme repression, and DNA and RNA damage. ROS at high concentrations causes cell death, and at low doses it causes mutations. When ROS production is induced by visible or ultraviolet light, it exhibits a photocatalytic property by nanomaterials. Titanium dioxide nanoparticles induce lipid peroxidation under near-UV, leading to respiratory dysfunction and E. coli apoptosis. Other effects of nanomaterials are direct inhibition of enzymes, induction of reactive nitrogen species (NRS), and apoptosis. The new therapies demonstrate the potential use of gold nanoparticles in preventing biofilm-related Klebsiella pneumoniae. Due to the strong biocidal effect against microorganisms, silver nanoparticles are also known as universal

antimicrobial substances. Silver nanoparticles' tiny particle size (10–100 nm) allows them to easily cling to the cell wall and penetrate into bacterium cells, aiding antibacterial action. Against a number of Gram-negative foodborne pathogens, silver nanoparticles demonstrate strong antibacterial activity. As a result, silver nanoparticles can be utilized to fight multidrug-resistant microorganisms (Li et al. 2010; Beyth et al. 2010; Loo et al. 2018).

1.6.5 Cells Stimulation for Mechano-transduction

It is well noted that several growth factors and bioactive molecules control the activity of the cell in the human body. Studies have reported that, besides these particles, mechanical forces also play a crucial role in deciding the function of the cell by affecting the mechano-transduction cascade (Orr et al. 2006). Various strategies have been utilized, comprising the development of shear stress by the bioreactors and stiffness of patterned substrates to mechanically regulate cell activity. Nevertheless, metallic nanoparticles have been reported to be better than all these approaches as they have the potential to be regulated spatially, temporally, and remotely via a magnetic field (Tseng et al. 2012). In the case of the microscopic level, the mechanism takes place as follows. Initially, the metallic nanoparticles are coated with a specific targeting antibody. The cells get accumulated towards the direction of the magnetic field once the magnetic field is enforced. Therefore, the receptor-mediated cell activity is altered based on the particular antibody used. The study by Mannix and colleagues reported attaining an elevation in the level of intracellular calcium ions, whereas Gopinath and colleagues were capable to activate the apoptotic cascade by utilizing these strategies (Mannix et al. 2008; Gopinath et al. 2010). These two major studies including several other analyses have reported how metallic nanoparticle integration can specify complete information about cell differentiation and its activities in regenerative medicine in a remote aspect.

1.6.6 Gene Delivery

The technology of gene delivery aiming at the stem cells or matured cells has evolved as a crucial issue within tissue engineering. Human mesenchymal stem cells are multipotent cells that displayed immunosuppressive characteristics and have an intrinsic potential to transform into several cell types, comprising osteoblasts, chondrocytes, adipocytes, and myocytes (Wang et al. 2012). For persuasive applications of gene therapy, it is pivotal to generate an appropriate vector system with reduced cytotoxicity, high distinction to unhealthy cells, and high efficiency of gene transfection.

1.7 Challenges and Future Perspective

Although nanoparticles have displayed a promising capability in the field of TE, but several challenges exist in imposing their use in clinical applications as depicted in Fig. 1.3. For instance, the first compelling need is the development of better tools and methods for proper assessment of nanoparticle toxicity, teratogenicity, and carcinogenicity. Subsequently, the teratogenicity, toxicity, and carcinogenicity of nanoparticles depend highly on the dose and exposure. For several purposes, the nanoparticles are utilized below their threshold levels which is usually noted as non-toxic. Moreover, it is well known that the nanoparticles can aggregate for a long course of time within the body which can cause serious harm to the body like toxicity to the cells, cancer, or severe effects on the reproductive system. Additionally, although there are several items accommodating nanoparticles existing in the market, there are still certain methodological and scientific gaps in the knowledge on particular risks of nanoparticles. Hence it is necessary discrete measures must be taken for the utilization of nanoparticles where there is a probability of chronic bio-aggregation.

1.8 Conclusion

For surface alterations, nanoparticles displayed well-established approaches and superior biocompatibility which has made them eminently efficient for various utilization in the field of biomedical sciences. Utilizing nanoparticles, the electric pairing between proliferation rates and decellularized cells on various tissues is also found to have elevated effectively. Nanoparticles are noticed to develop

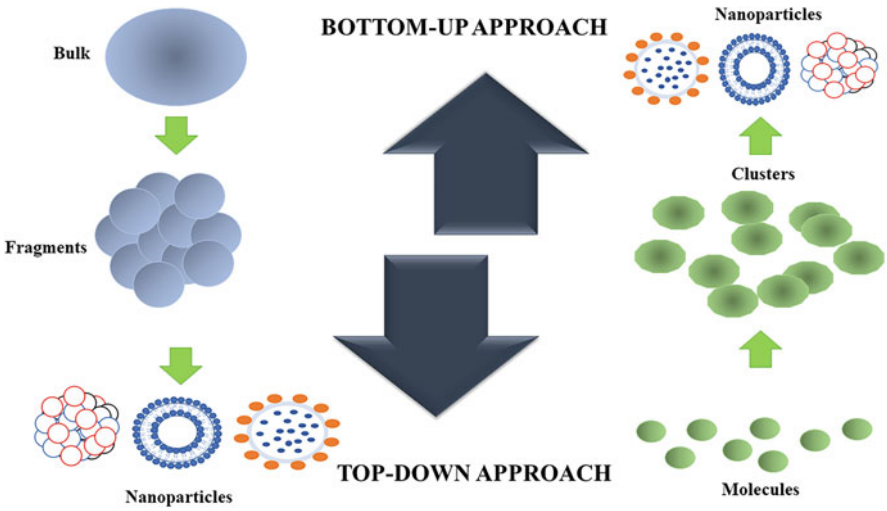

Fig. 1.3 Figure depicting the challenges encountered by nanoparticles

electrochemical, optical, and electrical sensors for DNA, molecules, and protein detection with greatly precise outcomes. By amending these applications, these biosensors could have an immense impact on the medical field. Though nanoparticles display an assuring future in tissue engineering applications, there is still inadequate evidence on in vivo experimentation, which is required to be done in order to confirm the broad array of promising outcomes from in vitro analysis. The nanoparticles could apprehend its utilization in the near future which can direct and engage the distinct molecules, targeting the specific regions in the body and govern the development of tissues in vivo.

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Nanoparticles and Bioceramics Used in Hard Tissue Engineering

2

U. S. Rahul Siddartha, R. Arun Kumar, and A. Moorthi

Abstract

Tissue engineering is an interdisciplinary field. Despite the name, most tissue engineering does not involve traditional engineering. Tissue engineering is about restoring tissue damaged by trauma or disease to its original condition. That in turn requires knowledge of the cellular structure of tissue and, in the case of musculoskeletal tissues like cartilage and bone, the extracellular matrix. Nanotechnology can be used in various ways for biomedical applications. The use of nanoparticles for site-specific, targeted drug delivery is widely reported by researchers. The major advantage is that you can target the drug to a specific site. Furthermore, you can control the release with the help of internal or external stimuli. The other highly explored area is to develop scaffolds for tissue engineering. Bio ceramics are basically inorganic materials with a mix of ionic and covalent bonding. These materials are an important source of biomaterial in biomedical engineering applications. The materials present the ideal properties for bone replacement and tissue engineering and are leading to a new era of regeneration materials. In this review, we discuss the various nanoparticles and bio ceramics that have been employed to upbring the field of hard tissue engineering to this current level.

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Keywords

Nano particles · Bio-ceramics · Nanotubes · Quantum dots · Bone tissue engineering

2.1 Introduction

Bone being a composite living connective tissue plays an imperative part in contributing primary structure, mechanical assistance, and pliability to the body (Sekaran and Ambigapathi 2013). Furthermore, bone is too engaged with mineral stockpiling and homeostasis particularly calcium and blood pH guideline and has other optional capacities (Copp and Shim 1963). Representing half of the ongoing persistent disorders in individuals more than 50 years of age, bone and its associated illnesses actually endure a significant clinical dispute (Brinker and O'Connor 2004). A convincing method to manage hard tissue design focuses to reestablish the capacity of infirm or injured bone tissue by incorporating isolated functional cells and biodegradable scaffolds arranged from engineering bioceramics (Oh et al. 2006). Cells, biomaterials, and growth factors are the fundamental principles of tissue engineering that are necessary to regenerate functional tissue (Langer and Vacanti 1993). Standard methodologies like interruption osteogenesis, bone vehicle, or distinctive bone grafting techniques like autografts, allografts, bone graft substitutes, or utilizing growth factors are helpful in the restoration or substitutions of damaged bone tissue (Smrke et al. 2013). This chapter is a work to sum up the various sorts of accessible synthetic bioceramics as well as nanoparticles utilized for bone recovery, either alone or in the blend.

2.2 Nanoparticles Used in Hard Tissue Engineering

Given the size-determined properties, nanoparticles (NPs) are at the cutting edge of nanotechnology having shown guarantee in conquering a significant number of the obstructions overlooked by tissue engineering (Hasan et al. 2018). GNPs and titanium oxide (Tio^2) are the most frequently used nanoparticles to improve cell proliferation rate in bone. Having shown prevalent biocompatibility and the capacity for surface modification, GNPs have bought fascinating biomedical applications (Boisselier and Astruc 2009). TiO2 nanoparticles have likewise been 3D printed with polymers, for example, poly (lactic-co-glycolic corrosive) (PLGA), to precisely match the nanostructured harshness of bone itself and altogether improve bone cell execution (Liu et al. 2006).

2.2.1 Organic Nanoparticles

With novel properties provided by their size, organic nanoparticles are placed at the front line of arising innovations. They are profoundly helpful in bioanalysis and essential science as model frameworks to contemplate single-molecule conduct, molecular acknowledgment, signal transduction, and protein-intervened film combination measures (Feracci et al. 2012). Specifically, organic nanoparticles with remarkable highlights including adaptable synthesis, easy processability, amazing biocompatibility, and low cytotoxicity have shown promising biomedical applications, like disease diagnosis, drug conveyance, bioimaging, and cancer treatment. Thus, impressive endeavors have been made to foster various methodologies for developing organic nanoparticles with particular morphologies and properties (Fang et al. 2020).

2.2.1.1 Liposomes

As described by Alec, 1995, liposomes are "simply the vesicles which form spontaneously when isolated natural cell membrane phospholipids are shaken in water" (Bangham 1995). Exploiting this trademark, the author utilized the liposomes as a model for the cell layer, be that as it may, these days these particles are likewise utilized in a wide scope of utilizations, including drug conveyance and imaging (Bozzuto and Molinari 2015). Regularly, liposomes are utilized as vehicles in conveyance as well as focusing on frameworks. With that in mind, it is important to fuse the ideal particles inside the liposome structure. In the event that the particle is hydrophobic, it is blended in with a natural dissolvable, and it will be coordinated inside the hydrophobic part. Be that as it may, when the load is hydrophilic, it should be added as a liquid composition, being held in liposome internal part (Gulati et al. 2021).

The benefits of liposomal-based medications ought to be more noteworthy dissolvability of the payload, expanded half-life, specific conveyance to the site of activity, and the capacity to triumph over resistance toward chemotherapeutics (Felice et al. 2014).

2.2.1.2 Polymeric Nanoparticles

Polymeric nanoparticles are circular with a width of roughly 100 nm; with their mass physical properties, tunable engineering, and biodegradability, polymers have been drawing much consideration for a long time (Nicolas et al. 2013). So far, polymers are being utilized to deliver polymeric micelles, nanofibers, and round NPs, either nanocapsules or nanospheres (Rao and Geckeler 2011). Another benefit of polymeric NPs is identified as their exceptional high medication stacking capacity. Consequently, these transporters are presently being utilized for the vehicle and conveyance of molecules in a few fields, like vaccination, cancer, aggravation, neurologic pathologies, and tissue healing (Nicolas et al. 2013).

2.2.2 Inorganic Nanoparticles

With silica NPs, metallic NPs (such as magnetic, gold, or silver NPs), bio-ceramic NPs, carbon nanotubes, and quantum dots as the most promising nanoparticles, inorganic NPs are helpful for biomedical applications (Pandey and Dahiya 2016). These inorganic nanoparticles are blended such that they are joined with ligands, antibodies, and other medication of interest which clears the way for likely applications in the field of biomedical designing and analytic imaging.

2.2.2.1 Silica NPs

Providing biomedical applications in drug delivery and imaging, silver is well known for biocompatibility, exceptional chemical stability, and characterized surface properties (Slowing et al. 2008). Imaging agents like fluorescent probes immersed at the core of a molecule are shielded by the silica shell to diminish the degree of photo-bleaching and empower the long-haul observing of the labeled material (Lee et al. 2012). Besides the cytotoxic studies stipulating that silica is non-toxic to the cells and appropriate for cell culture applications, it is also shown to enhance the wettability and coarseness of the fibers. Studies also indicate that the proliferation and attachment of osteoblast can be successfully improved by the nanoscaled surface structure created by the silica nanoparticles (Tang et al. 2013).

2.2.2.2 Metallic NPs

Displaying eccentric characteristics that are direct consequential of the quantum effect, metallic NPs are different from bulk material (Zhao et al. 2013). Among this type of nanoparticles, gold NPs have emerged as a possibility for enhancing deep tissue visualization as they can be adapted to absorb in the near infrared range during the bioimaging process (Vigderman et al. 2012). Likewise, the high nuclear number can actuate solid X-ray constriction bringing about an upgraded computed tomography contrast (Meir et al. 2014). Albeit gold NPs are considered more secure than other metallic NPs, it is as yet important to analyze their biocompatibility, biodistribution, bioelimination, and ecological effect before their mass production for a huge scope for in vivo applications (Murphy et al. 2008).

2.2.2.3 Bioactive Glass

Bioactive glasses are nebulous silicate-based materials that can shape a chemical bond with bone tissue (Hench et al. 1971). These NPs have been joined with characteristic and synthetic polymers shaping nanocomposite materials (Wang et al. 2014). Upon embedding the bioactive glass, the silicon part of the substance is delivered which helps in the development of calcium-deficient hydroxyapatite on the surface that connects with the collagen fibrils of the injured bone, permitting the bioactive glass to attach to the encompassing tissue (Hench and Paschall 1973). In order to deliver bioactive nanostructured polymeric composites with enhanced properties, bioactive glass NPs have been joined with various sorts of polymers, both engineered and normal (Leite et al. 2016).

2.2.2.4 Carbon Nanotubes

Carbon nanotubes (CNTs) have been reported as a likely essential tool for an assortment of utilizations in the biomedical field to assess their potential for drug delivery, theranostics, biosensing, microscopy, and support of composite materials (Zhang et al. 2010). CNTs are graphitic empty rounded designs, made by a sheet of carbon atoms with amazing electrical properties along with high mechanical and chemical firmness. Because of their tubular design, it is feasible to functionalize these fabrications both from inside and outside, with two typical atoms for two extraordinary purposes (Balasubramanian and Burghard 2005). As to applications, it is as yet important to perform further and normalized studies to comprehend their toxicity (Liu et al. 2013).

2.2.2.5 Quantum Dots

Quantum Dots (QDs) are colloidal semiconductor nanocrystals, going from 1 to 10 nm with a distinctive layout of core/shell construction of type II-VI, where the shell ensures and improves the optical properties of the core (Massey et al. 2014). Photostability and prolonged energized state lifetime, making them appropriate probes for following dynamic measures over the long haul and for long-term cell labeling are the extraordinary optical qualities of quantum dots (Li et al. 2012). In addition, these NPs are very helpful for multiplexed investigation and multicolor imaging, since it is feasible to concurrently excite and identify numerous colors of QDs utilizing a single light source (Deerinck 2008).

2.3 Bioceramics Used in Tissue Engineering

Bioceramics are termed as ceramics and used in the maintenance and transformation of injured or damaged parts of the musculoskeletal system of the body. They consist of alumina, silica, glass ceramic, titanium dioxide, zirconia, calcium phosphates, and bioactive glass (Hench 1991). In view of their positive interconnections with the human tissue, bioceramics are employed in various biomedical applications such as temporary bone space fillers after tumor excision, maxillofacial reconstruction, stabilization and augmentation of jaw bones, replacements for teeth, knees, hips, ligaments, and tendons, spinal fusion (Dearnaley and Arps 2005; Eslami et al. 2018).

Bioceramics are basically classified on origin basis, tissue response, and composition. A detailed description is given in below sections.

2.3.1 Classification Based on Origin

The origin of bioceramics can be natural and synthetic; those obtained naturally and from different living or deceased organisms are phrased as “Natural Bioceramics” that include biogenic silica, natural pearls, mollusk shells, bones, and teeth (Brundavanam et al. 2017). While the others that are manufactured industrially are phrased as “Synthetic Bioceramics” that comprise calcium phosphate-based

materials, hydroxyapatite (HA), zirconia, alumina, bioglass, etc. (Chevalier and Gremillard 2009).

2.3.2 Classification Based on Tissue Response

Bioceramics are relatively classified into bioinert ceramics and bioactive or surface reactive ceramics based on tissue response, which was first employed as an alternative to metallic materials to heave the biocompatibility of implants (De Aza et al. 2005; Utneja et al. 2015).

Bioceramics that don't incite any tissue responses on association with the physiological processes are named "Bioinert," e.g., Titania (TiO_2) (Benic et al. 2017), where the effect of zirconia-TiO_2 implants had exhibited new mineralized bone regeneration in the area of dental defect. On the other hand, bioceramics having the capability of inciting a specific tissue reaction upon contact with the physiological processes are named "Bioactive," (straightforwardly connects deep down by chemical bonding with the bone, forms a solid interface), e.g., Hydroxyapatite (HA), in light of their osteoconductive properties they go about as a scaffold to increase bone development on their surface (Merolli 2019).

2.3.3 Classification Based on Composition

2.3.3.1 Zirconium-Based Bioceramics

Zirconium is a profoundly dynamic metal and is impervious to corrosion by water, steam, mineral acids, alkalies, salts, and so on. Zirconium is exceptionally unmanageable and the metal is broken only by solid reagents and high temperatures (Nielsen et al. 2013). They exhibit mechanical properties like stainless steel with a good capacity to tolerate cyclic stresses (Piconi and Maccauro 1999). By enhancing the material strength and biocompatibility, zirconia has a high possibility to be utilized in various clinical applications (Boutin 2014).

2.3.3.2 Alumina-Based Bioceramics

A fine white powder which is the oxide of aluminum that contains traces of oxides of magnesium, silicon, sodium, calcium, and iron is termed "Alumina." Alumina is a fragile solid that breaks on getting a hard impact and displays hardness marginally not as much as diamond, making it difficult to cut or manage with some other metal aside from diamond. It displays poor flexural strength, notwithstanding, great compressive strength furthermore, superb dimensional steadiness against heat, pressure, and shock (Boutin 2014).

2.3.3.3 Carbon-Based Bioceramics

Besides the physical features closer to the bone, some other types of carbons like graphene, carbon nanotubes, and diamond-like carbon show good biocompatibility, thromboresistance, and good chemical inertia (De Aza et al. 2005). One of the

exceptional biological properties of carbon-based nanomaterials is to create multi-functional TE frameworks that persuade tissue recovery, while, for instance, at the same time forestalling contamination or taking into consideration the capacity to screen the tissue growth (Cha et al. 2013).

2.4 Properties of Nanoparticles and Bioceramic Materials

Uses of bioceramics in the clinical field include a trade for knees, hips, tendons, ligaments, as well as maxillofacial construction (Bunpetch et al. 2019). Bone space fillers after tumor extraction and spinal fusion are the new turns of events in ceramics utilization in bone tissue engineering (Ma et al. 2018). Sadly, a large portion of the implants like stainless steel, cobalt-chromium combination, or titanium amalgam lack bioactivity. These implant surface covering materials ought to be biocompatible, osteoconductive, and osteoinductive and should display adequate mechanical stability under physiological stacking without withdrawing from the implant surface and antimicrobial properties to limit prosthetic contamination threat (Tobin 2017; Zhang et al. 2014).

Bioceramic coatings are useful in articulating prostheses as they eliminate fragile breaks because of their intense metallic substrates, lower the creation of wear fragments due to more noteworthy hardness and wear opposition of ceramic coat, and limit hypersensitive responses because of dissolvable metal ions like cobalt, chromium, and nickel by acting as a boundary between metallic embed and human bone (Bhirde et al. 2011).

Nanoparticles can be utilized as devices for bone TE through a wide scope of uses—from drug delivery systems to the development of biomimetic various leveled frameworks. In addition, with nanotechnology, the cell labeling methods have gotten more explicit and withstanding, permitting non-intrusive in vivo approaches for cell tracking and monitoring.

The integration of nanotechnology in TE applications has been giving a wide scope of new chance for researchers and another expectation for future. For bone TE, research has been done generally in three territories showing promising outcomes: scaffolds improvement, nanocarriers, and cell labeling for imaging (Pourhaghgouy et al. 2016).

As of late, Pourhaghgouy et al. supported chitosan frameworks with bioactive glass NPs (10, 30, and 50 wt.%). The subsequent nanocomposite platforms, delivered by the freeze-projecting method, had a decent interfacial holding between the NPs and the polymeric lattice, which brought about construction with improved mechanical properties as to the compressive strength (Tang et al. 2013).

2.5 Current Challenges and Future Perspective

Bone tissue engineering is considered a rapidly growing field and the products based on the BTE are clinically utilized and are expected to be available for patient use in upcoming years. Albeit the competition to make BTE a clinical reality is all around justified, huge difficulties and restrictions in this field actually exist. Some of the challenges that are currently faced in the field of BTE are selecting the most effective cell type, scaffold type, and growth factors. At present, by far most announced precisely solid BTE scaffolds experience bone tissue recovery that is restricted to the fringe of the scaffold upon implantation, because of the absence of adequate and opportune vascularization of the construct. By incorporating growth factors via the scaffold or genetically modified cells, researchers are endeavoring to handle both improvised vascularization and inhibition of fibrous tissue formation which delivers increased levels of angiogenic vascular endothelial growth factor (VEGF). Perhaps the biggest challenges confronting bone tissue designing might be growing mechanically solid permeable scaffolds that hold legitimate vascularization and host integration, as the bone tissue structure and mechanical strength fluctuate by particular and dynamic stacking conditions, just as their location in the body.

Despite other tissues, bone is dynamic, distinctly vascularized tissue with an eccentric ability to heal and redesign without leaving a scar (Tomlinson and Silva 2013). Even though an extraordinary advance in the comprehension of bone science has been accomplished up to this point, there are few things to be considered to better comprehend what is expected to foster a materialistic tissue-engineered bone.

1. To additionally see how the growth factors collaborate with one another and with cells, what is their impact, which intracellular pathways are set off by them, and how they can be enacted/inactivated.
2. Material science—another age of biodegradable biomaterials—is at present being planned to evoke explicit cell reactions at the sub-atomic level. Another age of scaffolds is additionally required, with suitable porosity, degradation rates, and mechanical properties.
3. Lastly, scaffold processing technologies, and new quick prototyping philosophies, have appeared to survive a portion of the constraints of the current techniques, and are very promising later on for tissue designing applications. In particular, those that take into account the advancement of platforms with improved mechanical properties without impacting the porosity and interconnectivity ought to be contemplated and created (Fig. 2.1).

2.6 Conclusion

Designing bone tissue, nonetheless, isn't just founded on standards of cellular and molecular developmental science furthermore, morphogenesis, is particularly guided by bioengineering and biomechanics. Even though numerous BTE systems have been explored, so far, a couple has been endorsed for clinical use. These are

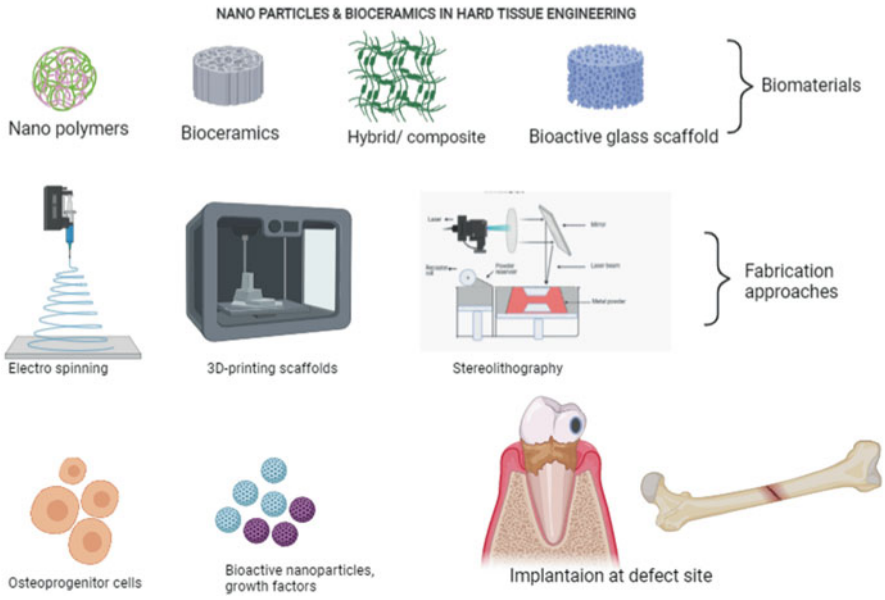

Fig. 2.1 Types and application of nanoparticles and bioceramics in hard tissue engineering

generally single-segment methodologies including cells, growth factors, or deformity filling materials. For BTE to become a far-reaching clinical reality, it should integrate the new advances that use all the important features (i.e., scaffolds, cells, furthermore, growth factors) for effective bone healing and regeneration. Besides, BTE may even posture as a well-being care trouble in its present structure, as it accompanies high assembling costs and shows restraint explicit. To expand productivity, patient-autonomous techniques should be considered. Likewise, more compelling cell isolation, culturing, and refined strategies should be created to smooth out the engineering process and diminish the safety hazards related to taking care of the builds during the pre-implantation time frame.

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Application of Nanoparticles in Soft Tissue Engineering

3

N. S. Raja and Manish Dash

Abstract

Tissue engineering is an interdisciplinary approach that integrates several fields of study such as biology, biochemistry, chemistry, nanotechnology, engineering, and material science. Three components are commonly found in tissue engineering: cells that are capable of tissue repair, a scaffold that supports the proliferation of the cells impregnated on them, and bioactive molecules that will synchronize the architecture of desired tissue. The challenging task of tissue engineering is a scarcity of suitable biomaterial for scaffold formation, below-par proliferation of the cells on the scaffolds, controlled delivery of bioactive components in accordance with the requirement of the cells and lack of techniques that enable the formation of the suitable 3D architecture of the tissue. Size-dependent unique physiochemical properties of the nanoparticles could potentially fill the gaps in tissue engineering research. Nanoparticles and the nanomaterials such as nanotubes, nanowires, and nanowhiskers have been successfully engineered to produce suitable scaffolds that provide a distinct environment for individual cell types to proliferate and differentiate into matured tissues. This chapter presents an overview of the application of nanomaterials and the challenges involved in the application of nanomaterials in tissue engineering.

Keywords

Soft tissue engineering · Nanofibers · Nanoparticles · Stem cells · Osteology

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3.1 Introduction

Tissue engineering and theranostics require constant innovations and technology development to provide solutions to increasingly complex biological problems. Advances in producing and controlling nanosized materials opened up new avenues in tissue engineering that heavily depend on mimicking biological architecture where most of the components are nanoscale dimensions (Mann 2008). The unique properties of nanoscale objects such as low bioreactivity, tuneable physiochemical properties, targeted delivery, and controlled release inside the biological tissues made them a favorite for tissue engineering applications. Tissue engineering requires accurate spatiotemporal controls and real-time monitoring. Various complex scaffolds tailored to suit the requirements of cell growth and tissue formation in two and three-dimension have been created using nanofiber production techniques such as electrospinning and electrospraying. Also, several composite materials are created using different combinations of natural and synthetic fibers to enhance the desirable properties. These scaffolds are often embedded with inorganic nanoparticles to fine-tune the properties. The addition of these nanoparticles enhances the biocompatibility and cell proliferation in the nanocomposite scaffolds. This chapter will discuss the different materials employed to produce nano-scaffolds like natural and synthetic polymers, and inorganic nanoparticles, and highlight their applications.

3.2 Nanofibers for Soft Tissue Engineering

The nanofiber materials are an excellent option for soft tissue fabrication, as the cellular microenvironment contains many materials that mimic nanofibers. The ultimate function of nanofibers is to imitate the mechanical and biochemical properties of real tissue. It aids soft tissue regeneration by providing a suitable environment. We classify nanofibers into two types: nature-derived polymers and synthetic polymers. In the cellular microenvironment of soft tissue, the extracellular matrix (ECM) often contains collagen fibers, peptidoglycans, and other polysaccharide polymers. Researchers used these components to develop nanofiber materials. Nature-derived nanofibers often contain biochemical cues such as extracellular matrix proteins, bioactive peptides, or cytokines. These components modulate physiological processes like cell reactions, biocompatibility, and integration with the niche.

Similarly, the mechanical characteristics of the real tissue dictate the behavior of the cell. A mismatch between the tissue and biomaterial leads to mechanical failure. Weak biomaterial induces inflammation and tissue damage, whereas stiff material prevents interfacial connections of the implant with the native tissue. Synthetic nanofibers offer the flexibility to develop highly controlled, reproducible compositions. Often they are chemically modified to produce the desired effect. The lack of intrinsic bioactivity and reduction of biocompatibility are the biggest challenges in the development. Natural biomaterials have a high degree of

biocompatibility and are physiologically similar to native tissue. But, they have weaker mechanical properties and are quick to degrade when compared with synthetic materials. The compositions of natural biomaterials also vary according to the source (Xia et al. 2018a).

3.2.1 Nature-Derived Nanofibers

Polymeric materials, both natural and synthetic in various combinations, were used to develop nanofibers. Zhong et al. prepared nano-dimension fibers made of natural ECM that resemble native ECM with a high surface area (Zhong et al. 2005). Cao et al. developed scaffolds containing nanofibers through freeze-drying collagen/chitosan/chondroitin sulfate incorporated with bFGF-loaded PLGA microspheres primarily for the controlled release of drugs. The electrospinning technique was used to prepare meshes of collagen and/or elastin from aqueous solutions. An increase in the elastin content increases the fiber's size from 220 to 600 nm (Cao et al. 2015). Kaplan et al. proposed a two-step self-assembly process of Silk–Elastin-like Protein Polymers (SELPs) in an aqueous solution. The Silk-to-elastin ratio in a SELP does determine the outcome. Different structures formed by varying the ratio are nanoparticles, hydrogels, and nanofibers. This could be achieved by precise tuning of the ratio of silk to elastin (Xia et al. 2011). Silk fibroin (SF) nanofiber nonwovens were synthesized by electrospinning method and fabricated for cell culture of human keratinocytes and fibroblasts. Silk fibroin nanofibers exhibited a circular cross-section with a smooth surface and had an average diameter of 80 nm (Min et al. 2004).

Amyloid fibrils are pathogenic, but they are also involved in hormone storage and biosynthesis. Amyloid fibrils are highly concentrated in semen and their exact function is not clear. These small peptides derived from the semen self-assemble into nanofibers. These nanofibers are used for gene transfer techniques and do not have any side effects. These nanofibrils could be customized into self-assembling peptides (Meier et al. 2014). Wang et al. have constructed oriented chitosan fibers from nonwoven nanofiber mesh tubes by electrospinning (Wang et al. 2009). Schwann cells are appropriately aligned in the mesh, and these tubes positively affect peripheral nerve regeneration. The physical blending of polyurethane (PU) with cellulose acetate (CA) and zein produced source material to develop nanofibrous scaffolds with diameters around 400–700 nm. These hydrophobic electrospun nanofibers were antibacterial and increased wound healing (Unnithan et al. 2014). Cellulose is the most abundant polymer on this globe. Plants and many microorganisms produce it. Bacterial cellulose (BC) has several physicochemical properties suitable for tissue engineering, and it is biocompatible. BC scaffold is prepared from the Acetobacter xylinum X-2 (A. xylinum X-2) cellulose and coated with bone morphogenetic protein-2 (BMP-2). Mouse fibroblast-like C2C12 cells are differentiated into osteoblasts when this scaffold is used for cell culture (Shi et al. 2012). Hyaluronic acid (HA), one of the abundant ECM components, was also tested for its ability to produce nanofibers. In a study, HA nanofibers were synthesized

using electrospinning with an average diameter of 33 ± 5 nm, 59 ± 12 nm, 79 ± 12 nm, and 113 ± 19 nm (Yao et al. 2013). Another group observed that polyvinyl alcohol/hyaluronan (PVA/HA) polysaccharide nanofiber enhanced cell differentiation in human-induced pluripotent stem cells (Deng et al. 2014). Demineralized bone matrix (DBM) is a naturally derived polymer devoid of any inorganic material. It contains adhesion ligands and osteoinductive signals. Leszczak et al. surveyed several solvents and solvent blends for electrospinning DBM into nanofibers. The solvent used for electrospinning and the cross-linking did not induce cytotoxicity (Leszczak et al. 2014).

3.2.2 Synthetic Nanofibers

Synthetic polymers are chemically defined and produced in a controlled environment. They have wide applications and compatibility for different types of tissues and cells. Three different polymers, polyethylene terephthalate, polytetrafluoroethylene, and polyurethane, were tested for their stability towards the fabrication of vascular grafts. Polyethylene terephthalate (PET), PET/chitosan, and PET/honey at different concentrations were electrospun to produce fibrous mats. Incorporating honey improved the processivity of the PET fibers (Arslan et al. 2014). At the interface between the tissue and material, adverse host reactions to implanted devices are induced. The nanofibrous PTFE (nPTFE) of 20–30 nm width, and 3–4 mm length coated on the glass slide, reduces the adverse reaction. This scaffold absorbs a high amount of BSA on its surface and this in turn increases the surface area and hydrophobicity (Ainslie et al. 2007). Nanofibrous polyurethane (PU) vascular grafts have mechanical properties and biocompatibility that suit the requirements of soft tissue engineering. In a study, 48 adult male beagle dogs were implanted with polyurethane (PU) or polytetrafluoroethylene (PTFE) grafts. PU group outperformed the PTFE group in the parameters such as endothelial cells proliferation and smooth muscle cells architecture. The patency rate was also higher in the PU group (Hu et al. 2012). Biodegradable polymers also were tested for their application in soft tissue engineering. Calcium silicate hydrate (CSH) nanowire/poly (l-lactide) (PLLA) nanocomposites with tuneable microstructures were developed as potential bone graft substitutes. The addition of CSH nanowires increases the hydrophilic nature of the PLLA films. It also increased apatite formation. Bone marrow stromal cells exhibited increased attachment and proliferation in the composites (Dou et al. 2012).

Hydroxyapatite (HAp) is biocompatible with soft tissues. An alginate-di-aldehyde (ADA) cross-linked gelatin (GEL)/nano-hydroxyapatite (nHAp) bio-scaffold was synthesized by lyophilization for tissue engineering application. The calcium-to-phosphorus ratio of HAp is 1.51, suitable for biomedical applications (Mehedi et al. 2018). Curcumin-loaded poly(lactic-co-glycolic) acids (PLGA) nanoparticles were synthesized and tested for curcumin release studies. The release profile follows the Korsmeyer-Peppas model. The researchers suggested that the drug release mechanism is a mix of surface drug dissolution and non-Fickian diffusion (Sampath

et al. 2014). Nelson and his group developed a platform for gene delivery in a multidisciplinary approach, combining gene delivery devices and an electrospun nanofiber mesh (NFM). The gene delivery vehicles used here are liposomes. Polycaprolactone is used for NFM production. Runt-related transcription factor 2 (RUNX2) is the protein that aids osteoblast induction. Expression of RUNX2 induces bone tissue regeneration. NFM impregnated with liposomes containing RUNX2 plasmid induced long-term gene expression in cultured osteoblasts. Ethicon in 1981 commercialized polydioxanone (PDX) as a biodegradable monofilament. Electrospun PDX mats have similar mechanical properties to that of ECM (Monteiro et al. 2014). Goonoo et al. reviewed the synthesis of PDX and its copolymers and its applicability in nanofiber synthesis (Goonoo et al. 2015). PDX matrix could be incorporated with different drugs and bioactive compounds to suit the requirements of soft tissue engineering. Polyvinyl alcohol-based nanofibers were also used for soft tissue engineering applications (Manjumeena et al. 2015; Song et al. 2012).

Tissue engineering involves combining synthetic or nature-derived materials. Different cell types and growth factors are embedded in a functional scaffold to substitute organs that are not functional. Nanoparticle-containing complex scaffolds are preferred in tissue engineering as they enhance cell attachment to the substrate and proliferation. Nanotechnology advances in the last two decades enabled the fabrication of inorganic nanoparticles, nanopatterned surfaces, and nanodevices with potential applications in the biomedical field. Inorganic nanoparticles are used in biomolecular delivery, bio-imaging, and theranostic applications relevant to tissue regeneration applications (Huang et al. 2013). Inorganic nanoparticles possess tunable physicochemical properties and are easily modified. It makes them attractive for soft tissue engineering applications. On the other hand, several studies on animals reported nanoparticle accumulation in the lung and other tissues, breach of the blood-brain barrier, and inflammation induction. Inorganic nanoparticles are also less soluble in aqueous solutions and have proven to be selectively targeted into the specific organelle. Any application of these particles requires clinical testing and long-term studies (Koutsopoulos 2012).

3.3 Inorganic Nanoparticles

Inorganic nanoparticles are prepared from metals and metal oxides. Gold, silver, zinc, aluminum, magnesium, iron, and titanium are popular metals for nanoparticle applications. Inorganic metal nanoparticles are incorporated into a matrix/scaffold to add additional functionalities such as antibacterial activity and controlled release of bioactive materials.

3.3.1 Silver Nanoparticles

Silver nanoparticles were known to aid bone and wound repair, enhance the potency of the vaccines (Asgary et al. 2014), and reduce diabetics (Saratale et al. 2017).

Antibacterial properties of silver nanoparticles are known for an extended period of history (Alexander 2009).

They are much more effective than other antimicrobial agents due to their multimodal action that prevents the development of resistance (Panáček et al. 2018). Silver nanoparticles also exhibit anticancer, antifungal, and anti-nematode activities, making them desirable candidates for soft tissue engineering. Chitosan-coated silver nanoparticles-agarose composite is synthesized using glutaraldehyde as a cross-linking agent. This composite has antibacterial activity and superior hemocompatibility (Kumar et al. 2018). Nanocomposite scaffolds containing chitosan, carboxymethyl cellulose, and silver nanoparticles at varying concentrations were fabricated using freeze-drying. This composite provided mechanical strength as well as antimicrobial activity. The addition of nanoparticle composite reduced the swelling capacity of scaffolds, and the scaffold degradation rate could be tuned to support angiogenesis and vascularization. These scaffolds also supported the adhesion of MG63 cells and their proliferation and improved biomineralization for bone growth (Hasan et al. 2018).

Silver nanoparticles loaded into chemically cross-linked cellulose-poly vinyl alcohol blend of the nanofibrous mat imparted antibacterial activity to it (Wadke et al. 2017). In a recent study, a novel polymeric nanocomposite (ARX-GO-nHAp/nAl2O3-AAc) was developed through free radical polymerization. The porous scaffolds were fabricated from the polymeric nanocomposite (ARX-GO-g-nHAp/n-Al2O3-AAc) via freeze-drying. A mixture of arabinoxylan-co-acrylic acid, nano-hydroxyapatite (nHAp), nano-aluminum oxide (nAl2O3), and graphene oxide (GO) yielded a porous scaffold through free-radical polymerization and freeze-drying technique. This scaffold was coated with silver (Ag) nanoparticles. Together, nHAp, nAl_2O_3, and GO control the physiological activities (Umar et al. 2021).

Neural and cardiac regeneration require electroactive biomaterials. An electroactive nanocomposite was synthesized from collagen fibrils and silver nanowires (AgNW). These materials are mainly composed of Type I collagen fibrils and have a charge storage capacity and possess mechanical properties similar to soft tissues (Wickham et al. 2016).

3.3.2 Gold Nanoparticles

Gold nanoparticles are used as a versatile tool for tissue engineering. Gold nanoparticles could be tuned to the requirement and possess different optical properties depending on their size. These particles could be easily functionalized. Gold nanoparticles are integrated into the scaffolding materials for varying tissue engineering applications. Tissue engineering scaffolds with improved properties could be formed by embedding gold nanoparticles into base materials. The choice of the base material is very important as it influences adhesion, proliferation, differentiation, and macroscale tissue properties.

The incorporation of gold nanoparticles increase the stiffness of the scaffold, as demonstrated by several studies (Deeken et al. 2011; Shevach et al. 2014;

Abdelrasoul et al. 2015). The increase in stiffness is because of the improved structural integrity as the electrostatic interactions between the AuNPs and the scaffold increases the stiffness (Yadid et al. 2019).

Electrically conductive scaffolds are used to create an interface that mimics the stimulation of the electrical environment of the tissues. Synthetic materials incorporated with AuNPs allow smooth modifications of the surface and programmable conductivity. Cardiac, muscular, and neuronal tissue engineering applications demand scaffolds of this kind (Umar et al. 2021; Ghasemi-Mobarakeh et al. 2011; Fabbro et al. 2011). An increase in the gold nanowires increased the conductivity of the scaffold and decreased the impedance. When used for cardiac patch engineering, these scaffolds improved cell organization and calcium transient potential propagation. A gap junction protein Cx43 also showed an increased level of expression (Shevach et al. 2014). Polycaprolactone-gelatine fiber scaffolds incorporated with 10 nm gold nanoparticles AuNPs provided positive results in their ability to induce differentiation in immature PC12 cells into neuron-like cells. These cells were forming neuronal networks also. Nevertheless, when raised on scaffolds without gold nanoparticles, researchers could not observe long neuritis, and the cell formed only limited neuronal networks (Qian et al. 2018).

Gold nanoparticle integrated materials were also used for tissue welding and cell adhesion. Matteani et al. created a nanocomposite composed of gold nanoparticles and chitosan, which absorb 810 nm laser and acted as an adhesive to rabbit tendon and porcine carotid arteries (Ratto et al. 2011). Polypeptides similar to elastin were cross-linked with gold nanoparticles and used for welding ruptured intestinal tissue under near-infrared radiation (Huang et al. 2013). Gold nanoparticles are also capable of influencing several cellular functions in stem cells. They modulate stem cell differentiation and proliferation. We still need to understand the pathways for the cellular behaviors exhibited by the stem cells before these techniques are translated into clinical applications. Studies have to be made on the toxic effects of gold nanoparticles, significantly how the gold nanoparticles affect the cell cycle. Teratogenic observed in specific stem cells also has to be addressed.(Yadid et al. 2019).

3.3.3 Iron Nanoparticles

Toxicity is the major limitation in the application of nanomaterials in soft tissue engineering. Metallic materials with biodegradable characteristics like iron (Fe) and their alloys can overcome this limitation. In general, the toxicity of iron nanoparticles increases with the interaction of cells with nanobeads, nanoworms, and nanospheres. The potential adverse effects of metal and metal oxide nanoparticles were reduced or removed by appropriate surface coatings (Yu et al. 2012). A 3D scaffold composed of poly(ε-caprolactone) matrix reinforced with iron-doped hydroxyapatite nanoparticles was synthesized and characterized using the Brazilian test. When bone marrow stem cells were grown on these scaffolds, a 33% increase in cell growth was observed. It is 2.2 folds higher than the cells grown without a magnetic field on the same scaffold (De Santis et al. 2015).

Magnetic nanoparticles were obtained from iron oxides (Fe_3O_4 or Fe_2O_3) and used in the biomedical applications field. They have low toxicity when compared with other transition metals. The particles were prepared by co-precipitation of Fe_3O_4 or γ-Fe_2O_3 (Gupta and Gupta 2005). Ferric and ferrous ions dissolved in basic solutions are mixed at 1:2 molar ratios at varying temperatures. These particles were used for cancer cell imaging and in vivo monitoring of stem cells and transplanted tissues. In the same way, these particles could also be used for the development of engineered tissues. Nanoparticles of less than 100 nm with narrow size distribution are the choice for biomedical applications. Generally, magnetic nanoparticles lose their magnetic property after the removal of the magnetic field, but the 10–50 nm size nanoparticles retain magnetism, and when surface coated with appropriate material, they possess long circulation times and could be controlled by an external magnetic field (Medeiros et al. 2011).

Neuron growth and differentiation could be improved by superparamagnetic iron oxide-Au core-shell nanoparticles decorated with nerve growth factor (NGF). PC-12 proliferated at a higher rate and was appropriately oriented in the presence of these nanoparticles when grown under dynamic rotating magnetic fields compared to static magnetic fields (Zhang et al. 2019). Dynamic and remote control of collagen fiber orientation was achieved in situ using magnetic nanoparticles (Antman-Passig and Shefi 2016). Neurons cultured in the magnetic 3D gels neurons differentiated into elongated morphology. They also displayed cellular viability and electrical activity. Regeneration of brain tissue was achieved when peptides and different growth factors conjugated iron oxides as iron oxides pass the blood-brain barrier (Pilakka-Kanthikeel et al. 2013).

3.3.4 Aluminum Nanoparticles

Anodic aluminum oxide is a material that could be easily engineered into nanoporous structures. Nanoporous anodic alumina coated with biocompatible materials is widely used in cell culture, functionalization of biomolecules, drug delivery, and biosensing. In vivo and in vitro studies to improve the biocompatibility of anodic aluminum oxide are still lacking (Davoodi et al. 2020). Anodic aluminum oxide could be formed into a scaffold directly or it could be used for preparing a composite scaffold with other polymeric substances (Ashammakhi et al. 2012). The oxidative anodization technique was used to generate two kinds of alumina surfaces. Their pore size also varies in diameters in the mean range of 16–30 nm and 65–89 nm (Mussano et al. 2018). Advanced techniques for surface modification of anodic aluminum oxide add new properties to the structures. The surface functionalization for anodic aluminum oxide involves wet chemical and gas-phase techniques (Md Jani et al. 2013). Two different types of alumina layers commonly found in the literature are the barrier anodic layer and the porous alumina layer.

Electrolyte pH determines the type of alumina formed during anodization. Both potentiostatic and galvanostatic conditions were used for the anodic porous alumina formation (Sulka 2008). Poly(3-caprolactone) (PCL) nanowires of anodic aluminum

oxide templates were synthesized by Bechara et al. for the culture of neuronal cells. Polylactide nanorods fabricated with alumina allowed the proliferation of fibroblasts similar to the natural cells (Grimm et al. 2010).

The crystal orientation of poly(ethylene oxide) nanotubes tuned to desired orientation was achieved by Liu et al. using aluminum oxide as a template. Several studies reported the use of nanoporous alumina as a mould for mammalian cell culture (Ashammakhi et al. 2012; Sarkar et al. 2007; Wang et al. 2010). Increased adhesion and proliferation of human aortic endothelial cells on anodic alumina functionalized with fibronectin was observed. But the functionalization has a minimal effect on the morphology of the cells (Formentín et al. 2018) when compared with the pore size of alumina. Biocompatibility of alumina is still a debate and it limits clinical trials. More in vitro and in vivo studies have to be conducted on the biocompatibility of aluminum oxides for tissue engineering applications (Davoodi et al. 2020).

3.3.5 Zinc Nanomaterial

Zinc oxide has multifunctional properties, and these compounds could be easily fabricated into different morphologies, like nanowires, nanorods, and nanoparticles (Laurenti and Cauda 2017). Zinc oxide nanomaterials with an average size of 30 nm promote osteoblast growth at concentrations of 30 and 60 μg mL^{-1}. These particles also promote the differentiation of mesenchymal stem cells into osteoblasts (Tahereh 2014). Ramesh and coworkers tested the in vitro biocompatibility of zinc oxide nanomaterials (Gopikrishnan et al. 2011). The biocompatible properties of zinc oxide nanomaterials were tested in rat lung epithelial cells. Cell viability was independent of zinc oxide nanomaterials concentration and time of exposure (Jones et al. 2008). Zinc oxide nanomaterials were capable of electrical stimulation of macrophages and osteoblast tumor cells. When these cells were cultured on ZnO nanosheet arrays the cells exert pressure on the underlying piezoelectric ZnO nanosheet array and an electric field is induced. This electric field influences the biology of the cells (Taccola et al. 2011).

Mammalian cell lines PC12 line and H9c2 line are electrically excitable. Ciofani et al. cultured these cells in a zinc oxide nanowire array as a model for muscle cells. Both cells exhibited high cell viability (Ciofani et al. 2012). A biomaterial should prevent the adhesion of macrophages and reduce the viability of the macrophage. Glass substrates coated with zinc oxide nanorods could achieve while used for the culture of fibroblasts and endothelial cells. The lamellipodia were absent, and the area of spreading of the cell was also reduced in the macrophages (Lee et al. 2008).

3.3.6 Magnesium Nanoparticles

Magnesium oxide nanoparticles are non-toxic and biocompatible, have a higher surface-area-to-volume ratio, and are insulated to heat and electricity. These nanoparticles have high absorptivity, antimicrobial activity, nontoxicity, and

biocompatibility (De et al. 2017; Song et al. 2016). Alginate nanofibrous scaffolds with MgO nanoparticles were synthesized by the electrospinning method. Spherical MgO nanoparticles were synthesized by the ex situ method. The MgO reinforced alginate nanofibrous scaffolds prepared demonstrated its potential as a substitute for extracellular matrices. These scaffolds hold excellent scope for biomedical applications (De et al. 2017). Two different magnesium oxide (MgO) nanoparticles-based composites were prepared using poly(l-lactic acid) (PLLA) and hydroxyapatite (HA) nanoparticle-PLLA.

The addition of MgO nanoparticles enhanced osteoblast adhesion and proliferation. The toxicity of MgO is reduced when osteoblasts were cultured in the degrading magnesium nanocomposites (Hickey et al. 2015). Film casting and polyvinyl alcohol leaching were the two common techniques used to prepare magnesium oxide (MO) nanoparticle-reinforced sodium alginate scaffolds. Sodium alginate nanocomposites embedded with magnesium oxide nanoparticles have desirable properties for tissue engineering. An increase in the nanoparticle concentration increased the antibacterial properties and reduced the degradation rates (Nasri-Nasrabadi et al. 2018). In another study, the in vivo implantation of magnesium-containing electrospun nanofibrous membrane enhanced bone regeneration in rat calvarial defect 12 weeks post-surgery (Xing et al. 2020).

3.3.7 Titanium

The effective concentration and the size of TiO_2 nanoparticles encourage cell migration to cell culture and animal studies (Hou et al. 2013; Abou and Knowles 2008). Tissue engineering scaffolds based on poly(vinylidene fluoride-trifluoroethylene) loaded with TiO_2 nanowires (TNW) were prepared using the wet-chemical method. These nanowires increased the adhesion and proliferation of osteoblast-like cells on the nanocomposite scaffolds. The developed poly(vinylidene fluoride-trifluoroethylene) scaffolds containing titanium oxide wires scaffolds possess superior qualities for tissue engineering (Augustine et al. 2019). Titanium isopropoxide (TiO_2) and hydroxyapatite nanoparticles were incorporated into the silk fibroin scaffolds using sol-gel synthesis. The salt-leaching process is used to fabricate aporous structure scaffold. Experiments revealed that the scaffold had improved osteoinductive property and proved that the osteogenic and mechanical property of the scaffold is greater than the naked silk fibroin scaffold (Kim et al. 2014). In vitro viability tests conducted on polycaprolactone (PCL)-based nanofibers functionalized with bare (ligand-free) titanium nitride (TiN) nanoparticles using 3T3 fibroblast cells provide positive effects, thus confirming the biocompatibility (Nirwan et al. 2021). Gerhardt et al. (Gerhardt et al. 2007) fabricated films composed of poly(D, L lactic acid) filled with 0, 5, and 30-weight percentage TiO_2 nanoparticles. Increasing the titanium oxide concentrations up to 100 μg/mL had no significant effect on the cell viability of MG-63 cells. Human fibroblast cells incubated for 7 days in the scaffold prepared by salt leaching of silk showed increased attachment and proliferation (Kim et al. 2014).

3.4 Nanomaterial Applications in Specific Areas of Tissue Engineering

3.4.1 Application of Nanomaterials for Soft Tissue Engineering in Dentistry

With the emerging advancement in nanotechnology and nano-biomaterials, their usage is implemented in the field of dentistry as well. There are several applications of nanomaterials in dental science ranging from periodontics, operation and restoration surgeries, prosthodontics to various personalized dental treatments when specifically focusing on soft tissue engineering. The same nanomaterials in dental applications include dental nanocomposites, modified resin nano-glass Ionomer cements, GIC formulations, nanorobots, nano-implants, nano-bioactive materials and nanoceramics which are broadly categorized either under organic, inorganic or carbon-based materials (Yushau et al. 2020).

These materials have antiviral, antifungal and antimicrobial properties and hold potential in dental therapeutics because of their resistance against biofilm formation, preventing micro-leakage and caries. Are they biocompatible? Studies suggest that they are biocompatible and have better bonding capacity with dentin and help better repair and recovery of damaged tissue (Yin et al. 2020; Raura et al. 2020; Song and Ge 2019).

Soft tissue engineering strategies for any field of specialization involve the injection of nanomaterial loaded vehicle or stem cell at the target site, induction and scaffolding.

Due to the sensitive environment of the buccal cavity, designing nanoparticles becomes crucial and targeting them to the affected site with safety and without infection is the utmost priority. Nanomaterials been designed to have already been shown to be very effective in preventing biofilm formation with the help of nanoapatites and demineralization of the enamel lesion. Targeted preventive measures for carious dentin disinfection use silver nanoparticle formulations that are shown to be effective against various infectious bacteria and their biofilm formation from many oral pathogens mainly *Enterococcus faecalis* and *Streptococcus* spp. (Allaker 2012; Allaker and Memarzadeh 2014). Nanomaterials like sodium trimetaphosphate (TMP) when added to fluorides in 1100 ppm F could enhance eradication of dentinal tubules and reduce dentin hypersensitivity capacity (Favretto et al. 2018; Danelon et al. 2015; Favretto et al. 2021). While adding *Ficus benghalensis* prop root extract to this mixture along with silver oxide nanoparticles the toothpaste becomes germicidal giving better protection to the oral cavity (Manikandan et al. 2017). Did you know that even toothpastes are manufactured with such recipes for preventive measures and that a variety of toothbrush available commercially releases a certain minuscule amount of silver NPs (Mackevica et al. 2017)?

Apart from the silver NPs and formulations, researchers have worked on other nanomaterials as well. Dental sealants that prevent tooth decay are of importance in soft tissue engineering applications. They are implied in surgeries or dental care

alongside tissue repair and regeneration procedures. These sealants are designed such that they have an antibacterial resin containing calcium phosphate amorphous nanoparticles (Ibrahim et al. 2018), varnish with fluoride in chitosan NPs (Wassel and Khattab 2017; Pichaiaukrit et al. 2019), propolis or miswak, zirconium dioxide or carboxymethyl chitosan conjugated with alendronate mixed with amorphous $Ca_3(PO_4)_2$ (Zhu et al. 2021). Ferumoxytol also have been shown to elucidate strong antibacterial effects and preventive measures against dental caries (Armijo et al. 2020).

This also brings us to NPs used for dental delivery. Successful tissue repair and regeneration therapies focus on precise and fast therapeutic delivery of the medicine to the target site. Active components like fluorides are loaded into biopolymers like chitosan, pectin or alginate for effective topical delivery (Nguyen et al. 2017). These methods also protect the acidic environment from sudden exposure to fluorides leading to chemical carcinogenesis. Carboxymethyl starch (CMS)–Chitosan (CS)–Montmorillonite (MMT) was engineered to deliver curcumin to caries or biofilm-formed site (Jahanizadeh et al. 2017). Encapsulation and delivery of chlorhexidine (CHX) via mesoporous silica NPs were also used to modify GIC which is nothing but dental glass ionomer cement that has a high potential to bind the soft tissue during soft tissue regeneration procedure (Yan et al. 2017; Akram et al. 2021). This formulation effectively prevented the formation of biofilms without any mechanical impact on the functioning of GIC. Similarly, hybrid NPs were also synthesized for the effective delivery of active agents to the affected site. Copper NPs with chitosan shells or nanocomposites with silica NPs containing spherical or anisotropic fillers allow lower agglomeration rate and better homogenous filler dispersion (Jayaramudu et al. 2019). Resin-based composites such as dicalcium phosphate dihydrate NPs were also shown to remove and resist biofilm formation and even secondary caries (Wang et al. 2021).

Toothlessness, edentulism or implant dentistry procedures are followed along with oral soft tissue repair procedures to prevent the formation of plaque and a future recession. Edentulism can be the result of old age or tooth decay but nanocomposites incorporating various techniques and materials help complete the fabrication of the tooth denture keeping in mind the safety of pulp. The materials are to be designed such that they do not cause mild to severe peri-implant mucositis. Materials like titanium dioxide nanocomposites or yttria-stabilized tetragonal zirconia polycrystals are being used for crown synthesis and tooth prostheses (Magalhães et al. 2020; Hu et al. 2019a). To increase the enhancement of the tensile strength, researchers added zirconium oxide along with polymethyl methacrylate to form a strong composite where the strength of the composite was directly proportional to the concentration of zirconium oxide (Gad et al. 2018). Similarly, to deliver silver NPs to the site of infection, titanium nanotubes (NT) or copper nanocubes (NC) were designed (Colino et al. 2021). For these purposes, researchers also built a hybrid design of both the NT and NC that provided high biocide efficacy against potential oral pathogens (MacIa et al. 2019). To make this design easier to manufacture, a two-step protocol involving silver sputtering was incorporated to form silver nanoclusters which are then used as composites for edentulism. The two-step

procedure was considered longer especially when researchers were able to develop a technique that just involved a single step of depositing silver NPs on titanium substrates. The efficiency and stability of gold NPs were evaluated and they were found to be more stable compared to silver and other nanomaterials. These NPs were easy to manufacture, environmentally friendly, and had potential application in regenerative medicine.

Nanomaterials are designed and used in endodontics as well as restoration procedures keeping in mind the soft tissue engineering strategies. The bioactive composites and mixtures help in repairing tissues and in the treatment of the complex causes of toothache, especially in case of severe infection. Methacrylate-type resins like urethane dimethacrylate, bisphenol A-glycidylmethacrylate, and other inorganic fillers help to develop performance composite resins to attain clinical restorations as they are highly durable and resistant to pathogen infection (Yadav and Gangwar 2019; Pratap et al. 2019). Similarly, nanomaterials have their role in periodontics as well. Chitosan-based risedronate or zinc hydroxyapatite intra-pocket dental film was developed for treating alveolar bone loss (Khajuria et al. 2018). Cerium oxide NPs treated on human dental pulp stem cells showed that it helped stem cells be protected from the reactive oxygen species enriched environment with potential for regenerative therapeutics (Mahapatra et al. 2017). Calcium phosphate cement scaffolds helped in osteogenesis and the osteogenic differentiation of human dental pulp stem cells (Wang et al. 2014; Xia et al. 2018b; Xia et al. 2019). In another study, the researcher added gold NPs to the calcium phosphate cement furthermore enhancing the differentiation capacities of the human dental pulp stem cells (Xia et al. 2018c). Mesoporous calcium silicate NPs are also used in dental regenerative therapeutics (Huang et al. 2017). There seems to be a lot of studies done and ongoing for better oral soft tissue engineering and regeneration with the help of nanomaterials. It is a potential field of research that has beneficial factors in clinical applications.

3.4.2 Applications of Nanomaterials for Soft Tissue Engineering in Stem Cells

Nanomaterials used in stem cell research are targeted either towards bioactive compound delivery for therapeutic purposes or for inducing expression of a specific phenotype. There is a wide scope of research being done in the field of stem cells in various categories. The general process of stem cell therapeutics in soft tissue engineering is the collection of biopsy from the donor and inducing a monolayer culture from it. As the monolayer cells are established, they are further induced to cell expansion and differentiation with the help of specific growth factors. This is then scaled up to culture the cells on the 3D scaffold to give rise to specific organoids that can further give rise to specific tissue grafts and implants. For example, nanomaterials used in adipose-derived stem cell research are studied for their role in angiogenesis and cancer for therapeutic deliveries; their applications extend to studying their role in neurodegenerative diseases and development of neural stem

cells, in understanding autophagy, to deliver complex gene editing tools like CRISPR-CAS9 system, for providing better organoid substrates in organ on chip research, for understanding the biological role of differentiating stem cells on lab on a chip, or even as conjugate-based therapeutics in case of COVID-19 or generation of humanized grafts and implants.

Researchers have studied the role of nanomaterials in regulating the differentiation and proliferation of various stem cells. Gold nanoparticles are widely used for drug or gene delivery, as conjugates with stem cell biomarkers for electron microscopy imaging and biosensors (Hu et al. 2020). Gold NPs are used in the form of nanorods and nanofibers for promoting static and dynamic culture conditions and enhancing cell-matrix interactions (Villanueva-Flores et al. 2020). Similarly, silver, titanium, graphene, polyethylene glycol, polymeric nanomaterials like chitosan, PLL, poly-diallyl-dimethyl-ammonium chloride, DNA nanostructures like DNA origami, DNA nanotubes, nucleic acid aptamers, antisense oligonucleotides, antimicrobial peptides, and metallic nanopillars are all variety of nanomaterials used in stem cell and biomedical research. They are biocompatible, low in cytotoxicity, low in elucidating immunogenicity, easy to modify, and have controllable surfaces. Their production and manufacturing cost is low and can modulate the phenotypic expression of stem cells in vitro as well as in vivo.

Cruz et al. showed that PLGA which is poly(lactic-co-glycolic acid) NPs can be used effectively in human pluripotent stem cells (HPSCs) to deliver CRISPR-CAS9 system without inducing cellular cytotoxicity. This method is faster and efficient enough in terms of generating transgenic knockout, and knockdown lines. These NPs were shown to escape the lysosomal degradation efficiently for gene editing of gamma-globin gene. The method was discussed further for developing a robust tool that can be used for in vivo targeted therapeutics (Cruz et al. 2021). Karkan et al. studied the angiogenic capacity of human endothelial cells seeded on PU/PCL or polyurethane-poly caprolactone scaffolds. This scaffold was shown to be very low in cytotoxicity and zero nitrosative stress and promoted attachment and growth of these human endothelial cells in static and dynamic culture conditions (Karkan et al. 2021). Karimi et al. designed a 3D hydrogel construct that could enhance the human olfactory mucosa stem cells' bioactivity. These alginate hydrogels were mixed with magnetic nanofibers to develop an ecofriendly environment mimicking the extracellular matrix. These also contained bioactive short magnetic nanofibers for olfactory ecto-mesenchymal stem cell encapsulation. The design showed enhanced neuronal regeneration and proliferation rate of these stem cells (Karimi et al. 2021). When discussing stem cells in biomedical research, nanoconjugate-based stem cell therapeutics have been developed for the SARS-COV2 infection management and mitigation that had been shown to be efficient in damaged tissue repair and regeneration. Researchers are looking into the role of exosomes and other nano-cargo delivery systems that can deliver the stem cell-based nanomedicine to the infected area for rapid tissue repair and regeneration in pneumonia-like symptoms as well as in other virus-induced lung infections like in COVID-19 (Pinky et al. 2021). Prakash et al. worked on another stem cell-based therapeutics that involved sivelestat-loaded nanostructured lipid carriers which can modulate the

inflammatory and oxidative stress and damage in MSCs and dental pulp (Prakash et al. 2021). DPSCs and MSCs exposed to oxygen-glucose deprivation were treated with this nanomedicine and it showed improvement in the survival rate of these cells henceforth providing an efficient strategy for rescuing stem cells from ischemic stroke. Liu et al. were able to enhance the angiogenic capacity of dental stem cells via hypoxia-mimicking cobalt-doped multi-walled carbon nanotubes (Liu et al. 2021a). This composite was designed by using the organic metal framework for the insertion of this nanotube structure into cobalt oxide polyhedra. This composite was able to regulate and stimulate the hypoxic condition of stem cells to be able to promote the endothelial cells' vessel formation and increase angiogenic potential. Reding et al. worked on Niobium carbide nanosheets to show that these nanostructures could protect the intestinal cells and stimulate the survivability of intestinal cells in cell lesion and damage conditions or while undergoing a medical treatment. The nanosheets were produced by HF etching and plasma sintering and were placed in intestinal organoids. These nanosheets were effective in their potential therapeutic action on low dosage administration rather than the higher dosage which caused cytotoxicity (Reding et al. 2021). Jasenka et al. designed novel composite films made of biocompatible polysaccharides with conducting polyaniline polymerization. The feature of these composite films was that they proved to be very efficient substrate for adhesion and proliferation of human-induced pluripotent stem cells. These composite films were shown to have excellent tissue engineering properties in case of stimuli-responsive tissue. These films were antibacterial and did not induce any cytotoxicity (Jasenská et al. 2021). Noormohammadi et al. showed that nanowhiskers made of cellulose presented enhanced capacities in a wide range of tissue engineering applications. They synthesized PCL_{500}-PEG_x-PCL_{500} block copolymer-based polyurethanes with cellulose nanowhiskers. This nanomaterial was shown to have the potential to alter cellular functioning for different responses that could be crucial in terms of tissue engineering applications (Noormohammadi et al. 2021).

Yang et al. used cadmium-free $CuInS_2$/ZnS quantum dots modified with polyethylene glycol (PEG) to check its effects on neuron like PC12 cells. They investigated the neurotoxicity of this nanomaterial and also checked the phenotypic effect it had on this cell type. They were able to show that using this nanomaterial neurite outgrowth was inhibited and in this process, the differentiation-associated factors like neurite growth factor, $p75^{NTR}$ and MAPK pathway were downregulated. This nanomaterial proved to be an effective tool in tissue engineering and biological applications (Yang et al. 2021).

Stem cell therapeutics-based tissue engineering also encompasses designing materials that can be used for the detection and diagnostics of an anomaly in the cell. Fan et al. used the CLDN18.2 with a stem-loop hairpin RNA structure to form a molecular beacon that can target gastrointestinal tumors. This system was optimized by 2'-*O*-methyl and phosphorothiolate for enhanced stability and efficiency. This molecular beacon could detect the *CLDN18.2* RNA with greater efficiency compared to other methods in circulating tumor cell assay. It was shown better to perform for RNA detection than the CLDN18.2 antibody detection test (Fan et al.

2021). Similarly, Chen et al. designed fluorescently doped silicon quantum dots that are completely biocompatible. These nanoparticles were extracted from wheat straw and could be used to detect heavy metal poisoning and imaging in biomedical diagnosis (Chen et al. 2021). NP-based biomedicines provide novel a approach to stem cell therapeutics and enhance the efficacy of this system. The nanomaterial system should be designed such that it should be capable enough for the delivery of novel therapeutics, to study its delivery and distribution into the target cells.

3.4.3 Application of Nanomaterials for Soft Tissue Engineering in Osteology

Nanomaterials have their application in bone regeneration, bone defects, artificial cartilage construction, composite tissue, and its engineering. Biomodified biomineralized 3D polycaprolactone nanofiber scaffold and silk fibroin were designed by Xiao et al. such that they can promote the regeneration of bones in rats. This model was developed as a successor to 2D nanofiber as the 2D nanofiber mats are usually dense and prevent cellular functions like growth and infiltration. These 3D structures are radially interconnected macrochannels and aligned nanofibers (Xiao et al. 2021). Carbon nanotubes are universal material to develop a biomedical nanomaterial. They have immense applications in bone regeneration and cartilage construction. Carbon, the most basic organic compound, which is the foundational element of the biological system. Nanotubes made of carbon backbone, especially materials like graphene exhibits excellent biocompatibility and biosafety with very low toxicity. Carbon nanotubes are used in the application of imaging and sensors, drug delivery systems, mechanical strength, and scaffolding for regenerative medicine (Pei et al. 2019). Carbon nanotube scaffold (CNTS) is used for designing CNTS block, porous block, alginate scaffold, and PDLLA/CNT/nHA scaffold and these are used as cellular adhesives being tissue compatible (Huang 2020; Siqueira et al. 2015; Stocco et al. 2019). Carbon nanomaterial composites (CNTC) are used to design PMMA-CNT bone cement and CNT/alumina ceramic composite for cellular adhesion and mechanical strength (Kim et al. 2019; Okolo et al. 2019). Carbon nanotube coated metals like CNT coated $TiAl_6V_4$ titanium alloy (Pandey et al. 2018). Carbon nanotubes are also used as therapeutics in the cure of tumors. CNT-based bio-therapeutics like gadolinium and other anticancer agents and various CNT-based fluorescent dyes are being used for sensor and imaging (Aoki et al. 2020; Barabás et al. 2020).

Tolba et al., on the other hand, developed the unique self-healing characteristics of medical cements in the lab by using amorphous polyphosphate and calcium carbonate NPs. They mixed these compounds in the ratio of 1:10 and incorporated the mixture into poly methyl methacrylate. This design tenfold times increased the self-healing of medical cement as well as its biocompatibility (Tolba et al. 2020). Bone regeneration is the main focus when developing novel nanomaterials for orthopedics and natural clay nanomaterials have been shown to have great potential in this field in applications like tissue engineering and osteogenesis. Novel

bio-scaffolds were 3D bioprinted by using natural bioactive attapulgite nanorods by binding them with bioadhesive and sintering. Polyvinyl alcohol was used as the bioadhesive and this scaffold was then shown to be highly biocompatible and efficient in cases of osteogenesis in osteogenic precursor cells by Wang et al. (Wang et al. 2020). In a histology analysis after in vivo experiments with the rat cranial defect model, it was found that bone formation took place following membranous ossification and bone angiogenesis took place within the 3D scaffolds after 8 weeks of the treatment. Silva et al. also developed a unique nanomaterial by using the electrospinning technique to develop a core-shell and monolithic fibers from biocompatible polyvinyl and lactic acid polymers (da Silva et al. 2019). Poly-L-lactic acid polymer-based nanosheets with fibroblast growth factors have been designed in the Murahashi lab which also showed efficient osteoblast differentiation, hence being a potential nanomaterial in bone regeneration (da Silva et al. 2019).

Pelin et al. used a unique material that is fish bone-derived bi-phasic calcium coat which can be directly applied to various bone regeneration and orthopedic applications. This calcium coating is deposited on the target surface via pulsed laser deposition. It was found that the coating formed was antibacterial and anti-inflammatory and could be used for preventing various nosocomial infections (Popescu-Pelin et al. 2020). Afewerki et al. did a bioprinting job of a unique orthopedic clay called synthetic smectic clay for various orthopedic applications. This clay was designed by mixing a commercially available nanomaterial called Laponite with suitable nanocomposites via cross-linking to make a bio-ink that can be used in bioprinter to give this clay a specific ortho-applied application (Afewerki et al. 2019). There are various Laponite and Laponite mixtures listed in previous literature. GelMA-silicate nanoplatelets (laponites) and tricalcium phosphate NPs used as growth factors for the regulation of osteogenic differentiations in a single medium (Gaharwar et al. 2013), Laponite nanoclay and silk fibroin and silanized hydroxypropylmethylcellulose mixed with laponite are used for designing composites that can improve mechanical properties without interfering with other cellular processes and viability (Su et al. 2016). Laponite, alginate, and methylcellulose mixtures are used for printing scaffolds that could be fabricated as per application requirement as well as for specific drug delivery; laponite mixed with Si-HPMC are used to develop an interpenetrating network for regenerating bone structure to provide suitable mechanical strength (Boyer et al. 2018). PEG blocks have also been devised to use with laponites that can be used to improve the viscoelastic properties of PEG hydrogels, and keratin, pluronic, and chitosan have also been mixed with laponites for various orthopedic applications (Maeda 2019). There are various 3D bioprinting techniques used for various applications, e.g., inkjet printing used the bio-ink drop by drop for standardizing muscle cells encapsulated in collagen and development of small bone fabric structures, extrusion bioprinting for the construction of intrinsic and large size structures that can mimic physiological bone and cartilage structures, stereolithography and projection patterning method to manufacture microdevices for bone density diagnosis and monitoring purpose and mostly adopted laser-based printing that is used for printing artificial skin and cartilage

substitute for implantations (Gungor-Ozkerim et al. 2018; Ashammakhi et al. 2019; Das and Basu 2019; Ning et al. 2020; Turnbull et al. 2020; Shamma et al. 2022).

Su et al. used graphene oxide (GO) doped electrospun polylactic-co-glycolic acid nanomembrane (PLGA filament) that can be applied to various ortho-surgeries for facilitating tendon-to-bone integration. The membrane design resulted in the rapid proliferation of bone marrow stem cells and their osteogenic differentiation as well. It showed good healing efficiency in rabbit models with improved collagen arrangement (Su et al. 2019). Egawa et al. used polyethylene terephthalate for artificial ligament reconstruction. This method was made novel with higher fold improvement by using silicate substituted strontium that displayed good osteogenic potential in healing and construction (Egawa et al. 2019).

Various nanocomposites are used along with hydrogels for restoration of degenerated load-bearing tissue and to increase its mechanical strength. But a disadvantage of these gels is the inability of monitoring them after their injection as they can create swelling and inflammation in the local tissue area. Henceforth, to monitor such activities, Shanks et al. developed a novel nanomaterial-based gel called core shell-shell nanoparticles that can be fluorescently imaged via near-infrared radiometric testing and hence giving the upper hand in understanding the situation and accordingly mitigating it (Shanks et al. 2019).

Complex ortho-surgeries like the replacement of intravertebral discs need nanogels like polyethylene glycol hydrogels that provide high mechanical strength with better performance in compressive and tensile fracture strain tests (Hu et al. 2019b). Hence, nanotechnology has to play a major role in designing nanomaterials that can be used in bone and cartilage regeneration, scaffolds and composites for better mechanical strength, and performance and nanomaterial-based therapeutics for treating musculoskeletal tumors.

3.4.4 Application of Nanomaterials for Soft Tissue Engineering in Cardiac Muscles

One of the main applications of nanomaterials in cardiac tissue engineering and therapeutics is in myocardial infarction and heart failure, and the application of nanomaterials is important here because of the low regenerative capacity of the human heart. Researchers have used the concept of modRNA or modified RNA that is non-immunogenic and robust in inducing cardiac regeneration post-myocardial infarction. Modified RNA technology is being exploited as the main model of cardiac repair and various approaches are being studied on daily basis. Genes that are involved in this system are VEGFa, IGF-1, EGFP, DN-IGF-1R, IGFR, and mutated FSTL1. These are either antiapoptotic or induce vascular regeneration, engraftment proliferation, decreased caspase activity, and increased efficient delivery of the therapeutic RNA. These RNAs are being delivered using materials like RNAiMAX, polyethylenimine-based NPs, formulated lipidoid NPs, exosomes, sucrose citrate buffer, or encapsulated alginate NPs (Chien et al. 2015).

Babiker et al. showed that G6 PAMAM (sixth generation cationic dendrimer of polyamidoamine) administration to non-diabetic hearts that were subjected to ischemia and reperfusion in a dose-dependent manner impaired the recovery of vascular dynamics and cardiac hemodynamics. This study was essential to show the nanotoxicological effects of G6 PAMAM NPs that are being used increasingly in the delivery of therapeutics in diabetic heart cells (Babiker et al. 2020). Similarly, nanoSiO_2 are widely used for biomedical and clinical applications but Lozano et al. showed that these amorphous silica NPs upon administration reduced the mechanical performance of the heart of rat. It promoted cardiac dysfunction by regulating the permeability of mitochondria in human cardiomyocytes. They also treated the same cells with Cyclosporin A and showed reduced apoptosis due to nanoSiO_2 and increased ATP production (Lozano et al. 2020). Hozayen et al. showed that popularly used silica NPs for drug delivery can increase reactive oxygen species production and imbalance redox potential in cardiomyocytes. The treatment leads to severe increment in the tumor necrosis factor and toxic lipid accumulation leading to histological modifications (Hozayen et al. 2019). These studies reflect upon the fact that there needs to be research done on NPs being used in biomedical research and clinical applications for their toxicological effects before using them in the field.

On the other hand, Bier et al. showed the natural method to deliver therapeutics in the case of Duchenne muscular dystrophy. They have used placenta-derived mesenchymal stem cells and their derived exosomes to treat mouse models and Duchenne patients. This method significantly showed downregulation of fibrosis in the diaphragm and cardiac muscles and reduced inflammation. They also showed that the method was able to upregulate the utrophin expression that helps in the repair and regeneration of damaged muscles (Bier et al. 2018). Liu et al. fabricated NPs based on PEGylated chitosan bioactive hydrogel that was embedded with TiO_2 that could be potentially used in cardiac muscle restoration and repair. This hydrogel had enhanced stability and reduced vascular swelling. It promoted cell adhesion due to proper cell and hydrogel interaction with no cytotoxicity and organization (Liu et al. 2018).

Sridharan et al. designed an electrospun scaffold that could be potentially used for cardiac tissue repair. This 3D scaffold was fabricated by aligning coaxial nanofibers comprising polycaprolactone core and gelatin shell. This scaffold was also used for seeding and culturing the cardiomyocytes derived from human induced pluripotent stem cells that gave rise to a well-functioning cardiac patches for cardiac repair applications (Sridharan et al. 2021). Aalders et al. could culture heart 3D organoids by suspending cells in a drop of medium and then encapsulating it with silica powder NPs. These NPs were treated with hydrophobic chemical hexamethyldisilazane (nHMDS) which resulted in the formation of microbioreactor giving rise to cardiospheres stimulating cell coalescence and 3D aggregation (Aalders et al. 2021). Liu et al. studied the properties of quercetin and its loading into mesoporous silica NPs for drug delivery and for studying myocardial ischemia-reperfusion injury. These studies were performed in rats and it was shown that this nanomaterial activated JAK2/STAT3 pathway inhibiting cell apoptosis and oxidative stress. It was

shown to reduce myocardial infarction size and enhance ventricular remodeling and vascularization. It recovered cardiac blood flow and other cardiac function-related biochemical attributes (Liu et al. 2021b). Li et al. did something novel with graphene hydrogels. They designed dopamine-reduced graphene oxide and doped it in gelatin methacrylate hydrogels which resulted into nanomaterial that can regulate cardiomyocytes cell growth in a controlled manner. They also stimulated the cells with an electrical impulses that facilitated the maturation of cardiomyocytes and improved orientation order with better intercellular adhesion and expression of gap junction proteins. This nanomaterial model could potentially be used for developing functional and mature myocardial layers and in drug screening and disease models in vitro (Li et al. 2021).

Nanomaterials have also been implied in diagnostic and investigatory studies apart from tissue engineering that in turn helps the tissue engineering and regeneration process. Rotenberg et al. devised and designed a nanomaterial-based diagnostic tool that can be easily applied to various cellular applications and for in vitro and in vivo investigations. They developed a cell-silicon hybrid using silicon nanowires that offered easy bioelectric and optical stimulation-based diagnostic tools (Rotenberg et al. 2021). In another study, they developed nanowire myofibroblast hybrids that are electrically coupled with cardiomyocytes and that the photostimulation of these nanowires could potentially perform subcellular resolution-based optical interrogation (Rotenberg et al. 2020).

There have been various nanomaterial-based scaffolds devised in the field of cardiomyocytes research used for in vitro culture, cell attachment and proliferation, alignment of focal adhesions, early differentiation of mESCs, improved attachment of myoblasts, increased alignment of heart cells and improved mechanical properties, regulated angiogenesis and remodeling, cardiogenic differentiation of MSCs, upregulation of myosin, b-tubulin, faster and longer contraction of cardiomyocytes, improved survival rate, synchronous heart rhythm and electrical coupling. GelMA and MeTro, PEG, PS, agarose, PGS, PHB, PLGA, BSA/PVA, PMGI+heparin binding peptide I, PGS/gelatin, PLGA+YIGSR along with aloe vera extracts in PEGDA hydrogels, alginate, chitosan and most importantly carbon-based nanomaterials like carbon nanotubes and graphene nanowires and NPs are the materials being used for such sophisticated functions in vitro and in vivo. Various techniques like photolithography-based micromodeling, laser ablation and assisted technique, electrospinning, plasma etching, 3D printing, 3D-MPE, microstereolithography, spray drying method, ionic cross-linking, decellularization process, film preparation, freeze- drying and hydrogel microelectrode array preparation are used for the nanomaterial synthesis and assembly (Cristallini et al. 2020).

3.4.5 Application of Nanomaterials for Soft Tissue Engineering in Neurology

The application of nanomaterials in neurology in soft tissue engineering is currently limited to biomedical research in the field. Both nanomaterials are being used in

diagnostics or research done on nerve regeneration and human embryonic stem cells derived neurons or drug delivery across the blood-brain barrier.

For nerve regeneration and developmental diagnostics, nanomaterials like AGulX NPs, NPs coated with K16ApoE, MNPs mixed with target-specific antibodies, fluorescent quantum dots or quantum rods, DA functionalized CuInS2/ZnS quantum dots, gold NPs with gelsolin, nano-biosensors, NP@SiO_2@FSLOH, Prp^c-GNPs, boron dipyrromethene biosensors, DNA nanomachine, etc. are being used. They are implied in various applications for better imaging and diagnostics especially for MRI, as pathological markers with low toxicity, for immunoassay and immunodetections, detection of marker protein with microscope and spectrometer in vitro, via NIR tissue imaging, or as DNA-based biosensors or on lab on a chip (Xu et al. 2021).

For drug delivery, nanomaterials like CNMs, NGF-$PSIO_2$, TiO_2 nanowired cerebrolysin with neprilysin and marker specific antibody, SERS, marker specific monoclonal antibody gold NPs, donepezil-PLGA NPs, CT/siRNA, exosomes, HupA Lf-TMC NPs, bFGF-STL NPs, PLGA-pb NPs, PLGA-CRT_S1, anthocyanin loaded PEG gold NPs, GH-CX NPs, PLGA-PEG-PPARgammaA, LMNs-PC, SM-EC, CRISPR-Cas9 NPs, and NT lipidoid NPs are being used along with scaffolds like silk nanofibers, polyacrylonitrile, chitosan or fibrin grafted polyurethane, PEG hydrogels, chitosan, 2-D plastic substrate, collagen nanofibers or PCL/Gelatin nanofibers for applications like inhibition of amyloid fibrils progression, reduced cytotoxicity, enhanced neuroprotection and improved behavioral functions, targeted delivery with high specificity, superior sensitivity and better delivery and detection range and efficient entry through blood-brain barrier and CNS (Teleanu et al. 2019; Arzaghi et al. 2020).

Apart from the nanomaterials, researchers are also developing microenvironment-based engineered systems that can mimic the actual physiology of the brain. Brain on chip models, organoid models, or blood-brain barrier platforms, these in vitro models mimic the actual physiology of the brain to provide us with answers to complex problems that tackle drug delivery, metabolic flux regulations, transport mechanism, and regeneration biology.

Ahn et al. developed a 3D on-chip system that mimics the blood-brain barrier physiology and function. This model provides astrocyte networks, gene regulatory elements and various other important cellular interactions that generally need an animal model to study upon. This on-chip model was developed to study the transport mechanism across the barrier and uptake of the drug to the targeted region (Ahn et al. 2020). Another similar study was done by Wevers et al. where they studied the blood-brain barrier function and specifically targeted the disease model with monoclonal antibody for studying its therapeutic efficacy (Wevers et al. 2018).

Losada et al., on the other hand, developed a 3D scaffold-based system for better neuronal differentiation. They studied various nanomaterials for this purpose in their study that included graphene oxide, full reduced graphene oxide, its partially reduced version and GO film. They studied these materials for their potential in cell proliferation, differentiation, maturation, survival and most importantly the bioenergetic function in the dopaminergic cell lines. Various bioreactor-based metabolic assays were

performed using tyrosine hydroxylase, dopamine transporter, synaptic proteins and glutamate-specific potassium channels. They also checked the efficacy of these materials in mitochondrial dysfunction and found that graphene oxide films among others performed better in providing a matrix to developing and differentiating cells. It performed better in cell survival assay and hence proved to be a potential scaffold for the neuronal cells to test drugs and drug delivery and in cell replacement therapy (Rodriguez-Losada et al. 2020).

Yang et al. studied the function of carbon nanotubes and their potential on neuronal cells as they provide a wide range of application in tissue regeneration therapeutic applications because of their unique biochemical and physiochemical properties. They studied its impact on brain microvasculature in endothelial cells and permeability of blood-brain barrier. They studied two forms of carbon nanotubes, pristine and carboxylated forms. Their study focused on endothelial permeability and paracellular flux on hemichannel activity to check for marker protein expression and its localization. This study enlightened the potential and impact of carbon nanotubes applications on brain endothelial cell rigidity, stability and permeability for downstream applications in targeted therapeutics (Yang et al. 2020). Various studies are also coming up studying the role of various nanomedicine in the case of glioblastoma. Researchers have tried transdifferentiating cancer stem cells into therapeutic models. This was demonstrated by the transdifferentiating ability of graphene and its derivatives and they were able to show its potential in promoting the differentiation of cancer stem cells into therapeutic models for studying targeted drug delivery of nanomedicines against glioblastoma (Martelli et al. 2020).

Similarly, researchers are working on many other topics that require attention like spinal cord injury. Nanomaterials are used in spinal cord repair and cell therapies for life-threatening neurological diseases like DMD. Many researchers devised combined cell therapeutics or binary therapy approaches to solve the puzzle of the brain and treatment of neurological disorders. But further research in this field needs to be done as this seems a promising approach to combining molecular medicine with nanotechnology.

3.5 Future Directions in Soft Tissue Engineering

There is a lot of work being done on soft tissue engineering in various biomedical disciplines involving nanomaterials. As discussed earlier, the nanomaterials being used in this discipline require to be bioinert, functionality based on surface bioactivity and resorption. They should be biomimetic promoting better cellular adhesion signaling and response, they should be able to prevent biofilm formation and infection fulfilling the criteria required to be a medical-grade material. The design and development of bio-nanomaterials depend upon the progress of nanotechnology and material science and how well these nanomaterials respond to the cellular and molecular level interface and interactions. The design of such materials needs a realistic approach of concepts for controlled targeted tissue and host response. The design of the nanomaterial should be done such that it is able to interact

efficiently with the biochemical pathways of the cell with necessary functionalities and therefore it is necessary to have an understanding of the nanomaterial being used for constructing scaffolds that can match the growth factors, cytokine and other biochemical functions with very low host immunogenicity against the nanomaterial in cell to produce efficient reintegration of engineered construct and host tissue (Parisi et al. 2018; Nie and Wang 2018; Mastrullo et al. 2020).

Apart from the nanomaterials tissue regeneration and engineering completely rely on how these materials are manufactured. Whether the techniques used in designing and manufacturing these nanomaterials involve simple or cumbersome processes and protocols and how well can they be scaled up for commercial manufacture and distribution? One of the promising design and manufacturing techniques is 3D printing which is also referred to as additive manufacturing. It gives freedom to simplistically design geometrically complex structures and allows rapid and low-cost development of patient-specific required tissue engineering construct. This also provides better biomimetics and functional aspects compared to other techniques and print constructs that are clinically validated (Arjunan et al. 2020). 3D printing involved organoid development and scaffold printing that can give rise to tissue-specific grafts with very low host immunogenicity (Maina et al. 2020).

Nanomaterials in soft tissue engineering provide a promising future to biomedical research and industry but still need a lot of work before implementing in the field as this is challenged by a lot of different factors like reproducibility, scalability and especially social acceptance with a regulatory layout to use them. Technical challenges obviously include biomimetics at the cellular and molecular level, vascularization and patient-specific requirements to provide personalized therapeutics that can help the local tissue to repair, survive and reintegrate (Auger et al. 2013). Work on these nanomaterials and scaffolds also concentrates on the material porosity gradients, growth factors and cytokines required for the controlled growth of organoids for tissue and graft generation (Pina et al. 2019).

A lot of focus is also been given to interface organization and remodeling. It is important that physical, chemical and biological interactions at the cellular junctions and interfaces like these be more studied for the development of a robust system in soft tissue repair and regeneration.

3.6 Conclusion

Regeneration of cells and tissue is highly coordinated in vivo. The interplay between various physiological and molecular processes involved in the process like blood circulation, immunological responses, hormonal controls, and bioenergetics influence the proliferation and maturation of tissues. Nanotechnology aids in the development of sophisticated scaffolds and precision-controlled delivery of drug and bioactive compounds. Advanced nanofibers and wires could create novel and biomimicking scaffold architecture to aid tissue engineering in 3D. Impregnation of novel nanoparticles into scaffold aid in the development of hitherto impossible environments like magnetic and electric fields for the cell growth, apart from the

usual cell proliferation aiding characteristics. However, cytotoxicity and biocompatibility of newly formulated materials are still a challenge. 3D printing of various biomaterials is another rapidly developing field with many possibilities in tissue engineering. Big data analysis, artificial intelligence, and machine learning will aid the process of design and development of different scaffolds and materials for tissue engineering. In the next decade, we will see the integration of computers, advanced material science, nanotechnology, and stem cell biology to develop highly advanced and accurate tissue mimics. Tissue engineers will transform into organ farmers.

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3D and 4D Nanoprinting for Tissue Regeneration

4

Anjali P. Patni, Dannie Macrin, Dibyo Maiti, and Arikketh Devi

Abstract

In recent years, to deliver an effective transplantation process and develop better disease models, considerable efforts are devoted to advancing innovative techniques and strategies for tissue regeneration. Nanotechnology has given rise to various molecularly engineered nanostructures (organic and non-organic) and nanoprinting that can be manipulated for therapeutic and diagnostic purposes. Moreover, a whole new class of nanodevices and nano-robots has evolved with three-dimensional (3D), and four-dimensional (4D) nanoprinting frameworks to unlock tremendous potential for tissue engineering. This book chapter highlights an exhaustive overview of 3D, 4D nanoprinting applications over the traditional scaffold production strategy in sustaining tissue viability and advancing functional maturation in building regenerative constructs. Challenges and emerging possibilities for viable nano-bioprinting tissues are also addressed, converging on future research in the field.

Keywords

Nano bioprinting · Scaffold synthesis · Biomaterials · Regenerative medicine

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4.1 Introduction

The field of biotechnology has witnessed significant advancements in the last decade, which has led to innovations, enabling scientists to explore new avenues in different areas. Some of these could be integrated, leading to multidisciplinary research involving researchers from different specializations. Nanotechnology and tissue engineering are emerging areas gaining imminence, and the incorporation of both can pave the way to open up novel possibilities in the field of regenerative medicine. It can assist in creating artificial organs and alleviate the problems of patients involved in organ transplant rejection. It can solve the issue of the shortage of organs available for donation. Thus, this chapter focuses on integrating nanoprinting for tissue regeneration and discusses the different techniques, advances, and challenges involving both areas to enable researchers to identify ways to address the demerits and complications associated with it.

The human organ comprises several types of cells, an extracellular matrix (ECM), and a range of signaling components that collectively form tissues. The ECM is a complex network that communicates biochemical/mechanical signals from the surroundings to drive cellular behavior into the cells. Tissue engineering strives to emulate the natural ECM by incorporating cells, growth factors, scaffolds, and several other components for tissue regeneration. Cartilage, bone, skin, blood vessel tissue, and nerve regeneration are primary research topics in tissue engineering. Biomimetic tissue printing, which involves synthetic approaches to mimic biochemical processes, plays a vital role in developing innovative technologies, including drug testing, tissue engineering, biomimetic sensors, and three-dimensional (3D) tissue models. Research in tissue engineering has reiterated the necessity to develop 3D biodegradable scaffolds; consequently, considerable work has been invested in constructing a synthetic ECM in vitro. Scaffolds should be naturally eliminated or degraded by the body into non-toxic materials to avoid the necessity for surgical removal. Unfortunately, since two-dimensional (2D) bioprinting technologies do not precisely control the scaffold's architecture, pore shape, porosity, or interconnectivity, they cannot drive or accelerate cell proliferation and tissue creation.

In contrast to traditional 2D culture techniques, the latest cellular regenerative constructs involving diverse biomaterials and scaffolds exhibit higher efficiency in enhancing the process of generating new tissue. Recently, nanostructured platforms like films, 3D scaffolds, and nanoparticles (NPs) have been employed to integrate a plethora of smart sensory delivery vehicles and nano-robots. With the advent of 3D nanoprinting technology, fabricated complex scaffolds are generated that mimic the structure and composition of living tissue by layering materials on top of each other to obtain the required features with precision coordination of architecture and spatial chemistry. Likewise, cell nanoprinting holds tremendous potential for implementing tissue and organ regeneration, which may also apply to human tissue development and repair (Kang et al. 2016). The possibilities of 3D and 4D nanoprinting strategies in tissue engineering for generating personalized scaffold constructs can assist in developing better and healthier organ transplants. This book chapter also

summarizes recent advances in 3D and 4D nano-bioprinting technology for tissue engineering, techniques employed in directing the biomaterials, advances in the nanoprinting methods, and 3D bioprinting applications in regenerative medicine involving implant device fabrication.

4.2 Bioprinting Techniques Using Biopolymers and Biomaterials

Bioprinting is a 3D tissue fabrication technique that allows for the creation of tissues with accuracy through layer-by-layer construction that incorporates biomaterials and biopolymers (Tamay et al. 2019). This technique prints intricate tissue constructs using hydrogels loaded with cells. Using this technique, a variety of transplantable soft tissues can be generated, which includes cartilage, bone, and skin (Mandrycky et al. 2016). Hydrogels were the very first biomaterials designed for human use, which were also found to be compatible with use in the human body. Hydrogels are water-retaining swollen structures capable of retaining 3D tissue structure and conformation (Kopeček 2007). Bioinspired materials used to construct tissues can undergo the transition from 3D bioprinting to 4D bioprinting. It could be a material producing a current, becoming bioactive, changing shape, or performing a particular bio function after exposure to an external stimulus (Tamay et al. 2019).

The main constituents of bio-nanoprinting are bioinks which are essential for printing 3D tissues. Bioinks should possess particular characteristics and match specific criteria to serve as printing materials in bioprinting techniques. They should be biocompatible, meaning that they should not cause an immune reaction in the recipient's body after transplantation, and robust, that is, be able to resist any physical and mechanical forces of the environment (Mosadegh et al. 2015). Today, the most commonly used bioinks are hydrogels preferably because of their cross-linkable, printable, biocompatible nature, along with a high swelling capacity (Mandrycky et al. 2016).

Hydrogels can be synthetic or natural in origin. Natural hydrogel mainly comprises polysaccharides and constituents of the extracellular matrix (ECM). Examples of polysaccharides include alginate and agarose. ECM consists of collagen, laminin, fibronectin, and gelatin (Zorlutuna et al. 2013). Collagen and gelatin are highly biocompatible with a unique property of promoting cell proliferation. GelMA, the methylated form of gelatin, is effortlessly printed using bioprinters and quickly stabilized by exposure to UV radiation (Zhang et al. 2017). Alginate is a linear polysaccharide derived from brown algae and is widely used in 3D bioprinting because of its biocompatibility, ability to promote cell proliferation, low costs, and ability to gel in calcium ion-containing solutions quickly. Agarose is another linear polysaccharide, which resides as a gel at room temperature and converts to a solution when subjected to temperatures beyond 37 °C.

4D bioprinting is the process of engineering and manufacturing tissue structures using smart biomaterials. They can self-transform into a predetermined shape or exert a predefined function in response to external stimuli (Pei 2014; Choi et al.

Table 4.1 Nanofiber scaffolds currently used in tissue engineering

Nanofiber scaffold	Cell type	Organ/ tissue application	Reference
Fibrinogen and Collagen	Chondrocytes	Cartilage	Xu et al. (2013)
Poly(L-Lactide) (PLLA), Gelatin and Agar			Gong et al. (2007)
Alginate and Nanofibrillated cellulose			Markstedt et al. (2015)
Weakened Native Cartilage with porous Polylcaprolaptone (PCL)	Adult Stem Cells (ASCs)	Cartilage	Garrigues et al. (2014)
PCL/collagen nanofibers containing TFG-β1 or gentamicin	Human Dermal Fibroblasts (HDFs)	Wound Healing	Albright et al. (2018)
Collagen-based construct with a PCL mesh loaded with fibroblasts	Keratinocytes	Human Skin	Kim et al. (2017)
Methacrylated gelatin and alginate	Human Umbilical Vein Endothelial Cells (HUVECs)	Cardiac	Colosi et al. (2016)
Methacrylated gelatin and Methacrylated hyaluronan	Human Aortic Valve Interstitial Cells (hAVICs)	Cardiac	Duan et al. (2014)
Cell-laden collagen core on alginate sheet	hASCs	Liver	Yeo et al. (2016)
Gelatin and Fibrinogen	hUVECs, hMSCs, Human Neonatal Dermal Fibroblasts (hNDFs)	Vascular	Kolesky et al. (2016)
Collagen-coated Nanofibrous Polyethylene terepthalate (PET)	Epithelial Cells from Colon Tissue (Caco-2)	Intestinal Epithelium	Patient et al. (2019)

2015). A biomaterial's response can be chemical or physical upon exposure to a stimulus, and this response or a change depends on time. The usual responses include curling, folding, twisting, contraction, extraction, color change, and degradation (Li et al. 2016). (Table 4.1).

4.3 Advances in 3D and 4D Nanoprinting Methods

The significant roadblocks in tissue engineering using stem cells are, insertion of the cells into the defected site and retaining the cells in the vicinity of regeneration (Shi et al. 2017b). In addition, stem cells directly inserted/injected into the defect site can cause teratomas if they colonize far-off organs (Gutierrez-Aranda et al. 2010). However, both the roadblocks mentioned above can be overcome by using a scaffold material that acts as a base for the regenerative cells to adhere, expand, and stay localized to the affected area (Ma and Elisseeff 2005). In general, a scaffold used for

tissue regeneration should have the traits such as being non-immunogenic, promoting cell growth, biocompatible, and self-degrading (Godier-Furnémont et al. 2011; Do et al. 2015).

Traditionally, scaffolds were synthesized using methods like leaching of particles, phase separation, phase inversion, gas foaming, bonding of fibers, and lyophilization (Pennarossa et al. 2021). Nanoprinting to synthesize 3D scaffolds addresses the multiple challenges traditional methods face, such as developing complex structures to match significant defects like bone fractures. It also enabled the synthesis of interconnected variable porosity scaffolds, essential for regenerating organs with complex architecture (Lee 2015).

4.3.1 Traditional Methods of 3D Scaffold Synthesis

4.3.1.1 Gas Foaming

Gas foaming was primarily used to synthesize 3D scaffolds as it prevents the usage of caustic or toxic solvents. Thus, it renders multiple benefits to the scaffolds by enhancing biocompatibility and permitting sensitive biomolecule utilization in the scaffold matrix (Zhu et al. 2008). In this method, a solid polymer disc formed by compression molding is incubated in a high-pressure gas chamber for a prolonged period. The gases in the chamber help create pores in the solid polymer disc, which are necessary for cellular colonization and tissue growth (Mooney et al. 1996) (Fig. 4.1).

4.3.1.2 Freeze-Drying

Freeze-drying is most commonly employed in synthesizing 3D scaffolds using soluble and phase separation polymers. Scaffold materials such as chitosan, carboxymethylcellulose, gelatin, and hyaluronan are made using the freeze-drying method (Sultana and Wang 2012; Wu et al. 2010; Wiwatwongwana and Pattana 2010; Tan et al. 2018). The process of freeze-drying involves the removal of solvent from a saturated solution of polymer material and solute. Under vacuum conditions, the solvent sublimates under negative temperature leaving the dry supersaturated structure behind. The porosity and other properties can be regulated by controlling the parameters such as temperature, time, and concentration (Ghalia and Dahman 2016) (Fig. 4.2).

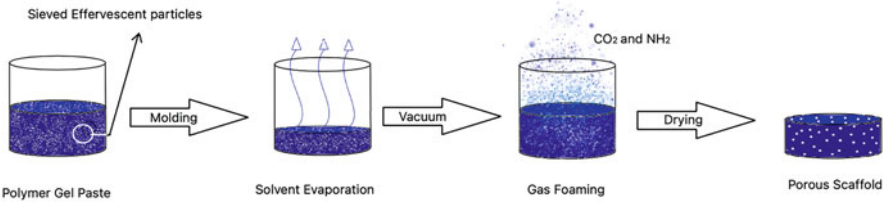

Fig. 4.1 Schematic representation of gas foaming process

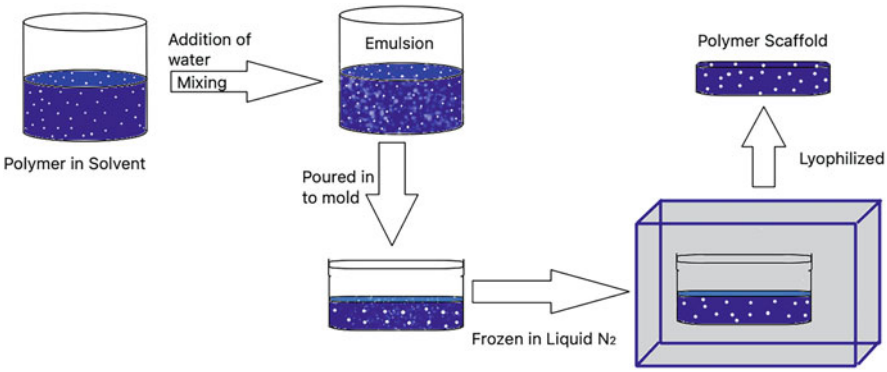

Fig. 4.2 Schematic representation of freeze-drying process

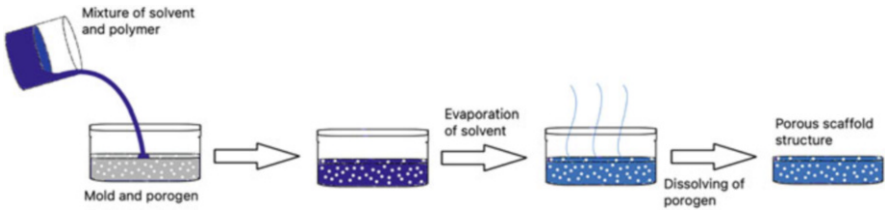

Fig. 4.3 Schematic representation of particle leaching process

4.3.1.3 Particle Leaching

The particle leaching method mainly aids in synthesizing composite scaffolds such as poly-lactic acid (PLA) and poly-ortho ester; there are two effective forms of particulate leaching methods, solvent casting, and melt molding. In the solvent casting method, salt particles are embedded within the polymer material and casted as a thin film (Zhou et al. 2005; Mi et al. 2014). After the cast solidifies, the salt particles leach out using an appropriate solvent leaving behind a porous scaffold. The melt molding method involves the embedding of solid porogen having a low melting point. The porous scaffold extraction occurs by melting off the porogen after the scaffold formation. The major advantage of particle leaching methods is the ability to have greater control over the pore size (Garg et al. 2012) (Fig. 4.3).

4.3.1.4 Fiber Bonding

Polylactic acid (PLA), polyglycolic acid (PGA) and poly(lactic-co-glycolic) acid (PLGA) fibers are some of the most successfully used scaffold materials to be formed using the fiber bonding method. In this method, the non-bonded fiber is immersed in a solution containing the bonding agent (Wake et al. 1996; Wald et al. 1993). The solution is evaporated, and the fibers are heated above their melting point, causing them to bond. The contact points of the bonded fibers are stabilized by the bonding agent leaving the fibers in interlocked structures. The advantage of this method is the large surface-to-volume ratio provided by fiber-bonded scaffolds.

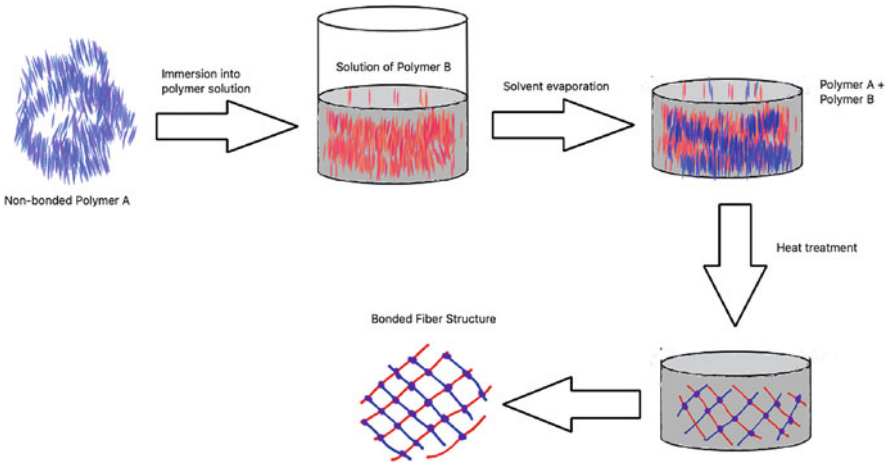

Fig. 4.4 Schematic representation of fiber bonding process

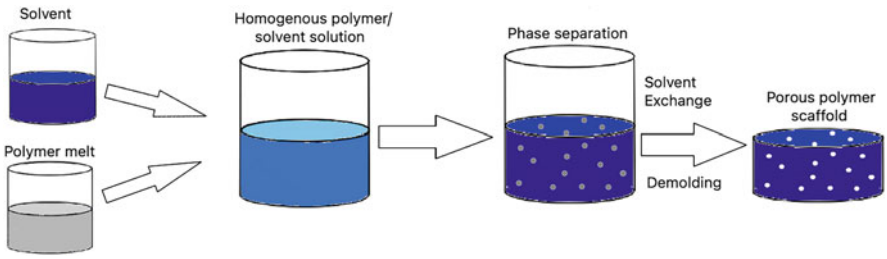

Fig. 4.5 Schematic representation of phase separation process

However, the production of complex 3D structures is difficult using the fiber bonding method (Kurtis Kasper and Mikos 2013) (Fig. 4.4).

4.3.1.5 Phase Separation

This technique involves using two solutions of varied polymer concentration and rapid temperature change. The solution having a lower concentration is called the lean phase and the one having the higher polymer concentration is called the rich phase (Li et al. 2004; Martinez Perez et al. 2011). A solid phase is obtained by quenching and separating the liquid-liquid phase, and the solvent is removed by evaporation or sublimation. The most critical parameters for the success of this method are the selection of the correct solvents and favorable temperatures. The main advantage of the phase separation technique is the possibility of dispersion of bioactive molecules in the solvent and the ability to control pore morphology (Kurtis Kasper and Mikos 2013) (Fig. 4.5; Table 4.2).

Table 4.2 Traditional methods of scaffold preparation and their advantages and disadvantages

Method	Advantages	Disadvantages	Citation
Gas Foaming	Avoiding caustic/toxic solvents	Low reproducibility, less control over pores	Zhu et al. (2008), Mooney et al. (1996)
Freeze-Drying	Use temperature sensitive bioactive components such as growth hormones	Less control over pore morphology and size	Ghalia and Dahman (2016)
Particle Leaching	Precise control over pore size and shape	Usage of caustic solvents such as leaching agents	Garg et al. (2012), Zhou et al. (2005), Mi et al. (2014)
Fiber Bonding	Large surface-to-volume ratio	Difficult to synthesize 3D structures	Kurtis Kasper and Mikos (2013)
Phase Separation	Greater control over pore morphology, dispersion of bioactive molecules	Requires precise temperature control, low reproducibility	Li et al. (2004), Martinez Perez et al. (2011), Kurtis Kasper and Mikos (2013)

4.3.2 Advanced Nanoprinting Methods for Scaffold Synthesis

4.3.2.1 Rapid Prototyping

Rapid prototyping (RP) is a free-form technique that involves computer-aided multilayer fabrication methods. This type of nanoprinting is preferred in scaffolds used for the localization of regenerative cells in large defect areas such as bone fractures. RP is preferred in these scenarios because of its ability to produce accurate three-dimensional scaffolds.

A series of cross-sections of a complex defect is generated by using CAD (3D Computer-Aided Model) and keyed down from the bottom up (Woodfield et al. 2009). RP is advantageous in forming biomimetic structures having more control over parameters like interconnectivity, shape, geometry, and branching design of a scaffold. The major challenge of using RP for scaffolds in tissue engineering is finding the right type of immunocompetent scaffold material that has a rapid setting property which is required for layer deposits. The current RP techniques also suffer from limitations in the resolution at which these scaffolds could be formed (Subia and Kundu 2010).

4.3.2.2 Two-Photon Absorption

Two-photon absorption (2PA) is a form of microstereolithography (MTSL). The MTSL system uses a photo-activated polymer that solidifies light absorption at specific wavelengths. In MTSL, the light waves are condensed by using multiple lens components to produce an ultra-low diameter light beam which increases the resolution of the printed scaffold structure (Kang and Lee 2004; Low et al. 2017).

Though MTSL can achieve micrometer-level resolution in the nanoprinted scaffolds, it has an inherent flaw. The liquid photo-activated polymer produces refraction of the condensed light beam causing polymerization or partial polymerization of the liquid polymer around the point coordinate. This is addressed by

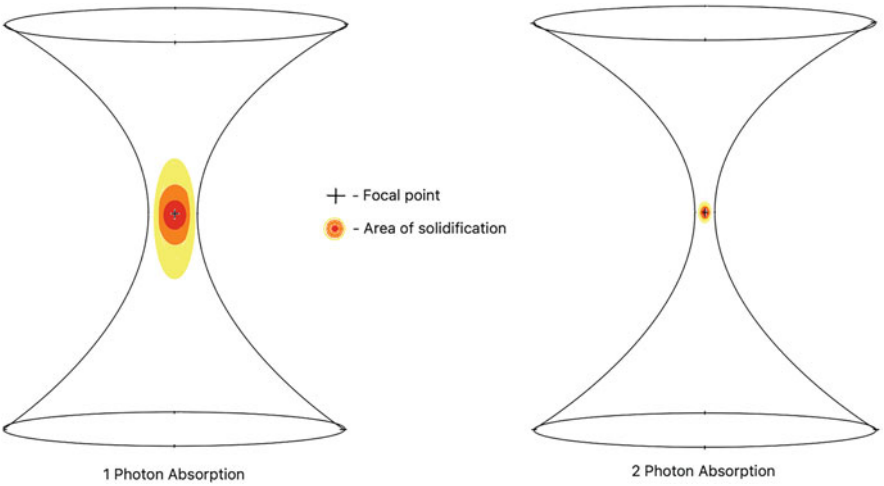

Fig. 4.6 Difference in resolution of 2 Photon Absorption and 1 Photon absorption 3D printing

2PA-based laser-based printing, which works on the principle of 2PP (2 photon polymerization) (Liska et al. 2007). In this case, polymerization requires added up energy from 2 photons absorbed by a molecule within a time frame of a few femtoseconds. For example, a photoinitiator requires a 1000 nm wavelength to polymerize and absorb two photons of a 500 nm laser simultaneously within a few femtoseconds. Any refracted laser around a point coordinate will always be below this total required wavelength which prevents polymerization, increasing the resolution of this technique to submicron levels (Xing et al. 2007) (Fig. 4.6).

4.3.2.3 Controlled Electrospinning

Electrospinning is a technique for 3D polymer synthesis that was proposed in 1934 (Huang et al. 2003). Controlled electrospinning uses a viscoelastic polymer solution to produce fibers collected in a specially designed container. The deposition process of the polymer is controlled and guided by a high-magnitude electric field generated between the solution outlet and the collection container guide (Boland et al. 2004). Fibers of a wide diameter range and thickness could be produced by varying parameters associated with the solution, device, and collection. Parameters like pH, concentration, and solvent could be used at the solution level.

Similarly, the device parameters such as distance, electric field, and nozzle could be varied. The collection could be by a stationary plate collection or a rotating mandrel. The main drawback of this method is that there is little control of the shape of the finished scaffold; it is more of a non-woven mat of randomly oriented fibers (Lee 2015) (Fig. 4.7).

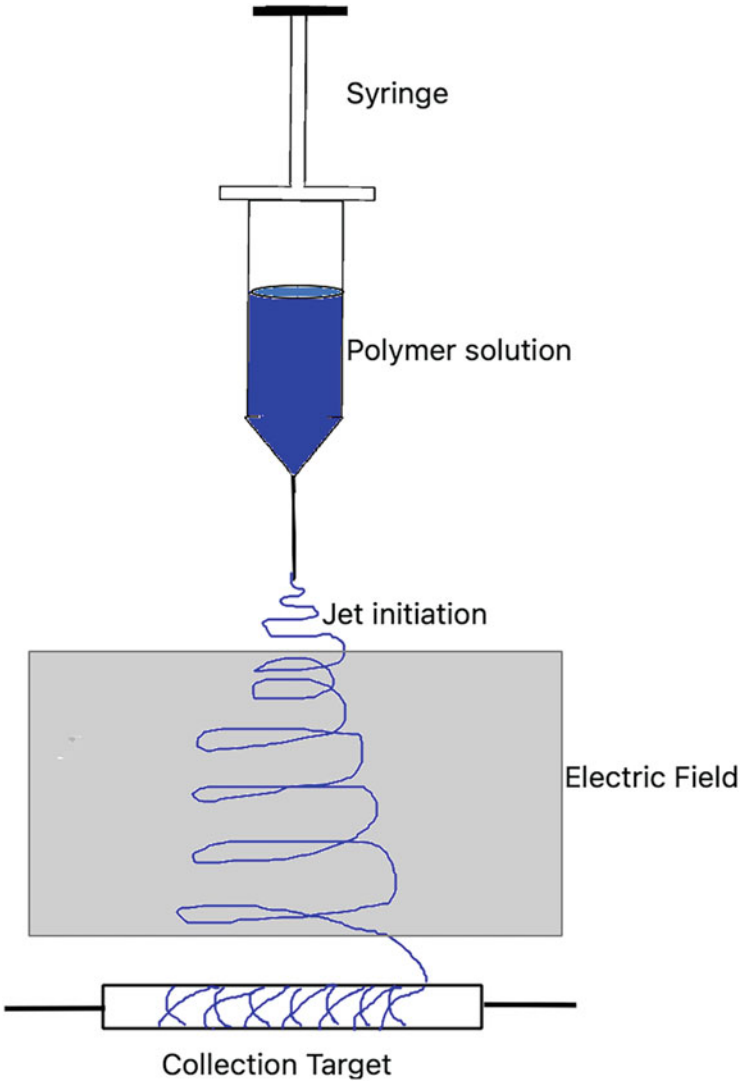

Fig. 4.7 Schematic representation of controlled electrospinning process of 3D printing

4.3.2.4 Charged Aerosol Jet

Aerosol jet nanoprinting is used for direct printing of 3D arrays of flexible high-resolution nanostructures. This novel technique uses dry atmosphere aerosol particles condensed through a di-electric mask to be deposited on a biased substrate made of silica. A nanoscale lens is formed by the ions that accumulate around the mask's holes, which focuses on the nanoscale aerosol jets. Complex 3D structures get printed by moving the silica substrate (Fig. 4.8).

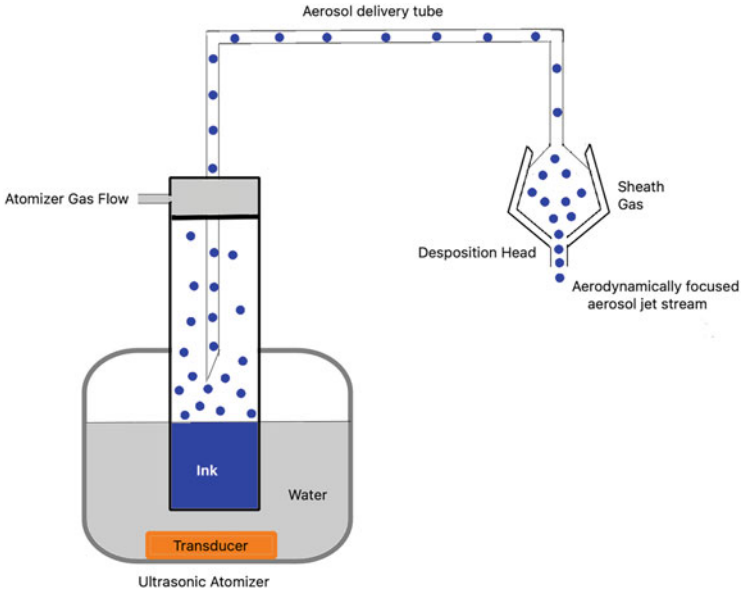

Fig. 4.8 Schematic representation of charged aerosol jet 3D printing

4.4 Advances in Nanoprinting of Cells, Tissues, and Organs

3D and 4D nanoprinting are particularly appealing for tissue engineering product creation since it allows for exceptional flexibility across the design and construction of the scaffolds. Cell type and density are crucial considerations in the nanoprinting of living cells (Maisonneuve et al. 2013). Long-term studies of cell viability and proliferation in vitro and in vivo are essential to understanding construct maturation and efficient tissue regeneration and integration. Prior to nano-bioprinting, the cellular expansion pattern of the developing tissue substitute must be optimized to give the required amount of cells inside the constructs, and attention must be given to the appropriate cell movement capabilities and adhesion features. The bioink employed for printing is one of the most significant components of 3D bioprinting, and it should be highly biocompatible and mechanically durable. During printing, it must give high resolution to incorporate live cells after nanoprinting (Gopinathan and Noh 2018; Gillispie et al. 2020). A study demonstrated that the physicochemical properties of plant-based natural gums in the form of 3D nanoprinted constructs offer consistency with the native ECM in 3D cell culture and can be used for tissue regeneration of various tissues such as skin, retinal, bone, cartilage, neural, and other tissues (Mohammadinejad et al. 2020).

4.4.1 Skin

Skin grafting and commercially available skin substitutes are two existing alternatives for managing severe skin wounds. Skin grafting is still plagued by a lack of donor tissue morbidity, while artificially engineered skin replacements prevent replicating the complex natural milieu essential for wound healing. The application of 3D nanoprinted dermal scaffolds as a therapy alternative for patients with critical skin wounds has appeared as a unique therapy that could address these issues (Singh et al. 2016). In several studies, artificial skin substitutes comprising natural or synthetic nanopolymers or nanofibers have improved efficacy for the wound healing process (Croitoru et al. 2020). Similarly, electrospun mats, 3D nanoprinted scaffolds, nanopolymer films, hydrogels, and several other types of dressing architecture impact biodegradability, mechanical qualities, air diffusion, and wound healing processes (Patil et al. 2016; Tamay et al. 2019; Chung et al. 2020). Lee et al. recently established a robust bioink that sustains printed cells' purpose of building up ECM components by fabricating and characterizing the biocompatible material, porcine skin powder (PSP)-ink 3D-construct via a decellularization approach (Lee et al. 2020). A recent study has reported that the choice of dressing architecture and polymer compositions employed in 3D printed scaffolds contribute to a more favorable healing outcome in wounds treated, including improved angiogenesis (Nun et al. 2020). Burn masks are an excellent treatment for hypertrophic scarring, a common consequence of skin burns. Akiki et al. have developed a unique burn mask that employs a 3D-printed positive mold to circumvent the arduous and uncomfortable process of directly placing alginate onto burned patient skin, which is especially difficult for younger patients (Akiki et al. 2020).

4.4.2 Bone and Cartilage

Bone is a mineralized tissue that is hard and stiff and has high mechanical strength, while cartilage is soft, flexible, and elastic, and their physiologies differ based on the anatomical positions. Furthermore, mesenchymal stem cells (MSCs) obtained from bone marrow or adipose tissue, and human umbilical vein endothelial cells (HUVECs) are the most often utilized cells in bone tissue engineering applications (Cui et al. 2016; Cunniffe et al. 2017). Current research focuses on printing cells and scaffolds together with various nano-biocomposites (collagen, agarose, alginate, gelatin, gelatin methacryloyl (GelMA), methacrylate hyaluronic acid (HAMA), and PEG dimethacrylate (PEGDMA) hydrogels) employed as bioinks to enhance the biomechanical features of the printed constructs (Bendtsen et al. 2017; Keriquel et al. 2017). Despite the noted benefit of electrospun nanofibrous scaffolds (chitosan, gelatin, collagen, hydroxyapatite, silk fibroin, and thermoplastic polyurethane) for bone, cartilage regeneration, the topography and architecture of these constructs still pose considerable obstacles to replicate the native ECM successfully.

In vivo regeneration may be limited by applying cell-free approaches in nanoprinting technology. Regardless, several clinics utilize cell-free implants for

bone and cartilage regeneration. Numerous studies have detailed the use of cell-free biocomposites such as hydroxyapatite (HAP), tricalcium phosphate (TCP), and Poly (ε-caprolactone) (PCL) in the fabrication of 3D printed substitutes for bone tissue engineering (Wang et al. 2016; Lee et al. 2016). Similarly, numerous growth factors, including FDA-approved bone morphogenic protein-2 (BMP-2) and others, have been utilized successfully in several clinical studies to aid nano-bioprinting of bone tissue constructions during bioink formation (Park et al. 2015; Huang et al. 2018). Bone regeneration has prospered with regulated patterns and biomimetic structures using 3D and 4D nanoprinted technologies, which have enhanced cell survival, proliferation, homing, osteogenic differentiation, vascularization, and host-specific integration (Qasim et al. 2019). Thus, integrating medical imaging, computational modeling, well-defined configuration, and 3D, 4D nanoprinting in tissue engineering is a leap into making structurally robust and functioning bone and cartilage replacements more efficient.

4.4.3 Retina

Although retinal degeneration has been a widespread problem, research is much more demanding since the retina comprises many delicate neural structures and layers. Shi et al. successfully created a 3D retinal pigment epithelial cell (RPE), and photoreceptors construct, simulating the functional interaction required for optimal vision (Shi et al. 2017a). They printed an ultrathin layer of ARPE-19 cells on a prepared membrane using an alginate/pluronic hybrid solution and added it to Y79 photoreceptor cells. While the technique currently needs an ultrathin membrane layer to print the RPE layer, the printing procedure did not affect the overall shape of the cells, and these cells were capable of continued growth. This hybrid 3D bioprinting method can facilitate research into these retinal complexes by adding intercellular interconnections. Likewise, Kim et al. developed polyethylene glycol (PEG)/Gellan Gum (GeG) hydrogels for retinal regeneration, and the impact of PEG levels on ARPE-19 cell adhesion and proliferation in PEG/GeG hydrogels were studied (Kim et al. 2019). Compared to just GeG hydrogels, PEG/GeG hydrogels exhibited better biocompatibility in cellular adhesion, proliferation and a favorable effect on retinal pigment epithelium (RPE)-specific gene expression. Another major issue in treating eye illnesses is the drug's short residence term, which occurs due to the eye's inability to accept more liquids, which is a critical challenge in treating eye disorders. Hence, to overcome this limitation, Gottel et al. established a novel ocular drug delivery method based on electrospun gellan gum/pullulan nanofibers administered in dry form and instantly creating a gel upon dosing (Göttel et al. 2020). Moreover, the retina and cornea are the primary targets of current research into 3D bioprinting of ocular models for future ocular tissue regeneration. The area of nanoprinting materials for ocular research is relatively emerging, where effective 3D printing of retinal and corneal cells has shown high cell survival. However, the overall efficiency and cellular density of the generated tissue remain modest.

4.4.4 Neural

Nerve regeneration is an intricate biological event where minor damage may repair itself, while severe damages necessitate effective surgical intervention. The field of 3D nano-bioprinting now emphasizes in vitro and in vivo modeling of neurodegenerative disease and the reparation of neural tissues. In nerve regeneration studies, primarily neural stem cells (NSCs) such as glial cells, Schwann cells (SCs), astrocytes, retinal ganglion cells (RGCs), and bone marrow stem cells (BMSCs) are integrated into printed constructs. Gu et al. established a novel 3D neural mini-tissue construct by micro-extrusion bioprinting of NSCs embedded in alginate, carboxymethyl chitosan, and agarose polysaccharide hydrogel that led to improved synaptic contacts, networks, and an increased calcium response (Gu et al. 2016). A recent study emphasizes optimizing methacrylated hyaluronic acid 3D bioprinting to develop in vitro testbeds for neural repair processes incorporating regeneration-promoting growth factors (Ngo et al. 2020). Several methods have been explored for major nerve repair, including peripheral nerve regeneration and spinal cord repair. Koffler et al. created complex 3D biomimetic CNS scaffolds out of polyethylene glycol-gelatin-methacrylate (PEG-GelMa) and engrafted them into spinal cord-derived cells, which lead to extended axons into the host spinal cord, restored synaptic transmission, and improved electrophysiological and functional outcomes (Koffler et al. 2019). These recent findings demonstrate that by combining ideally designed stem cells, scaffolds, and growth factors, the hostile environment of spinal cord damage may alleviate and restore a state of neural regeneration in the spinal cord. Based on the unique mechanical strength, unique surface chemistry, and desirable electrical conductivity, which have additional benefits in the field of nerve tissue engineering, carbon nano-biomaterials such as graphene and carbon nanotubes (CNT) have drawn interest over the years as tools to investigate and control the fate of neural cells (Ahadian et al. 2016).

4.5 Major Challenges Influencing the Bio-nanoprinting for Tissue Engineering

In the field of tissue engineering, creating patient-specific scaffolds is one of the most challenging tasks. The scaffolds that comprise a complex hierarchy of natural tissues are insufficient for tissue engineering applications. This problem is overcome by 3D bio-nanoprinting (Tamay et al. 2019).

3D printing techniques and their associated printing methods are impeded by the presence of a limited number of biocompatible materials, which restricts the number of usable thermoplastic materials (Chia and Wu 2015; Colasante et al. 2016). One such printing technique called Near-Field Electrospinning (NFES) requires the dipping of the probe tip into the polymer solution, which hampers the uninterrupted large-scale production of nano- and microfibers. Similarly, hydrogels derived from alginate are mechanically stiff and therefore do not lend themselves well to 3D bioprinting (Du 2018).

4.5.1 Factors Influencing Bio-nanoprinting for Tissue Engineering

Tissue regeneration through tissue engineering relies heavily on the extracellular matrix (ECM), which provides a three-dimensional matrix of various cell types and signaling molecules to bind and form a definite order (Marchand et al. 2019).

Fused deposition modeling (FDM) forms a bio-product whose resolution can be influenced by many factors, including print speed, number of layers, the distance between layers, the angle between adjacent fibers, and nozzle diameter (Yuan et al. 2017). It is challenging to find biocompatible materials that have the desired melt viscosity and are both low enough for extrusion and high enough for depositing simultaneously (Chia and Wu 2015). In 3D printing techniques, the distance between the tip of the nozzle and the collector plays a significant role in accurate fiber deposition. While the short space between the tip of the nozzle and the collector permits precise fiber deposition, it restricts the stretching and thinning of fibers resulting in the deposition of fibers with a thicker diameter (He et al. 2017). According to some research, this problem can be rectified by initiating slight modifications in spinning voltage and distance, solution concentrations, and collector speed (Tamay et al. 2019).

There are mainly five factors that influence and control the processes of 4D printing, namely, (1) type of stimulus, (2) kind of responsive material, (3) type of additive manufacturing process, (4) mechanism of interaction between material and stimulus, and (5) mathematical modeling of the transformation of biomaterials. Responsive biomaterials are crucial in 4D bioprinting since it is the biomaterial providing the fourth dimension that makes 3D bioprinting into a process that can be used to produce 4D bioprints in tissue engineering. The stiffness and elasticity of biological substrates, such as hydrogels, can significantly impact cell fate and differentiation (Engler et al. 2006).

4.6 Future Perspectives

Until now, it has been unclear how cell phenotype and gene expression alters in response to growing cells on surfaces that contain nanotopographic features. However, the advancement of nano-bioprinting technology holds the potential to transform the biological sciences arena by creating scaffolds/grafts for organ transplantation, drug screening, and regenerative medicine applications. By using nanoprinting methods, an accurate 3D groove imitating the structure of the ECM can be generated, and its features assist in investigating undiscovered cellular conducts, including adhesion, proliferation, differentiation, and regeneration. As it stands, researchers are still involved in developing novel nanocomposite materials and biological equipment for the use of 3D and 4D printing techniques. Biomimetic materials, particularly those designed to react to stimuli and programmable computer-based design methods, are often expensive. Thus, additional research is anticipated to resolve the current difficulties before 4D printing can be used to create

dynamic and hierarchical structures of natural tissues or organs, which would be groundbreaking.

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Strategies to Improve Delivery of Bioactive Agents

5

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Abstract

The delivery of bioactive agents/drugs in disease therapy and its improper disposal is one of the major causes of the constant rise in pollution levels. The majority of these factors depend upon alternative approaches and the development of new and beneficial technologies to combat the therapeutic complexities associated with improper drug use. The problem associated with drug-mediated disease therapy not only focuses on their improper disposal but also suffers from major deficiencies, the major one being the nontargeted impact of bioactive agents on the other organs causing diverse side effects. At the same time, the easy and quick removal of the bioactive agents with lower retention in the circulation hampers their therapeutic value. The emergence of nanotechnology and its vast progress has provided better opportunities to cope with the abovementioned problems, specifically in the case of the delivery of bioactive agents. In this chapter, we tried to briefly discuss the different strategies that could be employed to improve the efficiency of nanoparticles as carriers for the delivery of bioactive agents/drugs. This study demonstrates that nanoparticles can be employed in the field of nanomedicine by manipulating and improving their physical and chemical parameters, which makes them suitable for delivering the bioactive agents to their target. The proper choice of the nanoparticle surface

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properties and its modification with specific molecules like ligands, PEG, lipids, aptamers, etc. are of great concern to promote specific cellular internalization and localization, stability, immune clearance escape, and greater retention time, thus improving the delivery of bioactive agents.

Keywords

Nanotechnology · Drug delivery system · Nanomedicine · Therapeutics

5.1 Introduction

With the rapid increment in global population and economic development, the release of environmental contaminants is becoming a matter of concern. The planning and successful execution of different remedial techniques may be considered a promising challenge for a healthy environment. The proficiency of conventional methods to remove the environmental pollutants or contaminants is insufficient to stop their harmful effect. The classical way of energy resource uses, i.e., combustion of fossil fuel, coal, etc., is responsible to release greenhouse gases which are playing a causal role in global warming and climate change (Rani et al. 2018). Recent advancement in the area of nanotechnology and nanomaterials unwraps the opportunities to tackle these environmental and energy problems and disease therapy. Nanotechnology is the restructuration and application of matter on the nanoscale in various fields such as industrial, environmental, and energy conservation, pharmaceutical, and biomedical science, tissue engineering, and food and agro-industries. The use of nanoscience in the environmental field includes waste management, bioremediation, biosensor, and energy saving. With the advancement in this field, the key focus should include sustainable development, safe designing of nanomaterials with potential benefits, and promotion (Pathakoti et al. 2018).

Nanotechnology draws much attention due to its significant potency in the field of medicine and healthcare, including drug delivery systems, imaging techniques, and diagnostic tools, and also has leading edge treatments option for several diseases including cancer, cardiovascular diseases, microbial infections, diabetes, and neurodegenerative diseases (Sim and Wong 2021). The application of nanotechnology in the field of health science enabled more accurate and sustained drug delivery resulting in increased functional efficiency with minimal side effects. The progression in the field of nano-biotechnology enabled us to modify nanoparticles with desired attributes to bypass the major constraints for the successful treatment of several diseases including cancer. Stability, longer half-life, permeability, and intense bioavailability are the prime features to be kept in consideration during drug designing with the higher therapeutic efficiency. Composition, smaller size, surface chemistry, and high surface-to-volume ratio of nanomaterials draw much attention to develop nanomedicines with high pharmacokinetics, pharmacodynamics, and therapeutic index. Evolution in the field of nanomaterials enabled us to address the difficulties of target-specific drug delivery and their sustained release.

Here we try to summarize the different strategies used to improve the efficiency of nanoparticles emphasizing their therapeutic use.

A high accumulation of nanomedicines is crucial for cancer therapy. Neoplastic cell-induced angiogenesis is connected with aberrant blood vessel development comprising pores and fenestrations. Angiogenic blood vessels along with malfunctioned drainage systems induce the accumulation of extravasated nanomedicines within tumors. Although the permeability and accumulation of nanoparticles in the tumor cells mainly depend on the tumor type and disease stage. So, the designing of nanomedicine for cancer therapy requires the strategic development and modification of nanomedicine with enhanced permeability and retention (EPR) effects (Villaverde and Baeza 2019).

The size and shape are two main attributes of nanomaterial determining the acceleration of EPR effects, as the too small (<5 nm) or too large (>200 nm) nanoparticles are cleared by the renal system and hampered extravasation from aberrant vasculature system in tumors, respectively. The non-spherical nanorods have greater frictional resistance than the spherical ones with a similar diameters. This characteristic promotes the prolonged interaction between nanoparticles and vessel walls and therefore their extravasation. Non-spherical nanoparticles ranging between 12 and 60 nm generally have higher EPR effects (Stylianopoulos and Jain 2015). The size of the nanoparticles also influences the circulatory half-life as larger particles have smaller half-life as they are readily removed by the liver and phagocytic cells.

So, size and surface and surface structures are the key characteristics of nanodrug that influence the EPR effects mediated passive targeting (Mattheolabakis et al. 2012). The passive targeting of nanomedicine often lacks the specificity, consistency, and desired efficacy level at the target site. To overcome this defectiveness of passive targeting, nanoparticles are conjugated with specific ligand molecules having a greater expression of their corresponding receptor at the target sites. The active targeting of nanoparticles involves a vast range of molecules including aptamers, peptides, antibodies, proteins, etc. (Mattheolabakis et al. 2012). A set of unique chemical and physical properties make nanoparticle-mediated therapeutics smarter than traditional therapy. Size, shape, solubility, biocompatibility, surface, and optical properties made nanoparticles efficient and target molecule in diverse fields of biomedicine including imaging, diagnostics, novel drug formulation, cancer treatment, and tissue engineering.

5.2 Strategies for Improving Delivery of Bioactive Agents

As alternatives to the commercial strategies commonly employed for the delivery of bioactive agents, recently nanomaterials have gained wide attention as efficient carriers of bioactive agents. With the advancement of nanocarriers, several strategies have been employed in recent times to combat their insufficiencies and further improve their quality to ease the delivery of the bioactive agents aided by these nanocarriers. A promising and highly efficient strategy in this approach is the photocatalysis-based technique to improve the quality of these nanoparticles.

Several nanoparticles composed of different metal oxides are employed on a large scale as favorable agents of semiconductor photocatalysis. Semiconductor photocatalysis mainly relies upon solar energy to carry out redox reactions over its surface, which in turn is employed as potential photocatalytic agents (Yang et al. 2017). The unique feature of the semiconductor is mainly attributed to its surface electronic configuration where the gap between the valence electronic band and the conductance electronic band allows it to act as a potential photoactive agent. This, upon incidence of the photon by sunlight, excites and is followed by charge separation to play a major role as a photocatalytic agent (Shand and Anderson 2013). This property improves the efficiency of nanoparticles as carriers of bioactive agents.

Another potential strategy employed for improvement in the delivery rate of potential bioactive agents is the reduction in the size of the nanocarrier. The reduced size of the carrier molecule provides a greater surface area to accommodate more bioactive agents on the surface of the nanoparticle. The employment of several oxide-based nanoparticles in this regard as the carrier of bioactive agents is preferable; however, these delivery agents suffer from minimum surface defects, which could greatly hamper the desired surface characteristics as required for the nanocarrier. Thus, these photocatalysis-based modified nanoparticles are superiorly well established in their performance for the delivery of bioactive agents to the target sites. Treatment of the nanocarriers with elevated temperature for some period serves as a beneficial strategy to reduce the surface deformities while at the same time, facing major drawbacks such as reversion of phase from anatase and getting converted to rutile. This reversion of phase from anatase to rutile however greatly hampers the surface area of the nanoparticle, thereby ceasing the rate of the delivery of bioactive agents by these carriers. Hence, the nanocarriers must be chosen so that they are highly thermostable and do not easily undergo phase reversion. This provides greater surface area and quality performance as delivery agents. Metal ion doping is another alternative strategy for enhancing the thermal stability of nanocarriers owing to the binding of different groups such as oxide, carbon, and amine on the surface of the nanocarrier (Jing et al. 2013). These strategies of surface metal ion doping not only stabilize the surface to its anatase state but also prevent it from being rutile. These surface properties of the carrier serve efficient delivery of the bioactive agents to their target sites.

Several other strategies have been employed, which, despite the size, surface properties, surface charge, etc., promote better localization of the nanoparticle to the target sites and at the same time provide efficient delivery of the bioactive agents to their targets. One such strategy is the PEGylation of the nanoparticle used as a delivery agent (Jiang et al. 2017). In this strategy, the surface of the nanoparticles used as carrier molecules is coated with some types of polymers, viz. polyethylene glycol. The PEGylation of the nanoparticle forms a surface hydration layer, which acts as a sword of the nanocarrier and protects it from immune clearance (Tong and Kohane 2016). Employing nanoparticles with more elastic properties and asymmetric irregular shapes also provides improved delivery of bioactive agents by these nanocarriers as compared to the nonelastic and symmetrical ones. This is attributed to their greater ability to avoid immune clearance and greater retention in their target

sites owing to their properties and shape. Another important strategy to improve the delivery of bioactive agents by these nanoparticles mainly relies upon the surface composition of these nanocarriers. Nanoparticles with hydrophobic or hydrophilic surfaces are yet another major factor that greatly influences the delivery of bioactive agents mediated by these nanocarriers. Among them, nanoparticles with a hydrophobic surface are superior to those with the hydrophilic surfaces as it enables the nanoparticles to escape immune clearance and at the same time enhance their retention in circulation. The surface of the nanoparticle with engineered molecular ligands also provides a beneficial approach to retain the nanoparticle and hence improve the delivery of bioactive agents (Takizawa and Manz 2007). All these properties greatly contribute to the better delivery of the nanoparticles as carrier molecules and hence the delivery of the bioactive agents to their specific target sites.

5.3 Improvements in Nanoparticles for Enhanced Permeability and Retention (EPR) Effects for Delivery of Bioactive Agents

The cellular uptake of nanoparticles for delivering the bioactive agents is under the control of different physicochemical properties of the nanoparticles which greatly influence their uptake by the cell. However, several strategic modifications efficiently enhance the cellular internalization and localization of the nanoparticle-mediated delivery of bioactive agents, working upon several properties of nanoparticles like size, shape, surface composition, etc.

The use of nanoparticles as carriers of bioactive agents faces lots of challenges from being recognized as non-self to overcoming different types of barriers in order to reach their target sites. Several strategies have been employed to improve the nanoparticles to overcome these barriers, i.e., enhanced permeation and retention (EPR) effects which enhance the rate of delivery of bioactive agents mediated by these nanoparticles. These improved nanocarriers are also able to prevent the structural modifications and degradation of the bioactive agents, induced by the harsh conditions of the gastrointestinal tract, phagocytosis mediated by macrophages as well as delivery to nontargeted sites, and efflux out of the cell mediated by the efflux pump. At the same time, nanoparticles are also able to maintain the bioactivity and pharmacokinetics of the bioactive agents/drugs following its pre-adsorption to the target cell, uptake, and eventually bio-interaction with specific target molecules to exhibit their action (Azevedo et al. 2018).

5.3.1 Nanoparticle Improvements for Permeating Cell-Cell Barriers

The delivery of nanoparticle-mediated bioactive agents to the intracellular sites mainly follows either of the 2 pathways—paracellular or endocytic pathways. To cross over these paracellular pathways, the nanoparticles mainly regulate and open

the tight junctions (TJs), which keep the cells in tight contact with each other, forming a transcellular barrier. These transcellular barriers formed by the TJs restrict the nanoparticle from being transported inside the cells and hamper the delivery of the bioactive agents.

In order to overcome these transcellular barriers, one of the most vital strategies employed is the use of some high-molecular-weight permeation enhancers such as PPS (Dimethylpalmitoyl aminopropanesulfonate), lectin, chitosan, and polyacrylate. These permeation enhancers are linked over the nanoparticle surfaces and serve as the most suitable approach to keep the TJs reversibly open, and allow the cellular uptake of the nanoparticles, thereby crossing the transcellular barrier for delivering the bioactive agents. These enhancers are known to mediate their action by downregulating the associated proteins such as occludin and claudin, which are known to be one of the major components of TJs and also inhibiting them enzymatically. The enhancers such as polyacrylate linked over the surface of nanocarriers are also aided by strong binding with cations like Ca^{2+} ions and regulate the opening of the TJs to cross over the transcellular barrier.

Another strategy for overcoming the transcellular barrier is the use of nanoparticles coated with outer layers of thiomers such as thiolated anionic and cationic polymers. These thiolated nanocarriers are able to enhance their adhesion and retention on the mucosal layer of the cell surface. These thiol groups are then oxidized to disulfide bonds, linking the glycoprotein present over the mucosal surface to the nanoparticles. This, in turn, retains the nanoparticles carrying the bioactive agents in the mucosal layer. The reduced glutathione so formed by the thiomers on the nanoparticle surface also reduces the oxidized glutathione and inhibits the activity of the enzyme—protein tyrosine phosphatase (PTP) by binding to the active site of the enzyme with the aid of disulfide bonds so formed (Clausen et al. 2002). As the PTPs can no longer act, the phosphorylated condition of tyrosine residues in the TJs is maintained. This, in turn, keeps the TJs open and improves the nanocarrier to cross the transcellular barriers and enter the target cell (Chen et al. 2013). This is one of the effective strategies to improve the delivery of nanoparticle-mediated bioactive agents.

5.3.2 Nanoparticle Improvements to Overcome Blood–Brain Barrier (BBB)

Another physiological barrier that restrains the nanoparticles from the delivery of the bioactive agents is the blood-brain barrier (BBB). BBB is the cellular barrier that occurs in between the cerebrospinal compartment and the systemic circulatory system and hampers the movement of particles between them. This barrier also prevents the passage of drugs/chemicals to their target sites, owing to their inability to cross this barrier, which is aimed at the treatment of many physiological disorders and diseases, mainly tumors. One of the most vital and efficient strategies in the field of nanomedicine is the design of nanoparticles as carrier molecules and their

structural modification which can easily cross over these BBB and arrive at their respective target sites for delivery of bioactive agents.

The most vital approach to overcome this BBB is to design nanoparticles and coat its outer surface with surfactants. Target-specific peptides are also tethered over the surface of these nanocarriers to specifically interact with specialized molecules of the BBB and this interaction enables the nanoparticle to easily cross this barrier and reach their target sites to deliver the bioactive agents efficiently (von Roemeling et al. 2017). Many other strategies have emerged, such as the use of ultrasonic sound waves, whose frequency mechanically disrupts the BBB and enables the nanoparticle to cross the BBB and reach its target site (Carpentier et al. 2016). The use of ultrasound to aid nanoparticles to deliver the bioactive agents is one of the most inevitable improvements of nanocarriers. However, many improvements are still required to improve and enhance their functionality without hampering the cellular conditions.

5.3.3 Nanoparticle Improvements to Overcome Macrophage-Mediated Immune Clearance

One of the major drawbacks faced by nanoparticles in delivering the bioactive agents is their higher probability of facing immune clearance. Once the nanoparticles are released in the peripheral circulation, they are recognized as non-self, and several opsonins such as serum albumin and Ig are readily targeted to be adsorbed on their surfaces. These opsonins serve as molecular markers and are recognized by the receptors of the phagocytic cells as non-self/foreign and countenance immune clearance immediately by sequestering inside the phagocytes and reducing their circulation time (Jiang et al. 2017).

Some strategies have been employed to prevent their opsonization and sequestration by the phagocytic cells and in turn effectively enhance their circulation time in the peripheral blood; this helps the nanocarriers to reach and interact with their specific target cells and also effectively deliver the bioactive agents. One such strategy is the encapsulation of the nanoparticle surface with a coat of polyethylene glycol (PEG) polymers, i.e., PEGylation. This results in the formation of an outer hydration layer which interferes with the adsorption of opsonins over the outer surface of the nanoparticle. This in turn prevents the nanoparticle from being uptaken by the macrophage through serum absorption and thus the nanoparticle escapes immune clearance, successfully delivering the bioactive agents (Schöttler et al. 2016; Tong and Kohane 2016). Another important strategy to evade the immune clearance of nanoparticles as carriers of the bioactive agents is the modification or engineering of the surface of nanoparticle with specific molecular ligands like CD47, self-recognizing peptides, etc. which enables the nanoparticle to be recognized as self and hence serves as an alternate strategy to escape immune clearance and improve their circulation time. Primarily, the underlying basis of phagocytosis is by modulating the behavior of the cytoskeletal network system. The CD47 receptor over the surface of the nanoparticle interacts with the signal

recognition molecule on the macrophage and dendritic cell surface. This interaction in turn inhibits or suppresses the remodeling of the cytoskeleton and prevents the phagocytosis of the nanoparticle (Takizawa and Manz 2007). This strategy of artificially mimicking the phagocyte membrane is known as the biomimetic surface of nanocarrier which prevents their immune clearance and mediates better delivery of the bioactive agents to their target sites. Nanoparticles less than 6 nm in size are discharged by renal filtration but larger particles do not freely undergo renal filtration. Delivery of bioactive agents to the target sites in cancer patients, especially those with kidney or renal disorders or nephropathy, the problem of renal clearance of nanoparticles is mitigated due to renal insufficiencies (Schrier 2002). This follows that the size of the nanoparticle as a carrier of the bioactive agents must be chosen by keeping in mind their removal following glomerular filtration and excretion. All these strategies efficiently enhance the EPR effects of the nanoparticles and therefore improve the delivery of the bioactive agents to their target sites.

5.4 Strategic Improvement of Drug Delivery Systems

A drug delivery system is designed for the proper delivery and restricted release of drugs in the target site. The efficiency of the drug delivery systems depends on its size, structure, and surface property which affect the drug loading capacity and drug release capacity in the target location. Nanotechnology is widely used nowadays for controlled drug delivery systems. Inside the host body, the drug delivery system faces different challenges during the delivery of the therapeutic agents into the target sites. During transcellular movement, the tight junctions limit the movement of nanocarriers. To overcome this challenge, a few chemical surface modifications are required, which increase the hydrophilicity and allow these nanocarriers to pass through tight junctions during cellular uptake. Blood-brain barriers also disrupt the delivery of therapeutic agents in the brain by larger carriers. Small size nanocarriers coating with some suitable surfactant can be used to conquer this problem and to deliver drugs to central nervous systems (von Roemeling et al. 2017). In the circulatory system of body, there is also a chance for these nanocarriers to be engulfed by macrophages and cleared out by the body immune system. To avoid these circumstances, nanoparticles encapsulated in PEG can be used as a drug delivery system (Jiang et al. 2017). Improvement in the drug delivery system is essential to increase drug efficiency, loading capacity, longer retention and accumulation of drugs in a particular target location and to avoid unwanted toxicities in the nontarget sites of the body. Different nanoformulations for targeted delivery of bioactive agents have been shown in Fig. 5.1.

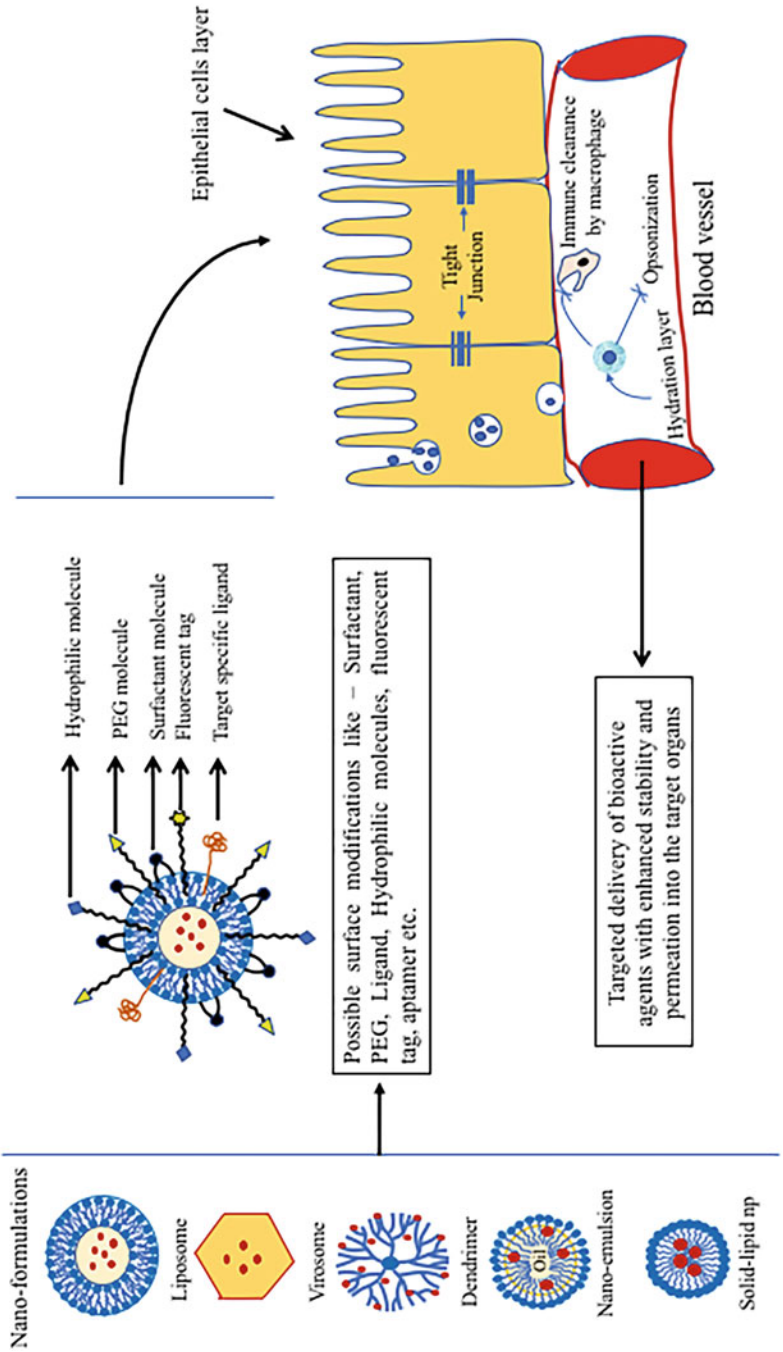

Fig. 5.1 Different types of nanoformulations and their surface modifications are required for transcellular movement, immune tolerance, stability, enhanced permeability, and targeted delivery of bioactive agents

5.4.1 Liposome-Based Drug Delivery

Liposomes are the most popular, versatile, and well-studied carrier for nanomedicine. Due to their capability to entrap both hydrophilic and lipophilic compounds, liposomes are used for a wide array of drug delivery, including small molecules, peptides, genes, and monoclonal antibodies. Biocompatible exterior surface and large aqueous central core of liposome favor the delivery of macromolecule (e.g., DNA, Protein) and molecular agents for imaging. The key characteristics of liposomes are depending on size, charge, surface modification, and lipid composition. Liposomal encapsulation protects the active drug agent from early inactivation and decomposition in the circulatory system. Liposomes lowered the toxicity of the encapsulated molecule through modification of pharmacokinetics and biodistribution to magnify the drug delivery in the targeted tissue. However, the main problem of liposomes as a drug carriers is their opsonization by macrophages and accelerated blood clarence through reticuloendothelial system (RES), therefore reducing its therapeutic efficacy. To overcome this limitation, sterically stabilized liposomes were developed which are more stable and have more circulation time in blood. To construct sterically stabilized liposomes, the hydrophilic polymer PEG is appropriate choice. The encapsulation of liposomes by such materials improves the efficacy of active agents (Saraf et al. 2020; Sercombe et al. 2015).

5.4.2 Virosome-Based Drug Delivery

The proper delivery of therapeutic agents in a controlled way in the target site is the key to success in different therapies like cancer treatment. To achieve this target, various drug delivery systems have been introduced in the medical field which can be classified as viral and nonviral drug delivery systems. Both these groups have some advantages and disadvantages over others. Viral-based drug carriers can transfer genes more efficiently than nonviral-based carriers but are not safe as they can leak viral genes and alter the host genome (Verma and Weitzman 2005). On other hand, nonviral based drug carriers are more safe than viral based but are not as efficient in drug delivery as viral ones. To overcome all these drawbacks a virosome-based drug delivery system was invented and introduced in medical therapies which comprise both viral and nonviral characteristics. Virosome has a cavity or hollow space at the core which is used to carry different therapeutic agents like protein and nucleic acids to the target site. The outer layer of the virosome comprises phospholipids bilayer on glycoprotein protruding viral surface and lacking viral genome which makes it safer for drug delivery. Different viruses like influenza virus and Sendai virus have been modified to use as virosome. Influenza-derived virosome is the most frequently used one; its outer surface layer has neuraminidase which helps in the virosome entry into the target cells. Virosome is prepared first by solubilizing the viral membrane using detergents and removing the nucleocapsids using ultracentrifugation, and finally lipid membrane is assembled on the outer layer (Stegmann et al. 1987). Using virosome drug delivery systems both hydrophilic and

hydrophobic bioactive agents can be delivered to the target sites. The sizes of virosomes are ranging between 150 and 500 nm which can be chosen depending on the desired target site and amount of loaded therapeutic agents. Sterilization of virosome can be done using membrane filtration before administration through different routes like oral, intramuscular, respiratory, and parenteral. Almost every type of bioactive agent can be delivered using virosomes. Their outer lipid bilayers prevent degradation and protect from the host body's defense mechanism. Virosome is biodegradable as well as biocompatible and it cannot replicate inside the host body, which makes it safe for use as drug carriers. Virosome is widely used in cancer immunotherapy, carrying tumor antigens and other therapeutic agents (Wiedermann et al. 2010). Besides all these advantages, virosome-based drug delivery systems have few drawbacks; e.g., they can stimulate the host immune defense system as their surface contains viral glycoprotein. Another drawback is that virosome degenerate easily in the circulatory system, which can be solved by making virosome more stable and making the drug delivery process quicker after administration in the host body (Liu et al. 2015).

5.4.3 Solid Lipid Nanoparticles Based Drug Delivery

Solid lipid nanoparticles as nanocarriers are gaining attention in drug delivery systems as they are considered nontoxic and biocompatible. Solid lipid nanoparticles are synthesized at high temperatures by immersing melted lipids in the water using a high-pressure homogenizer. Due to added surfactants (soy lecithin), fatty acids, and stabilizer (poloxamer), they remain solid at room temperature and are considered stable at body temperature, and are thus used to deliver therapeutic agents to the target site in the body. They provide stability to the loaded drugs and prevent the degradation of therapeutic agents while delivering to the target site efficiently (Schwarz et al. 1994). They have a high capacity to release the therapeutic agents in a controlled manner and penetrate cells easily (Basha et al. 2021). Solid lipid nanoparticles-based drug delivery is popular in the field of anticancer, antimicrobial treatment, and central nervous system disorder-related therapies. A few drawbacks of solid lipid nanoparticles are low loading capacity due to their small uniform crystalline structure (Lingayat et al. 2017).

5.4.4 Dendrimer-Based Drug Delivery

Nanomedicine is a promising field that uses nanosize engineered therapeutic agents and carriers to treat different diseases. Dendrimers are nanosized three-dimensional organic compounds having attached functional groups on the surface. Other properties of dendrimers, e.g., highly branched, water-soluble, and highly reactive, make them suitable to be used in several nanomedicine applications. The structure of dendrimers has a core molecule that affects their three-dimensional structure; interior branching also affects their morphology and internal voids to carry bioactive agents. They also have different functional groups on the surface which can be modified

depending on the target sites (Mintzer and Grinstaff 2011). Dendrimers can be hydrophilic as well as hydrophobic depending on their surface chemistry. Dendrimers are biocompatible and do not induce immune responses in the host body and show very less or no side effects. There are different types of dendrimers that can be manufactured by both divergent and convergent methods. In divergent methods the core is synthesized first, then the surface functional groups are synthesized from and around the core forming three-dimensional dendrimers. In convergent methods, the synthesis of dendrimers starts from the outer surface groups towards the core. There are other methods also like click chemistry for making carbon-rich dendrimers and double exponential growth methods (Arseneault et al. 2015; Kawaguchi et al. 1995). Due to their unique structural characteristics, dendrimers are used in various fields like photodynamic cancer therapies, magnetic resonance imaging, gene transfer, tissue engineering, and drug delivery. Dendrimers can carry both lipophilic and hydrophilic drugs. Drugs can be loaded inside the dendrimers by hydrogen bonds and also can be conjugated covalently with their surface functional groups. Polyamidoamine (PAMAM) and polypropyleneimine (PPI) are the mostly used dendrimers for drug delivery in the target site. Dendrimers increase the drug stability and retention time in the body and help in the controlled release of the drugs in the target site. Dendrimers are used as a carrier of different groups of drugs like non-steroidal anti-inflammatory drugs (NSAIDs) and anticancer drugs through transdermal, oral, and pulmonary routes. Due to the complex structure of dendrimers, they require multiple processing during their production which affects their large-scale production rate; to cope with this problem different techniques should be evaluated in the future (Nikzamir et al. 2021).

5.4.5 Nano-emulsion-Based Drug Delivery

Nano-emulsion carrier particulate size varies between 10 and 1000 nm and its surface is negatively charged and lipophilic. Nano-emulsion carriers are prepared by dispersing oil in water or water in oil in the presence of suitable surfactants or emulsifying agents. Nano-emulsion can be prepared by using both high-energy emulsifying methods, e.g., membrane and ultrasonic emulsification, high-pressure homogenization, and low-energy emulsifying methods, e.g., spontaneous emulsifications (Shakeel et al. 2008). Nano-emulsion-based drug delivery system is used in cancer treatment, enzyme replacement, and also in vaccination. It increases the stability of therapeutic agents and minimizes the toxic effects in nontarget sites. It increases the availability of oil soluble agents in the cell and tissue culture process and enhances the bioavailability of therapeutic agents in target sites (Jaiswal et al. 2015).

5.4.6 Mesoporous Silica Nanoparticles (MSNP) Based Drug Delivery

MSNP is gaining much interest in the field of medical biology, especially in drug delivery due to its various advantageous properties like larger surface area, uniform

pore size, and hollow structure. MSNP have a uniform porous structure made up of silica (SiO_2) and have larger pore volume which makes them ideal for high drug loading and delivery to target sites. The larger surface area of MSNP is enough stable and allows surface modification like adding different functional groups. MSNP is proved to be more versatile and advantageous than other types of drug delivery systems like liposomes, as they can carry the larger types of therapeutic agents in target sites (Tang et al. 2012). MSNP pores and surface can be modified by photocapping with AuNP and photocagging with o-nitrobenzyle for controlled delivery of drugs to the desired target site with higher efficiency. Light illuminations cleave the cationic linkers and release the photocage leaving the photocap of AuNP on pores of MSNP. Finally, the electrostatic repulsive force between MSNP and photocap opens the pore and leads to the release of loaded drugs. MSNP allows the co-delivery of two drugs at a time by using their both interior pore and external surface (Zhao et al. 2009). To improve the interaction between the therapeutic molecules and loading of various medications can be done in MSNP by attaching desired functional group to its pore surface. Loading capacity of hydrophobic amino acid was reported to increase by 10 times after modification of MSNP by different lengths of alkyl organic amines (Vallet-Regi et al. 2001). The manufacturing process of MSNP is also relatively simple and cost-effective, which is essential to accomplish future demands.

5.5 Conclusion

Delivering the bioactive agents to the target site without changing their actions and avoiding the unwanted toxic effects in the nontarget sites are major challenges in tissue engineering and other medical fields. Manufacturing and choosing a suitable carrier are very much essential to avoid wastage of all valuable therapeutic agents and other recourses and also to minimize their environmental contaminations. The proper functioning and stability of a carrier for bioactive agents inside the host body depend on its size, shape, and surface chemistry. Nanosize carriers to deliver bioactive agents are much more precise and stable than other conventional techniques. There are different nanocarriers having different properties mentioned in this chapter; those are liposome-based drug delivery systems, dendrimer-based drug delivery systems, nano-emulsion-based drug delivery systems, MSNP-based drug delivery systems, solid lipid nanoparticles based drug delivery systems, and virosome-based drug delivery system that are used nowadays to deliver different types of drugs to the target sites. All these nanocarriers can successfully deliver the bioactive agents to the target site in a controlled way, and also increase the functioning and stability of those agents inside the body. All these nanocarriers are biocompatible as well as biodegradable which shows very less or no toxicity in the body. Despite all these precious advantages, drug delivery systems need many modifications to improve a few drawbacks like drug loading capacity and proper release of the loaded drugs in a controlled way.

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Nanotechnology and Its Applications in Molecular Detection

6

Arikath Kirtana, Raziq Abdul, and S. Barathi

Abstract

Nanotechnology deals with the manipulation of single atoms and molecules to build devices of nano- or microscale to use for various purposes in several fields. Through its unique properties, it has unraveled an array of new opportunities in molecular diagnostics to explore. Using several particles and structures on the nanoscale exhibits different properties when compared to their bulk counterpart and such properties are appropriate to use in molecular detection. Lab-on-a-chip is an advanced technology that encodes nanodevices on a disposable chip which is very significant in biological detections. Gold on the nanoscale has appropriate properties that can be used for several biological processes. Similarly, magnetic nanoparticles react when a magnetic field is applied, which would have great applications in bio-separation. Other such technologies include quantum dot technology, nanobarcodes, and nanoparticle probes. Structures like nanowires are used in various ways to detect and separate the target analyte. Nanopore technology makes use of a pore (in nanoscale) in appropriate (Nanotechnology in molecular detection) substrates to detect anomalies in the target nucleic acid molecules. Cantilever arrays, nanosensors, and DNA nanomachines are also very useful technologies in biological detection. This field of study has a lot more to explore in the future.

Arikath Kirtana and Raziq Abdul contributed equally to this work.

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Keywords

Nanotechnology · Nanoparticles · Nanoparticle technologies · Molecular detection · Diagnostics · Nanoarrays

6.1 Nanotechnology and Molecular Detection: Importance of Nanotechnology in Molecular Detection

Nanotechnology is helping to improve many industries and technology sectors. The applications of nanotechnology include the detection of molecular disease markers, implant technology, molecular imaging, tissue engineering, and devices for drug, protein, and gene, and radionuclide delivery. By extending the limits of molecular diagnostics, nanotechnology has the potential to improve the performance of biological tests. Nanotechnology is being widely used in early disease detection, in vivo diagnosis, predictive medicine, and genomic technologies. Nanotechnology-on-a-chips are becoming more prevalent in diagnostic tests. They allow for faster, more sensitive, and flexible results. Magnetic nanoparticles can be used to label various molecules and organisms. They can also be used to detect a genetic sequence in a sample. For the investigation of nucleic acids, nanopore technology can be utilized to change over the strings of electrochemical nucleotides into electronic marks (Jain 2003). In this chapter, we have divided the major applications of nanotechnology in molecular detection into three main sections, nanotechnology on a chip, nanoparticle technologies, and other main inventions/technologies (Fig. 6.1).

6.2 Applications

6.2.1 Nanotechnology on a Chip

6.2.1.1 Microfluidic Chips for Nanolitre Volumes: Nanochip

A microfluid chip is a device that consists of multiple microchannels that are connected together to form a single cohesive unit. The microchannels are etched into materials such as glass, silicone, or polymer. These channels should be sealed tightly. The sample or liquid is injected into the chip via a syringe pump or a peristaltic pump. Microfluidic systems are used in various diagnostic procedures, nucleic acid detection, immunoassays, etc. Due to the development of processes like a deposition, electrodeposition, etching, bonding, injection molding, embossing, etc., the use of different materials (polymers, glass, silicon, etc.) for microfluidic chips has been possible. Microfluidic chips are widely used in many fields. The ability to integrate diverse medical tests on a single chip has proved to be useful in the biomedical field. These chips are also used in protein crystallization due to their

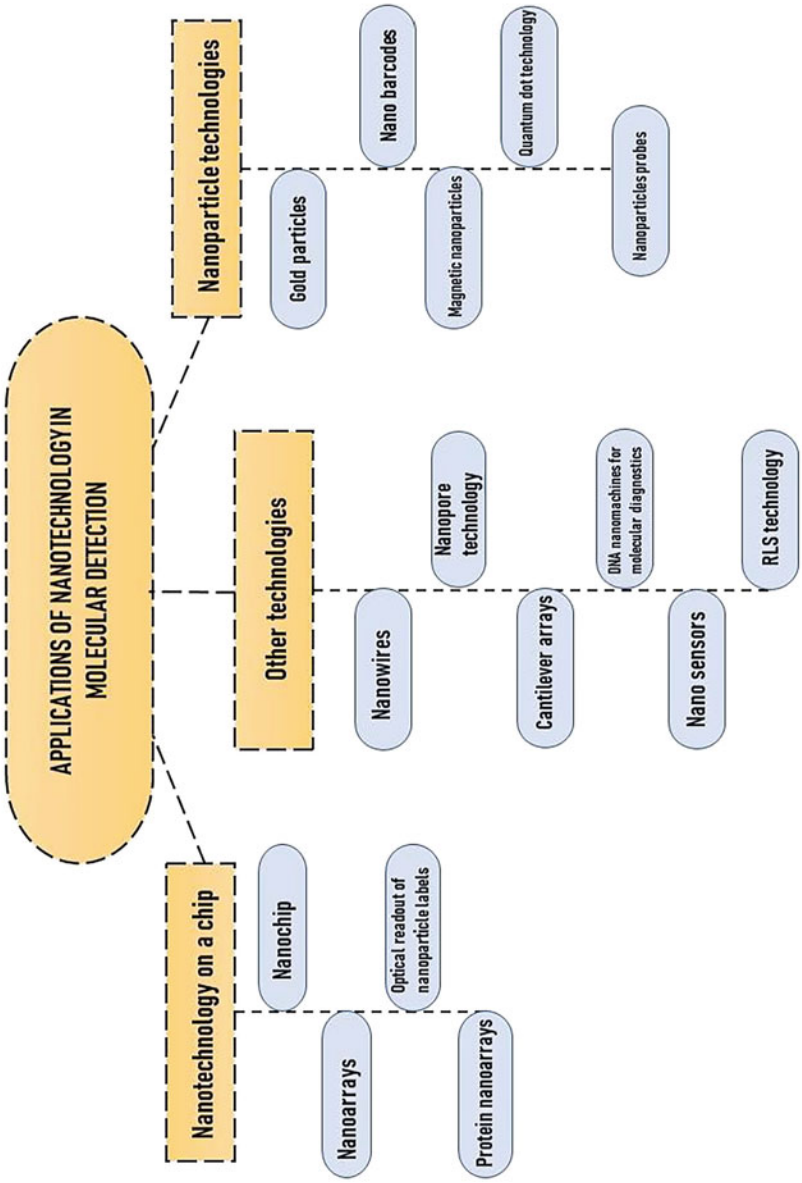

Fig. 6.1 Classification of applications of nanotechnology in molecular detection

ability to generate on a single chip a large number of crystallization conditions. As microchannels have the same characteristics as that of biological cells, it allows easy manipulation of single cells and rapid drug changes and thus have found their application in cell biology research.

The **NanoCHIP®** is a small, lab-on-a-chip silicon product that helps in the identification and analysis of DNA/RNA. NanoCHIP® (Nanogen, San Diego, CA) has integrated test sites for 100 hydrogel-coated electrodes containing streptavidin. Programming is performed on a biologically evaluated target-binding probe that moves to a specific electrode and remains bound to streptavidin when a positive charge is applied. The electric field also concentrates the target molecule on the electrode, accelerates hybridization, and removes the non-specific binding material. Fluorescence is used for final detection (Martel et al. 2005).

6.2.1.2 Optical Readout of Nanoparticle Labels

The study of molecular interactions with the help of optical readout of nanoparticles has emerged recently and it is essentially a parallel detection of DNA hybridization with the help of microarrays. The molecular interactions and binding events of DNA probes incubated with nanoparticle-labelled target DNA probes having a complementary sequence assembled on a glass chip are monitored optically using light. By counting on optical transmission and reflection, the process of detection is simplified to a great extent by stabilizing the samples. Using high light intensities for readout results in a much shorter detection time than using fluorescent dyes. Reanalysing the samples after months or years is possible due to the stabilization of the samples (Reichert et al. 2000).

6.2.1.3 Nanoarrays

Existing DNA microchips/microarrays use cumbersome detection tools, making sample amplification and labelling very difficult, increasing analysis costs, and slowing down the time to obtain results. By showing higher sensitivity and simpler methodology, nanoarrays have taken over microarrays. Sensitivity, specificity, speed, portability, throughput, and cost are advantages of using nanoarrays accorded due to their small size which accounts for a large surface-to-volume ratio. Nanoarrays have specific target-binding properties and are structurally sturdy and reduce the need for skilled manpower. A nanoarray is read using a Nanoreader, an atomic force microscope, which is needed because of the ultra-miniaturization of nanoarrays. The probe used in the microscope scans the surface, records the surface interactions and detects topography and chemical interactions. These nanoarrays are used in the field of diagnostics, proteomics and drug discovery by allowing large-scale screening of drug and molecular interactions. There are two main strategies for generating nanoarrays. First, one can change the properties of an area, such with the help of light to create different chemical functionalities. Second, chemical components can be locally deposited directly onto the surface, for example, by mechanical means such as Nanometre "pencil"—a strategy similar to "new generation lithography" (Nicolau et al. 2005). Carbon Nanotube Technology (CNT) is an ideal nanoelectrode array platform. Carbon Nanotube Technologies (CNTs) are

structurally equivalent to a two-dimensional sheet of graphene encased in a column-shaped carbon tube. It consists of a single-walled carbon nanotube or a multiwalled carbon nanotube and has high aspect ratios (the length of the microarray being many times greater than its width). CNTs being critical components of the nanodevices are incorporated either directly or indirectly during fabrication routes (Chen and LI 2007).

6.2.1.4 Protein Nanoarrays

A group of nanoscale protein domains on solid surfaces are protein nanoarrays and these are useful in ultra-miniaturized bioanalysis. The conception and program of protein arrays were presented in antibody arrays. The chip comprises a support surface such as glass, a nitrocellulose membrane, beads, and microtiter plates to which a chain of capture proteins are bound. A probe molecule labelled with a fluorescent dye is usually added to a matrix. The reaction occurring between the probe and the immobilized protein effuses a fluorescent signal which is scrutinized by a laser scanner. An aqueous environment is vitally important at all steps of chip manufacturing and functioning in order to inhibit protein denaturation. The sample buffer contains a high percentage of glycerol that helps to decrease the freezing point. The humidity of the production medium is also adjusted.

There are essentially three major types of protein microarrays—Analytical microarrays, Reverse phase protein microarray (RPPA), and functional microarrays. In Analytical microarrays, antibodies, aptamers, or affibody banks are placed on the surface of the holder. They are used to capture molecules as each molecule specifically binds to a specific protein. The array is probed with complex protein solutions such as cell lysates. Binding reactions obtained using an assortment of detection systems can be analysed to obtain information on the expression levels of specific proteins in a sample. Reverse (RPPA) arrays contain tissue lysates. Isolated cells from various tissues of concern are lysed and the lysate is set down on a microarray and probed with an antibody to the protein of interest. Chemiluminescence, Fluorescence, or colorimetry is used for detection. Control peptides are printed on slides to allow the quantification of proteins in sample lysates. In Functional microarrays, the target protein arrays are developed by immobilizing purified proteins. It determines the various interactions of proteins with DNA, RNA, phospholipids, other small molecules, and with other proteins too.

Researchers have fostered another kind of protein array for contemplating connections among proteins and different atoms on a nanoscale. The method used for the creation of the protein array is termed dip-pen lithography which uses an instrument to modify the gold film surface of the arrays. This method has proven to be highly sensitive and straightforward in the detection of DNA (Lee et al. 2002). A study done by Minsu Lee et al., using the dip-pen nanolithography (DPN) method, studied the single molecular nanopatterning and molecular interaction of proteins that were immobilized on the prolinker surface of gold-coated silicon wafer (Lee et al. 2002). Protein nanoarrays have been highly beneficial in proteomic and genetic screening, providing important leads for therapeutic agents in the pharmaceutical industry. Deviating from the mainstream applications of protein nanoarrays, a

protein-based light-harvesting system was established using protein nanoarrays that inspected the process of photosynthesis using enzyme-triggered protein nanosheets that resembled thylakoids (Zhao et al. 2017). The main advantage of using a protein nanoarray is that it has high throughput and can parallelly track a large number of proteins.

6.2.2 Nanoparticle Technology

6.2.2.1 Gold Particles

Introduction

Gold nanoparticles (AuNPs), also called colloidal gold, possess attributes like shape and size, large surface-to-volume ratio, multiple surface functionality, and low toxicity that make them widely compatible in several applications of bio-nanotechnology. AuNPs' unique surface chemistry and functionalization properties provide a versatile platform for nano-biological assemblies with several biological compounds like antibodies, proteins, and oligonucleotides, which makes AuNPs a promising candidate for molecular diagnostics. In diagnostics, AuNPs bind with the analytes which alters several physicochemical properties of the AuNPs like their resonance, conductance, and redox behaviour which can be studied to detect any particular analyte (Yeh et al. 2011).

Synthesis of AuNps

Generally, gold nanoparticles are suspended in a solvent, most often water, which gives it the name colloidal gold. A broad range of approaches has been developed in the past decades to control the size, shape, and functionality of AuNPs. Turkevich et al. developed an appropriate artificial method for synthesizing AuNPs, where citric acid was treated with hydrogen tetrachlorocuprate in boiling water. In this technique, citric acid has two important roles; it acts as a stabilizing agent and as a reducing agent and this redox reaction gives rise to AuNPS. This method was refined by altering the citrate-to-gold ratio to control particle size (Turkevich et al. 1951). This protocol was only feasible when the required product was a dilute solution of moderately stable AuNPs with a diameter of 10–20 nm. AuNPs synthesis with citrate stabilizing mechanism had a drawback—these AuNPs can undergo an irreversible aggregation with thiolate ligands during the functionalization process. Several strategies such as using a surfactant prior to the modification and using thiotic acid as an intermediate via two-step functionalization solved the problem of aggregation (Aslan and Perez-Luna 2002). Even after nullifying such drawbacks, this protocol was not effective when the product needs to be synthesized in large amounts because of the high dilution rates.

In 1994, Brust and Schriffin formulated an effective protocol to synthesize AuNPs by producing alkanethiol-stabilized AuNps through biphasic reduction with sodium borohydride ($NaBH_4$) as the reducing agent and tetraoctylammonium bromide (TOAB) as the transfer reagent. Low dispersive AuNPs of 1.5–5 nm were synthesized by this methodology and the size could be altered by altering gold-to-

thiol ratios. These alkanethiol-protected AuNPs were more stable when compared to citrate-stabilized AuNPs and this stability was due to the van der Waals attractions between the ligands and the synergic effect of the strong thiol-gold interactions (Yeh et al. 2011).

Properties of AuNPs

AuNPs synthesized for nano-biological purposes are generally spherical in shape. Spherical AuNPs possess several attributes: sufficient surface-to-volume ratio and improved biocompatibility and shape-related optoelectronic properties. Spherical AuNPs have the ability to quench fluorescence. They also exhibit the ability to emit a range of colours which is dependent on the size of the particle. The colours emitted range from brown, orange, and red to purple as the size of the particle increases from 1 to 100 nm and they have an absorption peak of 500–550 nm. This is called the "surface plasmon band" which indicates that the absorption band arises from the collective oscillation of conducting electrons due to the resonant excitation by the incident photons. This band is significant for nanoparticles because the band is absent in both small nanoparticles ($d < 2$ nm) and bulk materials. Other physical properties the except size like shape, solvent, surface ligand, temperature, and proximity of other particles contribute to the colour-emitting phenomenon.

Applications of AuNPs

Unique functionalization properties of gold nanoparticles form a versatile platform to form nano-biological assemblies with several biological compounds. These assemblies would alter the physicochemical properties of the nanoparticle, and these changes are detected to identify the analyte. This phenomenon makes AuNPs more effective in the field of molecular diagnostics. AuNPs also have applications in therapeutic drug delivery aided by the property of conjugation and applications in diagnostic imaging aided by its ability to quench fluorophores (Yeh et al. 2011).

6.2.2.2 Nanoparticle Probes

Nanoparticle probes are nanoparticles (generally gold) to which DNA is attached through a proprietary modification procedure. These probes help to signal the presence of the target DNA sequence. This technology makes use of the advantageous properties of the nanoparticle to which the DNA is attached. AuNPs are used in the technology because of their versatile functionalization property and their ability to emit different wavelengths of light when their sizes are varied. Technically this technology is an extension of AuNP (Jain 2003). Based on this extended application of AuNPs, several products have been developed for molecular detection, which includes:

- A method called nanosphere spot assay is used for colorimetric detection of amplified DNA sequences. The identification and differentiation of single nucleotide proteins (SNPs) are achieved through the specificity of nanoparticle probes.

- Gold nanoparticle probe assay is used for DNA target analysis. This technique eliminates the time-consuming process of amplification and hence proves to be cost-effective.

Nanoparticle probes are very advantageous in several other fields like detection of SNPs, infectious disease diagnostics and antibiotic-resistant bacterial infection diagnostics.

6.2.2.3 Nanobarcodes

Nanobarcodes are the miniature version of barcodes in terms of their applications. This technology was introduced by researchers at Nanoplex Technologies (CA, USA) who have produced submicrometric metallic barcodes using sequential electrochemical deposition of metal to produce stripping patterns. These adjacent stripes have differential reflectivity and this enables the identification of the unique stripping pattern by the light of fluorescent microscopy. The standout of this technology is that this procedure of readout mechanism does not interfere with the use of fluorescence for the detection of analytes bound to particles by affinity capture. With the technology being appropriate, Nanobarcodes are used for population diagnostics and in point-of-care handheld devices. This technology has a wide range of applications that are to be explored. However, SurroMed Inc. has attempted to use this technology to develop a phenotyping platform with access to a large clinical population. This attempt from the company shows us the potential of nanobarcodes and how they would enable the development of personalized medicine through bio-marker-based drug development (Jain 2003).

6.2.2.4 Magnetic Nanoparticles: Ferrofluid

Magnetic nanoparticles have a core made up of magnetic substances which are generally iron (Fe), cobalt (Co), manganese (Mn), or nickel (Ni). The core is surrounded by biocompatible polymer chains and this polymeric layer is coated with affinity molecules that have the ability to capture the biological targets (analytes) from blood or other fluid samples. The size of magnetic nanoparticles is generally around 25–100 nm and they behave in liquids as a solution, not as suspension. Magnetic nanoparticles have unique properties that support their use in molecular diagnostics, such properties include large surface-to-volume ratio, high saturation magnetization, and importantly they can be manipulated by magnetic fields (Khizar et al. 2020). Magnetic nanoparticles are synthesized in appropriately sized cores which can be altered along with diverse surface coatings; these coatings provide the functionalization to the nanoparticle. The organic or inorganic coating makes the nanoparticle biocompatible to target the biomolecule (analyte). The interesting property of magnetic nanoparticles is that they exhibit super magnetism when the size is reduced. Such magnetically labelled biomolecules can be isolated from their carrier fluid on application of a magnetic field. These properties make the magnetic nanoparticles appropriate for molecular detection. The nanoparticles which have captured the analyte from the bloodstream or any tissue system can be isolated by applying magnetic fields and can be detected in several ways. Magnetic

nanoparticle technology is used in several detection procedures like NMR and MRI in medical fields. A wide array of applications of magnetic nanoparticles in bio-nanotechnology is available in the fields of sensing, detection, cell sorting, gene/protein analysation, and microfluidic mixing; the unique properties of magnetic nanoparticles make them the right candidate for all such applications (Jain 2003).

6.2.2.5 Quantum Dot Technology

Quantum dots are generally nanocrystals made up of cadmium selenide coated with zinc sulphide and range from 2000 to 10,000 atoms wide in size. They are often referred to as tiny man-made semiconductor particles and their size normally does not exceed 10 nm. The extremely small size alters their optical and electronic properties which makes them different from the same material found in bulk. The frequency of the light emitted when irradiated with low-energy light is proportional to the size of the quantum dots. Quantum dots were observed to be unstable during their initial discovery but embedding the dots in the pores of latex beads solved the problem and made them more stable and ready for use. The majority of such nanoparticles can emit lights of specific wavelengths when excited with electricity or light; typically smaller dots emit shorter wavelengths generating colours like violet, blue or green. Bulk semiconductor materials exhibit the property of fluorescence but the quantum dots are scattered away from each other to create continuous conduction and valence bands. The fluorescence produced by the quantum dots can be readily controlled by changing their size during their synthesis, which makes their detection possible in any system. The emission wavelengths of quantum dots range from ultraviolet to infrared. Quantum dots also have high quantum yield, high photo stability, and high molar extinction coefficients. Recent studies also suggest that the fluorescent yield of quantum dots can be improved by building a "shell" of a larger band of semiconductor material around them. With their semiconductor-like properties, they have a wide application in photovoltaic devices and light-emitting devices. Their properties are also manipulated to use in bio-detection or bioimaging. In the generic process of bioimaging various types of organic dyes are used. However, these dyes suffer from low quantum yields and photostability. Quantum dots for their ability to have high quantum yields have been considered over the traditional dyes in many aspects. Quantum dots are found to be 100 times more stable and 20 times brighter than traditionally used fluorescent dyes. For bioimaging applications, the probes made of quantum dots in this case have to remain well dispersed and stable in the aqueous medium with extreme pH and ionic strength. In recent times, numerous techniques have been developed to make the quantum dots water-dispersible. Quantum dots are employed for *in vitro* and *in vivo* imaging, which in turn proves to be very important to diagnose many diseases and helps to understand embryogenesis and lymphocyte immunology (Jain 2003).

6.2.3 Other Nanoparticles

6.2.3.1 Nanowires

A nanowire is another type of nanostructure, with a diameter measuring in nanometres. Nanowires have similar structures to nanotubes, but they differ from nanotubes as they have different aspect ratios. The constraint that the nanowires follow is that their ratio of length to width is always larger than 1000. At such dimensions (like nanometre) quantum mechanical effects tend to be altered and are significant; for this reason, nanowires are also called quantum wires. Nanowires are manufactured by a variety of processes such as alternating current electrodeposition, chemical vapour deposition (CVD), and thermal evaporation (Mahbub and Hoque 2020). They also have high sensitivity and comparatively lower response times compared to other sensor systems. The properties of such structures when manufactured with non-toxic materials can serve a great purpose in bio-nanotechnology. Compared to their bulk counterpart, nanowires have significant electron transport properties and their charge carrier motions are improved (Rabbani et al. 2020). Nanowires are more efficient than nanotubes in several ways. One being the modifications in their design are controllable during the synthesis process to alter and manage operational parameters; another being many materials that are used to produce nanowires are compatible to open the cops for functionalization. For their property of small size and capability, they are appropriate for bio-detection of pathogens and biological chemical species. Wang and Joseph developed a biosensor to determine the existence of toxicants within the living cell where the sensor had optical fibers with nanowires covered with antibodies (Wang 2005). Cullum et al. synthesized nanowires over gold electrodes and coated ZnO for the detection of hydrazine by amperometric responses (Cullum et al. 2000).

6.2.3.2 Cantilever Arrays

Cantilevers are rectangular beams that are about 1 μm thick. These micromechanical cantilevers are functionalized with receptor molecules that adsorb or recognize molecules on their surface, which causes surface stress and bends the cantilevers. And a laser beam detects such nano bending of cantilevers. Their major advantage of such cantilever systems is that they can function in various environments like liquid, air, or vacuum. Such technology is utilized in molecular detection where silicon cantilevers are used to detect the analyte. In biological applications, the surface of each cantilever is coated with DNA which binds to the target sequence. These DNA-coated cantilevers are exposed to the sample and the surface stress bends the beam by some nanometres and this indicates the presence of the target in the sample. When these cantilevers are assembled in a microarray assembly, they have the ability to detect multiple unlabelled biomolecules simultaneously within minutes (Mckendry et al. 2002). The ligand-receptor interactions occurring on the microfabricated silicon beams lead to nano chemical bending and this is detected optically *in situ*. Differential measurements along with control cantilevers on an array of eight cantilevers can specifically detect DNA targets in 75-fold excess of non-matching DNA background. These are also used to investigate the

thermodynamic characteristic of the biomolecular interactions happening on the array. Because of such properties exhibited by the cantilever array system, they allow multiple binding assays in parallel and they have the ability to detect femtomoles of DNA at a background of a complex solution. One significant application of microcantilever is in the detection of prostate-specific antigen (PSA). The cantilevers are coated with PSA antibodies and are exposed to the sample. The bending due to surface stress is detected by the laser and this detection indicates the presence of PSA. The advantage of this detection technique includes the ability to detect PSA in clinically relevant concentrations in the background of other proteins and this technique is more cost-effective as it eliminates the requirement of labelling. This technique demonstrated lesser false positives which are caused due to irrelevant binding of foreign molecules to the PSA antibodies (lang et al. 2005). The development of such successful detection techniques using cantilever arrays attracts a lot of research to exploit the capacity of this technology. Rockville, Inc., MD, USA, is striving to develop a cantilever technology that would allow multiple tests to be performed. on a single disposable chip (Jain 2003).

6.2.3.3 DNA Nanomachines for Molecular Detection

One of the most advanced protocols for manipulating DNA has led to performing computational operations. A radio antenna is fixed to a specific DNA molecule and when the relevant radio frequency is captured by the antenna, the DNA molecule seems to be boosted with energy and as a result, the DNA molecule responds. The functional antenna attached to the DNA molecule is made up of a combination of metals and its size is around 100 atoms wide and 1 nm long. A radio signal sent to such a system of DNA attached to an antenna has been shown to cause dehybridization of the DNA strand (Hamad-Schifferli et al. 2002). The major advantage of this technique is that the switching process is reversible in certain conditions and it does not affect the neighbouring molecules without the antenna. This revolutionary demonstration opened a wide range of possibilities to explore. This technique should show promising results for proteins, peptides, or other biomolecules. In June 2001, this technology was licensed to EngeneOS, Inc. by the Institute of Technology (MIT, Cambridge) (Lee et al. 2002). Though the capacity of this technique is not exhausted yet, its applications in molecular detection are identified and they include biomolecular detectors and the direct electronic readout of biomolecular interactions.

6.2.3.4 Nanopore Technology

An advanced sequencing technique involving a nanopore is a traceable technique that enables real-time analysis of long DNA fragments without amplification. The basic principle of such a biosensing protocol involves the construction of a pore structure that is a few nanometres wide through a planar membrane that divides two chambers of saline solution. This setup is made in such a way that the only path between the two chambers is through the nanopore. When a relevant voltage is passed, ions from both the chambers move through the nanopore which thus creates a stable ionic circuit with an open nanopore. Generally, the transmembrane current is

of the order picoamperes (pA) to nanoampere (nA). In such a system, if we introduce a negatively charged DNA fragment to the chamber connected to the cathode, due to electrophoresis the DNA molecule tends to translocation from one chamber to another through the nanopore. While the translocation of DNA the transmembrane movement of the ions is decreased as the electrophoresis mobility of DNA is less than that of the ions. Due to this phenomenon, the transmembrane conductivity will be decreased and it produces a blockage signal which is recorded as the transmembrane current. This blockage signal helps us to detect the DNA molecule. In theory, each nucleotide has the ability to generate specific current blockage signals which could be detected and this detection could theoretically permit DNA or RNA sequencing. Agilent Laboratories is collaborating with Harvard University to develop such a technology (Jain 2003). The major advantage of nanopore sequencing is that it eliminates the need to amplify a DNA strand and instead can give out the desirable product even with a single DNA molecule. The thickness of Sin or SiO2 nanopore substrates is generally several tens of nanometres. But when a DNA strand travels through the nanopore, more than a single nucleotide is present in the nanopore which prevents the detection of each nucleotide and thus prevents sequencing. Graphene serves as an ideal substrate for single nucleotide resolution as it is a 2D nanomaterial with a thickness of a single atom. The current through a graphene field-effect transistor (FET) can be measured with the transmembrane ionic current. Radenovic et al. observed a change in carrier concentration of graphene nanoribbon due to the translocating DNA which thus led to spikes in drain current and transmembrane current of the graphene transistor. This technology can be exploited in biosensing and molecular detection. Nanopore technology has the capacity to distinguish and count a variety of specific biomolecules in a complex mixture. A biosensor has been designed that forms a DNA nanopore by covalently attaching one single DNA nucleotide to the lumen of α-haemolysin (α-HL) (Howorka et al. 2001). The binding of single-stranded DNA (ssDNA) to the tethered DNA strand causes a change in ionic signals. With this technique, we are able to discriminate between individual DNA strands up to 30 nucleotides in length (Shi and Fang 2018). This technology can also be applied to protein analysis. Nanopore technology's advantage of speed and simplicity has the potential to facilitate the development of molecular detection through nanotechnology.

6.2.3.5 Nanosensors

Nanosensors are high-sensitivity nanoscale biosensors that can convert physical quantities to detectable signals. Sensors play a main role in observing the unique changes of biomolecules that encode significant data that aids in interpreting basic biological processes. With an increasing number of studies in which intelligent nanosensors based on molecular markers are effectively applied in the pharmaceutical field, this superior technology has become a promising approach for pharmaceutical use. There are mechanical nanosensors and chemical nanosensors, both of which have different detection mechanisms. Currently, top-down lithography, bottom-up assembly, and molecular self-assembly are some of the ways used to make biosensors. Owing to their size-dependent physicochemical properties,

nanoscale materials have materialized as potential contenders for biosensor applications by providing unique information about real-time changes in crucial physiological parameters. Nanotubes, nanoprobes, nanoparticles, nanosensors having nanowires, cantilever involving nanosystems, and nano-electromechanical systems (NEMS) are a few different types of nanosensors (Shawon et al. 2020). Some of the advantages of nanobiosensors that make them useful and efficient in them detection of biomolecules include small size, high adsorption surface area and mobility of the particles, replicability of the particles, and greater surface-to-volume ratio, etc. The sensor can be electronically controlled in response to the binding of molecules. Prototype sensors have confirmed their ability to detect nucleic acids, proteins, and ions (Jain 2012). Fluorescence-based nanosensors, optode-based nanosensors, carbon-nanotube-based fluorescent nanosensors, and quantum dot-based fluorescent nanosensors are a few developments in the area of bio/nanosensors (Rong et al. 2019).

6.2.3.6 Resonance Light Scattering (RLS) Technology

Resonant light scattering (RLS) is elasticized scattering and occurs when the energy of the incident beam is immediate to the absorption band. First, we implemented RLS technology for studying biopolymers using traditional fluorescence spectroscopy. RLS has become a very interesting technique used to monitor molecular clusters and characterize chromophores. In recent years, RLS technology has been used to identify a variety of pharmaceutical and biological macromolecules such as nucleic acids, proteins, metal ions, and bacteria (Jiang et al. 2007). Incident wavelengths are particularly described in the absorption shell and amplified signals are being observed. Numerous RLS-derived approaches have been developed. The RLS technology has been used to study the aggregation of chromophores due to its simplicity and versatility. The observed RLS effect is an increase in the scattering intensity at the absorption wavelength of the aggregate molecule. If there is a strong electronic bond between the chromatophores, the effect can be improved many folds. RLS can benefit from particle size strategies established for current light scattering. DLS (Dynamic Light Scattering), a physics approach for determining the size distribution profile of tiny particles suspended in fluid or polymers in solution, measures the frequency spectrum of scattered light (Pasternack and Collings 1995).

6.3 Conclusion

With the advent of recent developments in the field of biotechnology, numerous fields of research have opened up new avenues for investigation. Nanotechnology is one such field and its applications in molecular detection can pave the way to identify, diagnose and treat diseases. In the near future, more advanced applications of nanotechnology could emerge facilitating a multitude of discoveries. The promise of non-invasive, increased sensitivity and specificity, small sample size and reduced

time highlights the vital role of this technology in nanodiagnostics and the field of scientific research.[1]

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Challenges and Future Prospect of Nanoparticles in Tissue Engineering

7

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Abstract

Tissue engineering (TE) is a process where the damaged tissues and organs are recovered in a natural process using the patient's physiological system. This involves fully repairing or regenerating damaged organs and tissues which also helps in restoring their functions. This process is established with the use of nanobiomaterials where the surfaces are developed with the required surface topography to accommodate biologically active substances that favor tissue growth. Nano-bioceramics represent the main constituent in human bones and offer new potential in the development of scaffolds and coatings for use in orthopedic and dental applications. The development of nano-bioceramic surfaces on implant alloys is always a challenge as the mechanical and biological properties of these coatings have to be optimized, which otherwise will result in the failure of the implants. Bioinspired nano-bioceramics coatings developed by electrophoretic deposition and dip coating for tissue engineering applications are presented along with their properties. Nano-bioceramic scaffolds and coatings with drug-loaded systems can be used to enhance the growth of bone grafts using tissue engineering which would offer new opportunities to biomedical scientists,

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engineers, and clinicians to come up with a new range of medical devices. Custom-made fabrication and large-scale production of nanomaterial-based tissue-engineered devices is the challenge that needs to be addressed.

Keywords

Bone tissue engineering · Scaffolds · Graphene · Nano-bioceramics · Implants

7.1 Nanobiomaterials and Tissue Engineering

The anatomical structure of the human body is made up of bones and tissues which are a group of cells that have similar structure and function together as a unit. The functioning of the human body is hindered when there is an injury to the tissues. The growth factor molecules released by the body in response to the injury take care of the automatic repair of the tissues and organs. In some cases, the damage and deterioration to the tissues are beyond the auto-recovery process resulting in irreversible damage that leads to the removal of the organ. The alternative to repair or replace these tissues is using biomaterials designed to regenerate new cells or organs, known as tissue engineering. The need for tissue engineering has increased due to alarmingly high demand for the treatment of congenital disabilities, trauma, multiple fractures, constructing cardiovascular, spinal cord, cancer-related tissue, and organ damages, etc., covering almost the entire human body from hair to toe. Tissue engineering is a promising and complex tool to reconstruct, repair, or remodel damaged organs. It can be widely applied in several aspects of organ transplants.

Grafting techniques like autografts and allografts are generally used to treat organ defects. Autograft is a standard gold method where the patient's tissue or bone, usually the iliac crest or the fibula, is taken to heal the damaged area. Even though this grafting is histocompatible, a second surgery is needed at the tissue harvested area. If the defect area is enormous, it is impossible to take the tissue from the patient's body. Allografts depend on the tissues or organs donated by another human. In this donor, the morbidity rate is higher, and pathogens are easily transferred to the patient.

Furthermore, the patient needs to wait for a longer time to get a suitable donor. Its source is minimal and also not economical. Even if grafting implantation techniques like autograft, allograft and xenograft are in long-term practice. But grafting has several limitations like transmission of diseases, less osteoinductive properties, different surgical procedures used at the tissue harvested area, donor site injury, etc. (Amini et al. 2012). The main disadvantage in the case of bone grafting is its limited source. The number of donors is significantly less compared to the number of patients on the waiting list for donors across the globe. Unlike the bone grafting technique, there is no extra surgery needed in tissue engineering. Moreover, the chance of rejection is nil because the cell source for tissue engineering is harvested from the patient itself. It will give a permanent solution for fractured bone, damaged tissues or organs, etc. The source will not be limited since the organs are not

harvested from the donor; it is grown artificially using the living cells of the human so that they can mimic the original one biologically as well as in functioning. The living cells in the scaffolds replicate and heal the damaged tissues.

The most alarming and uncontrollable fatal disease for both men and women is cardiovascular disease. Myocardial tissues have a limited regeneration capacity as myocytes proliferation rapidly ceases after birth. Hence, tissue engineering of the structurally and functionally complicated organ is essentially the direct approach of cardiac tissue engineering. It creates cardiac grafts, either whole heart substitutes or tissues that can be efficiently implanted in the organisms, regenerating the tissue and giving rise to a fully functional heart without side effects.

The future of tissue engineering is a most promising approach in the field of dentistry, as well as it restores function without the need for replacing the diseased tissue. Though several regenerative procedures have already been in clinical practice like bone grafts, guided tissue regeneration, enamel matrix derivative, etc., the outcome of all these procedures has limited success and poor clinical predictability. Hence, a new promising therapeutic approach using dental stem cells like dental follicle stem cells and apical papilla stem cells has been introduced, which has great potential for discovering new treatments. Tissue engineering techniques can recover the functions of tissues or teeth lost due to oral, maxillofacial, and dental pathologies of traumatic, inflammatory, and neoplastic origin, diseases of endodontia, periodontal, dental caries, and facial traumas.

The main plus of tissue engineering is that it can observe immunological, pathological, and healing changes in the human tissues within the scaffold without involving the patients. It is applied in the transplantation of failed organs or tissues such as bone, cartilage, blood vessels, etc., or to heal the damaged tissues due to trauma. Tissue engineering is a promising tool in plastic surgery for the wound healing process. Since the organs are complex and the techniques influencing their regeneration depend on many variables, nanobiomaterials are needed to understand their performance and solve the challenges so that patient safety is ensured.

The main drawback of tissue-engineered scaffolds is that they do not have their blood supply, which carries oxygen for the angiogenesis process. It is necessary to incorporate blood vessels in the implantation that should merge with the patient's blood vessels (Linda et al. 2002). These blood vessels supply the oxygen and growth factors to heal the damaged area in the body. The other major challenge is that a common standard does not exist for the preparation of an ideal scaffold as it changes with the nature of the tissue and the size of the implant (Xu et al. 2011).

Nanomaterials with their superior surface area properties have revolutionized the development of biomaterials and the tissue engineering field. Nature has constructed the human anatomical and physiological system to perform optimally at the nanoscale. Bone is a typical example of a nanocomposite structure that mainly consists of hydroxyapatite (HAP) in the form of nanorods impregnated in the collagen matrix. The nanocomposite bone structure is not only able to bear an entire load of a person but at the same time, it is able to auto-repair itself when fractures occur. Nanobiomaterials have inspired scientists to develop novel scaffolds to seed cells and develop tissue-engineered implants.

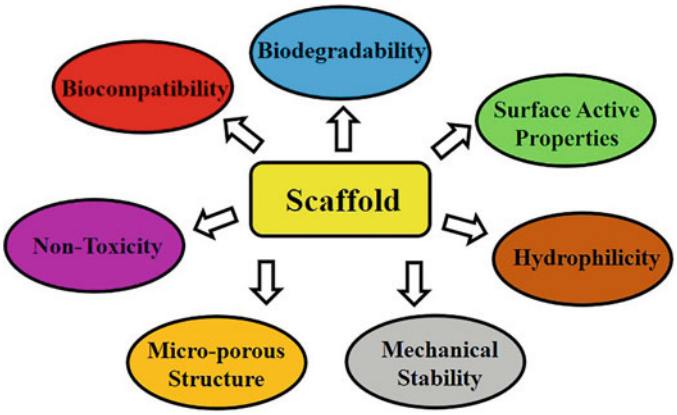

Fig. 7.1 Dynamic features of a newly developed nanomaterial scaffold

Nanobiomaterial-based tissue engineering is a multidisciplinary field that involves experts from medicine, chemistry, material scientists, biotechnologist, and engineers. For the development of tissue-engineered organs or tissues, it is necessary to have a biocompatible scaffold, immature cells to initiate tissue development on the scaffold, and biological growth factors to activate vascularization to supply nutrients and oxygen for the growth of tissues. The essential requirement for tissue engineering is the choice of the nanobiomaterials based on the required tissue properties, which could be a metal, polymer, ceramic, or composite material to construct a scaffold. These nanobiomaterial-based scaffolds are engineered for new cell growth to occur, wherein after implantation, the damaged tissue is reconstructed. This scaffold-implanted site restores the biomechanical and functional properties of cell growth (Stevens 2008; O'brien 2011).

Nanobiomaterials used for preparing scaffolds should possess the following prerequisites, namely biocompatibility, bioactivity, mechanical strength, surface wettability, porosity, non-inflammatory, and while on degradation, they should not release any toxic products. The dynamic features expected out of a developed nanoscaffold are given in Fig. 7.1. Scaffolds are commonly made of biopolymers with or without bioceramics rolled into a bed or in sheets with controlled porosity, enabling vascularization to occur.

7.2 Challenges with Nanoparticles for Biomaterials in Tissue Engineering

Essential parameters for designing nanobiomaterial-based tissue-engineered scaffolds are cell source selection, scaffold pore dimension, nanobiomaterial selection to formulate the scaffold and signaling molecules. Scaffolds should release the growth factors like angiogenic factors, vascular endothelial cell growth factor (VEGF), or fibroblast growth factor (FGF) that supply oxygen and blood for the

cells to grow and new tissues are formed, and the nanobiomaterial scaffolds will degrade slowly and replaced by the original tissues (Linda et al. 2002).

Cell source selection: Choosing the competent cells for growth in the in vivo environment is an important parameter that must be considered. Embryonic stem cells (ESC) and adult stem cells (ASC) are commonly chosen as source cells (Castells-Sala et al. 2013). ESC is also called pluripotent cells and ASC as multipotent cells. ASC cells are more likely to select tissue engineering because they show good histocompatibility once implanted in the body.

Designing Scaffold: Scaffolds provide the surface for the cells to grow. Scaffolds for nano tissue-engineered implants depend on the nature of the nanobiomaterial surface and their pore size.

Nature of nanobiomaterials: The nanobiomaterials must be biocompatible, biodegradable, or bioresorbable in physiological conditions. Tissue-engineered scaffolds are made to repair fractured bone, so the nanobiomaterial used in it should have a good load-bearing capacity and should be flexible, less brittle, and biocompatible. For instance, nano-hydroxyapatite, an important biomaterial used in bone scaffolds, is brittle, so its load withstanding ability is significantly less. Hence, it has to be reinforced with biopolymers, graphene oxide, etc., to improve its limitations to hold up in vivo stress and loading. Nanosized and porous-natured biomaterials used in scaffolds development enable the growth of cells in all directions. The nanobiomaterials have to possess suitable osteoinductive properties. It should be capable of removing the debris upon degradation. Finally, it has to reduce inflammation.

Scaffold pore size: Nano tissue-engineered implants consist of a substrate upon which the cells harvested from the patient can grow in vitro in a 2D fashion. On transplantation, the cells grow and heal the repaired tissues, and these implant surfaces will merge within the body, or if it is bioresorbable, the implantation will slowly get resorbed, and if it is biodegradable, it degrades slowly and gets removed. Nowadays the porous nanobiomaterials are used in tissue-engineered scaffolds, which allows the cells or tissues to grow in a 3D way, and in the physiological medium after implantation, the tissues start to grow in 3D, and the scaffolds will degrade slowly and heal the repaired tissue or organ (Laurencin et al. 1999). 3D cultured tissue-engineered scaffolds allow the tissues to grow in all three directions, and it mimics the in vivo microenvironment better than 2D culture. In 3D culture, it is necessary to consider the nanoporous nature of the extracellular matrix, chemical signals, and stiffness. The ECM nanopore dimension should be high enough to make the growth factors, proteins, enzymes, and adhesion factors attach to it and allow the tissues to grow in a 3D fashion (Castells-Sala et al. 2013).

Signaling molecules: Signaling molecules are factors that have the potential to alter cellular events in host tissue by stimulating or regulating the healing process. Signaling molecules form a covalent or non-covalent linkage with the scaffold, and it is constantly released at different intervals to initiate vascularization, which activates the development of attached immature cells and promotes cell proliferation and differentiation (Castells-Sala et al. 2013).

Thus, tissue-engineered nanoscaffolds must closely mimic the host tissue. It should have good mechanical strength and stiffness without degrading until the host tissues develop new cells in the scaffolds and get cured.

7.3 Bone Tissue Engineering

Bone forms the skeleton of the body. Bone is naturally made up of organic and inorganic constituents, which protect the body's vital organs. It helps in the locomotion of the human body. Bone gives mechanical support to the skeleton system, and it maintains the concentration of different ions through homeostasis. It aids the load-bearing capacity of to skeleton (Amini et al. 2012). It entraps the toxic metals which are present in the body. It supplies the elements needed to form blood cells; the process in which blood cells are formed is called hematopoiesis. Bone has 99% Ca, 85% of phosphate, and 55% Mg of the body. Bone consists of two parts, cortical and trabecular bone. Cortical bone is the outer layer that is dense and encloses the marrow cavity surrounded by long bones, and it is made up of an organic matrix. Trabecular or cancellous bone is present inside the cortical, and it has interconnecting meshwork filled with bone marrow. Trabecular bone is porous and consists of inorganic reinforcement.

A bone is a living tissue where old tissues are replaced with a new bone matrix. Three types of cells present in bone are responsible for this remodeling of bone. They are osteoclast, osteoblast, and osteocytes. Osteoclasts and osteoblasts are responsible for the remodeling of bone. Osteocytes are embedded in the bone matrix and respond to mechanical loading. Osteoclast degrades the old tissue by producing hydrogen ions to mobilize minerals, and lysosomal enzymes digest the organic matrix. Thus, the old bone gets resorbed. Whereas osteoblast forms bone by producing an organic matrix, it is reinforced with minerals like Ca, phosphate, and some amounts of carbonate, Mg, Na, K, etc. Hence, at the macroscopic level, bone consists of cortical bone as the outer layer which surrounds the inner trabecular layer.

The cortical and cancellous bone has three components at the microscopic level: organic matrix, inorganic reinforced constituents, and a tiny amount of water. Water is present as mobile pore water and collagen-bound water. The cortical bone comprises the organic matrix of the bone, which is made up of type I collagen where mineralized collagen fibers are arranged parallel to form lamellae, and inside these lamellae, nerves and blood vessels are present. The collagen fibers combine to form microfibrils in cortical bone, and microfibrils aggregate to form collagen fibrils. Non-collagenous proteins present in the organic matrix help in the cell signaling and bone mineralization process (Chao et al. 2019). Higher mineralization will increase the brittleness of bone and leads to bone fragility (Webster and Ahn 2006). Collagen-bound water is loosely held in it, and it is in higher concentration which gives high tensile strength, and yields stress and elastic toughness to the bone. The cancellous bone is made up of hydroxyapatite which is reinforced in the organic matrix, and it gives mechanical strength to the bone. The cancellous bone is porous, and it is filled with mobile pore water (Chao et al. 2019; Webster and Ahn 2006).

So nowadays, nanobone tissue engineering is preferable for transplantation and hydroxyapatite is widely used as nanobiomaterial for scaffolds due to its similar chemical composition as an inorganic mineral constituent of bone. HAP along with polymers can also be used. Nanobiomaterial scaffolds must have enough porosity for the cells to proliferate, vascularization to occur, and the nutrients to flow. If the scaffold is very compact, the growth of cells gets restricted due to an inadequate supply of nutrients. The scaffold pore size of 100–300 nm allows the cells to grow all over the scaffold, and also complete the transport of nutrients and blood throughout the scaffold. The nanosized biomaterials are chosen for scaffolds owing to their large surface area and surface roughness. If surface roughness and surface area increase, the osseointegration process also increases.

7.4 Orthopedic Implants

The selection of materials for the orthopedic implant is a very significant factor. The materials should be biocompatible and have to possess good mechanical strength. It should not undergo deformation when implanted. Materials used in the implant have to satisfy the FDA regulations (Laxmidhar et al. 2007). Different metals are employed in implants like Ti and its alloys, Co–Cr alloy, stainless steel, Mg alloys, etc. Ti and its alloys are of low weight, high resistance to corrosion, high fatigue, and yield strength. However, it has low abrasion resistance, and when implanted in the body, it may release debris materials that have a toxic effect on body tissues. Ti 6Al 4 V, which is Ti alloy when used in implantation V and Al ions present in it, has a toxic effect on the body. Moreover, no new Ti alloys are used in femoral stems and acetabular shells in total hip replacement (Sridhar et al. 2016). 316 L stainless steel is most commonly used in orthopedic implants due to its low cost, ease of fabrication, and flexible nature. Nevertheless, it is of low fatigue strength and wears resistance, so it undergoes stress corrosion and plastic deformation (Boccaccini et al. 2010). 316 LSS are used in cables and fracture fixation devices (Sridhar et al. 2016). The Co-Cr alloy has excellent wear, fatigue, corrosion, and abrasion resistance. Leaching of Co and Cr causes toxic reactions to the local tissues. Co-Cr alloys are applied in orthopedic prostheses (Laxmidhar et al. 2007). Mg alloys are employed in load-bearing orthopedic implants but their large-scale commercial use is facing tremendous hurdles. Most of the implant alloys have Ni, which will also cause allergic reactions in patients.

7.5 Challenges in Surface Modification of Orthopedic Implants Using Nanobiomaterials and Tissue Engineering

Orthopedic implants should repair or reestablish the damaged tissues and restore the affected area. However, metals and the alloys used in the implants do not aid new bone growth or deposition of HAP in physiological conditions but their failure due to corrosion results in the release of toxic metal ions, revision surgery, and failure of

implants. The life span of metal implants is also significantly less. For children, the implants have to be removed after some period which needs extra surgery to be performed in the implanted area. To avoid these complications, metallic scaffolds are synthesized through the selective layer sintering method (Turnbull et al. 2018). Here, heat is required to incorporate the biomaterials in metals which may degrade the biomaterials (Turnbull et al. 2018). So surface modification of metals can be chosen for bone tissue engineering. Coating the metal surface with biomaterials avoids the leaching out of metal ions from the surface. It also improves its fatigue resistance and increases its wear, abrasion, and corrosion resistance.

7.6 Nanobiomaterials for Orthopedic and Dental Implants

Biomaterials chosen for surface modification have to be nanosized, which increases the surface area and enhances the bioactivity. Nanomaterials give structural support for the cells to grow in the scaffold and increase their biological response. Several bioceramic coatings are applicable to improve the chemical, mechanical, and biological response of the implants. The bioceramic coatings will protect the implant and not allow the corrosion products to come out and react with the nearby tissues, thereby increasing the implant's life span (Boccaccini et al. 2010).

Most commonly employed nano-bioceramics are calcium phosphates, hydroxyapatite, tricalcium phosphates, and biphasic calcium phosphates (Turnbull et al. 2018). All these nano-bioceramics are biocompatible, and when the orthopedic implants coated with these nano-bioceramics are implanted in the body, they release calcium and phosphate ions which initiate and promote bone growth. Out of several bioceramics applied in orthopedic implants, nano-hydroxyapatite (n-HAP) is most commonly used to modify orthopedic implants' surfaces. n-HAP due to its noncytotoxic nature, biocompatibility, and osseointegrating behavior make the implants bind with the surrounding tissues, thereby fastens the curing process.

n-HAP are used in the preparation of 3D scaffolds. Nevertheless, when the implant is fixed in the body, these coatings are stable in the physiological medium and possess adequate load-bearing capacity but this needs to be further improved. So the nano-bioceramics have to be reinforced with mineral ions, polymers, etc., to improve implants' stability and life span. Most studies revealed the use of the carbon-containing compound is reinforced by several nano-bioceramics to overcome its limitations. Graphene oxide (GO) or reduced graphene oxide (rGO) which is also a nanomaterial is incorporated in n-HAP coatings on orthopedic implants which has increased the corrosion resistance and implant stability in the physiological medium. When GO and rGO are incorporated in nHAP they will act as nanofillers and reduce the brittleness of nHAP. They have low toxicity toward human osteoblasts and also increase cell activity. They also possess good antibacterial properties and initiate the apatite mechanism.

Polymers can also be incorporated with bioceramics to improve their limitations. Natural polymers like chitosan, collagen, and hyaluronic acid are used. Synthetic polymers like polylactic acid (PLA), polyglycolic acid (PGA), polyethylene glycol

(PEG), etc., are also used. All synthetic polymers are hydrophobic natured, so it reduces the cell proliferation in implantation. So this nature of the synthetic polymers has to be modified to employ them as implants. Hydrogels are modified synthetic polymers, which are cross-linked polymer chains. Hydrogels are hydrophilic, which absorb water and promote cell growth. Chitosan and collagen, when incorporated in bioceramics, increase their bioactivity. Natural polysaccharides increase the antibacterial properties in implants and increase the adhesive nature of bioceramics to the implants (Turnbull et al. 2018).

Several implant failures result from bacterial adhesion on the implant surface, which can cause infection and inflammation in the bone-implant interface. Bacteria proliferate and form biofilm on the implant surface and destroy the bone tissues within the implanted surface. It is necessary to avoid bacterial infection in the implanted area by adding nanobiomaterials having antibacterial activity. Ag nanoparticles, TiO_2 nanoparticles, chitosan, GO, rGO, etc. have promising antibacterial activity. They are incorporated into nano-bioceramic coatings to improve antibacterial behavior.

7.7 Nano-bioceramic Coating Methods for Tissue Engineering Applications

The distribution of nano-bioceramic particles and the porosity of the coatings are the key factors in determining the growth of tissues, their adherence, mechanical strength, and integration with the human body. The nano structured-biomaterial-based coatings on metallic implants can be altered by changing the concentration, pH, and temperature of the suspension. Nano-bioceramics can be coated by a host of techniques and among them electrophoretic deposition (EPD) and dip coating are discussed.

Electrophoretic deposition (EPD) works on the principle of deposition of nano-bioceramic powders on implant metal and alloy surfaces from a colloidal suspension preferably in an organic solvent medium. A DC-regulated power supply ranging from 1 to 120 V is used and adhesion is developed by sintering at high temperatures from 600 to 800 °C. Sridhar et al. (2016) have developed EPD protocols to deposit thin layers of nano-bioceramics like nHAP and doped nHAP, Tri Calcium Phosphate- α and β forms (TCP), Zirconia, Yttria Stabilized Zirconia (YSZ) on the surface of type 316L SS, Ti and its alloys.

The advantages of this technique are that the porosity of the coatings can be varied from nanometer to micrometer to enable the seeding of cells, for the growth of new tissues. The thickness of the coatings can be varied by optimizing the coating potential and time. EPD can be designed to develop free-standing graded coatings with each layer having different properties with the topmost layer of porous materials allowing infiltration of new materials (Vinodhini and Sridhar 2019).

EPD is a two-step process in which charged particles in suspension move toward an electrode of opposite charge due to the impact of an electric field and then deposit to form a compact film. EPD is a flexible method that can be applied to nanomaterials in powdered form that forms a stable suspension. A typical EPD

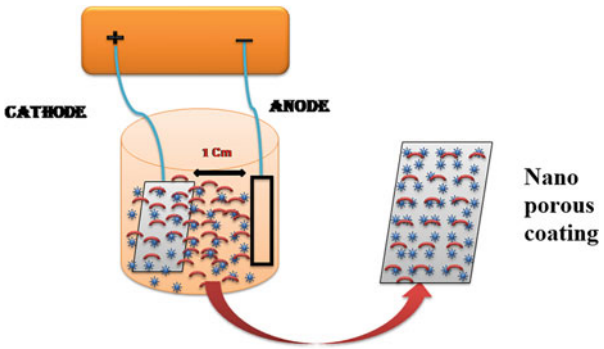

Fig. 7.2 Schematic representation of electrophoretic deposition setup

setup consisting of a power supply and electrodes is given in Fig. 7.2. EPD requires the use of a suitable medium for the stable dispersion of particles. Organic solvents such as alcohols and ketones are commonly deployed due to their relatively high density, good chemical stability, and low conductivity. The disadvantages are they help in drying the coatings within approximately 5 min leaving behind a nanoporous coating. EPD also gives important advantages in the deposition of complex compounds and ceramic laminates. The degree of stoichiometry in the electrophoretic deposit is controlled by the degree of stoichiometry in the nano powder used.

Electrophoretic deposition from settling suspension will lead to the gradient in deposition, i.e., thinner above and thicker deposit at the bottom when the deposition electrode is placed vertically. In electrophoretic deposition when larger particles are used, then a very strong surface charge must be obtained or the electrical double layer region must increase in size; hence nano particles are preferred as they are easy to deposit. The zeta potential of particles is a key factor in the EPD process. It is imperative to achieve a high and uniform surface charge of the suspended particles. It helps in (1) stabilizing the suspension by determining the intensity of repulsive interaction between particles, (2) determining the direction and migration velocity of the particle during EPD, and (3) finding out the green density of the deposit. The overall stability of a system depends on the interaction between individual nano particles in the suspension.

Optimized nano-zirconia-coated 316L SS possesses uniform and crack-free coatings on 316L SS using simple and inexpensive EPD techniques (Mohandoss et al. 2020). The surface morphological analysis with AFM confirms the presence of nanoporous, uniform distribution of nano-zirconia coatings on the whole surface of 316L SS as given in Fig. 7.3. The electrochemical studies in artificial saliva revealed that the nano-zirconia layer exhibited higher corrosion resistance than uncoated 316L SS. Electrochemical studies indicate that the nano-zirconia layer acts as an active barrier against corrosive ions attack and resists ions penetration from artificial saliva to the metal surface. The MTT assay results confirmed that nano-zirconia coatings on 316L SS are nontoxic and more cell viability was observed for nano-zirconia-coated sample when compared to 316L SS and the micrographs are presented in Fig. 7.4. The nano-zirconia-coated 316L SS opens up a new way to

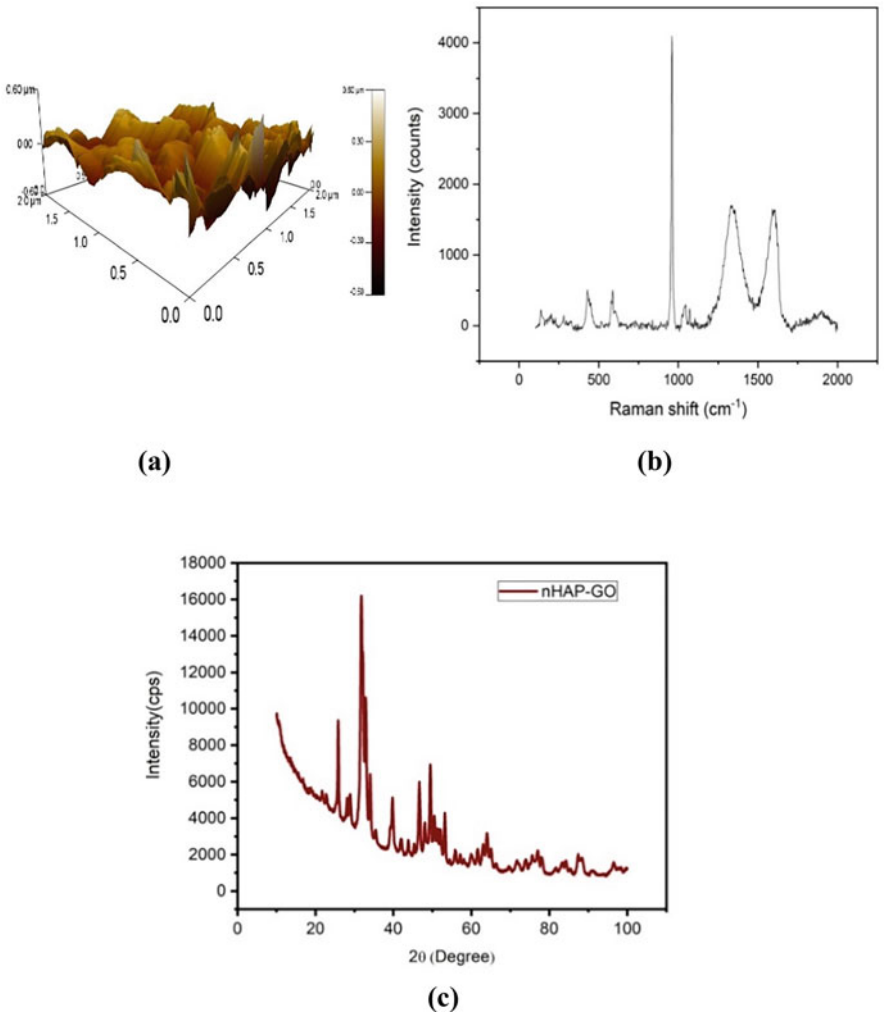

Fig. 7.3 Nano biocomposite coating of nHAP-GO on 316L SS and its characterization. (**a**) AFM, (**b**) Raman spectra, and (**c**) XRD patterns

fabricate nano coated bioceramic dental implants which enables the growth of tissues.

Nano-bioceramic composite coatings of nHAP-GO on 316L stainless steel developed by EPD are given in Fig. 7.6. The nanotopography of the nHAP and GO composite coated on stainless steel was characterized by AFM. A typical nanoporous rough surface of the coating required to promote the bone growth in the implanted area is given in Fig. 7.6(a). Raman spectrum confirms the presence of functional groups in the nanocomposite coatings (Fig. 7.6b). Raman spectroscopy usually determines the

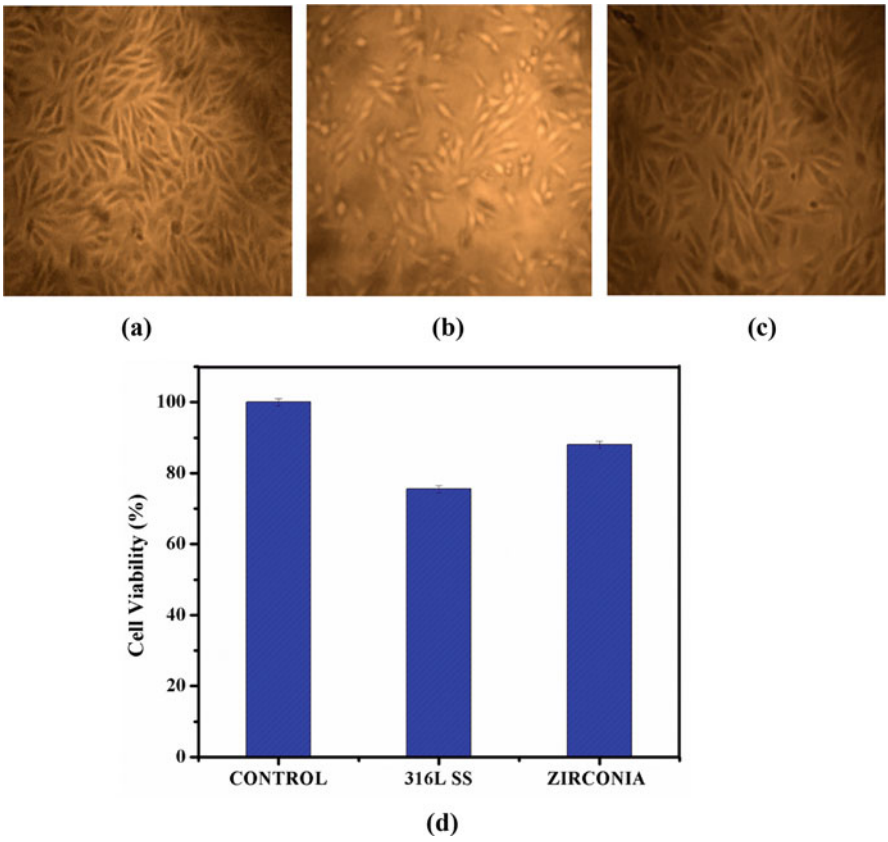

Fig. 7.4 (**a**–**c**) Microscopic images of cell for control cell line, uncoated, nano-zirconia-coated 316L SS and (**d**) its corresponding bar graph of cell viability by MTT assay

D and G band of carbon atoms present in GO and this further confirms the incorporation of GO in the nHAP coating. XRD determines the crystal structure of the nanomaterials (Fig. 7.6c). The XRD patterns indicate the presence of n-HAP phases present in their pure form with the incorporation of GO.

Dip-coating method is another commonly used liquid-phase deposition process for the formation of nano thin films. The nanoporous layers are formed by immersing an implant surface in a beaker containing nano-bioceramic particles blended with polymeric solutions to form a composite mixture followed by dipping the substrate into the mixture at a run rate. This process is schematically represented in Fig. 7.5.

After the liquid component of this solution is dried, a solid thin film is endured on the substrate. Thus, the predominant adhesion and cohesive forces between the dipped substrate and the solution are relevant in the process.

Cohesive forces affect intermolecular forces (hydrogen bonding and Van der Waals forces, for instance) which are interconnected with the tendency in liquids to resist separation. These types of forces are named attractive forces, and they exist

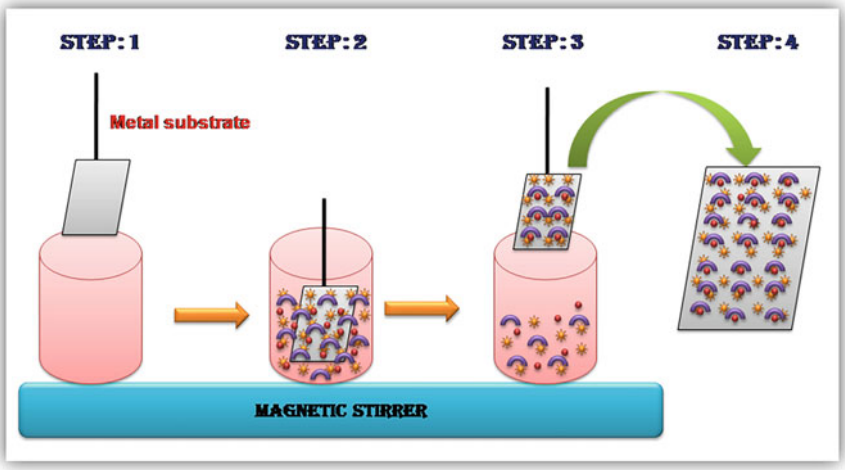

Fig. 7.5 Schematic representation of a typical dip coating process

between molecules of the same substance. On the other hand, adhesive forces are also attractive forces, but unlike molecules. Finally, forces of other nature such as mechanical and electrostatic forces are tangled leading to a wetting process, which is caused by the effect of additive forces and makes the spreading of a liquid on a surface. Instead, if cohesive forces are high enough, the liquid may divide into a number of small and roughly spherical beads, keeping minimal contact with the surface. A nanoporous graphene oxide–hydroxyapatite–poly ethylene glycol (GO/HAP/PEG) coating on Ti6Al4V alloy by dip coating is given in Fig. 7.6.

Figure 7.6(a) shows the Raman spectra of GO/nHAP/PEG-coated on Ti6Al4V substrate; for this composite-coated substrate, the peak at 238 cm^{-1} is due to the skeletal deformation mode and 840 cm^{-1} exhibits the skeletal formation of PEG (Poly Ethylene Glycol). The peaks at 1229 cm^{-1} are due to the C-H twisting vibrations and 1603 cm^{-1} exhibits the bending mode of O-H group; above all the peaks represent the PEG. The peak at 1131 cm^{-1} arises for asymmetric stretching mode υ_3, which confirms the presence of nano HAP, 1332 and 1473 cm^{-1} denoted for D and G band for graphene oxide (GO). Figure 7.6b shows the uniform and crack-free coatings which are PEG tightly bound with GO and nHAP. Figure 7.6c shows the contact angle data for GO/nHAP/PEG-coated on Ti6Al4V substrate respectively. GO/nHAP/PEG coated on Ti6Al4V substrate has higher contact angle (114.4°). An important consideration of this investigation was to evaluate the importance of nano surface preparation of implant materials with regard to wettability and create the driving force for the liquid to spread on the solid surface, thereby enabling the growth of bone cells and tissues on the surface. This technique can be used to develop nano surface coatings with a wide range of thickness, textures, and hardness to produce ideal surfaces for nano bone tissue engineering.

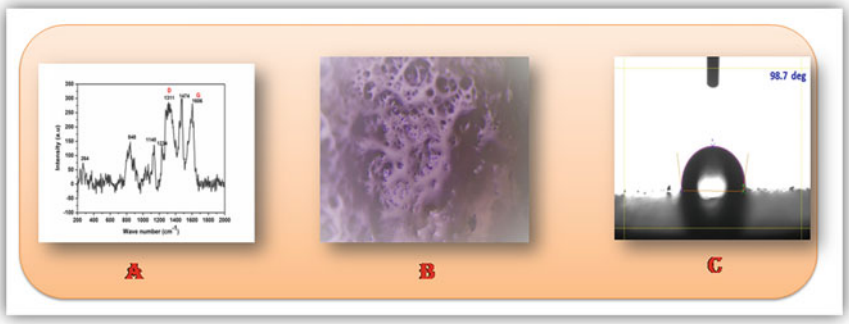

Fig. 7.6 (**a**) Raman spectra (**b**) microstructure (**c**) contact angle measurements of nanoporous GO/nHAP/PEG coated on Ti6Al4V alloy by dip coating process

7.8 Future Aspects of Tissue Engineering

The Covid-19 pandemic and its waves have changed the sphere of medical devices requirement and priorities in surgery. The need for scaffolds and coatings on implants is ever increasing and tissue engineering offers the promise of the next generation of nanobiomaterial-based clinical engineering. This interdisciplinary engineering technology has attracted much attention as a new therapeutic means to overcome the drawbacks of artificial organs and organ transplants. In the case of coatings, the nature of the surface determines the cell attachment and adhesion of the coatings to the metal substrate. Optimization of coating thickness with nanobiomaterials and integration with seeding of tissues would result in the production of medical devices. Scientists have made several advances in this field but the biological and clinical evaluations need to be done systematically with every modification to prove its efficacy and prove its success in the patient treatment. A large number of clinical samples have to be evaluated across age groups, sex, and comorbidities to check the randomization of the nano surfaces, matrix strength, and mimicking properties for tissue regeneration in comparison with a control group.

Drug delivery with these tissue-engineered scaffolds and nanobiomaterial composite-based implants and medical devices is another challenging area that is the need of the hour. This would save the patients from drug toxicity in the form of tablets and injections, and at the same time, the implanted area would heal at a faster pace increasing the success rate of the surgical procedures.

Another major area of work is in the development of custom-made prosthesis and medical devices using the CT and MRI scans of the damaged organs and tissues to develop implants using additive manufacturing and 3D printing matching the required size and shapes. The biomechanical properties are expected to be functional in all these devices.

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