

Review Article

Poornima Ramburrun, Riaz A. Khan*, and Yahya E. Choonara*

Design, preparation, and functionalization of nanobiomaterials for enhanced efficacy in current and future biomedical applications

<https://doi.org/10.1515/ntrev-2022-0106>

received January 17, 2022; accepted March 17, 2022

Abstract: Biocompatible, attuned, and congruent stimuli-responsive polymers of synthetic and plants and animals-based origins are essential raw materials for constructing bio-matching scaffolds, devices, and vehicles for payload deliveries, tissue engineering, therapeutics, and diagnostic purposes, and also as fill-in materials at biosites for various biomimetic applications. The nanobiomaterials for nanobiotechnology, nanopharmaceuticals, and nanobioengineering requires to have fit-in intrinsic chemical, physical, physico-chemical, mechanical, biological, and chemo-biological characteristics. The developed nanomaterials are also required to be receptive and biomimicking in quality, and they must not be toxic at any stage of use or implantation, minimally or of zero order degradation together with aging and use-related degradation, and last a life-time. The biomaterial at the upgraded scale needs to possess intricate characteristics equivalent to the biological materials, and compatible to the biological-sites during transportation, delivery, and site-unloading, and use. Recent developments in nanobio-material substrate designs and structure, characteristics, design requirements, chemo-biological interface related bio-systems response-compatibility, biomaterial synchronized, contemporaneous, and evolving applications

in health care, therapeutics, nanomedicine, bio-hazard prevention, toxicity and ways of toxicity-control are outlined. The structure-function relationship, scope and limitations, structural and functional classes, and diversified biological outreach are discussed.

Keywords: nanobiomaterials, designs and preparation, structural knitting, functional efficiency, contemporary and prospective uses

1 Introduction

The term “nanobiomaterials” encompasses two words: “nanomaterials” and “biomaterials.” Nanomaterials are defined as functional materials consisting of particles with at least one dimension below 100 nm [1]. Biomaterials are defined as biological materials, natural or synthetic substances, which can be used to treat, support, or replace damaged tissue, organs, or bodily function [2]. Nanotechnology is the use of matter and structures at the nanoscopic scale (1–100 nm) for inventing practical applications in medicine, biology, chemistry, physics, and engineering [3,4]. Essentially, nanobiomaterials use nanomaterials and nanotechnology to treat a condition within the human body. Nanostructured biomaterials such as nanoparticles, nanofibers, nanosurfaces, nanowires, and nanocomposites have attracted attention for a vast range of biomedical applications in tissue repair and regeneration, drug and gene delivery, cancer therapy, medical imaging, and theranostics [5].

The nanoparticle forms the building block of all nanobiomaterials. They can be used as a surface for molecular assembly and can consist of polymers (synthetic or natural) and inorganic materials (metals or ceramics). Nanobiomaterials occur in various shapes such as rods, needles, platelets, and polygons, with spheres being the most common. Preparation techniques produce nanobiomaterials in different forms: deposited layers, dispersions, colloids, suspensions, or agglomerates [6].

* **Corresponding author: Riaz A. Khan**, Medicinal Chemistry and Pharmacognosy Department, College of Pharmacy, Qassim University, Qassim 51452, Saudi Arabia, e-mail: riaz.a.kahn@gmail.com

* **Corresponding author: Yahya E. Choonara**, Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, e-mail: Yahya.Choonara@wits.ac.za

Poornima Ramburrun: Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Unlike conventional bulk materials, the size and shape of the nanobiomaterials influence their physical, chemical, electrical, mechanical, and optical properties for improved modulation and performance. Similarly, the size and shape of the nanobiomaterials depend on their intended purpose, application, and desirable interaction with the cell membrane. This makes the parameters of narrow size distribution and shapes extremely critical components for precision control in the design and synthesis of nanobiomaterials [7].

Treating disease and tissue defects has evolved from the use of bio-inert to bio-active materials. A typical cell is approximately 10 μm and the array of smaller organelles and biological molecules within it are sub-micron sized. Nanobiomaterials offer an attractive approach to probe the nanoarchitecture of organs, tissues, and cells for enhanced cellular internalization and interactions. Thus, the focus of treatment shifts from replacing defective tissues towards regenerating cells and biomimicking physiological functions and biological structures at the molecular level. Nanobiomaterials are used in implant applications, such as dental implants, mechanical heart valves, intraocular lenses, hip joints, knee joints, and shoulder joints. They are also used for culturing cells, analyzing blood proteins in clinical laboratories, processing biomolecules for biotechnology applications, implants for regulating the fertility of cattle, and diagnostic gene arrays [8]. For these abilities, surface modification and functionalization of nanobiomaterials with monolayers of biocompatible material and linker molecules are important to allow the particles to attach to various surfaces such as drug molecules, polymeric carriers, fluorophores, antibodies, cell membranes, and other biological components as required for the specific application.

Introducing any kind of foreign material, including nanobiomaterials, in the body elicits a host immune response which affects the performance of that material. The degree of this reaction depends on the shape, size, design, surface chemistry and roughness, electric charge, porosity, composition, hydrophobic or hydrophilic nature, sterility, contact duration, and degradation profiles of the implanted biomaterial [9]. Due to the nature of the nanobiomaterials, the concept of nanotoxicity and biopersistence is a rising challenge regarding safety concerns of the body tissues which internalize and conjugate to nanoparticles. Advancing the design of non-toxic multifunctional nanobiomaterials for biosensing and bioresponsive capabilities to support the focus of treating the body at the nanoscale level, requires revisiting the design criteria and parameters for nanobiomaterials [10]. This article discusses the evolving design criteria, major utility areas,

and challenges to clinical translation of nanobiomaterials for emerging applications in nanomedicine.

2 Classification

Nanobiomaterials may be classified according to several chemical or physical properties, such as composition and electrical, optical, or magnetic properties, depending on their application. Commonly, nanobiomaterials are classified according to composition, dimensionality, and morphology. In biomedical and pharmaceutical applications, these properties govern drug loading and release kinetics, cellular interactions, biocompatibility, and cytotoxicity. Composition, dimensionality, and morphology are only a few of the properties which influence the *in vitro* and *in vivo* behavior of nanobiomaterials. This review will focus on these three classifications as the basic elements to commence the design, preparation, and fine-tuning of nanobiomaterials. The surface chemistry and modification of surface properties are extensively studied for features such as immune system evasion, reduced cytotoxicity, improved cellular targeting, and internalization [11]. Likewise, dimensionality and the morphological effects of size, shape, and aspect ratio on biodistribution, tissue penetration, cellular uptake, and cytotoxicity require further attention and studies as well [11].

2.1 Composition

Nanobiomaterials can be composed of (1) organic, (2) inorganic, (3) carbon-based, or (4) composite-based materials in various morphologies of size and shape (Figure 1). The composition of nanobiomaterials encompasses the core of the material, its shell, and any surface modifications. Generally, the first property to be modified for biocompatibility and reduced cytotoxicity is the chemical composition of nanobiomaterials.

Organic nanobiomaterials are composed of carbon-containing organic materials derived from synthetic or natural polymers. Polymeric nanobiomaterials are widely investigated and used in pharmaceutical applications for controlled or sustained drug delivery, targeted drug release, and tissue engineering strategies. Nanobiomaterials in this class include nanoparticles, dendrimers, cyclodextrins, liposomes, micelles, and solid lipid nanoparticles [6]. The malleability of most polymers further allows fabricating of nanofibers, nanofilms, and nanocoatings. The

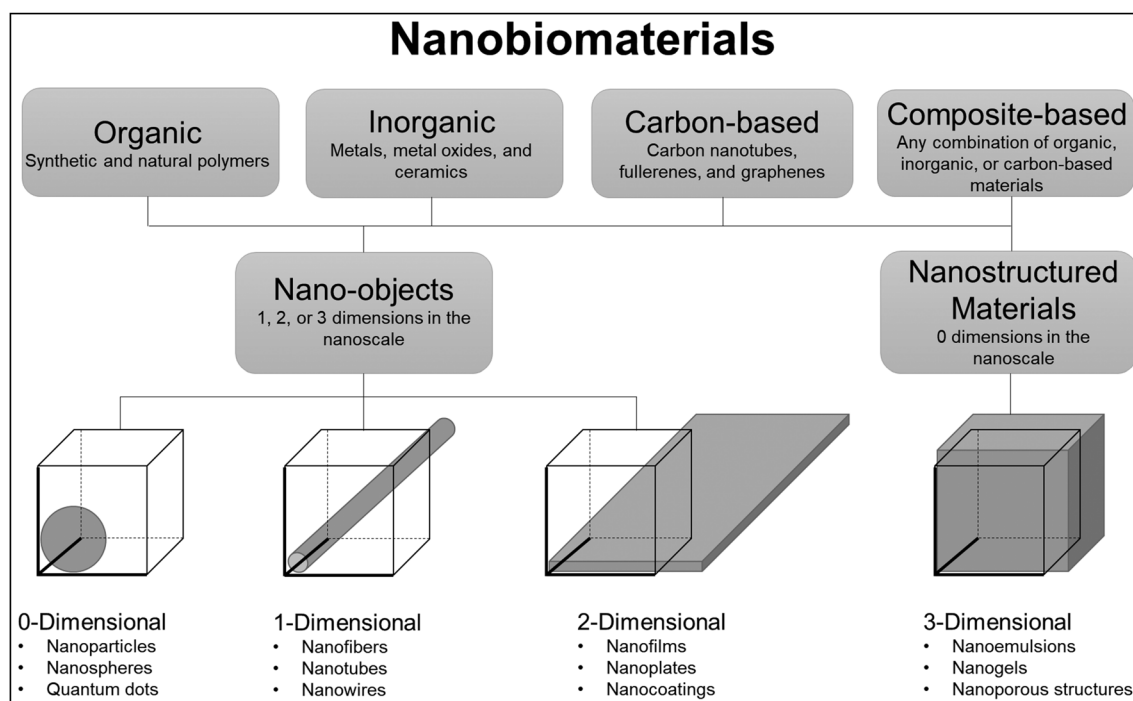


Figure 1: Schematic representing the classification of nanobiomaterials according to composition and dimensionality, where the dimensions of the cube line drawings are 100 nm in all directions.

highly favorable properties and versatility of synthetic polymers, polylactide-co-glycolide acid (PLGA) and polycaprolactone (PCL), and natural polymers, chitosan, and silk have endorsed its use for synthesizing nanobiomaterials for various applications in drug delivery, tissue regeneration, and diagnostic applications [12,13]. Polymers such as PLGA, PCL, chitosan, and silk have low cytotoxicity, high biocompatibility, and tunable biodegradability and mechanical properties, hence their appeal for modulating drug release kinetics, cytotoxicity, and cellular interactions.

Inorganic nanobiomaterials lack the carbon element and include metals (metallics, bimetallics, and metal oxides) and nonmetals (ceramics and silicates). Metallic nanobiomaterials that have been studied include silver, gold, copper, and iron nanoparticles for applications in diagnostics, drug delivery, restorative dentistry, and orthopedic repairs. Metallic nanobiomaterials have unique optical, electrical, and magnetic properties, compared to the bulk derivatives. Metal oxides, such as iron oxide, are investigated for their magnetic properties in guided diagnostics and drug delivery strategies, whereas titanium dioxide is a common material for constructing synthetic prosthesis and implants as it is lightweight and mechanically strong with good biocompatibility and antibacterial properties. Ceramics are commonly investigated in hard tissue engineering for teeth or bone repair and include hydroxyapatite and zirconia. Natural

hard tissues, such as bone and teeth, comprise inorganic materials like calcium and phosphorus in the form of hydroxyapatite crystals, hence, the feasibility of investigating such inorganic compounds for hard tissue engineering [14]. Likewise, nanoclays (thin platelet-like layers of silicate materials of sizes 1 nm in thickness and 70–150 nm in width) are promising biocompatible nanomaterial candidates for mechanically modifying scaffolds and sustaining drug release in bone and cartilage tissue regeneration [15]. The classes of nanoclays include montmorillonite, bentonite, kaolinite, hectorite, and halloysite – several of which are common as inert mineral fillers in topical and oral pharmaceutical products [16].

Carbon-based nanobiomaterials include fullerenes, carbon nanotubes (CNTs), graphene and its derivatives (graphene oxide), nanodiamonds, and carbon-based quantum dots. Their unique structural dimensions and excellent mechanical, electrical, thermal, optical, and chemical properties have attracted tremendous interest in biomedical applications, such as drug delivery, tissue engineering, imaging, and biosensing applications [17]. Carbon-based nanobiomaterials offer excellent optical properties, high surface areas, great mechanical strength, and high electrical conductivity, which make them ideal candidates for theranostic applications [6,17]. Despite these unique properties, biocompatibility, cytotoxicity,

and biodegradation need to be well established before implementing any carbon-based biomedical modalities in clinical applications [18].

Composite nanobiomaterials may comprise any combination of organic/organic, inorganic/inorganic, or organic/inorganic materials to increase the range of desirable properties, to compensate for weaker properties of a specific material, or to create multiphase materials [19]. The combination of several different materials is capable of producing composite or hybrid materials for customizable mechanical strength, biocompatibility, or biodegradation rate, depending on the type of biomedical application required [6]. For example, synthetic and natural polymers are commonly combined to improve the weaker mechanical strength of the natural material, whereas natural material is used to enhance biocompatibility and cellular interactions of the synthetic material. Likewise, metal nanoparticles may be combined with natural polymers to improve drug loading capacity, biocompatibility, and cellular interactions. An example is the improved biocompatibility and maintenance of antibacterial properties of hydroxyapatite and zinc oxide nanoparticles embedded in an alginate matrix to create a biphasic hybrid nanobiomaterial for bone tissue engineering [20].

2.2 Dimensionality

The dimensional properties of the nanobiomaterials refer to the number of external dimensions (height, width, and depth) that lie in the nanoscale division (Figure 1). Zero-dimensional (0D) nanobiomaterials have all three external dimensions in the nanoscale, and comprise basic solid or hollow nanoparticle shapes and quantum dots; one-dimensional (1D) nanobiomaterials have two external dimensions in the nanoscale, and comprise elongated shapes like nanofibers, nanowires, or nanotubes; two-dimensional (2D) nanobiomaterials have one external dimension in the nanoscale and include nanofilms, nanoplates, nanocoating, and nanolayers; three-dimensional (3D) nanobiomaterials do not have any external dimensions in the nanoscale as they are usually bulk or multiple arrangements of nanobiomaterials, and may also contain internal structures in the nanoscale (such as nanopores or nano textures) [6,21]. Recent developments in nanomedicine for cancer diagnosis and therapy have been proposed including a fourth dimension – time. This observes the ability of nanobiomaterials to change over time, specifically to degrade rather than accumulate in the body [22]. Rapidly progressing as a promising

class of nanomaterials in drug delivery, tissue engineering, biosensing, diagnostics, and antibacterial materials, are the 1D and 2D nanomaterials. The planar topography and high surface-area-to-volume ratio of the 1D and 2D nanomaterials enhance biomolecular and cellular interactions at the material interface [15,23].

2.3 Morphology

The classification of morphology is subdivided into the properties of size, shape, and aspect ratio. Several studies have demonstrated the effects of nanoparticle size on tissue penetration and biodistribution [24,25]. It is considered that small-sized particles with greater surface-area-to-volume ratios present greater toxicity profiles [26]. Unlike macro- or micro-sized bulk materials, a nanosized particle is perceived as a molecular signal by cells [26]. An early study by Pan and co-workers showed that 15 nm gold colloids were non-toxic; however, 1.4 and 1.2 nm gold nanoparticles caused cell death *via* necrosis and apoptosis, respectively, within 12 h – thus demonstrating the size-dependent cytotoxicity of the nanomaterials [27]. Due to its smaller size and greater surface-area-to-volume ratio, nanomaterials may penetrate and accumulate anywhere in the body, including cell organelles and cellular subunits, or a fetus.

Over the last decade, the potential of cytotoxicity and genotoxicity resulting from cellular interactions with high aspect ratio nanomaterials (HARNs) [28] has been of growing interest. Aspect ratio is the ratio of length to width of a particle and HARNs comprise nanoparticles with a length many times their width. This usually produces particle shapes of an elongated linear, tube-like, or cylindrical nature, such as nanorods, nanotubes, nanowires, nanofibers, or nano chains – typical 1D nanobiomaterials [29–31]. Low aspect ratio nanomaterials (LARNs) comprise spherical shapes and the cellular uptake of nanospheres is well explored [32,33]. HARNs interact with cellular entities differently compared to LARNs and thus require further investigation. Compared to LARNs, HARNs tend to display improved durability, longer blood half-lives, and persistence due to slower uptake of these particles by macrophages [30,31]. One of the fastest-growing groups of HARNs for drug delivery, biomedical imaging, and diagnosis are CNTs and gold nanorods [31,34]. The larger surface-area-to-volume ratio of HARNs provides greater space for multiple types of surface functionalization of nanobiomaterials for improved cellular targeting or internalization [30].

3 Need for nanobiomaterials: major utility areas

Nanotechnology is a fundamental part of modern medicine, in particular nanomedicine and precision medicine. Nanoparticles with much smaller scales, larger surface areas, new varieties of structures, and a wide range of properties, associated with the nanoscale dimension, offer unique functionalities of manipulability and biomimicry to enhance biological interactions and targeting (Figure 2). This allows liberty to modify structural architecture, tunable properties, microenvironmental interactivity, nano-biointerfacial functions, and the prospect of developing nanorobots for performing cellular level activities [35]. Advances in the physicochemical properties of nanobiomaterials have shown improved biocompatibility, stability, biodegradability, and tunability, hence, its extensive and emerging applications, science research, and clinical applications such as in drug delivery, oncology, diagnostics, tissue engineering, and the development of antimicrobial and antifouling biomaterials. This section discusses the importance of nanobiomaterial applications in the identified major utility areas.

3.1 Drug delivery

The primary barriers and innate defenses of the body (such as the skin, mucous membranes, mucus, tears,

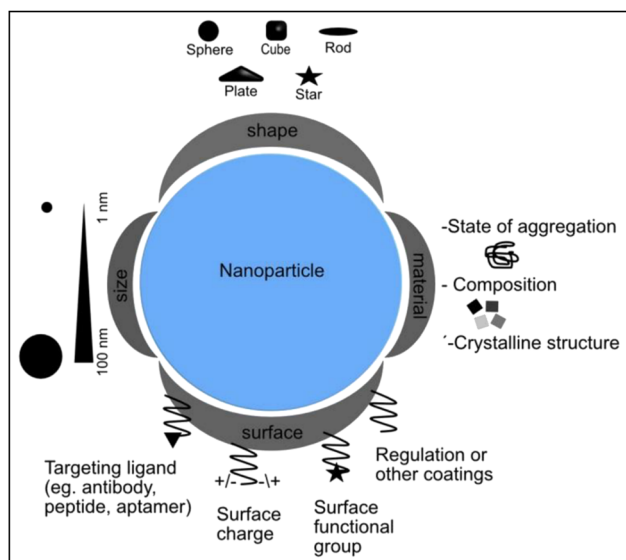


Figure 2: Schematic illustration of the main physicochemical properties of nanoparticles governing interaction mechanisms in biological systems [36]. Reproduced with permission from ref. [36], 2019 © Creative Commons CC BY 4.0.

stomach acid, and blood–tissue barriers) make the targeted delivery of therapeutic drugs difficult to achieve. The pharmacological and therapeutic effect of drugs is often reduced, once a drug is administered into the body and bloodstream, due to various blood–tissue barriers which bar the entry of drug molecules into the target tissues. The epithelial cells forming the blood–tissue barriers have dense intercellular tight junctions, a small number of pinocytotic vesicles, and selective ion and small molecule transporters. As a result, macromolecules are unable to traverse the barrier [37].

Nanobiomaterials formed into different nanocarriers perform the critical function of carrying therapeutic drug molecules across the blood–tissue barrier and into the target tissues where a pharmacological response is expected. The minute size ranges and unique properties of the nanobiomaterials offer an attractive approach to bypass the body's barriers to effectively deliver drug molecules to the required site of action. Nanocarriers, constructed from a range of nanobiomaterials, can be loaded with both hydrophilic or hydrophobic drugs *via* physical entrapment within the nanomaterial core, and attachment and adsorption through non-covalent or covalent interactions to the nanomaterial surface *via* degradable or non-degradable bonds for optimized drug use, targeted drug delivery, and controlled release functions [37,38]. Classes of nanocarriers include nanoparticles, nanospheres, nanocapsules, liposomes, micelles, and dendrimers [39]. The tunable size, shape, and surface properties of the nanobiomaterials enable the production of nanopharmaceuticals with high stability, solubility, and biocompatibility in the presence of biological fluids [38].

These nanotechnological approaches eradicate the older techniques that used the forceful opening of the tight junctions and disrupted the barrier function and integrity for increased permeability to foreign substances. Some of the main routes of nanocarrier drug delivery include oral, skin, intraperitoneal, and parenteral delivery where the mode of nanoparticulate entry into the target area occurs *via* two mechanisms: passive targeting and active targeting [40]. Passive targeting accumulates the nanobiomaterial or nanodrug in the affected area, and this is highly dependent on how long the drug can survive in the bloodstream [40]. Active targeting uses physical methods to enhance the permeability of the nanocarriers through cell membranes to reach their target site [40]. Examples of blood–tissue barriers that have benefited from nanotechnology-based drug delivery systems are the blood–brain barrier (drug delivery for the treatment of neurodegenerative disorders and infections), depicted in Figure 3, and the blood–retinal barrier (BRB; drug

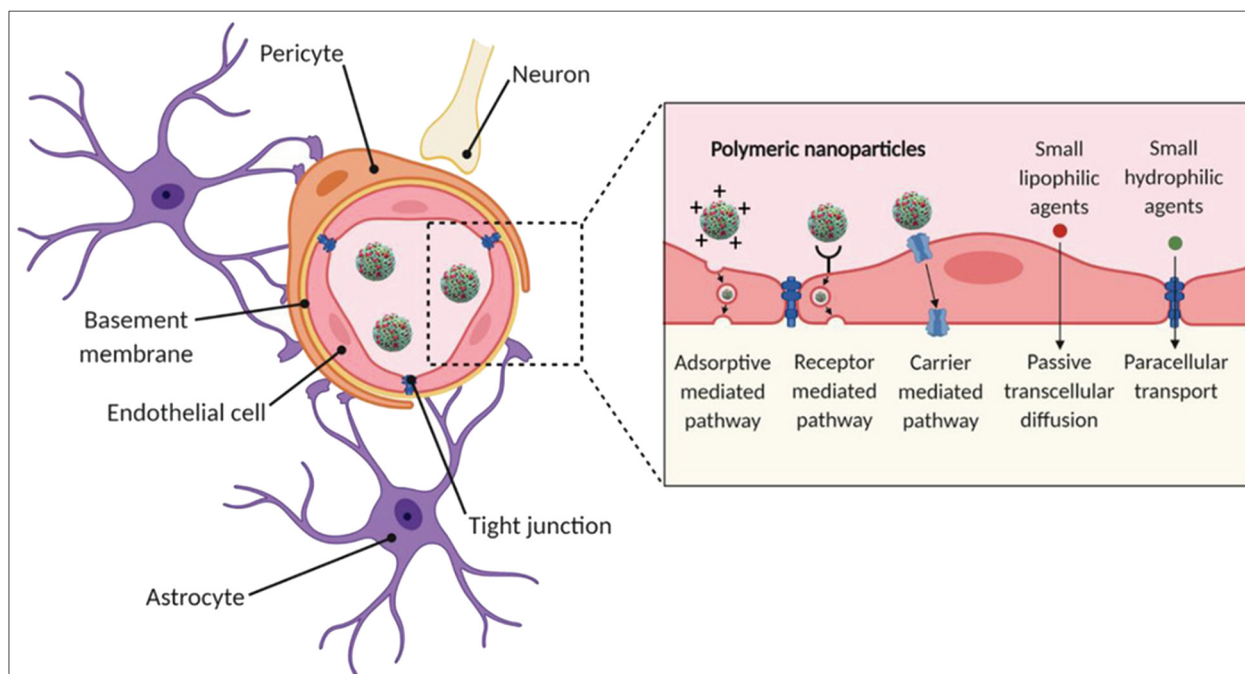


Figure 3: Schematic depicting the methods of transport of polymeric nanoparticles across the endothelium and tight junctions of the brain via carrier-mediated, receptor-mediated, and adsorptive-mediated pathways [42]. Reproduced with permission from ref. [42], 2021 © Creative Commons CC BY 4.0.

delivery for the treatment of glaucoma, age-related macular degeneration, diabetic retinopathy, and infections of the posterior segment of the eye) [41]. This in turn has optimized the therapeutic efficacy of drugs, eliminated possibilities of drug resistance, and reduced undesirable side-effects and toxicity [39].

3.2 Oncology

In oncology, nanobiomaterials are most frequently investigated for drug delivery to improve the *in vivo* performance of chemotherapy drugs in terms of bioavailability, specificity, and safety. Nanobiomaterials enable the design and synthesis of highly efficient, multifunctional drug delivery nanosystems which target the tumor site using a variety of moieties, such as tumor-specific ligands, antibodies, cytotoxic agents, and imaging probes [43]. It is well accepted that a successful cancer treatment strategy relies on early detection and rational drug therapy. The major challenge to this is the biological barriers that prevent the entry of therapeutic and diagnostic elements into the cancerous tissues and cells. The conventional techniques of cancer detection (such as X-ray, magnetic resonance imaging (MRI), ultrasound, endoscopy, and computed tomography) do not detect cancerous transitions until macroscopic changes to the tissues are evident [44]. Without the penetration of

therapeutic and diagnostic elements into the circulating tumor cells – the early detection and treatment of cancer cannot be achieved. Nanotechnology and nanobiomaterials have been shown to effectively address the challenge of overcoming biological barriers associated with cancer: the mononuclear phagocyte system, intratumoral pressure at the extracellular matrix (ECM), and permeation through the tumor cell membranes [35].

The current role of nanobiomaterials is to perform a dual function, on the same nanoparticulate, to simultaneously deliver therapeutic drugs and genes to the cancerous site, and to diagnose the molecular changes arising from tumor metastasis – this encompasses the emerging field of theranostics. Additional issues to effective cancer therapies include low cellular uptake, lysosomal escape, and systemic toxicity associated with current chemotherapy regimens. Nanocarriers, designed in various sizes, shapes, and with specific surface functionalities, can conquer these issues by offering increased blood circulation times, increased surface area for high drug loading capacities, efficient tumor targeting, and high cellular uptake [35]. Nanobiomaterials designed for cancer drug delivery (Figure 4) include solid lipid nanoparticles, liposomes, micelles, dendrimers, organic and inorganic nanoparticles, carbon nanoparticles and nanotubes, nanodiamonds, nanoemulsions, viral nanocarriers, and polymeric and peptide nanoparticles [38].

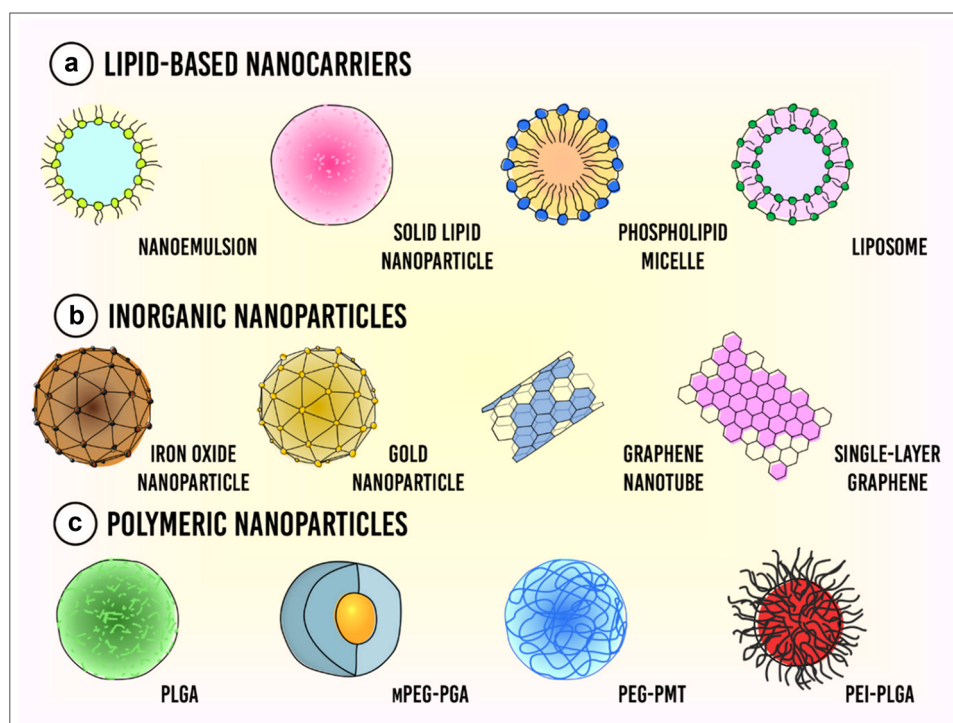


Figure 4: Types of nanocarriers composed of different nanobiomaterials for cancer drug delivery applications: (a) lipid-based nanocarriers, (b) inorganic nanoparticles, and (c) polymeric nanoparticles [46]. Reproduced with permission from ref. [46], 2021 © Creative Commons CC BY 4.0.

The established effectiveness of nanocarriers has led to the statutory approval and clinical use of several nanobiomaterial-based formulations (using liposomes, albumin, or PEGylation) for improved delivery, solubility, and circulation half-lives of commercially available chemotherapeutic agents, such as doxorubicin, daunorubicin, paclitaxel, vincristine, mifamurtide, and irinotecan [38,45].

Having achieved some success in nano chemotherapeutic drug delivery, research endeavors concerning the diagnostic function of oncology theranostics are striving for commercialization and clinical application of nanobiomaterials in the early detection of cancers. Nanomaterials offer the advantage of nanoscale size and an enlarged surface-area-to-volume ratio which enables its surface to be densely populated with a variety of moieties, such as antibodies, peptides, small molecules, and aptamers to identify and bind specific cancer molecules. Nanobiomaterials are designed to capture cancer biomarkers, exosomes, circulating tumor DNA, and circulating tumor cells. Quantum dots, polymer dots, gold nanoparticles, and magnetic nanoparticles are the most common nanobiomaterials investigated as cancer diagnostics [47]. Although there are no clinical applications of nanobiomaterials for cancer diagnostics, the prospective is well perceived considering the development of other nanodiagnostic tools.

3.3 Diagnostics

Nanobiomaterials used in diagnostics include detection, imaging, and biosensing applications. As with any foreign material introduced into the body, nanobiomaterial-based diagnostic tools should be biocompatible, non-toxic, non-immunogenic, and biodegradable. Nanostructures exist as 0D, 1D, and 2D materials. These dimensions also reflect the size range in which biomolecules, such as nucleic acids, proteins, and microbes exist at the nanoscale. *In vitro* diagnostics involve the use of materials to detect biochemical changes, activities, and concentrations of specific substances in samples of biological solutions taken from the body to detect a disease or medical condition [39]. Current *in vivo* diagnostics include more invasive procedures to monitor biomolecules (such as skin tests for antigen detection or biopsies for cancer detection), and for imaging purposes to track the occurrence or progress of a disease (such as X-ray and MRI for the visualizing pulmonary tuberculosis infections or tumors in various regions of the body, respectively).

Efficient medical imaging depends on sophisticated probes to detect biological processes and disease progression on a cellular and molecular level. Nanoscale probes show superior performance, compared to single molecular contrasting agents, for improved contrast and

biomarker visualization, increased circulation times, and large surface area for high loading capacity and conjugation with fluorophores, or drug molecules for theranostic functionality [7,48]. Likewise, the objective of the nanobiomaterials for biosensing applications is to offer highly sensitive, specific, and efficient analytical techniques for disease detection by relying on the increased surface-area-to-volume ratios of the nanoparticles [49]. Nanoparticle probes such as fluorescent nanobeads and quantum dots improve signal brightness, photostability, and offer multiplexing capabilities compared to conventional organic dyes and fluorescent molecules [50].

Biosensors are defined as analytical devices used for the detection of a chemical analyte or substance by converting a biochemical or biological reaction into a quantifiable and measurable physicochemical signal. They are used in medicine to detect biological molecules, (for example, blood glucose), pathogens (such as bacteria or viruses), or other disease-causing agents (such as cells or foreign material and pollutants) [51,52]. A biosensor device consists of two components, a bioreceptor and a transducer component which both make use of nanobiomaterials. The bioreceptor comprises moieties such as enzymes, microorganisms, antibodies, DNAs, aptamers, or cells that recognize the chemical target. The transducer comprises semiconducting nanomaterials, and an electronic system with a signal amplifier, processor, and display that converts the response into a measurable signal [52]. Nanobiomaterials, such as metal nanoparticles, magnetic nanoparticles, and carbon-based nanostructures are investigated as carriers for enhanced signal amplification. Carbon-based nanomaterials and metal nanoparticles are effective in promoting direct electron transfer between the biomolecules and electrode surfaces [50,52]. Key requirements for biosensing nanobiomaterials include chemical stability, high electrical conductivity, robust mechanical strength, high surface-to-volume ratio, and biocompatibility [52].

The introduction of nanobiomaterials for biosensing applications and the optimization of their interaction with biological recognition elements will contribute to the development of highly sensitive and specific point-of-care technology. This will allow the conception of high-performance devices with low system complexity, enhanced sensitivity, and reduced analysis time for clinical use in non-laboratory or resource-limited settings [53].

3.4 Tissue engineering

Tissue engineering is a rapidly developing field that aims to repair living tissues and organs by using synthetic or

natural materials as scaffolds to replace or regenerate defective tissues and organs. The scaffold should be a stable substrate that allows cells to proliferate and differentiate [5]. The perfect scaffold ought to have openings of sufficient size for cells and blood vessels to enter and bond with bones. Polymers like polylactic acid and polyglycolic acid and polyglycerol sebacate elastomers have been used for tissue engineering applications. Synthetic hydrogels are also being investigated for the development of scaffolds because of their high biocompatibility, hydrophilicity, and tissue-like characteristics [5]. An example of synthetic/natural combination nanophase system is collagen/calcium phosphates for biomimicry of bone nanostructure [48]. To recapitulate the bioelectrical properties of ECM, cardiac, neuronal, bone, and skeletal muscle tissues, electroconductive nanomaterials are being investigated as the current generation of tissue engineering materials [54]. Nanobiomaterials are less than 100 nm in dimension, and due to their comparable size to biomolecules and other biological micro and nanostructures, they can interact with and be internalized by cells to induce a cellular response [48]. Due to their miniscule size and biomimetic characteristics, nanobiomaterials can stimulate cell receptors and act as signals to provide instructions to initiate specific cellular behaviors [55].

The goal of tissue engineering is to develop a biomimetic biological substitute capable of restoring, maintaining, or improving the innate function of the defective tissue. Traditional scaffold fabrication techniques often fail to mimic the three-dimensional microstructure of ECM to provide the optimal environment for cell adhesion, proliferation, and differentiation. To overcome this limitation, nanobiomaterials promote interest in design endeavors for superior nanostructured tissue engineering scaffolds for optimal bioactivity, biomimetic, and biomechanical properties. Different combinations of natural and synthetic polymers and various nanofabrication techniques are used to create nanofibrous scaffolds that resemble natural ECM. Nanoporous, fibrous meshes are used to recreate the fibrillar and porous nanoscale topography of ECM using fibers of appropriate diameters arranged in a highly interconnected, porous architecture to allow mass transfer and waste removal from the developing tissues [56]. In addition, tissue regeneration scaffolds may be combined with nanoparticles as drug delivery systems for bioactive molecules and growth factors that can facilitate and accelerate the regeneration process – thus the provision of a multifunctional tissue repair system [57]. Manipulating biomaterials to create nanoscale surfaces and structures (nanobiomaterials) emulate the native micro and nanoenvironment (chemical and structural) of tissues

and cells to induce cell adhesion, growth, proliferation, and differentiation for optimal tissue healing [54].

When biological material is implanted in the body, it causes a foreign body reaction. The extent of this reaction depends on the shape, size, design, surface chemistry and roughness, electric charge, porosity, composition, hydrophobic or hydrophilic nature, sterility issues, contact duration, and degradation properties of the implanted device [9,58]. Understanding such nanobiomaterial properties as well as the ability to control them, directs the interactions which occur between the nanobiomaterial interface and the tissues and cells. The following sequence of events is believed to occur at the interface between the biomaterials and cells: (i) proteins from blood and tissue fluids adsorb onto the nanobiomaterial surface; (ii) tissues and cells of the target organ or inflammatory cells approach the material; (iii) possible targeted release of matrix proteins from the nanobiomaterial and selected adsorption of specific proteins; and (iv) adhesion of cells and commencement of subsequent cell functions, such as proliferation, differentiation, or phagocytosis [48]. In addition, material responses to the host, like material swelling, degradation, and release of monomers, also occur at the cell–biomaterial interface. All these interfacial events are critical for the success of nanobiomaterials (in their application as implants, nanobiomedical tools, or drug delivery systems) since they correlate to material cytocompatibility and host immune responses that determine its efficacy and safety *in vivo* [5,48].

3.5 Antimicrobial and antifouling nanobiomaterials

Medicine, in the past and nowadays, is dependent on the availability of effective antibiotics to manage infections, particularly in invasive surgeries. The irrational use of antibiotics has resulted in the emergence of antibiotic-resistant microbes, and hence, necessitates the innovative design of antimicrobial and antifouling biomaterials to prevent biofilms from forming on the surfaces of medical materials and implants. The increasing use of medical implants (artificial joints and tissue engineering constructs), medical devices (pacemakers), and medical materials (catheter and dental materials) provide foreign surfaces in the human body for microbial attachment, thereby increasing the risk of infections and the need for antibiotic treatment [59]. Accompanying the developments in material science and nanotechnology is the generation of novel nanobiomaterials with intrinsic antimicrobial and

antifouling properties to ease microbial management in biomedical applications. The field of dentistry is particularly gaining momentum in the use of antimicrobial and antifouling nanobiomaterials for the prevention of biofilm formation and recurrent caries in dental restorative, endodontic, and tissue regenerative applications of the oral cavity.

Nanomaterials have a large surface-area-to-volume ratio, which offers a higher degree of the active contact surface. Studies have shown that creating surface nanotopographies and inscribing nanopatterns confer antimicrobial and antifouling properties, and this feature is enhanced when HARNs are employed to control the spatial patterning of microbes on the surfaces [59,60]. The size, shape, and pattern of the surface nanostructures determine the bacterial response [61]. Other than antibiotic-releasing materials, the chemical composition of nanobiomaterials may also possess intrinsic antimicrobial and antifouling activity, such as metallic nanoparticles, polymeric biocides, and biocidal polymers, which exert their antimicrobial and antifouling action upon direct contact with the microorganism. The antimicrobial and antifouling mechanisms of the nanobiomaterials are depicted in Figure 5. Metallic nanoparticles (such as silver and metal oxides of iron, zinc, and titanium) disrupt microbial membranes and generate reactive oxygen species (ROS) which cause mitochondrial damage, cell membrane damage, and protein denaturation [62]. It is suggested that composite scaffolds containing silver nanoparticles could regulate bacterial infection during reconstructive bone surgery and act as a coating for protection against subsequent infection, sepsis, or malfunctioning of implants [63]. Other mechanisms of action include inhibition of metabolic processes, disturbance of the electron transport system, oxidation of macromolecules, and inhibition of DNA replication [64]. Unlike antibiotics, nanobiomaterials provide an opportunity to limit microbial growth before the onset of infection, and therefore, reduce the potential of developing microbial resistance [60].

4 Evolving design criteria and functional outreach of nanobiomaterials

Over the past decades, nanotechnology, the design of nanobiomaterials, and its applications have rapidly evolved, necessitating new design criteria thereof. The first generation of

nanomaterials was composed of single, nonfunctionalized, and as-synthesized, non-modified basic polymeric or metallic nanoparticles, and quantum dots with limited properties and biomedical uses [35]. To address complicated medical issues and fulfill the requirement for biocompatibility, nanobiomaterials were then functionalized with various moieties (such as anticancer or anti-infective drugs, biological molecules, fluorescent dyes, tumor-specific ligands, and genetic entities) to enable diagnostic and therapeutic use in clinical applications – giving rise to the second generation of multifunctional nanobiomaterials [35]. These multifunctional nanobiomaterials were then built with specific compositions and structures to create smart nanosystems which adapt to the physiological microenvironment and respond to changes in pH, light, or electrical field – the third generation of nanobiomaterials [66]. Further advancements of such stimuli-responsive nanobiomaterials are anticipated to produce the upcoming fourth generation of nanobiomachines and nanobots which could function, independently or cooperatively with innate biological systems, as bionic cells to sense and relay cellular signals, perform metabolic tasks, and control proliferative processes of regenerating tissues [35,67].

4.1 Pertinent properties for evolving nanobiomaterials

The use of nanobiomaterials in medical and therapeutic applications requires several characteristics to be considered

during the preformulation and development phase. Particle size, particle shape, surface area, solubility, and polymorphism are some of the basic physicochemical properties to be considered when formulating a drug for effective drug delivery, tissue regeneration, or diagnostic uses. Zahin *et al.* outlined additional characteristics, such as surface charge and hydrophobicity, that affect drug release kinetics [68]. To fully harness the power of nanotechnology for evolving applications in current times, it is important to understand how the physicochemical properties of the nanobiomaterials associate with the biological interactions and functions. Table 1 summarizes the key properties and features required for the design and preparation of nanobiomaterials for current biomedical applications, as well as addressing specific issues or knowledge gaps, where necessary. This section discusses evolutions in particle size, shape, and surface properties that affect the next generation of nanobiomaterials.

Particle size is an important physicochemical property as it influences the physical, chemical, and biological properties of the nanobiomaterials, as well as its surface area, and movement dynamics. This determines the fate of nanobiomaterials and the host tissue responses when the nanobiomaterial is introduced into the body. Size, and modifications thereof, determine the toxicity, *in vivo* distribution, targeting ability, drug loading capacity, drug stability, drug release kinetics, cellular interactions with nanobiomaterials, and elimination from the body, thereof [68]. Particles greater than 500 nm in diameter

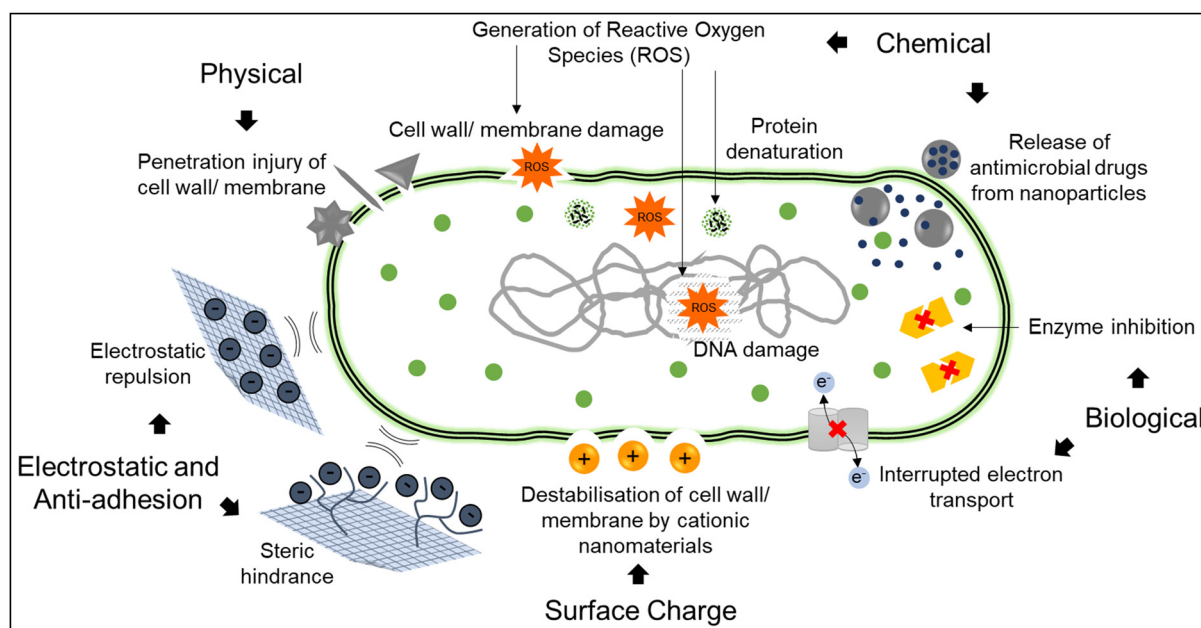


Figure 5: Schematic representation of the antimicrobial and antifouling mechanisms of nanobiomaterials in terms of physical, chemical, biological, surface charge properties, and electrostatic and anti-adhesion properties [65]. Reproduced with permission from ref. [65], 2021 © Creative Commons CC BY 4.0.

readily accumulate in the liver and spleen, whereas particles less than 5 nm in diameter could be eliminated *via* the kidneys [77]. Particle size and surface area are indirectly proportional variables, and this is one of the merits of nanobiomaterials. Due to their small particle size, nanobiomaterials have a large surface area which increases the capacity for functionalizing and loading various therapeutic drugs or biomolecules for different applications. This size attribute of nanobiomaterials enables passage across blood–tissue barriers that would normally be inaccessible to larger particles [78].

Similarly, particle shape has been shown to affect both the particle pharmacokinetics and biodistribution *via* several mechanisms. The geometry and size of the nanoparticles affect the physicochemical properties, and interactions with biological systems, to mediate effects such as cytotoxicity, uptake, biodistribution, and pharmacokinetics [69]. Truong *et al.* reviewed the influence of nanoparticle shape on drug delivery while comparing spherical and non-spherical shapes [79]. Spherical micelles are the mainstay of nanodrug delivery due to their drug delivery properties, ease of synthesis, and minimal testing difficulties experienced compared to other drug shapes [79]. A spherical low-dimension nanoparticle shape has minimum surface area per unit volume, compared to non-spherical high-dimension structures (for example, nanorods) and thus, non-spherical 2D nanobiomaterials have the desirable properties and benefits of increased drug loading capacity for the future of nanodrug delivery. Another aspect of nanobiomaterials' shape that affects *in vivo* performance is permeation – without the ability to permeate tissues, cell membranes, and blood–tissue barriers, site-specific, and targeted drug delivery would be impossible. A study that investigated the role of nanoparticle shape, size, and surface chemistry revealed higher cellular uptake of rod-shaped nanoparticles in a co-culture of intestinal cells compared to spheres, regardless of the presence of active targeting moieties [80]. Likewise, the increased surface area of 2D nanobiomaterials, such as filamentous structures, wires, tubes, and rods, provides increased contact points for cellular adhesion and proliferation in tissue engineering strategies. The provision of greater surface area and enhanced cellular uptake of high-dimensional nanobiomaterials is attractive for a variety of drug delivery, diagnostic, and tissue engineering applications.

Surface properties of the nanobiomaterials such as surface charge and surface hydrophobicity influence drug delivery by affecting drug distribution, drug circulation, and drug-protein adsorption [68]. Surface charge is directly related to drug adsorption to plasma which affects drug distribution [68]. Negatively charged nanobiomaterials

have been shown to ultimately increase blood circulation time by reducing the undesirable reticuloendothelial system (RES) clearance which in turn improves compatibility with blood and results in targeted anticancer drug delivery [68]. Negatively charged nanoparticles also show lower cellular uptake and lower cytotoxicity compared to neutral and positively charged nanoparticles [66,72]. Positively charged nanoparticles decrease the RES acceptance of particles and are cleared much slower as a result, which ensures better functionality and compatibility with the long-circulating delivery systems [68]. However, positively charged nanoparticles are limited by their cytotoxicity [72]. Coating nanoparticles with a zwitterionic structure of polymers improves stability, prolongs circulation time, and therefore, enhances drug compatibility [68]. It is suggested that the cell membrane possesses a negative charge and cell uptake is driven by electrostatic attractions. Studies have demonstrated that these electrostatic attractions between the cell membrane and the positively charged nanoparticle interface, promote cellular adhesion and uptake of the nanoparticle [66].

Surface hydrophobicity is an integral part of opsonization [68]. Opsonization is an immune process that uses opsonins to tag foreign pathogens for elimination by phagocytes [81]. *In vivo* hydrophobic particles are coated in plasma proteins and immunoglobulin complement proteins, to form a corona, which results in further protein adsorption at the surface of the nanoparticle and increased surface hydrophobicity [68]. Since surface charge affects corona composition, studies comparing positively and negatively charged nanoparticles are required to investigate the effects of corona formation and composition on drug release and tissue regenerative properties [66].

4.2 Bone and cartilage transplants

The focus of tissue engineering is to prepare biological materials that can “replace, regenerate, or repair damaged cells or tissues.” These materials must provide adequate support while serving as a network for cell adhesion, movement, and tissue growth. In the context of bone tissue regeneration, these biomaterials must have biocompatibility and biodegradability over a specific timeframe, provide sufficient mechanical support, and have osteoinductive and osteoconductive properties [82].

Biodegradable polymeric materials and ceramics have been actively studied as bone tissue engineering materials; however, they have not yet been able to fully resolve all types of bone defects, especially large bone defects. Nanoparticles can aid in the improvement of the regenerative

Table 1: Design and preparation requirements of nanobiomaterials using contemporary physicochemical properties and features for biomedical applications

Nanobiomaterial properties and features	Function, importance, and issues	Ref.
Size, shape, and aspect ratio	i. Fundamental properties affecting physical, chemical, and biological characteristics ii. Size and shape influence particle movement dynamics and flow iii. Affects nanobiomaterial tissue/cellular permeation, orientation, and direction of internalization and distribution kinetics iv. Smaller nano-sized structures and shapes of high aspect ratio provide greater surface areas for conjugation of bioactive/therapeutic molecules in drug delivery applications v. Bioaccumulation and biopersistence in tissues/cells lead to nanotoxicity	[35,69]
Composition and structure	i. First-line modification for reduced cytotoxicity, improved biocompatibility, and biomimicry of nanobiomaterials ii. Affects bioaccumulation, elimination, circulation half-life of nanoparticles, and biodegradation rate iii. Improving circulation half-life and elimination kinetics should be balanced for prolonged delivery of therapeutics iv. Biodegradation should match the rate of tissue regeneration to avoid occlusion or hindrances in tissue healing v. Adjusting dissolution properties of polymeric nanocarriers for optimizing drug release rates and elimination from the body vi. Vesicular and membrane-bound nanoparticles offer a simple structural template for artificial cell development vii. Structural variations provide opportunities for different modes of drug loading and surface modifications, <i>i.e.</i> , empty core/hollow nanostructures for drug encapsulation, solid core and membrane-bound nanoparticles for surface conjugation of biomolecules or ligands	[35,37,38,70]
Surface morphology and topography	i. Nanotopographies such as surface roughness and nanopatterns provide increased contact surface area to encourage cell adhesion and directional guidance of regenerating tissues ii. Increased surface area may allow for unfavorable microbial adhesion upon contamination	[61]
Agglomeration stability	i. Nanofluid systems for bio-fluidic replacements or nanoparticles as cell substitutes in blood should be non-agglomerating to mimic the normal rheological flow of biological fluids. ii. Modification of surface chemical composition, nanoparticle size and shape, and surface texture for colloidal stability and biocompatibility	[71]
Surface properties	i. Hydrophilic surfaces enhance cellular adhesion of regenerating tissues and prevents microbial adhesion ii. Superhydrophobic surfaces prevent both microbial and cellular adhesion of regenerating tissues iii. Surface charge and hydrophobicity influence distribution, drug circulation, and drug-protein adsorption of nanocarriers iv. Negatively charged nanoparticles show lower cellular uptake and lower cytotoxicity compared to neutral and positively charged nanoparticles v. Studies comparing positively and negatively charged nanoparticles are required to investigate the effects of corona formation and composition	[66,68,70,72–74]
Electrical, optical, and magnetic properties	i. Electrical properties essential for tissue engineering applications concerning electrically conductive tissues: bone (osteoconductive properties), cardiac tissue (myocontractility), and nerve tissue (neural electrical signaling) ii. Electrical properties of nanomaterials may assist with understanding and utilizing the concept of bioelectricity for synthesizing artificial hybrid cells iii. Electrical and optical properties are essential for developing biosensing nanobiomaterials for use in diagnostics	[52,75,76]

(Continued)

Table 1: Continued

Nanobiomaterial properties and features	Function, importance, and issues	Ref.
Antimicrobial and antifouling properties	iv. Optical properties of light absorption and bioluminescence of quantum dots, nanophosphors, and metallic nanoparticles within the biological window, <i>i.e.</i> , near infra-red range, is pertinent for biomedical optical imaging	[59,63]
	v. Nano-objects with bimodal magnetic and optical properties offer comprehensive imaging using both magnetic resonance and optical imaging technology	
	vi. Magnetic properties for magnetic-guided drug delivery and therapies	
	i. To prevent microbial contamination and biofilm formation in long-term tissue regenerative nanobiomaterials, medical implants, and dental applications	
	ii. To replace or reduce the need for antibiotic treatment in invasive surgical procedures <i>via</i> coating scaffolds and implants with antimicrobial releasing nanomaterials or antimicrobial metallic nanoparticles	
	iii. Cytotoxicity and biocompatibility to host tissues require further studies	
	iv. Potential for developing microbial resistance require elucidation	
	v. Duration of antimicrobial or antifouling activity to be investigated	

capacity of these and other biological materials by allowing for more precise control of surface and mechanical properties. Incorporating nanoparticles into biomaterials can also improve their biological features, such as improving cell adhesion, differentiation, and stem cell integration with their surroundings. The bioactive surfaces of nanobiomaterials mimic those of natural bones to promote greater amounts of protein adsorption to efficiently stimulate new bone formation compared to the conventional bulk materials (Figure 6) [83]. Furthermore, the drug delivery capabilities of the nanoparticles provide greater possibilities to enhance the properties of the biomaterials in regenerative medicine [82].

4.3 Optical uses

The eye is an ideal research tool for nanotechnology, due to the presence of the BRB. It is generally believed that the impact of nanomedicine injected into the eye on systemic circulation is negligible. There are several other ophthalmic barriers such as the tear film, corneal, blood-aqueous, and vitreal barriers. Therefore, drug delivery through conventional nanocarriers generally shows poor targeting ability, resulting in unsatisfactory therapeutic effects and unexpected side effects, especially for treating ocular diseases in the posterior segment of the eye [84]. Several new nano-platforms have been designed with different strategies to overcome these limitations (Figure 7). Smart nano-matrices have been widely used in the treatment

of various ocular diseases, by improving drug permeability, prolonging drug bioavailability, reducing dosing frequency, and improving patient compliance to improve treatment efficacy and outcomes [84].

4.4 Audio systems

Many diseases affect hearing and cause hearing loss, such as noise-induced or autoimmune hearing loss, exposure to ototoxic substances, genetic diseases, and ear infections [86]. One of the most common diseases that affect approximately 90% of hearing loss cases is sensorineural hearing loss. Due to the lack of non-invasive targeted delivery systems, current biological treatment options are limited. Barriers to drug administration to the inner ear include anatomical inaccessibility, limited blood supply, blood–labyrinth barrier (BLB), instability of biological therapy, and nonspecific administration, which result in sub-therapeutic concentrations of drug [86,87]. Advances in nanotechnology for the delivery of otoprotective drugs to the inner ear can provide solutions to these obstacles. Nanocarrier drug delivery systems commonly investigated for inner ear targeting include lipid, phospholipid, polymeric, metallic, and metal oxide nanoparticles, due to a variety of properties such as antibacterial activity, high drug loading capacity, biodegradability, and biocompatibility [88]. Nanoparticles can stabilize and transport biological materials across the round and oval window membranes to the inner ear compartments *via* intratympanic injection (as shown in

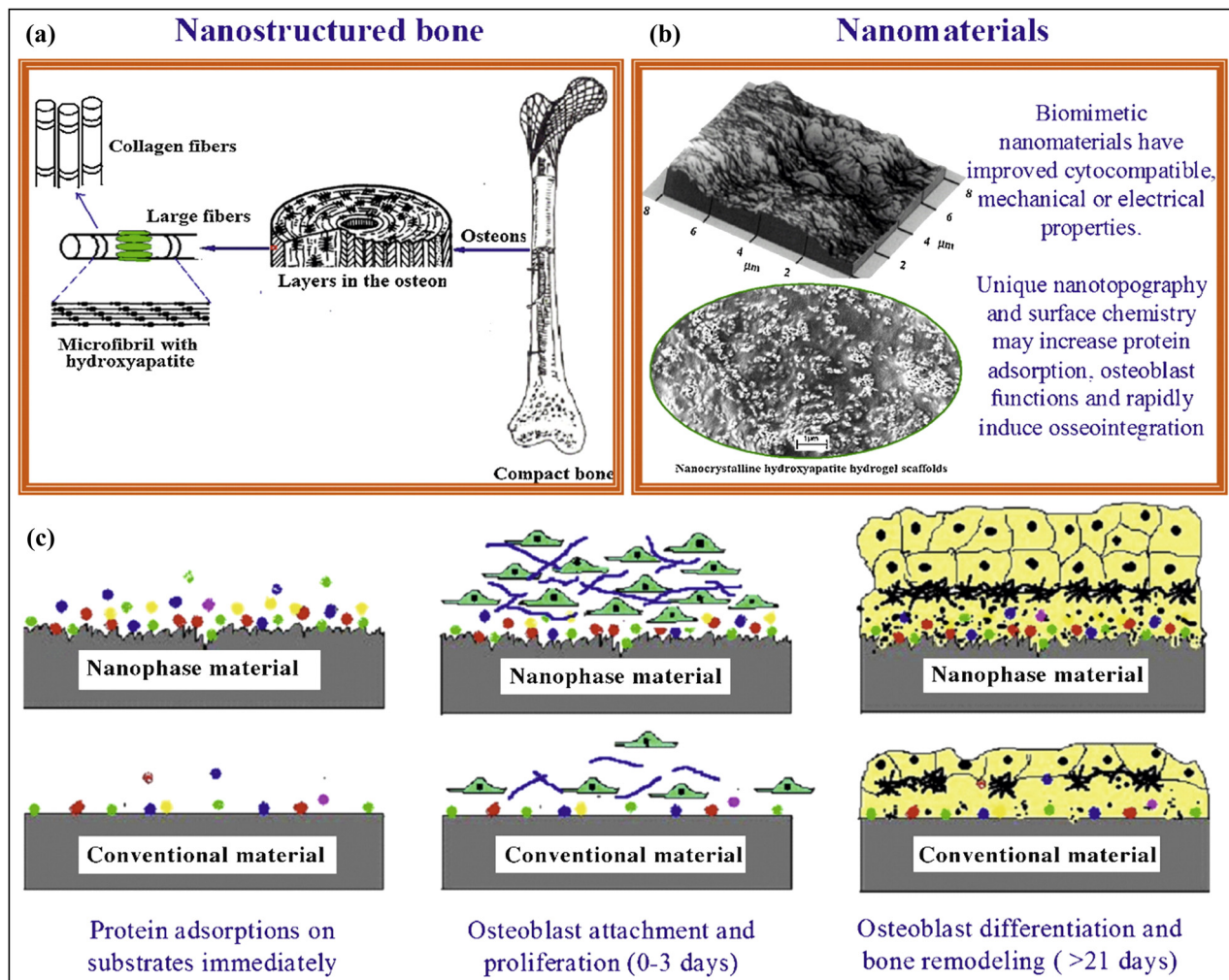


Figure 6: Schematic depicting the biomimetic advantages of nanobiomaterials. (a) The nanostructured hierarchal self-assembly of bone. (b) Nanophase titanium (top, the atomic force microscopy image) and nanocrystalline HA/HRN hydrogel scaffold (bottom, the SEM image). (c) Schematic illustration of the mechanism by which nanomaterials may be superior to conventional materials for bone regeneration [83]. Reproduced with permission from ref. [83], 2009 © Elsevier.

Figure 8), whereas surface modification of the nanoparticles using biologically coupled ligands or functional groups may allow for a specific orientation and targeting the cochlea. These attributes of nanoparticles for inner ear drug delivery have been shown in studies investigating PLGA polymeric nanoparticles for round window administration, PEG-PLA polymeric nanoparticles for dexamethasone delivery, and solid lipid nanoparticles for glucocorticoid delivery for auditory protection against cisplatin-induced ototoxicity [89–91]. Differing from nanoparticles, one study showed the ability of a thermosensitive poloxamer 407 hydrogel for sustained intratympanic delivery of *N*-acetylcysteine over 24 h [92]. The exploration of these individual systems leads to the realization that the application of nanoparticles alone, without the use of a bioresponsive matrix system for controlled nanoparticle delivery, may remain inefficient for inner ear delivery

and targeting. A study by Lajud and co-workers demonstrated that a thermosensitive chitosan-glycerophosphate hydrogel could withstand physiological conditions to sustain the release of incorporated liposomes to the inner ear for two weeks, when administered in the middle ear [93]. Thus, new nanohydrogel technologies can provide sustained and non-invasive biotherapeutic delivery to specific cells of the inner ear by merging hydrogels with nanocarriers for a nanocomposite approach. Such nanocomposites featuring *in situ* forming hydrogels with nanocarriers has accumulated recent interest for local drug delivery to the middle and inner ear compartments for treating hearing loss, tinnitus, and otitis media infections [94]. In addition to drug delivery, nanohydrogels could be used for inner ear dialysis as a potential treatment for sensorineural hearing loss caused by ototoxicity.

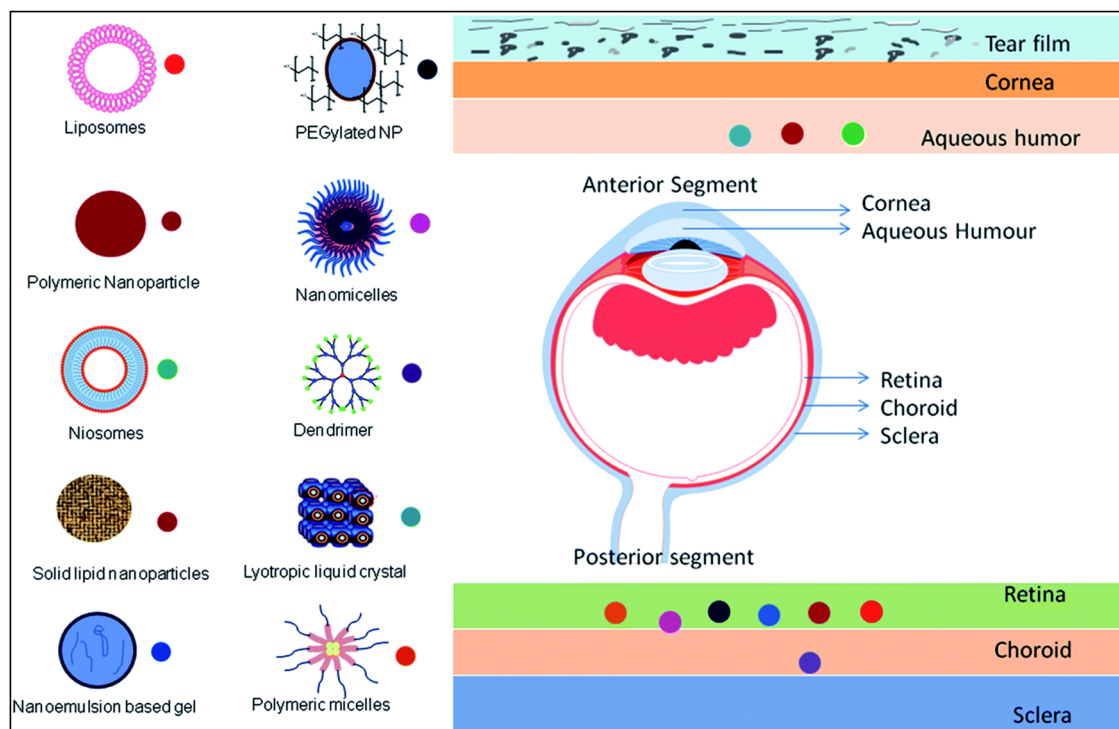


Figure 7: Schematic representation of different nanocarrier systems and their targeting ability. The permeation of nanomedicines across the ocular barrier on topical administration for the treatment of eye diseases [85]. Reproduced with permission from ref. [85], 2020 © Creative Commons CC BY-NC 3.0.

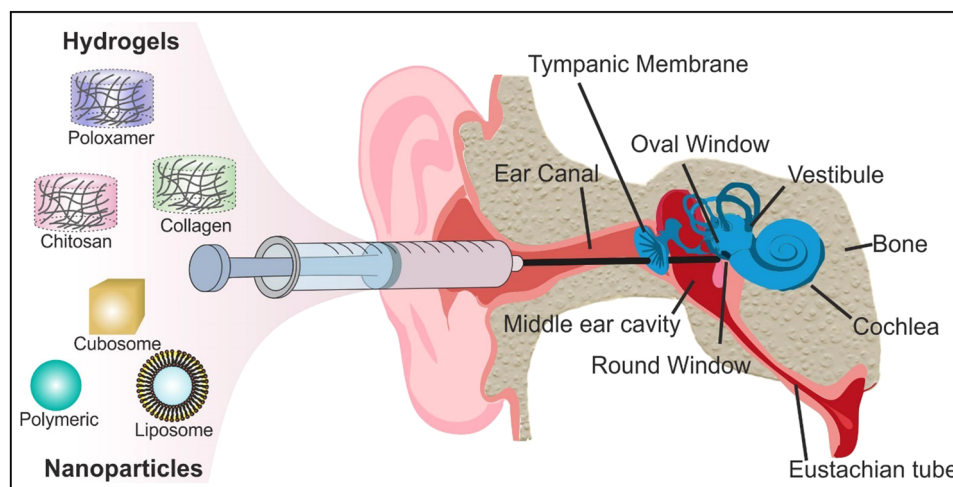


Figure 8: Representation of various injectable nanobiomaterial drug delivery systems to the inner ear [95]. Reproduced with permission from ref. [95], 2019 © Creative Commons CC BY 4.0.

4.5 Locomotion and neuronal sensor

The fundamental goal of employing nanotechnology to treat nervous system illnesses is to clearly understand how the nervous system functions, and how neurons connect and organize in an orderly network under varied

activities and mental states [96]. Nanotechnology and neuroscience together cover a wide range of concepts, such as drug delivery, cell protection, cell regeneration and differentiation, imaging, and surgery, thus giving rise to new clinical methods in the study of neuroscience. The possible use of nanotechnology in optogenetics and

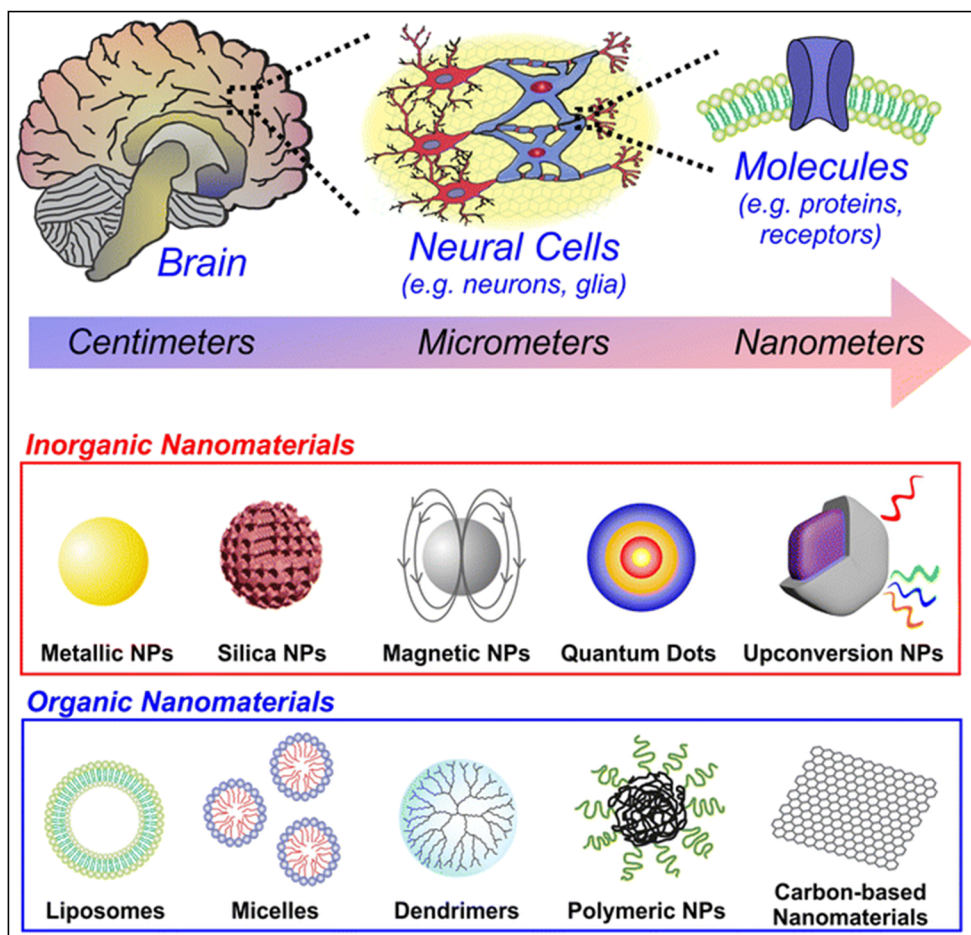


Figure 9: Representation of the nanobiomaterials toolkit for neuro-engineering applications [97]. Reproduced with permission from ref. [97], 2016 © Creative Commons CC BY 4.0.

piezoelectric effects further demonstrates its application prospects in neuroscience [47,96]. Different types of nanoparticle-based platforms and their roles in neuroscience applications are depicted in Figure 9. In neurology, these nanoparticles have been extensively studied for their potential applications in the diagnosis, treatment, and monitoring of a variety of neurological and neurodegenerative illnesses.

4.6 Touch and test sensation perceptibility

The strategy for designing an “active structure” touch sensor includes a “capacitance-based device” composed of a series of capacitor units to produce a signal in response to local strain [98]. Capacitor elements as small as $50\text{ }\mu\text{m}$ square have been manufactured using on-chip signal circuits to notice

fine textures, such as fingerprints. Although using conductive films for energy transduction is a very promising way, the current technology has two drawbacks: the dynamic range is limited, *i.e.*, the composite material’s response range to stress is limited, and the resolution is inadequate [98]. Recent studies have shown that nanocomposites with aligned CNTs can overcome these two obstacles. CNTs have been proven to be “highly pressure-sensitive materials.” However, when replaced by molecular devices composed of a molecular monolayer of pressure-sensitive molecules, nanomaterials (such as nanotubes or nanowires) can achieve even greater miniaturization [99]. Likewise, piezoresistive nanocomposites may offer similar attributes for tactile sensing in electronic skin devices, such as the recently developed magnetic field-controlled assembly of urchin-shaped conductive magnetic nanoparticles for improved performance during stretching strain [100].

5 Advanced nanobiomaterials: Emerging and future applications

5.1 Molecular, sub-cellular, and cellular grafting

Nano topography changes cell response by controlling “cell growth, protein deposition, and increasing osteoblast adhesion and proliferation,” which has been shown in recent studies [10]. Enhanced cell and protein binding ability is attributed to increased surface area and altered surface energy [10]. Nanomaterials are over a hundred times smaller than cells, and therefore, nanomaterial-based interfaces can be used for intracellular sensing, controlling cell biological interactions, drug delivery, or possibly replacing deficient or malfunctioning components at the cellular and sub-cellular level to target structures such as the cell membrane and its microdomains, cytoplasm, lysosomes, nuclear membrane, or the nucleus [101].

The development of artificial “living” cells from non-living synthetic or biological molecular materials presents a great challenge in the current era of scientific advancements. By studying the architecture of biological living cells, an approach to designing an artificial cellular system entails the formation of a lipid vesicle, and a lipid bilayer membrane containing cholesterol and proteins [102]. A traditional method for the synthesis of artificial cells involves combining non-living components with living cells to form hybrid cells. Non-living components include artificial cell-like molecular structures such as enzymes, proteins, DNA, and nanoparticles [103]. The synthesis of nanomaterials such as nanoparticles, nanocapsules, liposomes, and polymersomes rely on the basic modification of introducing a membrane around the nanostructure, thus offering a very simple structural outline of a typical cell. However, living cells have complex metabolic functions and interconnected organelles which simple synthetic subcellular structures cannot completely emulate [104]. In the study of molecular, subcellular, and cellular replacements, is the growing research interest in the development of hemoglobin-based oxygen carriers (HBOCs) and artificial blood cells (erythrocytes, leukocytes, and thrombocytes) for potentially creating blood substitutes and artificial blood. Artificial blood is in its preliminary stages of development and there are no FDA approved artificial blood substitutes or HBOCs with a minimal side-effect profile for safe clinical use [105]. Advancements in nanotechnology are concentrated towards preparing artificial hemoglobin (Hb) and enzymes as nano-complexes [70]. One

of the earliest nanobiotechnological approaches for synthesizing artificial Hb involved crosslinking of Hb tetramers into PolyHb complexes of nanodimension thickness using glutaraldehyde. Nanodimensional PolyHb could then be incorporated into or conjugated onto synthetic cellular mimics – lipid vesicles, biodegradable polymeric membranes (using PEG or PLA), or nanocapsules loaded with Hb and erythrocyte enzymes [70,106]. The addition of PEG to a nanoparticle to form a PEG-lipid-polymer membrane structure was shown to increase circulation times of synthetic HBOCs after injection, despite the smaller nano-size range compared to typical micro-sized erythrocytes [106].

5.2 Bio-fluidic replacements

Biological nanofluids may be defined as fluids containing suspended nanodroplets (nanoemulsions) or nanoparticles (nanosuspensions) of size range >100 nm. The main type of nanofluid explored for biomedical applications, drug delivery, imaging, and diagnostics are nanosuspensions constituting solid nanoparticles [71]. Similar to the progress of artificial cells, biomedical and medical applications of nanofluids are far from its conception in the laboratory through to its implementation in the clinical setting. The following key properties of nanofluids require further *in vitro* and *in vivo* investigations into nanofluid stability, agglomeration state, and biocompatibility: composition, size, crystallinity, and morphology [71].

Nanoparticles usually have surfaces that are responsive to creep. When exposed to liquids containing biological macromolecules, their surfaces will be quickly covered by dissolved components, especially proteins. The physical properties of protein crowns are largely controlled by the surface properties of the nanoparticles and the type and quantity of biomolecules in biological fluids [73].

To successfully design nanoparticles for biomedicine, researchers must predict the subsequent interactions within biological systems that could alter the surface of nanoparticles and determine *in vivo* characteristics such as, cellular uptake, pharmacokinetics, biodistribution, and toxicity. Nanoparticles functionalized with targeting ligands (such as antibodies, peptides, sugars, and proteins) can lose their ability to target when they form a crown of proteins on their surface and block these ligands. In addition, the body's immune system has powerful molecular tools that can change the surface that is recognized as a foreign body. The combination of antibodies and

corona can improve the recognition and clearance of the body's natural immune system [73].

Blood is a specialized body fluid that is considered a colloidal suspension of blood cells (erythrocytes, leukocytes, and platelets) in its normal biological state. When blood is considered a base fluid for nanoparticulate suspensions for synthesizing nano-blood fluids, mathematical studies and derivatizations are required for assessing the changes in blood flow properties and associated heat transfer rates attributed to nanoparticle size, shape, and morphology [107]. These parameters are important when designing nanofluids for drug delivery or blood replacements. Although blood vessel walls may be elastic, flexible, and permeable, non-Newtonian blood flow in both healthy and stenosed arteries must be probed to ensure biocompatibility of nanofluid systems [108]. There is great anticipation and curiosity concerning the role of nanotechnology and nanobiomaterials to drive the development of blood substitutes and artificial blood for the creation of a complex multifunctional nanofluid system to alleviate challenges in blood donations and transfusions [70].

5.3 Cartilage and bone applications – fillings and replacements

Bone cells naturally associate with surfaces of a high degree of nano-roughness. Placing biomolecules on the surface through ligand/receptor, DNA hybridization, or antigen/antibody interactions can significantly improve the biocompatibility and performance of the implant. It has been proposed that the Ti surface coated with spiral rosette nanotubes (HRN) can mimic the environment in which bone cells interact with HRN. It is a kind of biologically inspired new nanoscale nanotube self-assembly organic material [10].

Bone grafts are an example of a typical new method to produce nanoparticles with extreme quality (high purity and crystallinity), which can enhance the quality of the existing hydroxyapatite-based medical devices. Biocompatible and hydrophilic inorganic nanomaterials such as bio-inert nano grade alumina is used for coronary replacement, maxillofacial reconstruction, coupling of knee prostheses, *etc.* [10]. While studying the adhesion of osteoblasts to inorganic calcium phosphate nanoparticles with different Ca/P ratios, Ergun *et al.* showed that with an increase in the Ca/P ratio, the mean nanocrystalline grain size, porosity, and mean pore size decreased; and the adhesion of osteoblasts to calcium phosphate with a high calcium-phosphorus

ratio increased [109]. This demonstrated the effects of mineral ratio variation, crystallinity, and size dimensions, in inorganic nanoparticulate systems for improving osteoblast interactions in bone regeneration.

Another emerging inorganic class of nanobiomaterials showing optimistic potential in bone and cartilage engineering are nanoclays. Literature reports laponite, montmorillonite, and halloysite as the most studied nanoclays for biomedical applications [15]. Nanoclays can be incorporated as fillers into polymeric hydrogels (which typically present with weak mechanical properties) and other polyester-based polymeric scaffolds, for improved mechanical strength and rheological properties in hard tissue engineering applications. The multi-layered tubular formation of halloysite nanotubes offer an attractive approach for drug entrapment or drug protection in tissue regenerative applications, with the advantages of ecompatibility and biocompatibility [15,16,110]. Upon interaction with biological fluid, the exfoliation of the layered silicate morphology of nanoclays releases biologically relevant ions which may assist the repair process of bone tissue. Laponite nanoplatelets could induce osteogenic differentiation of stem cells in the absence of exogenous growth factors – thereby demonstrating its potential use as a bioactive agent [111]. The rheological properties, mechanical strength, and degradation stability of gelatin methacrylate-nanoclay composites can be customized for both cartilage or bone tissue regeneration by adjusting the concentration of laponite solid dispersion as a filler, at 6% w/v and 8% w/v, respectively, in gelatin methacrylate solutions of 6–15% w/v concentration [112,113]. Nanoclays present an exciting class of multifunctional nanobiomaterials that offer: (1) bioactive properties and cellular signaling, (2) mechanical and rheological modification of bulk materials, and (3) capacity for drug loading and sustained release.

5.4 Neuro-sensory applications and information-carriers

“The potential applications of nano neuroscience provide a valuable way for future research on miniaturization and improving the performance of small artificial devices” [114]. Collaboration between nanomaterial science and neuroprosthetic innovation has given gigantic conceivable outcomes for more secure and superior brain embedded medications for patients enduring neurological illnesses such as loss of motion, visual impairment, and epilepsy. With the help of nanotools, a broader understanding of neural signal formation and transmission at the cellular level can enhance

the design and development of brain-like computing based on recent interdisciplinary developments [114].

Due to the complexity of neuronal cells and the mammalian nervous system, the clinical application of nanotechnology in neuroscience is still at an early stage of development. Despite these obstacles, the expansion of multidisciplinary teams in the field of nano-neuroscience, the synergistic teamwork with engineers, physicists, materials scientists, and clinicians, help to push nano-neuroscience to a higher level [114].

5.5 The COVID-19 pandemic

The COVID-19 pandemic has also created the need for new developments in nanobiomaterials, including potential use in the therapeutics of the disease as they can solve complex medical problems in this area [40]. The problems solved using nanobiomaterials in the COVID-19 pandemic include point of care diagnostics, surveillance and monitoring, therapeutics, and vaccine development [40]. The research of organs *in vitro* has brought significant progress to these developments and tissue regeneration in COVID-19 patients has advanced in a way that eliminates the need for invasive surgery [40]. This is because nanobiomaterials show mechanical characteristics that mimic implantation tissue [40]. The use of electrospun polymer fibers can cue cell signaling and immunomodulation, preventing the damage caused by the COVID-19 virus in the lungs and related tissues [40]. Some developments include controlled drug delivery, which allows COVID-19 therapeutics to act over a prolonged period of time [40].

6 Challenges to the clinical translation of nanobiomaterials

6.1 Nanotoxicity

Although great progress has been made in the application of nanotechnology, the potential hazards associated with nanomaterial exposure and subsequent results have become an important research area in the field of health and toxicology risk assessment [10]. The unique properties that make nanoscale materials useful are the same properties that make them potentially dangerous in some way.

“Nanotoxicity” refers to the potential adverse effects of exposure to new nanomaterials on human health. Regarding the safety of the engineered nanomaterials, there are many important unresolved issues. The correlation of the surface properties of the nanophase with its stability, cytotoxicity, and biodistribution is essential for *in vivo* applications. The size, shape, surface chemistry, and degree of aggregation of nanomaterials affect the generation of free radicals and subsequent oxidative stress. Oxidative stress can cause inflammation, which can lead to genotoxicity. Nanoparticles can redistribute from their deposition sites, evade normal phagocytic defenses, and can change the structure of the proteins. There are reports that nanoparticles are toxic to the liver, spleen, kidneys, lymph nodes, heart, lungs, and bone marrow [10].

More realistic and long-term studies to determine safe and non-toxic nanoparticle doses are required. New standards are needed to analyze the interface characteristics of nanoparticles and nanofabrication technologies [115]. In orthopedics, particles are usually related to the wear of artificial joints, which is the main factor in the aseptic loosening of orthopedic implants. As we all know, nano-sized wear particles are highly inflammatory, can cause osteolysis, and can also migrate to other parts of the body, thereby causing potential adverse effects. Most reported nanotoxicity studies are based on short-term durations and thus, should be extended to predict possible long-term effects.

Current research trends emphasize the importance of fully validated analytical methods and a thorough understanding of the nanotoxicity mechanisms, soon, to enable the safe use of nanobiomaterials as diagnostic and therapeutic instruments [10]. Some long-term developments could be geared towards green nanobiotechnology for the fabrication of nanobiomaterials with enhanced biocompatibility and non-toxicity to natural tissues [116].

6.2 Regulatory considerations

Designed nanobiomaterials have minimal regulatory guidance because they are incredibly difficult to regulate. Regulatory bodies such as the FDA and the European Science Foundation base their safety guidelines on data collected from the performance of the bulk material rather than the nanobiomaterial in its nano state [117]. This proves to be a challenge because their actions can be vastly different in pharmacodynamics and pharmacokinetics, and their bioactivity status is different [117].

This suggests that the data may not reflect what happens in a clinical setting and thus, forming regulations on safety and efficacy parameters has proved to be an issue [117]. As for the testing of bioactivity of nanobiomaterials, traditional animal testing is considered unethical, unworkable, and expensive, therefore, *in vitro* testing is currently the gold standard in nanobiomaterial testing [117]. *In vitro* testing has proven to be less expensive with greater control on the environment around the experiment – even though this method may lack the nuances that come with working with a living organism [117]. Despite the efficiency of *in vivo* methods, there is significant preclinical safety and adverse effect data required before the approval of new nanobiomaterials for clinical use [117]. Regulatory bodies as well as academics and clinicians all have a role to play in the determination of guidelines for

nanomedicines. Without clear leadership and guidance, the current efforts will not lead to new products entering the market [117].

6.3 Commercialization

Nanobiomaterials approved and currently available for clinical use, or nanomedicines, including antifungal, anticancer agents, and pain management agents have proven to have a better risk to benefit ratio (Figure 10), where some of the risks include toxicity, and concerns about the accumulation and clearance of nanomedicines [117]. The lack of clinical information regarding nanobiomaterials makes it difficult to regulate, therefore, the

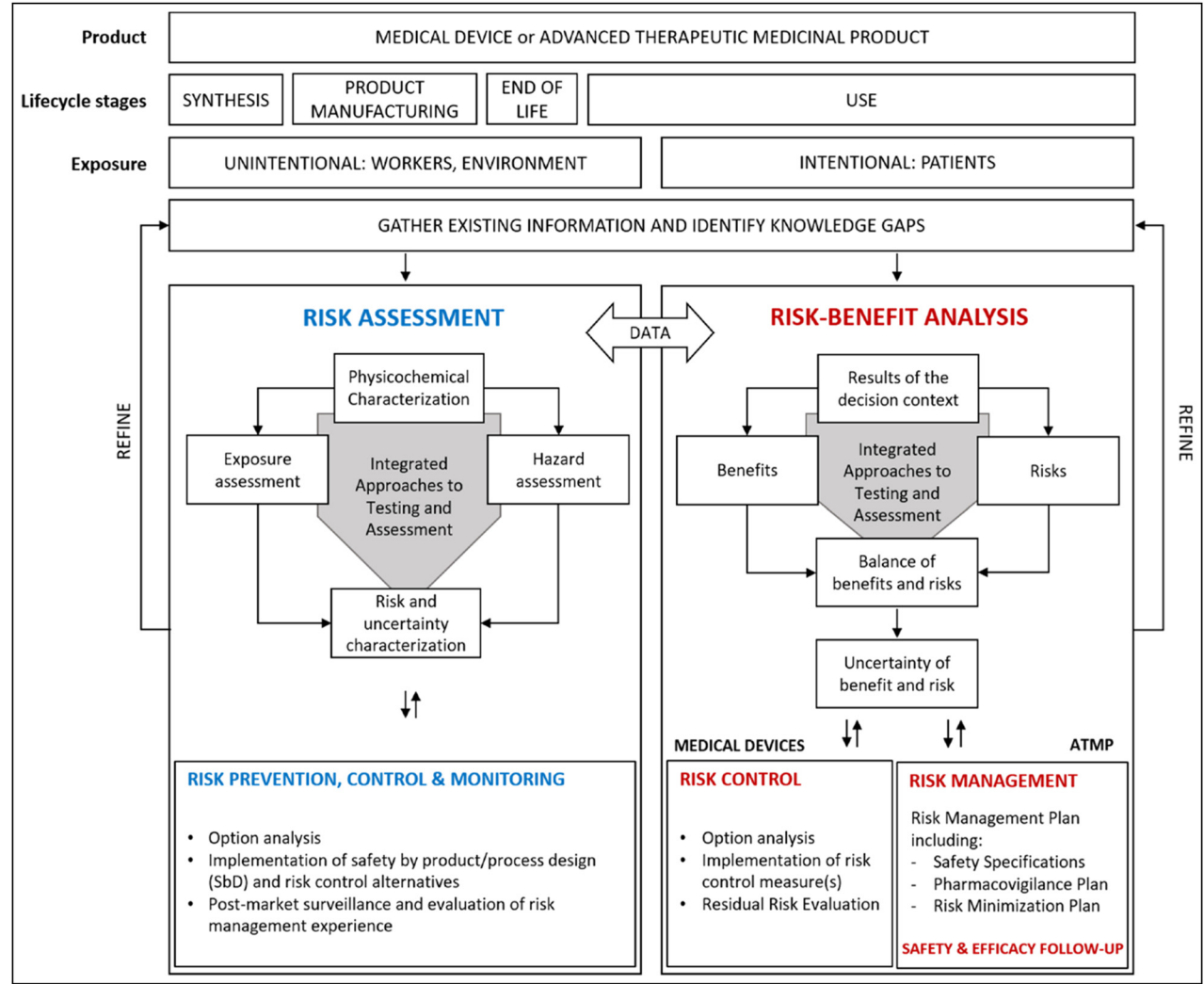


Figure 10: The risk-management framework for nanobiomaterials [118]. Reproduced with permission from ref. [118], 2020 © Creative Commons CC BY 4.0.

design of customized testing of nanobiomaterials is required [117]. Nanobiomaterial stability and toxicity are some of the important aspects of nanomedicine that need to be studied. With the lack of information in this area, near future biomedical and clinical requirements include more studies to improve clarity overregulation for the clinical use of nanobiomaterials. This results in the global issue of lack of formal regulation and the regulatory authorities in different countries have come to very different conclusions regarding what is acceptable and not, in nanomedicine and nano-medical devices [117]. In addition, economical constraints are a contributing factor to the slow advancement of nanobiomaterials in clinical use. The methods to produce nanobiomaterials and nanomedicine can be costly. The combination of nanobiomaterials with 3D printing processes creates a unique opportunity to produce nanomedicines for regenerative therapy at a lower cost [55].

7 Conclusion and future directions

Cytotoxicity and biocompatibility persist as issues seeking resolutions in the much-anticipated use of nanobiomaterials in the clinical setting for precision and personalized medicine – especially when manipulating or modifying nanomaterial structure, composition, and functionality. To avoid nanotoxicity, the elements of nanomaterial size, shape, and chemical composition require constant readjustment and reformulation to offer tailored and balanced yet predictable outcomes for circulation half-life, biodegradation, and elimination. Improving circulation half-life of nanocarriers for prolonged therapeutic effects may interfere with attempts to reduce biopersistence leading to nanotoxicity. The escalating concerns of nanotoxicity require standardized protocols for *in vivo* analysis and clinical trials. This may be the driving force needed to progress the use of nanobiomaterials in the clinical setting. For the benefits of nanomedicine to be fully harnessed in the clinical setting, regulatory guidelines need to be established to ensure the safe, reliable, and rational use of nanomedicines and nanopharmaceuticals. In addition to nanotoxicity, the added challenge to the commercialization of personalized nanomedicines is the pharmacokinetic and pharmacodynamic variability anticipated across different individuals or populations. Nanobiomaterials have sufficiently proved their value and success in drug delivery, imaging, and diagnostic applications, particularly in oncology. The feat of nanobiomaterials in eliciting essential tissue regenerative cues is initiating curiosity for and realization of the next level of tissue engineering

efforts – creating artificial hybrid cells and specialized biological fluid replacements, such as artificial blood, lymph, or synovial fluids. The emerging trend of multifunctional nanobiomaterials featuring biosensing capabilities using optical and electrical properties, antimicrobial/antifouling properties, and improved biocompatibility and biomimicry hold promise for the future generation of hybrid-bionic cell systems, or nanobiomachines and nanobots to perform intricate cellular functions, sense, diagnose, and release necessary therapeutic agents to target tissues or the blood stream. Investigation into the use of electroactive, piezoelectric, and optical nanobiomaterials combined with nanohydrogel technology may aid in progressing research endeavors in applications relying on biosensing and bioelectric functionality – this is either lacking or in its very early stages of research. Research geared towards these properties may advance biomedical applications for auditory systems to treat age-related and ototoxic-induced hearing loss, artificial sensory tissues and organs for touch and sight perception, and artificial neural transmitters for initiating or intercepting neuronal signals for potential in managing neurodegenerative diseases.

Nanobiomaterials are shown to be a great asset in the biomedical field and have increased in their impact over the past decades. This is because they are easy to fine-tune and have maintained compliance in the biomedical sector [40]. Nanobiomaterials can achieve a level of precision due to their small size, and therefore, form powerful tools in the treatment of disease compared to conventional medicines, to improve the quality of life of patients. Their drug targeting abilities significantly reduce adverse effects caused by certain drugs, such as cytotoxic chemotherapeutics [40]. These characteristics of nanobiomaterials can help solve many challenges related to human health and have been incorporated into modern medicine in two major areas namely, tissue engineering and the treatment of novel viruses such as COVID-19 [40]. Nanobiomaterials can be made efficient, dependable, and compatible with human tissue which makes them highly valuable in the biomedical field [40]. However, more clinical trials and research are needed to establish concerns of nanotoxicity, bioelimination, and translations to clinical applications. This propagates the demand for global action towards establishing standards, certifications, and regulations for the anticipated wave of nanomedicines to ensure the reliable and safe use of these products [119].

Acknowledgments: The researchers would like to thank the Deanship of Scientific Research, Qassim University, for funding the publication of this project.

Funding information: The authors state no research funding involved.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

References

- [1] Hochella MF. There's plenty of room at the bottom: nanoscience in geochemistry. *Geochim Cosmochim Acta*. 2002;66(5):735–43.
- [2] Hulbert SF. Ceramics in clinical applications: past, present and future. In: Vincenzini P, editor. *High tech ceramics*. Amsterdam: Elsevier; 1987. p. 189–213.
- [3] Thakur S, Agrawal R. Application of nanotechnology in pharmaceutical formulation design and development. *Curr Drug Ther*. 2015;10(1):20–34.
- [4] Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. *Molecules*. 2020;25(1):112.
- [5] Singh N, Sharma UC. Introduction to nano-biomaterials. In: Navani NK, Sinha S, editors. *Nanotechnology vol. 11 biomaterials*. Texas: Studium Press LLC; 2014. p. 1–12.
- [6] Saleh TA. Nanomaterials: classification, properties, and environmental toxicities. *Environ Technol Innov*. 2020;20:101067.
- [7] Salata OV. Applications of nanoparticles in biology and medicine. *J Nanobiotechnol*. 2004;2(1):3.
- [8] Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. *Biomaterials science: an introduction to materials in medicine*. 2nd edn. Elsevier; 2004.
- [9] Balasundaram G, Webster TJ. Nanotechnology and biomaterials for orthopedic medical applications. *Nanomedicine*. 2006;1(2):169–76.
- [10] Saji VS, Choe HC, Yeung KW. Nanotechnology in biomedical applications: a review. *Int J Nano Biomater*. 2010;3(2):119–39.
- [11] Schaeublin NM, Braydich-Stolle LK, Maurer EI, Park K, MacCuspie RI, Afroz ARMN, et al. Does shape matter? Bioeffects of gold nanomaterials in a human skin cell model. *Langmuir*. 2012;28(6):3248–58.
- [12] Viran M, Miricescu D, Radulescu R, Sabliov CM, Totan A, Calenic B, et al. Organic nanomaterials and their applications in the treatment of oral diseases. *Molecules*. 2016;21(2):207.
- [13] Operti MC, Bernhardt A, Grimm S, Engel A, Figdor CG, Tagit O. PLGA-based nanomedicines manufacturing: technologies overview and challenges in industrial scale-up. *Int J Pharm*. 2021;605:120807.
- [14] Kumar P, Saini M, Dehiya BS, Sindhu A, Kumar V, Kumar R, et al. Comprehensive survey on nanobiomaterials for bone tissue engineering applications. *Nanomaterials*. 2020;10(10):2019.
- [15] Murali A, Lokhande G, Deo KA, Brokesh A, Gaharwar AK. Emerging 2D nanomaterials for biomedical applications. *Mater Today*. 2021;50:276–302.
- [16] Massaro M, Cavallaro G, Colletti CG, Lazzara G, Milioto S, Noto R, et al. Chemical modification of halloysite nanotubes for controlled loading and release. *J Mater Chem B*. 2018;6(21):3415–33.
- [17] Patel KD, Singh RK, Kim H-W. Carbon-based nanomaterials as an emerging platform for theranostics. *Mater Horiz*. 2019;6(3):434–69.
- [18] Maiti D, Tong X, Mou X, Yang K. Carbon-based nanomaterials for biomedical applications: a recent study. *Front Pharmacol*. 2019;9:1401.
- [19] Sen M. Nanocomposite materials. In: Sen M, editor. *Nanotechnology and the environment*. London: IntechOpen; 2020. p. 107–12.
- [20] Turlybekuly A, Pogrebnyak AD, Sukhodub LF, Sukhodub LB, Kistaubayeva AS, Savitskaya IS, et al. Synthesis, characterization, *in vitro* biocompatibility and antibacterial properties study of nanocomposite materials based on hydroxyapatite-biphasic ZnO micro- and nanoparticles embedded in Alginate matrix. *Mater Sci Eng C*. 2019;104:109965.
- [21] Liu Y, Yu Q, Chang J, Wu C. Nanobiomaterials: from 0D to 3D for tumor therapy and tissue regeneration. *Nanoscale*. 2019;11(29):13678–708.
- [22] Torresan V, Forrer D, Guadagnini A, Badocco D, Pastore P, Casarin M, et al. 4D multimodal nanomedicines made of nonequilibrium Au–Fe alloy nanoparticles. *ACS Nano*. 2020;14(10):12840–53.
- [23] Liu M, Fakhruddin R, Novikov A, Panchal A, Lvov Y. Tubule nanoclay-organic heterostructures for biomedical applications. *Macromol Biosci*. 2019;19(4):1800419.
- [24] Yokota J, Kyotani S. Influence of nanoparticle size on the skin penetration, skin retention and anti-inflammatory activity of non-steroidal anti-inflammatory drugs. *J Chin Med Assoc*. 2018;81(6):511–9.
- [25] Meng Q, Meng H, Pan Y, Liu J, Li J, Qi Y, et al. Influence of nanoparticle size on blood–brain barrier penetration and the accumulation of anti-seizure medicines in the brain. *J Mater Chem B*. 2022;10(2):271–81.
- [26] Jahnke-Dechent W, Simon U. Function follows form: shape complementarity and nanoparticle toxicity. *Nanomedicine*. 2008;3(5):601–3.
- [27] Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, et al. Size-dependent cytotoxicity of gold nanoparticles. *Small*. 2007;3(11):1941–9.
- [28] Kim JS, Lee K, Lee YH, Cho HS, Kim KH, Choi KH, et al. Aspect ratio has no effect on genotoxicity of multi-wall carbon nanotubes. *Arch Toxicol*. 2011;85(7):775–86.
- [29] Bauer LA, Birenbaum NS, Meyer GJ. Biological applications of high aspect ratio nanoparticles. *J Mater Chem*. 2004;14(4):517–26.
- [30] Fratila RM, Rivera-Fernández S, Jesús M. Shape matters: synthesis and biomedical applications of high aspect ratio magnetic nanomaterials. *Nanoscale*. 2015;7(18):8233–60.

- [31] Luanpitpong S, Wang L, Davidson DC, Riedel H, Rojanasakul Y. Carcinogenic potential of high aspect ratio carbon nanomaterials. *Environ Sci Nano*. 2016;3(3):483–93.
- [32] Yi X, Shi X, Gao H. A universal law for cell uptake of one-dimensional nanomaterials. *Nano Lett*. 2014;14(2):1049–55.
- [33] Sanchez VC, Weston P, Yan A, Hurt RH, Kane AB. A 3-dimensional *in vitro* model of epithelioid granulomas induced by high aspect ratio nanomaterials. *Part Fibre Toxicol*. 2011;8(1):17.
- [34] Wan J, Wang J-H, Liu T, Xie Z, Yu X-F, Li W. Surface chemistry but not aspect ratio mediates the biological toxicity of gold nanorods *in vitro* and *in vivo*. *Sci Rep*. 2015;5(1):11398.
- [35] Wang Y, Sun S, Zhang Z, Shi D. Nanomaterials for cancer precision medicine. *Adv Mater*. 2018;30(17):1705660.
- [36] Auría-Soro C, Nesma T, Juanes-Velasco P, Landeira-Viñuela A, Fidalgo-Gomez H, Acebes-Fernandez V, et al. Interactions of nanoparticles and biosystems: microenvironment of nanoparticles and biomolecules in nanomedicine. *Nanomaterials*. 2019;9(10):1365.
- [37] Fröhlich E. Aufbau und funktion von blut-gewebe-schranken. *Dtsch Med Wochenschr*. 2002;127(49):2629–34. (German).
- [38] Bregoli L, Movia D, Gavigan-Imedio JD, Lysaght J, Reynolds J, Prina-Mello A. Nanomedicine applied to translational oncology: a future perspective on cancer treatment. *Nanomed Nanotechnol Biol Med*. 2016;12(1):81–103.
- [39] Ugwu MC, Amadi NC, Okolo CR, Dada MO, Egejuru CCJ, Ogbodo EC, et al. Nanomedicine: application of nanotechnology. *European J Biomed Pharm Sci*. 2017;4(12):136–43.
- [40] Melchor-Martínez EM, Torres Castillo NE, Macias-Garbutt R, Lucero-Saucedo SL, Parra-Saldívar R, Sosa-Hernández JE. Modern world applications for nano-bio materials: tissue engineering and COVID-19. *Front Bioeng Biotechnol*. 2021;9:393.
- [41] Khiev D, Mohamed ZA, Vichare R, Paulson R, Bhatia S, Mohapatra S, et al. Emerging nano-formulations and nanomedicines applications for ocular drug delivery. *Nanomaterials*. 2021;11(1):173.
- [42] Zhang W, Mehta A, Tong Z, Esser L, Voelcker NH. Development of polymeric nanoparticles for blood–brain barrier transfer – strategies and challenges. *Adv Sci*. 2021;8(10):2003937.
- [43] Riggio C, Pagni E, Raffa V, Cuschieri A. Nano-oncology: clinical application for cancer therapy and future perspectives. *J Nanomater*. 2011;2011:17.
- [44] Zhang Y, Li M, Gao X, Chen Y, Liu T. Nanotechnology in cancer diagnosis: progress, challenges and opportunities. *J Hematol Oncol*. 2019;12(1):137.
- [45] Anselmo AC, Mitragotri S. Nanoparticles in the clinic: an update. *Bioeng Transl Med*. 2019;4(3):e10143.
- [46] Lôbo GCNB, Paiva KLR, Silva ALG, Simões MM, Radicchi MA, Bão SN. Nanocarriers used in drug delivery to enhance immune system in cancer therapy. *Pharmaceutics*. 2021;13(8):1167.
- [47] Zhang Q, Yu H, Barbiero M, Wang B, Gu M. Artificial neural networks enabled by nanophotonics. *Light: Sci Appl*. 2019;8(1):42.
- [48] Yang L, Zhang L, Webster TJ. Nanobiomaterials: state of the art and future trends. *Adv Eng Mater*. 2011;13(6):B197–B217.
- [49] Shafiei-Irannejad V, Soleymani J, Azizi S, KhoubnasabJafari M, Jouyban A, Hasanzadeh M. Advanced nanomaterials towards biosensing of insulin: analytical approaches. *TrAC - Trends Anal Chem*. 2019;116:1–12.
- [50] Lei J, Ju H. Signal amplification using functional nanomaterials for biosensing. *Chem Soc Rev*. 2012;41(6):2122–34.
- [51] Holzinger M, Le Goff A, Cosnier S. Synergetic effects of combined nanomaterials for biosensing applications. *Sensors*. 2017;17(5):1010.
- [52] Hwang HS, Jeong JW, Kim YA, Chang M. Carbon nanomaterials as versatile platforms for biosensing applications. *Micromachines*. 2020;11(9):814.
- [53] Carneiro P, Morais S, Pereira MC. Nanomaterials towards biosensing of Alzheimer's disease biomarkers. *Nanomaterials*. 2019;9(12):1663.
- [54] Mostafavi E, Medina-Cruz D, Kalantari K, Taymoori A, Soltantabar P, Webster TJ. Electroconductive nanobiomaterials for tissue engineering and regenerative medicine. *Bioelectricity*. 2020;2(2):120–49.
- [55] Di Marzio N, Eglin D, Serra T, Moroni L. Bio-fabrication: convergence of 3D bioprinting and nano-biomaterials in tissue engineering and regenerative medicine. *Front Bioeng Biotechnol*. 2020;8:326.
- [56] Dvir T, Timko BP, Kohane DS, Langer R. Nanotechnological strategies for engineering complex tissues. *Nat Nanotechnol*. 2011;6(1):13–22.
- [57] Hudita A, Galateanu B, Costache M. Chapter 1 – Nanobiomaterials for tissue engineering. In: Holban A-M, Grumezescu AM, editors. *Materials for biomedical engineering*. Amsterdam: Elsevier; 2019. p. 1–21.
- [58] Nikolova MP, Chavali MS. Recent advances in biomaterials for 3D scaffolds: a review. *Bioact Mater*. 2019;4:271–92.
- [59] Wang J, Vermerris W. Antimicrobial nanomaterials derived from natural products – a review. *Materials*. 2016;9(4):255.
- [60] Ogunsona EO, Muthuraj R, Ojogbo E, Valerio O, Mekonnen TH. Engineered nanomaterials for antimicrobial applications: a review. *Appl Mater Today*. 2020;18:100473.
- [61] Dickson MN, Liang EI, Rodriguez LA, Vollereaux N, Yee AF. Nanopatterned polymer surfaces with bactericidal properties. *Biointerphases*. 2015;10(2):021010.
- [62] Sánchez-López E, Gomes D, Esteruelas G, Bonilla L, Lopez-Machado AL, Galindo R, et al. Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials*. 2020;10(2):292.
- [63] Hasan A, Morshed M, Memic A, Hassan S, Webster TJ, Marei HE-S. Nanoparticles in tissue engineering: applications, challenges and prospects. *Int J Nanomed*. 2018;13:5637–55.
- [64] Hemeg HA. Nanomaterials for alternative antibacterial therapy. *Int J Nanomed*. 2017;12:8211–25.
- [65] Ramburrun P, Pringle NA, Dube A, Adam RZ, D'Souza S, Aucamp M. Recent advances in the development of antimicrobial and antifouling biocompatible materials for dental applications. *Materials*. 2021;14(12):3167.
- [66] Albanese A, Tang PS, Chan WCW. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu Rev Biomed Eng*. 2012;14(1):1–16.
- [67] Srivastava SK, Yadav VG. Bionic manufacturing: towards cyborg cells and sentient microbots. *Trends Biotechnol*. 2018;36(5):483–7.
- [68] Zahin N, Anwar R, Tewari D, Kabir MT, Sajid A, Mathew B, et al. Nanoparticles and its biomedical applications in health

- and diseases: special focus on drug delivery. *Environ Sci Pollut Res.* 2020;27(16):19151–68.
- [69] Yadid M, Feiner R, Dvir T. Gold nanoparticle-integrated scaffolds for tissue engineering and regenerative medicine. *Nano Lett.* 2019;19(4):2198–206.
- [70] Alam F, Yadav N, Ahmad M, Shadan M. Blood substitutes: possibilities with nanotechnology. *Ind J Hematol Blood Transfus.* 2014;30(3):155–62.
- [71] Sheikhpour M, Arabi M, Kasaeian A, Rokn Rabei A, Taherian Z. Role of nanofluids in drug delivery and biomedical technology: methods and applications. *Nanotechnol Sci Appl.* 2020;13:47–59.
- [72] Fröhlich E. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. *Int J Nanomed.* 2012;7:5577–91.
- [73] Nienhaus K, Wang H, Nienhaus GU. Nanoparticles for biomedical applications: exploring and exploiting molecular interactions at the nano-bio interface. *Mater Today Adv.* 2020;5:100036.
- [74] De-la-Pinta I, Cobos M, Ibarretxe J, Montoya E, Eraso E, Guraya T, et al. Effect of biomaterials hydrophobicity and roughness on biofilm development. *J Mater Sci Mater Med.* 2019;30(7):77.
- [75] Saberi A, Jabbari F, Zarrintaj P, Saeb MR, Mozafari M. Electrically conductive materials: Opportunities and challenges in tissue engineering. *Biomolecules.* 2019;9(9):448.
- [76] Mnasri W, Parvizian M, Ammar-Merah S. Design and synthesis of luminescent lanthanide-based bimodal nanoprobe for dual magnetic resonance (MR) and optical imaging. *Nanomaterials.* 2021;11(2):354.
- [77] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol.* 2015;33(9):941–51.
- [78] Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurodegenerative diseases. *J Control Release.* 2016;235:34–47.
- [79] Truong NP, Whittaker MR, Mak CW, Davis TP. The importance of nanoparticle shape in cancer drug delivery. *Expert Opin Drug Deliv.* 2015;12(1):129–42.
- [80] Banerjee A, Qi J, Gogoi R, Wong J, Mitragotri S. Role of nanoparticle size, shape and surface chemistry in oral drug delivery. *J Control Release.* 2016;238:176–85.
- [81] Thau L, Asuka E, Mahajan K. Physiology, opsonization. *StatPearls [Internet].* Treasure Island: StatPearls Publishing; 2021. p. 1–5.
- [82] van Rijt S, Habibovic P. Enhancing regenerative approaches with nanoparticles. *J R Soc Interface.* 2017;14(129):20170093.
- [83] Zhang L, Webster TJ. Nanotechnology and nanomaterials: promises for improved tissue regeneration. *Nano Today.* 2009;4(1):66–80.
- [84] Lyu Q, Peng L, Hong X, Fan T, Li J, Cui Y, et al. Smart nano-micro platforms for ophthalmological applications: the state-of-the-art and future perspectives. *Biomaterials.* 2021;270:120682.
- [85] Gorantla S, Rapalli VK, Waghule T, Singh PP, Dubey SK, Saha RN, et al. Nanocarriers for ocular drug delivery: current status and translational opportunity. *RSC Adv.* 2020;10(46):27835–55.
- [86] Mittal R, Pena SA, Zhu A, Eshraghi N, Fesharaki A, Horesh EJ, et al. Nanoparticle-based drug delivery in the inner ear: current challenges, limitations and opportunities. *Artif Cells Nanomed Biotechnol.* 2019;47(1):1312–20.
- [87] Li L, Chao T, Brant J, O'Malley B, Tsourkas A, Li D. Advances in nano-based inner ear delivery systems for the treatment of sensorineural hearing loss. *Adv Drug Deliv Rev.* 2017;108:2–12.
- [88] Dindelegan MG, Blebea C, Perde-Schrepler M, Buzoianu AD, Maniu AA. Recent advances and future research directions for hearing loss treatment based on nanoparticles. *J Nanomater.* 2022;2022:7794384.
- [89] Cai H, Wen X, Wen L, Tirelli N, Zhang X, Zhang Y, et al. Enhanced local bioavailability of single or compound drugs delivery to the inner ear through application of PLGA nanoparticles via round window administration. *Int J Nanomed.* 2014;9:5591–601.
- [90] Sun C, Wang X, Zheng Z, Chen D, Wang X, Shi F, et al. A single dose of dexamethasone encapsulated in polyethylene glycol-coated polylactic acid nanoparticles attenuates cisplatin-induced hearing loss following round window membrane administration. *Int J Nanomed.* 2015;10:3567–79.
- [91] Cervantes B, Arana L, Murillo-Cuesta S, Bruno M, Alkorta I, Varela-Nieto I. Solid lipid nanoparticles loaded with glucocorticoids protect auditory cells from cisplatin-induced ototoxicity. *J Clin Med.* 2019;8(9):1464.
- [92] Gausterer JC, Saidov N, Ahmadi N, Zhu C, Wirth M, Reznicek G, et al. Intratympanic application of poloxamer 407 hydrogels results in sustained *N*-acetylcysteine delivery to the inner ear. *Eur J Pharm Biopharm.* 2020;150:143–55.
- [93] Lajud SA, Nagda DA, Qiao P, Tanaka N, Civantos A, Gu R, et al. A novel chitosan-hydrogel-based nanoparticle delivery system for local inner ear application. *Otol Neurotol.* 2015;36(2):341–7.
- [94] Magdy M, Elmowafy E, Ellassal M, Ishak RAH. Localized drug delivery to the middle ear: Recent advances and perspectives for the treatment of middle and inner ear diseases. *J Drug Deliv Sci Technol.* 2022;69:103149.
- [95] Rathnam C, Chueng S-TD, Ying Y-LM, Lee K-B, Kwan K. Developments in bio-inspired nanomaterials for therapeutic delivery to treat hearing loss. *Front Cell Neurosci.* 2019;13:493.
- [96] Berger M. Nanotechnology for neuroscience [Internet]. Nanowerk; 2017 [cited 2021 Dec 7] Available from: <https://www.nanowerk.com/spotlight/spotid=48348.php>
- [97] Shah S. The nanomaterial toolkit for neuroengineering. *Nano Converg.* 2016;3(1):25.
- [98] Maheshwari V, Saraf R. Tactile devices to sense touch on a par with a human finger. *Angew Chem - Int Ed.* 2008;47(41):7808–26.
- [99] Berger M. Nanotechnology skin to rival human touch [Internet]. Nanowerk; 2008 [cited 2021 Dec 7] Available from: <https://www.nanowerk.com/spotlight/spotid=7497.php>
- [100] Yu Z, Ying WB, Pravarthana D, Li YY, Mao GY, Liu YW, et al. Stretchable tactile sensor with high sensitivity and dynamic stability based on vertically aligned urchin-shaped nanoparticles. *Mater Today Phys.* 2020;14:100219.
- [101] Mendes PM. Cellular nanotechnology: making biological interfaces smarter. *Chem Soc Rev.* 2013;42(24):9207–18.

- [102] Jeong S, Nguyen HT, Kim CH, Ly MN, Shin K. Toward artificial cells: novel advances in energy conversion and cellular motility. *Adv Funct Mater.* 2020;30(11):1907182.
- [103] Elani Y. Interfacing living and synthetic cells as an emerging frontier in synthetic biology. *Angew Chem Int Ed.* 2021;60(11):5602–11.
- [104] Zhu Y, Guo X, Liu J, Li F, Yang D. Emerging advances of cell-free systems toward artificial cells. *Small Methods.* 2020;4(10):2000406.
- [105] Moradi S, Jahanian-Najafabadi A, Roudkenar MH. Artificial blood substitutes: first steps on the long route to clinical utility. *Clin Med Insights Blood Disord.* 2016;9: CMBD.S38461.
- [106] Chang TMS. Artificial cell evolves into nanomedicine, biotherapeutics, blood substitutes, drug delivery, enzyme/gene therapy, cancer therapy, cell/stem cell therapy, nanoparticles, liposomes, bioencapsulation, replicating synthetic cells, cell encapsulation/scaffold, biosorbent/immunosorbent haemoperfusion/plasmapheresis, regenerative medicine, encapsulated microbe, nanobiotechnology, nanotechnology. *Artif Cells Nanomed Biotechnol.* 2019;47(1):997–1013.
- [107] Khan I. New idea of Atangana and Baleanu fractional derivatives to human blood flow in nanofluids. *Chaos.* 2019;29(1):013121.
- [108] Eid MR, Alsaedi A, Muhammad T, Hayat T. Comprehensive analysis of heat transfer of gold-blood nanofluid (Sisko-model) with thermal radiation. *Results Phys.* 2017;7:4388–93.
- [109] Ergun C, Liu H, Webster TJ, Olcay E, Yılmaz Ş, Sahin FC. Increased osteoblast adhesion on nanoparticulate calcium phosphates with higher Ca/P ratios. *J Biomed Mater Res A.* 2008;85A(1):236–41.
- [110] Lisuzzo L, Cavallaro G, Milioto S, Lazzara G. Halloysite nanotubes as nanoreactors for heterogeneous micellar catalysis. *J Colloid Interface Sci.* 2022;608:424–34.
- [111] Gaharwar AK, Mihaila SM, Swami A, Patel A, Sant S, Reis RL, et al. Bioactive silicate nanoplatelets for osteogenic differentiation of human mesenchymal stem cells. *Adv Mater.* 2013;25(24):3329–36.
- [112] Dong Y, Wu X, Chen X, Zhou P, Xu F, Liang W. Nanotechnology shaping stem cell therapy: recent advances, application, challenges, and future outlook. *Biomed Pharmacother.* 2021;137:111236.
- [113] Hu H, Dong L, Bu Z, Shen Y, Luo J, Zhang H, et al. miR-23a-3p-abundant small extracellular vesicles released from Gelma/nanoclay hydrogel for cartilage regeneration. *J Extracell Vesicles.* 2020;9(1):1778883.
- [114] Kumar A, Tan A, Wong J, Spagnoli JC, Lam J, Blevins BD, et al. Nanotechnology for neuroscience: promising approaches for diagnostics, therapeutics and brain activity mapping. *Adv Funct Mater.* 2017;27(39):1700489.
- [115] Grainger DW, Castner DG. Nanobiomaterials and nanoanalysis: opportunities for improving the science to benefit biomedical technologies. *Adv Mater.* 2008;20(5):867–77.
- [116] Patra JK, Baek K-H. Green nanobiotechnology: factors affecting synthesis and characterization techniques. *J Nanomater.* 2015;2014:219.
- [117] Foulkes R, Man E, Thind J, Yeung S, Joy A, Hoskins C. The regulation of nanomaterials and nanomedicines for clinical application: current and future perspectives. *Biomater Sci.* 2020;8(17):4653–64.
- [118] Giubilato E, Cazzagon V, Amorim MJB, Blosi M, Bouillard J, Bouwmeester H, et al. Risk management framework for nano-biomaterials used in medical devices and advanced therapy medicinal products. *Materials.* 2020;13(20):4532.
- [119] Talebian S, Rodrigues T, das Neves J, Sarmiento B, Langer R, Conde J. Facts and figures on materials science and nanotechnology progress and investment. *ACS Nano.* 2021;15(10):15940–52.