Recent Advances in Nano-Biomaterials: Synthesis Strategies, Characterization Techniques, and Biomedical Applications

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Abstract

Nano-biomaterials are a rapidly evolving class of technologies that combines nanoscale engineering with inherent biological compatibility. This review offers a comprehensive examination of green synthesis, evolving categories, and expanding biomedical applications. Particular attention is made on ecologically friendly manufacturing processes that employ plant extracts, microorganisms, and biopolymers as long-term reducing and stabilizing agents, allowing nanoparticles to be produced with regulated morphology and specific functionality. Synthetic techniques are evaluated critically in terms of pH, temperature, and precursor concentration to determine their impact on nucleation, growth behavior, and overall particle architecture. Characterization techniques, including as TEM, SEM, XRD, FTIR, UV-Vis spectroscopy, and DLS, are reviewed in terms of determining physicochemical, structural, and surface-related properties required for biomedical performance. The review delves deeper into significant application categories, covering advancements in drug delivery, antimicrobial methods, wound healing, cancer therapies, biosensing systems, and tissue-engineering scaffolds. Current issues in cytotoxicity, biodegradation processes, standardization, and regulatory limitations are investigated to identify hurdles and potential for future clinical translation.

Overall, this article pursues to be an authoritative resource for scholars in chemical sciences, nanotechnology, pharmaceutical science, and biomedical engineering by providing an integrative perspective on the design principles and translational potential of nano-biomaterials.

Keywords: nano-biomaterials; biomedical applications; drug delivery; biocompatibility.

1. Introduction

Nano-biomaterials have developed as a transformational class of materials at the intersection of nanotechnology and biomaterials science, providing unprecedented control over physicochemical and biological interactions on the nanoscale (1-100 nm). Their high surface-area-to-volume ratio, variable surface chemistry, and size-dependent optical, electrical, and catalytic capabilities allow for better cellular absorption, targeted delivery, and therapeutic efficacy as compared to traditional biomaterials [1,2]. Recent discoveries in nanomedicine have shown that rationally created nano-biomaterials can affect biological processes at the molecular level, leading to better disease diagnostics, therapy, and tissue regeneration results [3].

Nano-biomaterial fabrication strategies are broadly classified into top-down approaches (e.g., lithography, mechanical milling) and bottom-up methods such as chemical precipitation, sol-gel processing, hydrothermal synthesis, and biological routes. Green synthesis techniques based on plant extracts, bacteria, fungus, and biopolymers have received a lot of attention in recent years because of their sustainability, low toxicity, and compatibility with biomedical applications [4-5]. These bio-assisted approaches reduce the need of toxic reagents while simultaneously introducing intrinsic surface functionalization via phytochemicals and biomolecules, which improves biocompatibility and therapeutic potential.

Polymeric nanoparticles such as Poly(lactic-co-glycolic acid) (PLGA) for controlled drug release, plasmonic nanostructures such as Gold nanoparticles (AuNPs) for cancer theranostics, and metal oxide nanoparticles such as Zinc oxide (ZnO) and iron oxide for antimicrobial activity and regenerative medicine [6-10] are among the most extensively researched systems. Because of their variable size, shape, and surface functionalization, these materials exhibit improved pharmacokinetics, imaging capabilities, and bioactivity. Comprehensive physicochemical characterisation is essential for establishing structure-function links and maintaining clinical safety. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering

(DLS), and zeta potential analysis provide detailed insights into particle morphology, crystallinity, surface chemistry, colloidal stability, and dispersity—parameters that directly influence biodistribution, cytocompatibility, and therapeutic performance [11-14].

Figure 1 depicts an overview of the synthesis, characterization, and biological uses of nano biomaterials.

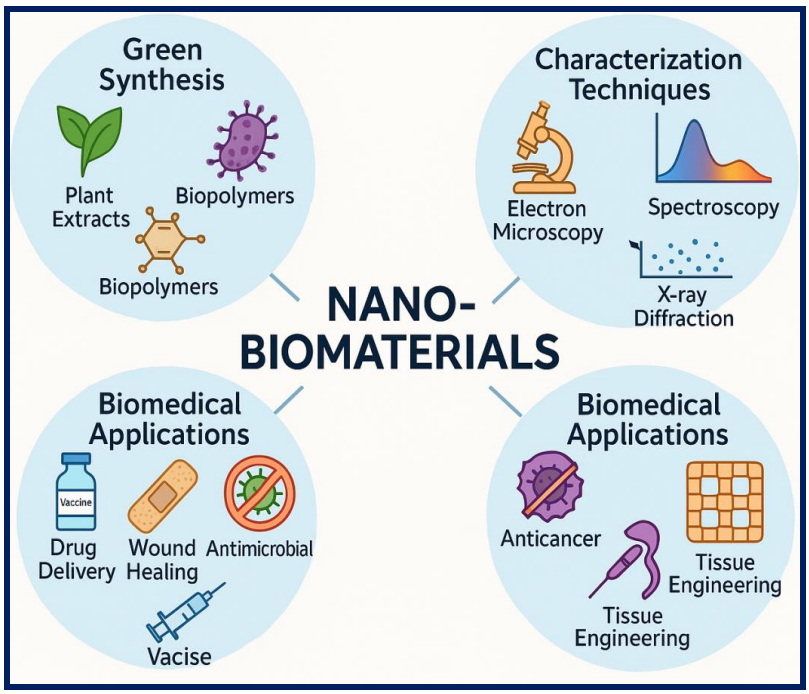

Figure 1 a Diagrammatic illustration of the synthesis, characterisation, and biomedical applications of nano-biomaterials

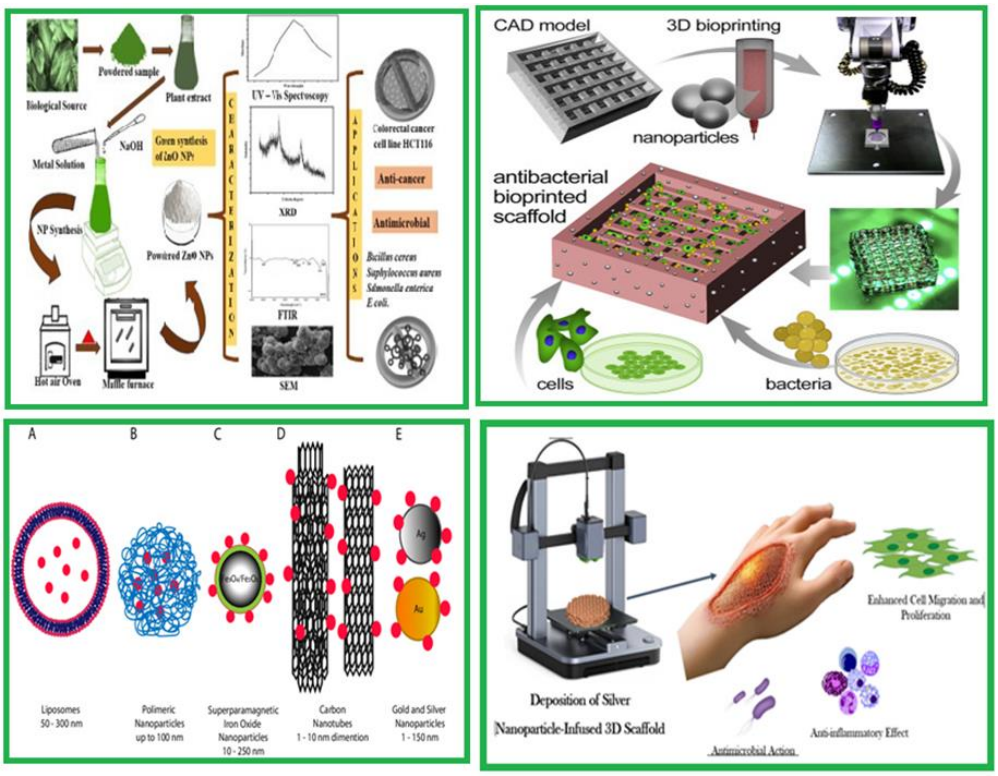

Figure 1 b. Diagrammatic illustration of the synthesis, characterisation, and biomedical applications of nano-biomaterials. **James C. L. Chow et al,** *Nanomaterials* 2025, Alberto Bacilio Quispe Cohaila *Nanomaterials* 2025 , Pronama Biswas, 2025

Despite significant advancements, long-term toxicity, biodegradation mechanisms, immunological responses, large-scale repeatability, and regulatory approval remain barriers to wider clinical translation [15-20]. To address these issues, synthesis methodologies, improved characterisation technologies, and high-impact biomedical applications must be carefully evaluated. The purpose of this review is to, critically analyze modern synthesis strategies, with a focus on green and controllable fabrication routes, discuss advanced characterization techniques that define nano-bio interface interactions; and evaluate selected high-impact nano-biomaterial systems in drug delivery, cancer theranostics, antimicrobial therapy, and tissue engineering, highlighting translational challenges and future perspectives.(21-25)

2. Synthesis of nanoparticles

Nanoparticles, which typically range in size from 1 to 100 nm, have sparked widespread interest due to their distinct physicochemical features, which include a high surface-area-to-volume ratio, variable reactivity, and quantum effects. These properties make them useful in a variety of sectors, including medicine, energy, electronics, catalysis, and environmental clean-up [26-29]. The synthesis process influences nanoparticle size, shape, stability, surface chemistry, and overall functioning. In general, nanoparticle synthesis methodologies are characterized as top-down or bottom-up [30-32]. Top-down ways include physically breaking down bulk materials into nanoscale particles, such as milling or lithography, whereas bottom-up methods use chemical or biological processes to create nanoparticles from atomic or molecular precursors. **Table 1** summarizing the common synthesis methods of nano-biomaterials, including the method, advantages, and limitations:

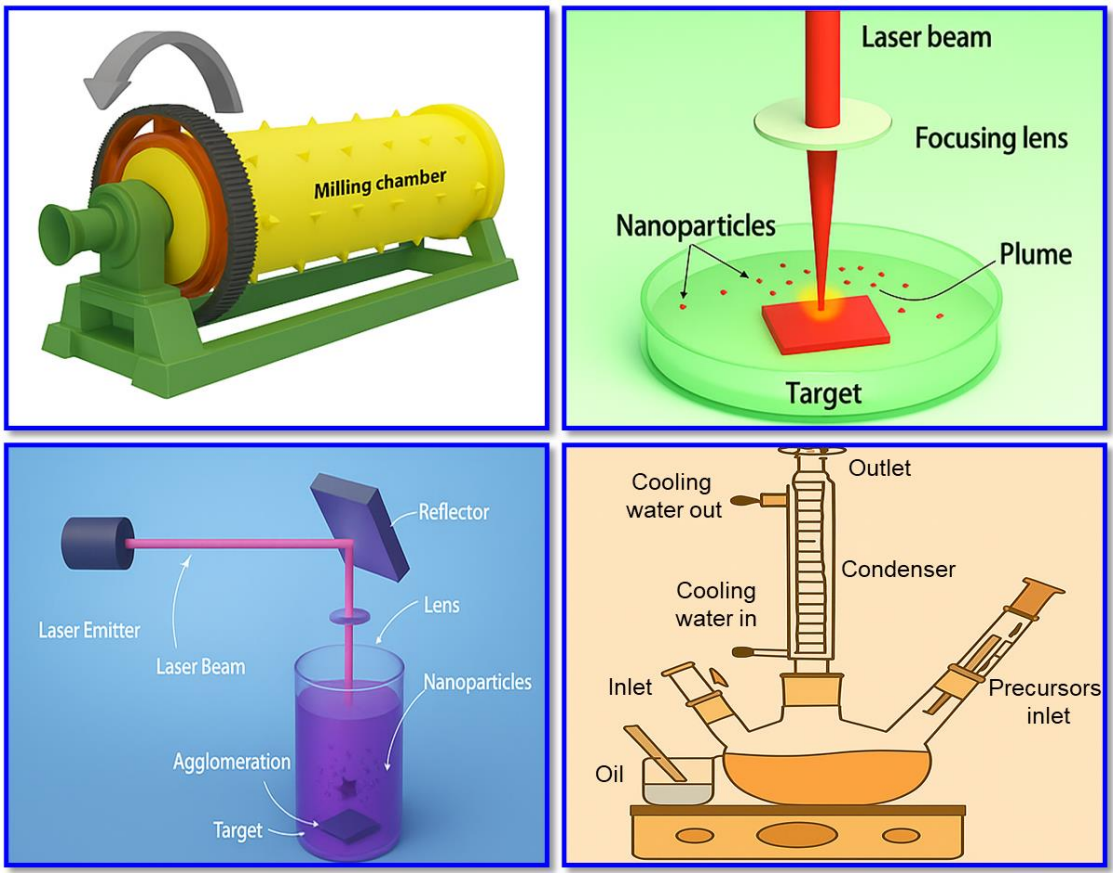

Figure 2. The different methods of synthesizing nano-biomaterials.

Table 1. Common synthesis methods of nano-biomaterials

S. No	Synthesis method	Examples	Advantages	Limitations	References
1.	Physical methods	Laser ablation, ball milling	High purity, controlled size	High energy cost, complex equipment	[33]
2.	Chemical methods	Sol-gel, chemical reduction, microemulsion	Scalable, tunable properties	Use of toxic chemicals, waste generation	[34]
3.	Green synthesis	Plant extracts, bacteria, fungi, algae	Eco-friendly, biocompatible, low cost	Limited control over size/shape, slower kinetics	[35]
4.	Biological template-based	Protein scaffolds, DNA origami	Highly specific, bioinspired structure	Costly biomolecules	[36]

2.1. Top-up method

The top-down method for producing nanoparticles involves reducing bulk materials into tiny particles using mechanical or physical means. Top-down techniques use external forces like mechanical machining, lithography, or laser ablation to reduce the size of larger structures, whereas bottom-up approaches build nanoparticles atom by atom or molecule by molecule [37]. This approach is extensively utilized because it is simple, scalable, and suitable for manufacturing huge amounts of nanoparticles. Despite its advantages, the top-down technique may result in poor control over particle shape and size distribution, as well as the introduction of flaws or contaminants during the milling or grinding process [38]. Nonetheless, it is still an important method, especially for applications that require mass production or where precise control over chemical composition is less vital.

2.1.1 Mechanical milling

Mechanical milling is a widely used physical approach for creating nanoparticles by mechanically reducing bulk materials. In this approach, a revolving milling vessel holding grinding balls causes repeated collisions and friction with the material, resulting in particle fracture and nanoscale refinement. The mechanical energy imparted by the milling media determines the transformation pathway, reaction kinetics, and final particle size distribution [39].

2.1.2 Nanolithography

Nanolithography is the process of creating tiny patterns with dimensions that typically range from 1 to 100 nm. To create highly controlled nanostructures, a variety of lithographic techniques are used, including photolithography, electron beam lithography, focussed ion beam lithography, nanoimprint lithography, soft lithography, and scanning probe. These techniques are frequently used in nanoelectronics, sensors, and innovative materials because of their precision and reproducibility [40].

2.1.3. Laser ablation

Laser ablation is a physical process that produces nanoparticles by irradiating a metal target with a high-energy laser beam in a liquid or gaseous media. The laser-material interaction generates a plasma plume of ionized atoms and clusters, which then cool and nucleate to form nanoparticles. This method is regarded as a quick and ecologically friendly technique because it avoids the use of toxic chemicals and complex synthesis techniques. Laser ablation also allows for exact control of nanoparticle purity, content, and size, making it ideal for biological, catalytic, and sensing applications [41-42].

2.1.4. Thermolysis

Thermolysis is the thermal breakdown of precursor chemicals to create nanoparticles. Typically, the precursor solution is heated under reflux conditions to encourage nucleation and controlled particle development. Prolonged heating improves crystallinity and particle homogeneity, but post-synthesis aging improves stability and size distribution. The nanoparticles are next cleaned by centrifugation and solvent washing to remove any remaining precursors or byproducts, followed by drying to produce nanopowders suitable for further characterisation and

applications. This approach is especially useful for producing metal and metal oxide nanoparticles with high purity and controlled shape [43].

2.2. Bottom-up method

Bottom-up synthesis is the creation of nanostructures from atomic or molecular precursors using chemical or biological processes. Top-down procedures break down bulk materials into nanoscale structures, whereas bottom-up methods assemble nanoparticles atom by atom or molecule by molecule. This method provides exact control over particle size, shape, content, and crystallinity, making it ideal for nano-biomaterials and biomedical applications. This approaches, which replicate natural processes like biomineralization, frequently yield very uniform and monodisperse nanoparticles with improved surface functionality. Sol-gel synthesis, chemical vapor deposition (CVD), co-precipitation, hydrothermal processing, and biologically based green synthesis are all common approaches. These methods enable molecular-level functionalization and increased purity, which are beneficial for applications in medicine, catalysis, electronics, and energy devices (Figure 3 [1-3]).These techniques allow for composition, size, and form control by generating nanostructures from atomic or molecular precursors. Green synthesis utilizes biological entities in an environmentally benign manner, whereas chemical and hydrothermal processes maintain structural precision.

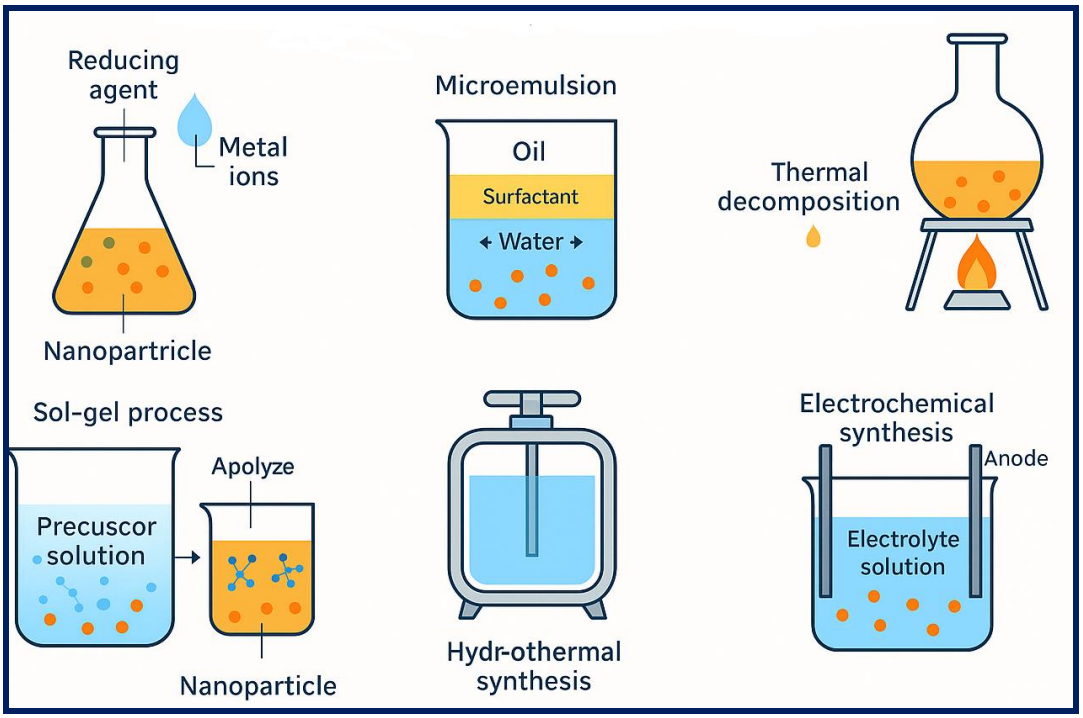

Figure 3. Schematic representation of bottom-up synthesis methods for nano-biomaterials.

2.2.1. Sol-gel method

A sol is a colloidal system made up of uniformly dispersed solid particles suspended in a liquid medium, whereas a gel is a semi-solid material composed of a three-dimensional network that traps the liquid phase. The sol-gel process is a popular chemical approach for producing nanomaterials, metal oxides, and ceramic materials. Precursors such as metal salts (chlorides, nitrates, or sulfides) or metal alkoxides (M(OR)n_nn) are hydrolyzed and condensed in the presence of acid or base catalysts to produce a homogenous sol. The sol progressively turns into a gel due to polymerization processes, resulting in a solid network containing solvent molecules. The resultant gel is then aged, separated by sedimentation or centrifugation, and dried to produce nanostructured metal oxides with regulated content and morphology [44, 45].

2.2.2. Spinning

The Spinning Disk Reactor (SDR) is a high-intensity thin-film reactor that works via centrifugal force. In this technique, precursor solutions are placed into a fast revolving disk, where centrifugal forces cause the liquid to spread into a thin film. This structure allows for efficient mixing, rapid heat and mass transport, and precise nucleation conditions, making SDR ideal for nanoparticle synthesis.

The thin film's high shear pressures encourage quick supersaturation and uniform nucleation, resulting in the creation of nanoparticles by processes like reduction, precipitation, or thermal breakdown. Controlling particle size, morphology, and crystallinity involves adjusting operating parameters such as disk rotation speed, precursor feed rate, temperature, and reactor environment. The generated nanoparticles are collected from the reactor output and sorted by filtration or centrifugation before drying [46,47].

2.2.3. Chemical vapor deposition (CVD)

Chemical Vapor Deposition (CVD) is a popular process for creating thin films and nanostructured materials by chemical interactions with vapor-phase precursors. In this technique, volatile precursor gases are injected into a reaction chamber with a heated substrate. The precursors are thermally decomposed, reduced, or oxidized near the substrate surface, leading in

the formation of a solid film and the removal of gaseous byproducts from the system. CVD allows for the production of high-purity, uniform, and conformal coatings on complicated surfaces. The structural and functional properties of deposited materials, including crystallinity, morphology, grain size, and electrical properties, can be precisely tailored for applications in electronics, optoelectronics, and nanomaterial synthesis, by controlling process parameters such as temperature, pressure, gas flow rate, and precursor composition [48].

2.2.4. Pyrolysis

Pyrolysis is a thermal decomposition process used for nanoparticle In synthesis, precursor materials in liquid or solid form are heated to high temperatures in a combustion or furnace system. During heating, the precursors decompose, oxidize, or burn, resulting in nanoparticles in a hot gas stream. The generated nanoparticles are transported by a carrier gas, such as air or nitrogen, and then separated using a particle classification or collection device. Pyrolysis has various advantages, including single-step synthesis, high production rates, and the capacity to generate nanoparticles with precise composition, crystallinity, and size distribution. This process is commonly used to produce metals, metal oxides, and composite nanomaterials [49,50].

2.2.5. Biosynthesis

Biosynthesis, also known as biological synthesis, is an environmentally benign method of creating nanoparticles from naturally occurring biological resources such as plants, fungi, bacteria, and yeast. In this approach, biomolecules included in biological extracts function as reducing and stabilizing agents, allowing metal ions to be converted into nanoparticles. Unlike traditional chemical procedures, biosynthesis does not require harmful chemicals and functions under gentle circumstances such as physiological temperature, pressure, and pH. Although chemical approaches may result in larger yields and faster synthesis, biological methods are more environmentally friendly, cost-effective, and biocompatible. Biosynthesized nanoparticles have shown great promise in biomedical applications such as medication transport, diagnostics, antimicrobial therapy, and nanomedicine, making green synthesis a crucial technique for achieving sustainable nanomaterial production [51, 52].

2.2.6 Nano-Biomaterial Synthesis Strategies: Comparative Analysis, Mechanistic Insights, Translational Feasibility, and Biological Performance Correlation

2.2.6.1 Comparative Analysis,

Table 2 provides a comparative overview of major nano-biomaterial synthesis strategies, namely top-down, bottom-up, and green/biological approaches. [53-60)

Table 2. Comparative Analysis of Nano-Biomaterial Synthesis Approaches

S.No	**Parameter**	**Top-Down Approaches** (e.g., milling, lithography)	**Bottom-Up Approaches** (e.g., sol–gel, chemical precipitation, hydrothermal)	**Green/Biological Approaches** (plant, microbial, biopolymer-mediated)
1	Principle	Mechanical/physical breakdown of bulk materials.	Atomic/molecular assembly via nucleation and growth	Bio-reduction and stabilization using phytochemicals, enzymes, or microbial metabolites
2	Particle Size Control	Moderate; possible polydispersity	High precision via reaction kinetics and precursor control	Moderate; influenced by extract composition and biomolecule concentration
3	Morphology & Crystallinity	Limited control; surface defects possible	Excellent tunability of shape, phase, and	Good control but variability may occur

			crystallinity	
4	Requires post-synthesis modification	Requires post-synthesis modification	Can be engineered during synthesis	Intrinsic capping by biomolecules enhances biocompatibility
5	Purity & Defects	Risk of contamination and structural damage	High crystallinity; chemical residues possible	Low toxic residues; organic impurities may remain
6	Scalability	Industrially scalable	Scalable with optimized parameters	Limited large-scale reproducibility
7	Environmental Impact	Energy-intensive processes	May involve toxic solvents/reagents	Eco-friendly, sustainable, low toxicity
8	Cost Consideration	High instrumentation cost	Moderate	Generally low-cost and sustainable
9	Biomedical Relevance	Suitable for robust inorganic nanostructures	Ideal for engineered drug delivery systems and precision nanocarriers	Highly promising for antimicrobial, wound healing, and regenerative applications
10	Key Limitation	Surface damage and limited fine control	Potential chemical toxicity	Batch variability and standardization

				challenges

2.2.6.2 Mechanistic Insight Strengthened

In particular, we now discuss the physical fragmentation mechanisms for top-down approaches in mechanical milling and lithographic processes, where bulk materials experience shear, compression, and impact forces that result in lattice strain, size reduction, and possible surface defect formation [61-63]. Based on conventional and non-classical crystallization theories, we offer a thorough description of nucleation–growth kinetics for bottom-up techniques, emphasizing how temperature, reaction time, supersaturation, and precursor concentration control particle size, morphology, and crystallinity [64-66]. We also go over controlled assembly procedures that allow for fine structural tweaking, like hydrothermal crystal formation pathways and sol–gel hydrolysis–condensation reactions.

We now discuss the phytochemical- or biomolecule-mediated reduction mechanisms for green synthesis techniques, in which polyphenols, flavonoids, proteins, and enzymes produced from plants serve as both reducing and stabilizing agents at the same time. These biomolecules affect size distribution, surface charge, and biological compatibility by promoting metal ion reduction, nanoparticle nucleation, and surface capping [67, 68].

2.2.6.3 Critical Evaluation of Translational Feasibility

Table 3: Illustrate a Critical Evaluation of Translational Feasibility of namely top-down, bottom-up, and green/biological approaches.

S.NO	Synthesis Approach	**Major Strengths**	Key Limitations	**Translational Feasibility**	Representative References
1	**Top-Down Methods** (e.g., milling, lithography)	High scalability; industrial compatibility; suitable for	Surface defects, structural damage, possible contamination,	Favorable for large-scale production but requires post-	[69,70]

		robust inorganic nanostructures	limited fine size control	processing to improve surface quality and biocompatibility	
2	**Bottom-Up Methods** (e.g., sol–gel, hydrothermal, precipitation)	Excellent control over particle size, morphology, and crystallinity; reproducible under optimized conditions	Potential use of toxic solvents/reagents; purification steps required	Highly suitable for precision drug delivery and engineered nanocarriers; regulatory compliance depends on purification efficiency	[71,72]
3	**Green/Biological Methods** (plant, microbial, biopolymer-mediated)	Eco-friendly; reduced toxicity; intrinsic surface functionalization; improved biocompatibility	Batch-to-batch variability; limited precise size control; scale-up challenges	Promising for biomedical and antimicrobial applications; requires standardization protocols for clinical translation	[73,74]

2.2.6.3.Application-Oriented Correlation Between Synthesis Strategy and Biological Performance

The choice of synthesis process directly affects the physicochemical properties of nano-biomaterials and, in turn, their biological performance, according to an application-oriented viewpoint. Top-down methods, which are frequently employed to create inorganic

nanostructures like metal oxides and nanoceramics, provide mechanical robustness and scalability; however, if not properly controlled, the induced surface defects and wider size distribution may change surface reactivity and possibly impact cytocompatibility [75,76].

Bottom-up approaches, on the other hand, provide exact control over nucleation and growth processes, enabling fine-tuning of crystallinity, morphology, and particle size. For polymeric nanocarriers like Poly(lactic-co-glycolic acid) nanoparticles and plasmonic systems like gold nanoparticles, where size uniformity and surface functionalization greatly affect cellular uptake, drug release kinetics, and photothermal efficiency in cancer theranostics, this degree of control is especially beneficial [77,78]. In the meantime, phytochemical-mediated surface capping is introduced by green synthesis techniques, which are frequently used in the manufacturing of metal oxide nanoparticles like zinc oxide, improving intrinsic biocompatibility and antibacterial activity [79,80]. Variability in extract composition can impact size precision and repeatability, even if these biologically derived coatings enhance regenerative and wound-healing efficacy. All things considered, the synthesis pathway controls both structural characteristics and interactions at the nano–bio interface, which has a significant impact on translational potential in biomedical applications. Fig. Represent the Comparative Analysis of Nano-Biomaterial Synthesis Strategies: Comparative Analysis, Mechanistic Insights, Translational Feasibility, and Biological Performance

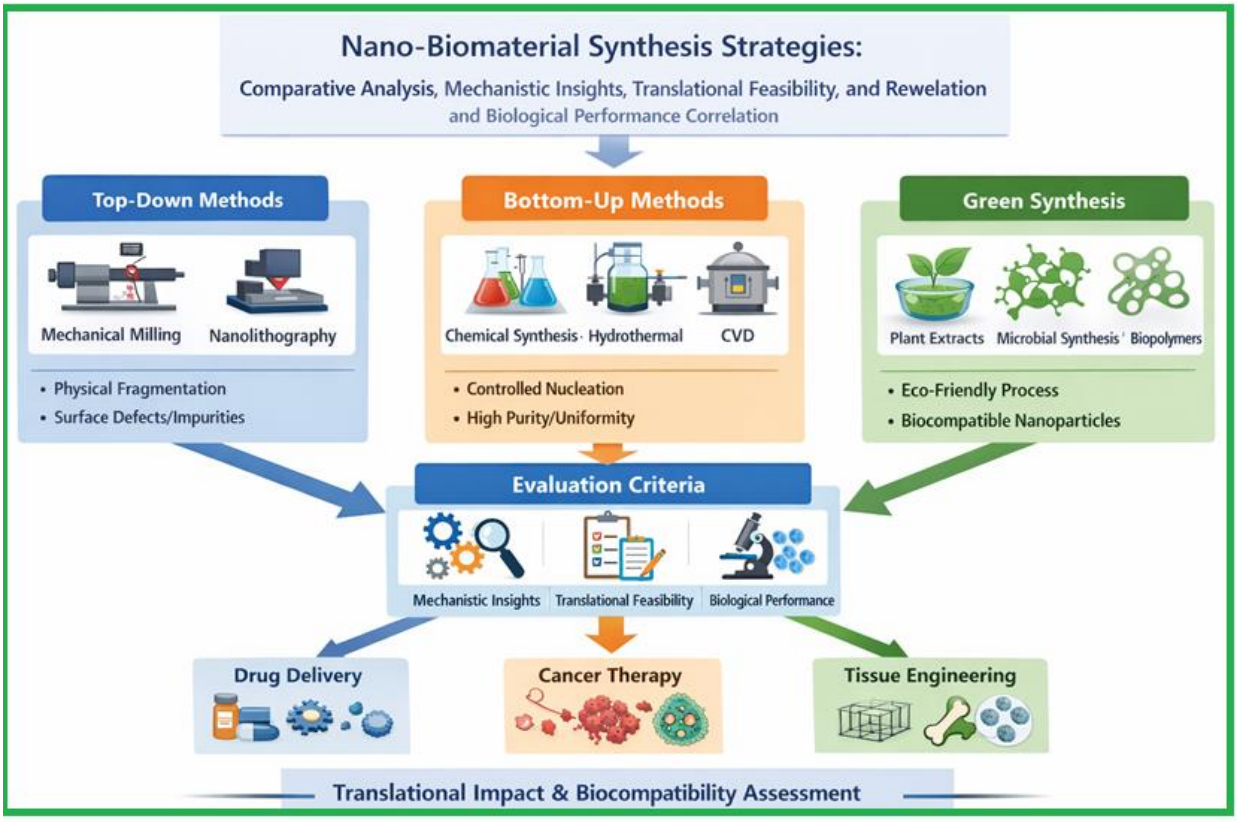

Figure 4. Represent the Comparative Analysis of Nano-Biomaterial Synthesis Strategies: Comparative Analysis, Mechanistic Insights, Translational Feasibility, and Biological Performance

3. Green Synthesis of nanoparticles

3.1. Bacteria in nanoparticle biosynthesis

Bacteria have lately gained popularity in nanoparticle production due to their rapid growth rates, ease of cultivation, and well-understood genetic and metabolic pathways. Bacterial systems provide a low-cost and environmentally benign platform for generating a wide range of metallic and metal oxide nanoparticles, including silver (Ag), gold (Au), zinc oxide (ZnO), and cadmium sulfide (CdS), among others. One of the most important factors influencing bacterial nanoparticle formation is the pH of the growth or reaction medium.

The pH plays an important role in regulating the morphology (shape and size) and location (intracellular or extracellular) of produced nanoparticles [81-83]. Bacteria can combine a wide range of inorganic nanomaterials via both internal and external mechanisms. One well-studied example is the production of silver nanoparticles (AgNPs) using bacterial strains, in which metal ions are reduced to nanoscale form by bioreduction processes. This procedure involves adding

silver ions (Ag^{+}) to a bacterial culture or cell-free supernatant. Microbes help convert Ag+ to metallic silver (Ag°) via a variety of metabolic processes. Extracellular reductase enzymes, which are released by bacterial cells into the surrounding medium, play an important role in this transformation. These enzymes act as biological reducing agents by catalyzing the conversion of ionic silver into nanometer-sized particles. In addition to reductases, additional microbial metabolites such as peptides, proteins, flavonoids, or polysaccharides may participate in the reduction process or act as stabilizing and capping agents, avoiding agglomeration and altering the size and shape of the final nanoparticles [84]

3.2. Yeast in biosynthesis of nanoparticles

Yeast-based systems offer several advantages for nanoparticle biosynthesis due to their rapid growth, ease of laboratory cultivation, and ability to grow on simple and inexpensive nutrient media. These properties make yeasts a scalable and environmentally friendly platform for the sustainable production of metal nanoparticles. Yeast species such as *Candida glabrata* and *Schizosaccharomyces pombe* have been widely investigated for their ability to biosynthesize intracellular metal nanoparticles. In this process, metal ions are absorbed by yeast cells and reduced by cellular metabolites, reductase enzymes, or thiol-containing biomolecules, leading to the formation of nanoparticles such as cadmium sulfide (CdS), silver (Ag), selenium (Se), titanium (Ti), and gold (Au). The formed nanoparticles are often stabilized by proteins or peptides, which improve their stability and reduce toxicity [85].

In addition to intracellular synthesis, extracellular biosynthesis has also been reported, particularly for CdS nanoparticles. In this approach, metal ions are reduced in the culture medium by enzymes or metabolites secreted by yeast cells, allowing nanoparticles to form outside the cell. This simplifies recovery through centrifugation or filtration [86].

3.3. Plants in biosynthesis of nanoparticles

Using biological techniques, metallic nanoparticles in a variety of sizes and shapes are synthesized from the stem, root, fruit, seed, callus, peel, leaves, and flower of plants. Through its bioactivity, an extract with durable medicinal and antioxidant qualities creates the ideal dispersive medium for stabilizing nanoparticles. Its features also work in synergy to improve its effects on its targets. For example, a plant extract might have antimicrobial qualities. Combining

nanoparticles with this potential has a synergistic effect that enhances action while lowering cytotoxicity and disposal effects. Precursor consumption is reduced because the combined movements of the nanostructure and the plant extract, they are connected to will support the nano system [87]. When biosynthesizing metallic nanoparticles using plant extract, three essential components are (1) metal salt, (2) a reducing agent, and (3) a stabilizing or capping agent to control the size of the nanoparticles and prevent them from aggregating. Plant biomolecules, such as proteins/enzymes, amino acids, carbohydrates, alkaloids, terpenoids, tannins, saponins, phenolic compounds, reducing sugar, and vitamins, may be involved in the bio reduction, synthesis, and stability of metal nanoparticles. The reduction potential of ions and the capacity of plants that depend on the presence of polyphenols, enzymes, and additional chelating agents have a substantial influence on the creation of nanoparticles [88].

Numerous researches have shown the production of metal nanoparticles using plant leaf extracts and their potential applications. Researchers have investigated the bio reduction of gold and silver ions using *Azadirachta indica* and *Pelargonium graveolens* leaf broth. Additionally, authors investigated *Cymbopogon flexuosus* (lemongrass) extracts formed triangular gold nanoprisms. They discovered that a process involving rapid bio reduction, assembly, and room-temperature sintering of "liquid-like" spherical gold nanoparticles appeared to be responsible for the development of the nano-triangles. Stable gold nanotriangles could also be produced quickly by using Tamarindus indica (tamarind) leaf extract as a reducing agent. Their electrical and optical properties were significantly restored by the shape of metal nanoparticles. They have also shown how to use plant extracts from Aloe vera to synthesize gold and silver nanoparticles in a range of sizes and forms, including spherical and triangular [99]. Since most plants are readily available, cost-effective, and nontoxic, the usage of plant extracts has grown significantly. Additionally, Plant extracts contain a wide variety of capping and reducing agents. As a result, these techniques may generate nanoparticles in a variety of shapes and have a significant potential for expanding further. Differences such as temperature, concentration of the extract, concentration of the metallic ions, and pH can impact the size and morphology of the nanoparticles [90]. **Table.4** provides a comparative summary of the common synthesis methods utilized to generate nano-biomaterials.

Table 4. An overview of the synthesis techniques for nano-biomaterials.

S. No	Method	Approach	Principle	Materials	Advantages	Limitations	References
1.	Mechanical milling	Top-down	Mechanical force grinds bulk to nanoscale	Metals, ceramics	Simple, scalable	Broad size distribution, surface defects	[91]
2.	Laser ablation	Top-down	Pulsed laser vaporizes bulk in a liquid medium	Au, Ag, ZnO	High purity, no chemical precursors	Equipment-intensive, limited control	[92]
3.	Photolithography / E-beam	Top-down	Nano-patterning via UV or electron beam	Silicon, polymers	Precise, useful for sensors	Expensive, low throughput	[93]
4.	Chemical precipitation	Bottom-up	Supersaturation leads to nucleation and particle growth	Hydroxyapatite, TiO_2	Low-cost, scalable	Poor shape control	[94]
5.	Chemical precipitation	Bottom-up	Supersaturation leads to nucleation and particle growth	Hydroxyapatite, TiO_2	Low-cost, scalable	Poor shape control	[95]
6.	Sol–gel process	Bottom-up	Hydrolysis/condensation of metal alkoxides into gels	Silica, bioactive glass	Uniform structure, high purity	Slow drying, sensitive to environment	[96]

7.	Self-assembly	Bottom-up	Molecules spontaneously organize into nanostructures	Liposomes, micelles, DNA	Mild conditions, versatile	Sensitive to pH, ionic strength	[97]
8.	Hydrothermal / Solvothermal	Bottom-up	Crystallization under pressure/heat in closed vessels	Metal oxides, carbon dots	Crystalline products, morphology control	Requires autoclaves/reactors	[98]
9.	Green synthesis (Plant extracts)	Green / Bio-inspired	Phytochemicals reduce metal ions to nanoparticles	Ag, Au, ZnO	Eco-friendly, biocompatible	Batch variability, scale issues	[99]
10.	Microbial synthesis	Green / Bio-inspired	Microbes mediate intracellular/extracellular nanoparticle formation	CdS, Ag, Se	Sustainable, safe	Slow, biosafety required	[100]
11.	Enzyme-assisted synthesis	Green / Bio-inspired	Enzymes catalyze nanoparticle nucleation and stabilization	Metallic nanoparticles	Mild, specific reactions	Enzyme cost, purification complexity	[101]

3.6. Characterization for nano-biomaterials

Establishing structure–property–function linkages and guaranteeing repeatability, safety, and translational reliability in biomedical applications need thorough characterization of nano-biomaterials. Atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), which offer nanoscale insights into surface topography, particle size distribution, internal structure, and crystallinity, are frequently used to assess morphological and structural features [102–110,111–116]. X-ray photoelectron spectroscopy (XPS) allows surface-sensitive analysis of elemental composition and oxidation states, which is especially important for doped, core-shell, and surface-functionalized nanoparticles [117–123]. X-ray diffraction (XRD) determines crystalline phase identification, lattice parameters, and grain size.

Dynamic light scattering (DLS) and zeta potential analysis, which measure hydrodynamic diameter, polydispersity index, and surface charge—important factors influencing protein corona formation, biodistribution, and cellular uptake—are frequently used to evaluate colloidal stability and dispersion behavior under physiological conditions [124–128]. UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Raman spectroscopy, including surface-enhanced Raman scattering (SERS), are used to characterize optical and surface chemical properties. This allows for the confirmation of nanoparticle synthesis, plasmonic behavior, and biomolecular conjugation [129–135].

Advanced analytical methods improve the assessment of nano-biomaterials even further. For the investigation of protein–nanoparticle interactions and core–shell structures, small-angle X-ray scattering (SAXS) offers ensemble structural information in solution [136-138]. In pharmacokinetic and toxicological investigations, very sensitive metal content quantification and biodistribution profiling are made possible by inductively coupled plasma mass spectrometry (ICP-MS) and single-particle ICP-MS (SP-ICP-MS) [139-143]. Superconducting quantum interference device (SQUID) magnetometry is used to evaluate magnetic properties related to MRI contrast enhancement and hyperthermia applications in magnetically responsive systems [145,146]. To ascertain elasticity and tensile strength, mechanical characterisation of nanofibrous scaffolds and biomimetic matrices is carried out employing MEMS-based testing and

nanoindentation techniques [147-150]. New operando and in situ spectroscopic techniques improve the predictive evaluation of biological performance by enabling real-time monitoring of chemical transformations and nano–bio interfacial dynamics under functional settings [151-155].

3.7. Biomedical applications of nano-biomaterials

Nano-biomaterials have emerged as transformative tools in biomedical science due to their unique physicochemical properties, high surface-area-to-volume ratio, and ability to interact with biological systems at the molecular level. These materials—including nanoparticles, nanofibers, nanotubes, nanogels, and nanocomposites—are increasingly engineered for applications in diagnostics, therapeutics, regenerative medicine, and biosensing. Their nanoscale size enables efficient cellular uptake and targeted delivery of therapeutic agents, thereby reducing off-target effects and improving treatment efficacy (156-160). One of the most significant applications of nano-biomaterials is in drug delivery systems. Nanocarriers such as liposomes, polymeric nanoparticles, dendrimers, and metallic nanoparticles can encapsulate drugs and release them in a controlled and targeted manner. In cancer therapy, these nanocarriers can accumulate in tumor tissues through the enhanced permeability and retention (EPR) effect, thereby reducing systemic toxicity and improving therapeutic outcomes. Additionally, stimuli-responsive nanocarriers that respond to internal signals (pH, enzymes, temperature) or external triggers (light, magnetic fields) are being developed for controlled drug release. Nano-biomaterials also play an essential role in biosensing and diagnostic imaging [161-165]. For example, Graphene as a Multifunctional Nano-Biomaterial, raphene as a Multifunctional Nano-Biomaterial and Hydroxyapatite Nanoparticles in Bone Tissue Engineering. Graphene and its derivatives, including graphene oxide (GO) and reduced graphene oxide (rGO), have gained significant attention as nano-biomaterials due to their exceptional mechanical strength, high electrical conductivity, large surface area, and good biocompatibility [166-170]. These properties enable graphene-based materials to be used in various biomedical applications such as tissue engineering, drug delivery, biosensing, and antimicrobial coatings. The two-dimensional honeycomb structure of graphene allows efficient adsorption of biomolecules and drugs through π–π interactions, facilitating controlled drug release and targeted therapeutic delivery. Graphene-based scaffolds have also been reported to enhance cell proliferation and osteogenic differentiation, making them promising for bone tissue engineering[171-173].In addition,

graphene nanocomposites exhibit antibacterial activity through membrane disruption and oxidative stress generation (Sharma et al., 2023; Singh et al., 2024). Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, is a calcium phosphate mineral that closely resembles the inorganic component of human bone. Due to this similarity, hydroxyapatite nanoparticles are widely used in orthopedic and dental applications. At the nanoscale, HA exhibits enhanced surface area and reactivity, improving cell adhesion, proliferation, and differentiation for bone regeneration. Nano-hydroxyapatite is commonly used as a coating material for metallic implants and is incorporated into polymeric scaffolds such as chitosan, collagen, and polycaprolactone to mimic natural bone structure. It can also serve as a carrier for therapeutic agents, enabling controlled drug delivery for bone-related diseases (Bose & Tarafder, 2023).

Silver nanoparticles (AgNPs) are widely studied because of their strong antimicrobial, antifungal, and antiviral properties. Their high surface-to-volume ratio enables effective interaction with microbial cell membranes, causing structural damage and increased permeability. AgNPs exert antimicrobial activity through several mechanisms, including the generation of reactive oxygen species (ROS), release of Ag^+ ions, disruption of cellular respiration, and inhibition of DNA replication. As a result, they are widely used in wound dressings, medical coatings, drug delivery systems, and antimicrobial textiles. Silver nanoparticle-based nanocomposites have shown strong activity against multidrug-resistant pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Rai et al., 2023).

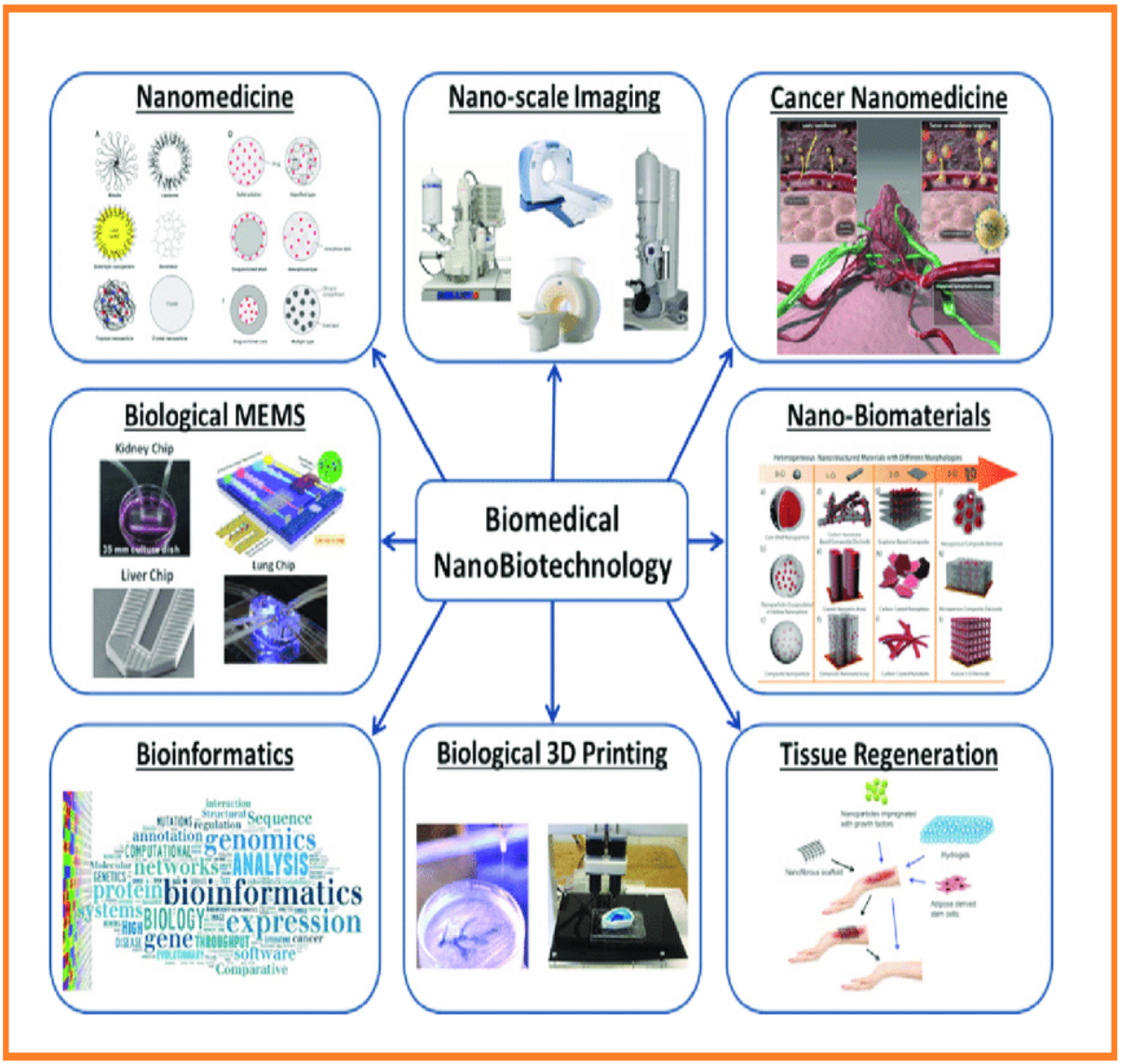

Figure 5. Nano-biomaterials and its applications. Reproduced from Singer et al., 2005 from The EuroBiotech Journal .

Figure 5 shows that nano-biomaterials play a crucial role in biomedical applications. Silver, copper oxide, zinc oxide, and chitosan nanoparticles exhibit strong antibacterial qualities and are being added to dressings for wounds, dental materials, implants, and surgical instruments to reduce the risk of infections [174, 175]. Nano-biomaterials also exhibit significant antibacterial activity through multiple mechanisms, including disruption of bacterial cell membranes,

interference with microbial DNA and proteins, and the generation of reactive oxygen species (ROS), which induce oxidative stress and cellular damage in pathogens. In addition to antimicrobial applications, nanocarriers are widely used in gene therapy for the delivery of nucleic acids such as DNA, RNA, and CRISPR-Cas components to specific target cells. Lipid nanoparticles (LNPs) have emerged as one of the most effective delivery platforms for messenger RNA (mRNA) therapeutics and vaccines, including those developed against COVID-19. Furthermore, nano-biomaterials are being integrated into bioelectronics and implantable devices, such as nano sensors for continuous glucose monitoring or neural interfaces for brain–machine communication. Their flexibility, biocompatibility, and sensitivity make them ideal candidates for wearable health monitoring systems and smart implants [176, 177]. The wide range of these applications is summarized in **Table 5**, highlighting their roles, advantages, and examples in modern biomedicine.

Table 5: Biomedical applications of nano-biomaterials with functions, advantages, and representative examples.

S. No.	Applications	Nanomaterials	Function/Mechanism	Advantages	References
1.	Drug delivery	PLGA, Au, Liposomes	Controlled and targeted release	Improved bioavailability	[178]
2.	Cancer therapy	Au, Ag	Photothermal, chemotherapy delivery	Tumor-specific cytotoxicity	[179]
3.	Imaging and Diagnostics	Quantum dots, SPIONs	Fluorescence, MRI contrast	High-resolution imaging	[180]
4.	Gene delivery	Cationic lipids, Dendrimers	Nucleic acid transport	Enhanced transfection efficiency	[181]
5.	Antibacterial agents	Ag, ZnO, CuO	Cell membrane disruption	Broad-spectrum activity	[182]
6.	Bone tissue engineering	Hydroxyapatite, TiO_2	Osteointegration support	Biocompatibility and regeneration	[183]
7.	Wound healing	Chitosan, Ag	Antibacterial and healing promotion	Faster tissue recovery	[184]
8.	Anti-inflammatory	Curcumin, Polymeric	Cytokine suppression	Reduced inflammation	[185]

9.	Neurodegenerative treatment	Polymeric, Nanogels	BBB penetration	Targeted CNS therapy	[186]
10.	Vaccines	Lipid, Virus-like particles	mRNA and antigen delivery	Rapid immune response	[187]
11.	Dental applications	ZnO, TiO_2	Enamel repair, antimicrobial	Caries prevention	[188]
12.	Cardiac regeneration	Gold, Graphene oxide	Electrical conductivity enhancement	Myocardial repair	[189]
13.	Ophthalmic delivery	Chitosan, Liposomes	Sustained drug delivery	Improved ocular retention	[190]
14.	Antiviral therapy	Ag, Si	Inhibition of viral replication	Reduced infection rate	[191]
15.	Skin care and cosmetics	ZnO, TiO_2	UV protection, antimicrobial	Enhanced skin compatibility	[192]
16.	3D Bioprinting	Hydrogel, Bioinks	Scaffold building	Personalized tissue engineering	[193]
17.	Hemostatic agents	Cellulose-based, Silica	Promote clotting	Fast bleeding control	[194]

18.	Osteoarthritis treatment	Magnetic, Chondrocyte carriers	Anti-inflammatory, targeted	Pain relief, regeneration	[195]
19.	Diabetes monitoring	Glucose-responsive	Biosensing	Non-invasive glucose monitoring	[196]
20.	Antioxidant delivery	Cerium oxide	Free radical scavenging	Neuro/cardioprotection	[197]

3.7. 1. Biomedical Applications of Nano-Biomaterials: Critical Insights into Performance, Challenges, and Limitations

The clinical performance of nano-biomaterials is still heavily reliant on biological complexity and material design, despite its transformative potential in drug transport, imaging, antimicrobial therapy, regenerative medicine, and bioelectronics. Through the increased permeability and retention (EPR) effect, nanocarriers like liposomes, PLGA nanoparticles, dendrimers, and metallic nanoparticles enhance pharmacokinetics, bioavailability, and tumor accumulation in drug delivery [198–199]. Recent research, however, highlights how highly variable the EPR effect is in human malignancies, which frequently leads to inadequate and uneven nanoparticle accumulation in therapeutic settings [200,201]. Therapeutic efficacy is greatly diminished by rapid opsonization, protein corona formation, early drug leakage, restricted endosomal escape, and clearance by the mononuclear phagocyte system [202,203]. Moreover, batch-to-batch repeatability, formulation stability, and scalable production continue to be significant translational obstacles [204].

Quantum dots, gold nanoparticles (AuNPs), and superparamagnetic iron oxide nanoparticles (SPIONs) improve contrast in fluorescence imaging, SERS platforms, and magnetic resonance imaging (MRI) in cancer imaging and theranostics [205–207]. Long-term biodistribution and toxicity issues still exist despite increased sensitivity. For instance, inorganic nanoparticles frequently build up in the liver and spleen as a result of sluggish biodegradation, whereas cadmium-based quantum dots may produce harmful ions [208]. Under physiological circumstances, SPION aggregation can change magnetic responsiveness and lower imaging accuracy [209].

Through the production of reactive oxygen species (ROS), breakdown of membranes, and interference with DNA, antimicrobial nano-biomaterials including silver (Ag), zinc oxide (ZnO), and copper oxide (CuO) nanoparticles have broad-spectrum bactericidal effects [210,211]. However, safe dosage ranges are limited by the potential for oxidative stress caused by nanoparticles and cytotoxicity toward mammalian cells at bactericidal quantities [212]. Additional mechanistic research is also necessary for emerging adaptive resistance mechanisms.

Lipid nanoparticles (LNPs) have transformed nucleic acid transport in gene therapy and vaccine delivery, especially in mRNA vaccines [213,214]. Although LNP systems aid in endosomal escape and shield mRNA from enzymatic destruction, inflammatory reactions,

complement activation, restricted tissue targeting beyond hepatic accumulation, and repeated-dose safety issues continue to be current research difficulties [216].

For tissue engineering and regenerative medicine, nanofibrous scaffolds, nano-hydroxyapatite, graphene oxide, and hydrogel-based bioinks mimic extracellular matrix (ECM) architecture and promote cell differentiationLipid nanoparticles (LNPs) have transformed nucleic acid transport in gene therapy and vaccine delivery, especially in mRNA vaccines [217,218]. Although LNP systems aid in endosomal escape and shield mRNA from enzymatic destruction, inflammatory reactions, complement activation, restricted tissue targeting beyond hepatic accumulation, and repeated-dose safety issues continue to be current research difficulties [219]. [220]. However, clinical translation is hampered by inadequate vascularization, mismatched biodegradation rates, mechanical instability, immune-mediated fibrosis, and challenges with large-scale production [221]. Many scaffolds enabled by nanotechnology still require a standardized long-term in vivo safety evaluation.

Although long-term device stability, biofouling, signal drift, and fibrotic encapsulation limit operational lifespan, applications in bioelectronics and implantable nanosensors show promise in glucose monitoring and neurological interfacing [222]. Overall, even though nano-biomaterials have many benefits, such as multifunctionality, tunable physicochemical properties, and targeted delivery, systematic biological interaction optimization, long-term toxicity profiling, scalable production methods, and regulatory standardization are necessary for successful clinical translation [223]. Reproducibility, clinically relevant performance validation, and mechanism-driven design should be given top priority in future development.

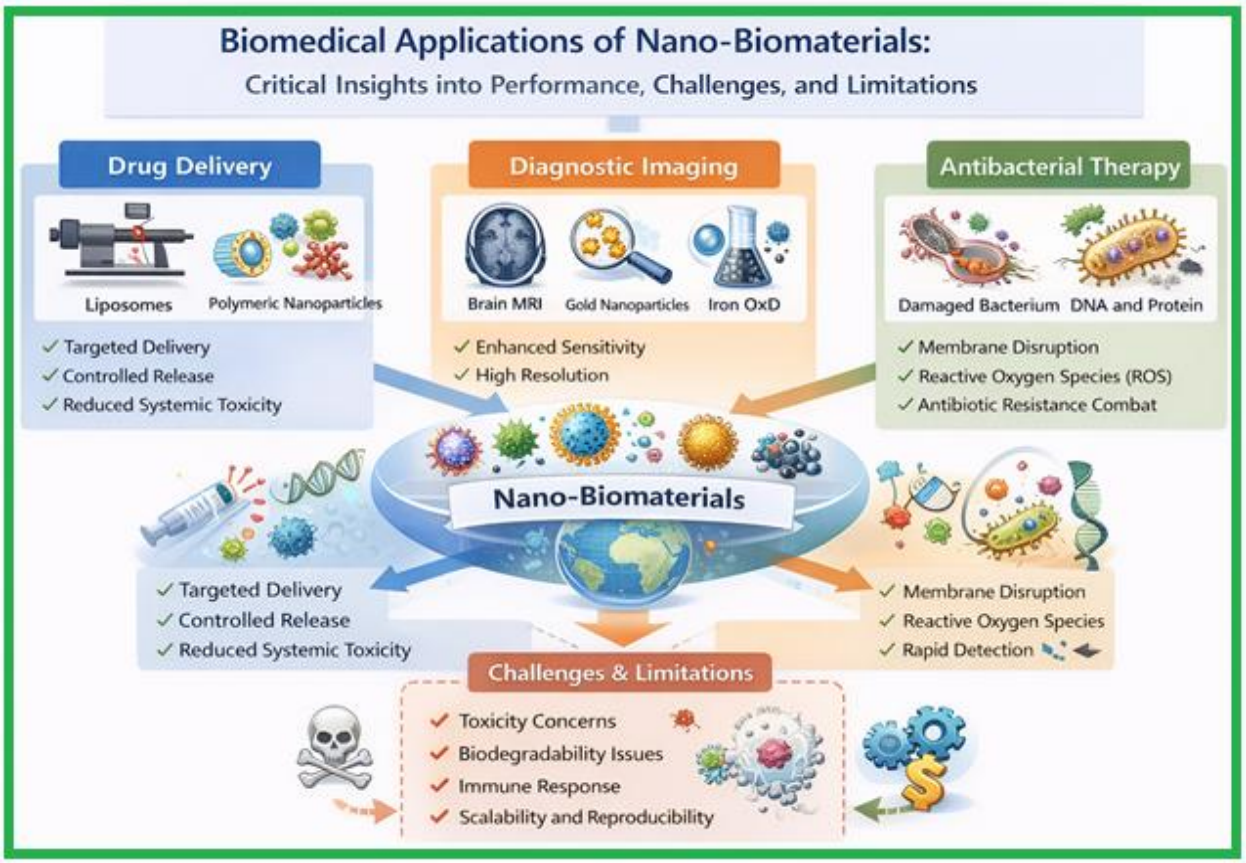

Figure.6. Biomedical Applications of Nano-Biomaterials: Critical Insights into Performance, Challenges, and Limitations

4. Challenges and future perspectives

This review main objective is to give a summary of nano-biomaterials on many different types of applications. Despite substantial advances, numerous major difficulties continue to impede the clinical translation of nano-biomaterials. Long-term biocompatibility and toxicity are still key concerns, as nano-bio interactions are heavily influenced by size, shape, surface chemistry, and dosage. Protein corona formation, immunological activation, unexpected biodistribution, and inadequate biodegradation can jeopardize safety and therapeutic efficacy. Standardized toxicological techniques and dependable in vitro-in vivo correlation models are consequently required. Reproducibility and large-scale production can pose significant challenges. The variability of synthesis processes, particularly in green and biologically derived systems, has an impact on consistency, stability, and regulatory acceptance. Commercialization requires the establishment of scalable, GMP-compliant production procedures as well as agreed characterization criteria.

Future research should concentrate on mechanism-driven design, a better understanding of nano-bio interface dynamics, and the integration of intelligent, stimuli-responsive, and multifunctional theranostic systems. Greater interdisciplinary collaboration, regulatory clarity, and long-term clinical validation will be critical for transforming nano-biomaterials from experimental platforms to safe and effective medical treatments.

5. Conclusion

Nano-biomaterials have developed as very adaptable platforms at the intersection of nanotechnology, biotechnology, and materials science, with important implications for diagnostics, therapeutics, antimicrobial applications, and regenerative medicine. The evolution of green and biologically inspired synthesis techniques has improved their biocompatibility, sustainability, and functional flexibility. Advanced characterisation techniques have allowed for precise control of size, shape, surface chemistry, and stability, improving the link between physicochemical qualities and biological performance. Nano-biomaterials outperform traditional systems in terms of targeting efficiency and therapeutic results due to their high surface area-to-volume ratio, variable surface functionality, and ability to manage therapeutic loading and release. Despite these encouraging improvements, many obstacles remain, including repeatable large-scale production, long-term toxicity evaluation, biodistribution and clearance profiling, immunogenicity assessment, and regulatory standardization. Addressing these restrictions through mechanism-driven design, thorough safety validation, and interdisciplinary collaboration will be critical for advancing nano-biomaterials from experimental research to clinically reliable technology. Overall, nano-biomaterials have revolutionary potential for next-generation biomedical applications, as long as future efforts prioritize safety, scalability, and translational practicality.

Authors Contributions

Monogar Priya: Conceptualization, Writing - original draft; **Raja Venkatesan:** Data curation, Investigation, Writing - review & editing; **Seong-Cheol Kim:** Supervision, Project administration, Funding acquisition, Writing - review & editing; All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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