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Review

Magnetic Nanocomposites and Imprinted Polymers for Biomedical Applications of Nucleic Acids

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Abstract: Magnetic nanocomposites (MNCs) combine the features of magnetic nanoparticles and a second material, which provide distinct physical, chemical, and biological properties. The magnetic core for nanocomposite synthesis is extensively used due to its high saturation magnetization, chemical stability, large surface area, and easy functionalization. Moreover, magnetic nanoparticles (MNPs) have great potential for magnetic resonance imaging (MRI), magnetic particle imaging (MPI), hyperthermia, and targeted drug and gene delivery by an external magnetic field. Numerous composing units exist, which leads to the outstanding application of composites. This review focuses on nucleic acid-based bioapplications of MNCs with polymeric, organic, inorganic, biomolecules, and bioinspared surface coating. In addition, different forms, such as core–shell, doping, multilayer, yolk–shell, and Janus-shaped hybrids, are discussed, and their unique properties are highlighted. The unique types of nanocomposites as magnetic molecularly imprinted polymer (MMIP) properties are presented. This review presents only the synthesis of MNCs using ready-made magnetic cores. These restrictions are associated with many materials, the quantitative and qualitative magnetic core composition, and synthesis procedures. This review aims to discuss the features of nucleic acid-based MNC information available to researchers in this field and guide them through some problems in the area, structure variation, and surface functionalization possibilities. The most recent advancements of MNCs and imprinted polymers in nucleic acid-based therapy, diagnostics, theranostics, magnetic separation, biocatalytic, and biosensing are introduced.

Keywords: magnetic nanoparticles; magnetic nanocomposites; nucleic acid; smart nanocarriers; imprinted polymers; magnetic molecularly imprinted polymer; cancer treatment; nucleic acid delivery; toxicity of magnetic nanoparticles; biomedical applications of nanoparticles

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1. Introduction

Magnetic nanocomposites (MNCs) are multiphase solid materials where one phase of a nanoscale material is magnetic, and another is varying. Such a combination allows for the creation of new materials with different properties from the initial nanoparticles. Various building blocks of MNCs may be small molecules (tannic, lauric, myristic, oleic acids, etc.) [1–4], organic polymers (polyethylene glycol, polyethylene imine, tween, etc.) [5–11], inorganic metals (gold, platinum, etc.) [12–15], oxides (silica) [16–19], salts (calcium carbonate) [20,21], or bioinspired materials (proteins, carbohydrates, nucleic acid, aptamers, polydopamine) [5,8,22–28] (Figure 1). A number of reviews highlight the possible features of such a surface stabilization and functionalization [12,22,25,29–31]. The magnetic core of MNCs has become vital for a wide range of applications, including material science, hyperthermia, the contrast agent area for magnetic resonance imaging (MRI) and magnetic particle imaging (MPI), and theranostics [32–48]. Manipulation with an external magnetic field is essential for magnetic separation, drug and gene delivery, biomass processing, and biosensing [39,42,49–51].

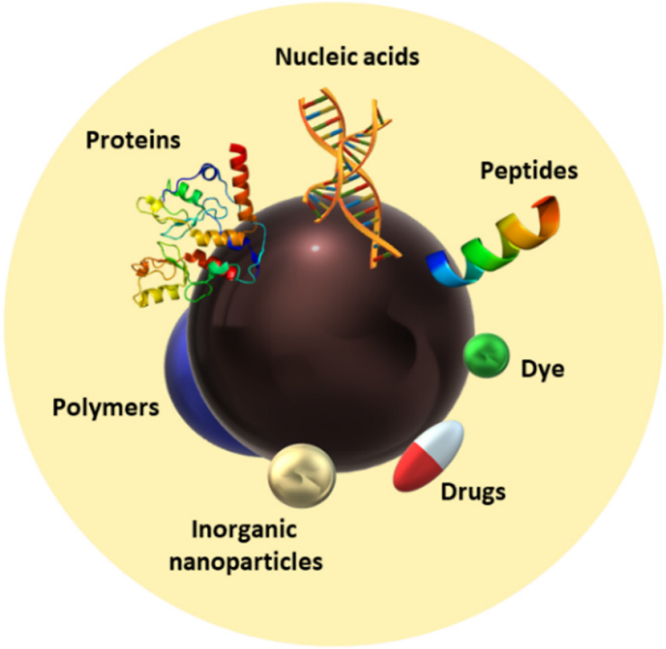

Figure 1. Schematic illustration of MNCs with different coatings, target ligands, drugs, etc. Molecules can be embedded in the coating or adsorbed on the surface.

The first reported iron nanoparticles were synthesized in 1984 [52]. However, about thirty years were required to be able to synthesize stable magnetic nanoparticles (MNPs) with optimal physical properties. One of the most common MNPs for biomedical applications is magnetite (Fe_3O_4) due to its ferrimagnetism, high stability, and cost-effectivity [31]. Many papers were produced which showed the use of magnetite MNPs for different applications [30,39,40,43,53–57].

One of the milestones was the synthesis of silica-coated MNPs in 1995, which opened the era of MNCs. Silica-coated and functionalized MNCs represent a significant doorway for nucleic acid (NA) separation, purification, and detection [50,58–74]. NA is one of the most important substances in cells and has an enormous number of biological functions. Functionally integrating NA and MNPs produces a rich variety of MNCs which, in many cases, display unique or augmented properties due to the synergistic effect. The NA component may be in various forms, such as plasmid DNA, single-stranded (ssNA) or double-stranded (dsNA) DNA or RNA, small interfering RNA (siRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), antisense oligonucleotides, etc. [75,76]. NA may be adsorbed on the MNCs or chemically bound to the surface, which requires preliminary modification. Thus, the design of NA-based MNCs is a challenging task and depends on the ultimate purpose. The NA component typically provides sequence-based addressability for probes, and complex spatial architecture (e.g., DNA origami, aptamers, G-quadruplex, triplex form, etc.) for specific binding with biopolymers, biological selectivity, or disease treatment [63,70,74]. Numerous NA-based MNCs have been developed for disease analysis purposes, which exhibit more advantages than traditional systems [63,69,74]. MNCs containing gene vectors possess unique advantages for their rapid transfection, isolation, and elevated gene shuffling into the cells [77–80]. The NA-based MNC tools provide diverse applications, which include targeted delivery, therapy, imaging, biosensing, and diagnostics [59,63,69,70,72,74,78,81–83]. This review highlights only the synthesis of MNCs using ready-made magnetic cores. These restrictions are associated with many materials, the quantitative and qualitative magnetic core composition, and synthesis procedures. This review is divided into two main sections. Each section provides a brief overview of some relevant NA-based MNC properties. The first section describes the structural types of MNCs with organic, inorganic, and biomolecule coatings. The second section is focused on NA-based MNC applications, highlighting multifunctional and bioinspired materials,

probes, smart constructions, and devices. Some key issues of an MNC structure and the importance of advanced materials for various applications are proposed. The outlook on the future directions and challenges are discussed.

2. Magnetic Nanocomposite Types

MNCs are multiphase systems that can combine the properties of their component materials. Many research articles focused on the synthesis and MNP coating procedures to obtain the desired morphology, particle sizes, and physicochemical properties [43,53]. The surfaces of MNPs used for biomedical applications are usually modified with various coatings and biological molecules to acquire a good aqueous stability, colloid stability, low toxicity, biocompatibility, and recognition by tissues or cells. Small organic molecules [1–4], artificial and natural polymers [5,8,22–26], and inorganic substances [12–19] are used for coating procedures [84–88].

The main structural types of nanocomposites are divided into core–shell [89–91], Janus [92–94], assembly [95,96], yolk–shell [97,98], multicomponent [99], multilayer shell [100], etc. (Figure 2) [101,102]. The core–shell structural type is generally biocompatible and is well tolerated in vivo [44]. The non-toxic properties appear due to a good magnetic core surface protection and low interaction with a solvent. The core–shell MNC usually has a well-controlled structure and tunable physicochemical properties, depending on the surfactant, coating type, and biocompatibility, and possible surface functionalization [89–91,102]. They primarily do not have a problem with magnetic core toxicity but may have with surfactant polymer biocompatibility. For example, PEI coating exhibited a significantly higher cell uptake but may cause severe cytotoxicity through multiple mechanisms. However, replacing PEI with a biocompatible coating may easily solve the problem [44]. Nowadays, core–shell MNCs are used in numerous potential issues such as biocatalysis, magnetic separation, biosensing, and nanosorbents [90,102]. Nevertheless, the synthesis procedure is not simple to yield a monodisperse and controlled shell thickness [90]. Yolk–shell nanocomposites are a subtype of the core–shell structure. The only difference is an interior void is located between the core and the shell [97,98,102]. An example of such a structural type is an MNP coated with a silica interlayer and porous gold outer shell, followed by removing the silica template [102]. The yolk–shell structure's extra feature is the diffusion possibility into and out of the shell. The cavity of the yolk–shell is a host place for drugs, adsorbed molecules, and catalytic activity [97,98,102]. Janus particle is a type that is divided into two parts, one of which is a repulsive core and the other is a highly interactive surface. The resulting MNC combines two sets of properties (magnetic core and various coating), which, unlike the core–shell composites, possess two types of properties. This technology may be useful for biosensors, protonics, imaging, and nanomedicine applications [93,94,97]. This section is divided into three subsections that highlight organic molecules, polymers, biomolecules, inorganic compounds, and other types of MNP coatings.

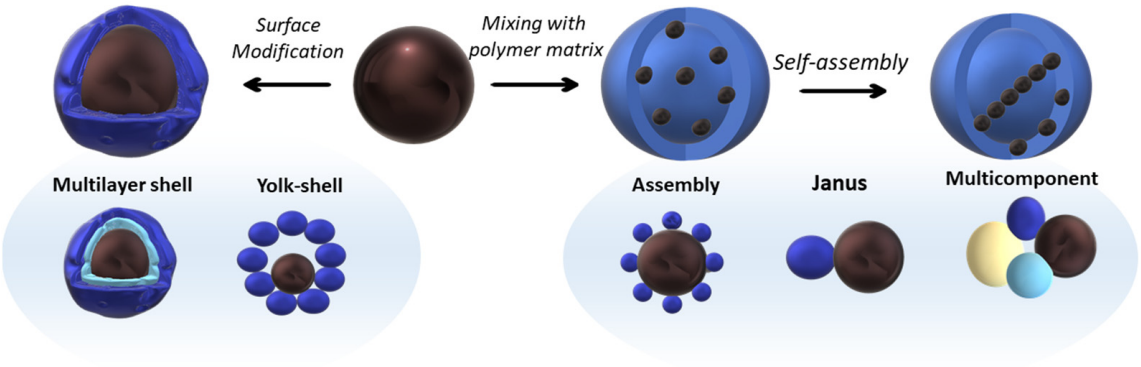

Figure 2. MNC structure types. Multilayer shells and yolk–shell are the subtypes of core–shell MNCs. Assembly, Janus, and multicomponent are self-assembly structural types.

2.1. Organic Molecules and Polymers-Coated MNPs

MNPs have a high surface energy and become unstable, as well as having a tendency to aggregate. The properties of MNPs' surface critically influence the overall performance of the material. The small organic molecules and a polymer coating may shield the MNPs' core from oxidation by the harsh chemical environment. Moreover, this enhances the colloidal stability of nanoparticles and their biocompatibility in the physiological media [22,29]. Small organic molecules or a polymer coating may be hydrophilic, hydrophobic, amphiphilic, or have a charge which highly restricts further application. A modern approach uses fatty or amphiphilic compounds, such as carboxylic acids (polyacrylic, lauric, myristic, or oleic) [103], alkylsulfonic acids, alkylphosphonic acids, polyphenols (tannic acid), alcohols or ethers (polyethylene glycol (PEG), polysorbate (Tween), polyvinylethanol) [5,104], carbohydrates or their derivatives (hyaluronic acid, dextran, chitosan) [5], amines (polyethyleneimine, PEI), polyamides (nylon 6), etc. [2–4,6–11,19,25,26,83,88,102,105–115]. Especially, polymers represent an excellent class of compounds for the high-capacity binding of biopolymers such as nucleic acids, peptides, enzymes, proteins, and lipids [112]. Structure variability and functional groups of polymers make the great diversification of MNCs' properties possible. The charge, solubility in water or organic solvents, viscosity, film-forming ability, pH-, ionic strength, and temperature stability may be changed. In addition, polymer-coated MNCs usually have a little tendency to aggregate and a higher colloidal stability, which significantly increases their applicability (Figure 3). For example, one of the most common polymeric ligands, PEG, usually increases the colloidal stability and solubility in water and removes non-specific protein adsorption and phagocyte uptake [7,88,116–120]. Tween family surfactants consist of a hydrophilic head group and a different-length hydrophobic alkyl chain [7,121]. Therefore, the desired hydrophile–hydrophobic tween molecule may be used for coating. However, the organic functionality is typically used to make the nanoparticles hydrophilic and biocompatible and to endow their colloidal stability in water. Small organic molecules and polymers allow simple nucleic acids and their derivatives' adsorption on the surface due to the positive charge and hydrophobic–hydrophilic balance [61,70,72,102,120,122]. The MNCs provide a simple and quick nucleic acid separation from the supernatant by applying a magnet.

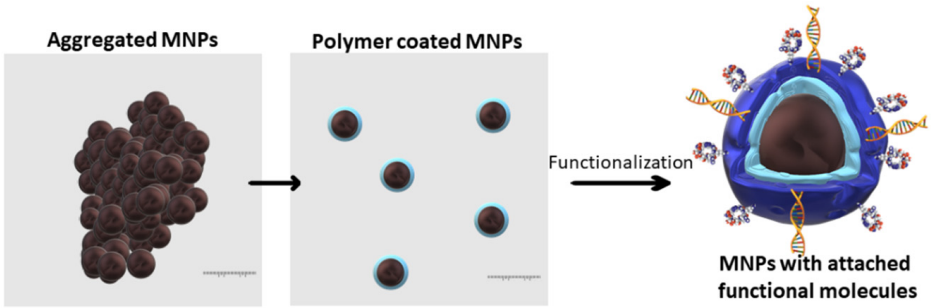

Figure 3. Schematic representation of polymer-coating features. Polymer shell usually provides higher colloidal stability and possible surface modification by functional, reporter, and targeting molecules (nucleic acids, antibodies, vitamins, etc.).

PEI is one of the widely used positive-charged polyamines [11]. Linear PEI contains only secondary amines. The branched PEI has primary, secondary, and tertiary amine groups. It can be easily modified by address, imaging, or therapeutic groups [123,124]. PEI is used for colloidal stabilization in water [120], NA extraction, detection, and gene delivery [38,61,62,82,114,120,124–126]. Furthermore, PEI-MNC is the most efficient sorbent for ssDNA isolation in comparison to gold, silica, and graphene derivatives [114]. Due to the high positive charge in an aqueous solution, PEI interacts with the negatively charged NA and covers it. It is suitable not only for NA transport but protection from extracellular degradation by hindering its interaction with NAses [125]. An MNC coated by PEI is one of the best candidates for magnetofection, magnetic transfection, or technology [61,114].

PEI-coated MNCs could be lyophilized and are stable for not less than 4 months at room temperature, which enables the mass production of nanocomposites for various applications.

Surface functional groups provide an attractive ionic, hydrogen bond, or hydrophobic interaction with the desired target. In addition to the physical sorption, the chemical modification makes any covalent binding with the reporter, fluorescence, or "pull-out" groups probable (Figure 3) [25,81,127,128]. One of the most popular is the acylation of amino and hydroxyl group-containing polymers forming stable amide and ester bonds. However, the UV-induced immobilization of the oligonucleotides on the polymers with the primary amino groups, such as nylon-6, is possible [19,129]. The presence of ten thymidine fragments in the oligonucleotide structure allows for the easy covalent capture on the nylon-6 surface with a good yield. Such an approach was used to target NA capture from the mixture with a high specificity [19]. The possibility of selective NA isolation provides rich information about the organism in health and disease. The polymer chemical modification by "pull-out" groups represents numerous biomedical applications of NA systems [19]. For example, aptamers for various targets can be non-covalently adsorbed or chemically bound with the polymer coating. Aptamers are short sequences of NA that bind a specific target molecule. Such artificial NA is widely used in biological research laboratories and medical tests. They can show strong binding to their target, which is the reason for their name "chemical antibodies". Aptamers can be used to identify various disease markers, be part of the drug delivery system, or have a therapeutic application [130].

Polymer-based MNCs may have various structures, which are presented in Figure 4. Core–shell, magnetic fibers, self-assembly, and doping MNCs are the most popular. Core–shell types are used everywhere, from biosensors to the theranostic area [16,44,47,131–135]. Polymer-based MNC nanofibers can be readily customized to adapt to different applications. It may be a small-length material with a magnetic inclusion, which may be useful for NA capture procedures due to its easy precipitation and good magnetization [19]. Furthermore, the fibers can be long, with a good porous structure and high surface area for drug absorption. Such MNCs demonstrate potential for cell and tissue adhesion and drug-loading for the formation of electrospinning biocompatible material [113]. Despite the procedure's high costs, expensive equipment, and laborious nature, it has an excellent potential for tissue engineering approaches. The sufficient magnetization of MNCs is essential for the formation of the dimensional fiber structure. Moreover, a controlled drug release is possible via an external magnetic field through the hyperthermia effect.

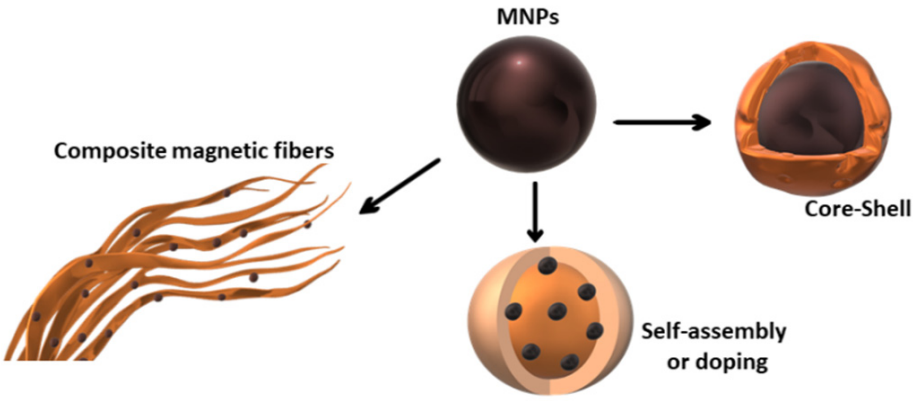

Figure 4. Primary polymer-coated MNC structures.

2.2. Biomolecule and Biopolymer-Coated MNCs (Bioinspired MNCs)

Nanocarriers provide new possibilities in NA and gene delivery. Recently, novel strategies such as bioinspired surface coating, coating functionalization with address molecules, cell-penetrating peptides, and reporter groups have emerged [44,136]. Biomolecules and biopolymers provide selective delivery, specific binding, high biocompatibility, biodegradation, no immune response, etc. Furthermore, biopolymers have amino, carboxyl, and hydroxyl groups, which can be successfully used for additional modification toward materi-

als with better properties and performance than the initial. Despite the advantages, MNCs are complex and may not be adapted for mass production, have a high price, low synthesis yield, and structure reproducibility. Moreover, bioinspired MNCs are not required for any bioapplication related to NA. For example, NA is easily isolated by polymer- or silica-coated MNCs. We believe that bioinspired material has more possibilities for the human organism-related areas. Among these possibilities are NA delivery, tissue engineering, in vivo diagnostics, therapy, and theranostics.

Polysaccharides are very popular for MNC surface modification [5,70,120,136,137]. The most common polysaccharides are chitosan, hyaluronic acid, heparin, starch, cellulose, agarose, and dextran. Chitosan is one of the most widely used biopolymers for MNP core stabilization [137]. It is a non-toxic, biocompatible, and biodegradable biopolymer with proven antiviral, anti-inflammatory, and antibacterial activity [11,137]. Free amine groups of chitosan allow for easy acylation, yielding the material with fluorescence, reporter, or other type groups. Due to the positive charge of amino groups, chitosan can interact with negatively charged NA and cell membranes [137,138]. ChitosanMNCs are the most widely used polysaccharides for MRI, gene delivery, NA extraction, and drug/NA systems for therapy [137,138]. Other polysaccharides, such as alginate, dextran, hyaluronic acid, heparin, mannan, pullulan, and starch, have neutral or negative charges (-OH and COOH groups), which are not suitable for efficient NA interaction. Surface chemical modification or positive polymer coating is required for magnetofection [130,137].

Protein coating provides biocompatibility, biodegradability, and less immunogenicity of MNCs [23,25,139–143]. For example, human serum albumin (HSA) [144–146] coating increases the colloidal stability in an aqueous solution, prolongs blood circulation time, prevents aggregation, and non-specific adsorption of blood components [23,25,143,147–151]. As one of the major plasma proteins, albumin corona forms on all nanoparticles in the bloodstream [152]. HSA increases the efficiency of tissue and cell targeting [153–155]. Albumin interaction with various receptors provides targeted delivery to tumors [25,143]. Moreover, HSA may be chemically modified by vitamins [156], cell-penetrating peptides, antibodies [157,158], imaging probes [159–162], NA, and drugs [156,161] for enhanced tumor delivery and theranostics [25]. AlbuminMNCs have been employed to deliver plasmids, oligonucleotides, and siRNAs for therapeutic applications [163,164].

Polydopamine (PDA) is a very important polymer that can be easily coated onto MNPs to form a uniform core–shell nanostructure [28,165]. PDA is the final oxidation product of dopamine or other catecholamines, attracting much attention as a versatile coating for MNPs [27,28,165–171]. PDA shows structural flexibility and strong adhesion to all types of substrates due to various functional groups. Moreover, PDA's chemical groups allow for decoration with biomolecules and various reporter residues, achieving hybrid smart systems. Various PDA-MNCs have been reported, and their applications in bioareas have been discussed [28]. PDA is an ideal candidate for environmental remediation (the removal of pollutants) [27,28], biomedicine and imaging [28], drug and gene delivery [28,168], DNA extraction and detection [169,171], and cell tracking [167].

In recent years, there has been a growing interest in the synthesis of MNCs with biomolecules for various applications [136]. The binding of proteins, polysaccharides, lipids, peptides, and nucleic acids onto MNCs provides a bioinspired interaction with a living organism. Among the nanocomposites, biopolymer-coated MNCs are one of the most seen in various biomedical applications [25,40,102,128,136].

2.3. Inorganic Compound-Coated MNPs (Noble Metals, Silica, Calcium Carbonate, Carbon, etc.)

One of the main categories of MNCs is inorganic material coated. Multiple structures, including core–shell, Janus, and dumbbell-shaped, may be formed using different types of inorganic compounds. We present the primary coating as a silica and noble metal (usually gold) and some rare calcium carbonate, carbon, and metal–organic frameworks regarding NA-based applications.

Silica coating on MNPs is the most popular due to its low cost, stability in aqueous solution, and biocompatibility [66,102,125,172–175]. Silica shells are primarily synthesized through simple sol–gel reactions. The most common silicate precursor used in silica coating is tetraethyl orthosilicate (TEOS) (Figure 5). For the reaction, MNPs, ammonia or NaOH, and TEOS are used. Many factors, such as surfactant, solvents, temperature, and stirring speed, influence the silicaMNCs' type, size, shape, and porosity [176]. Silica coating highly increases the stability of the magnetic core and reduces the interaction with oxygen and the formation of the reactive oxygen species' toxicity [16,102,177,178]. Due to the porous structure, MNCs are used for drug delivery and NA capture without modification [62,66,102,114,125,176]. However, MNCs can be easily functionalized by a standard procedure, forming amino groups on the surface (Figure 5). As mentioned above, the positive charge of amino groups may be useful for NA capture [114]. A further amino group modification greatly extends the applicability of MNCs [179].

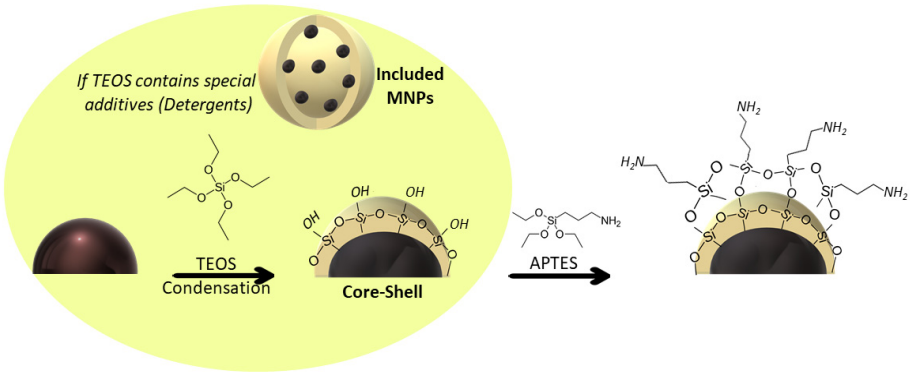

Figure 5. Synthesis of two types of silicaMNCs. Tetraethoxysilane (TEOS) forms a silicon dioxide shell. The simple hydrolysis of TEOS leads to a core–shell type structure. The presence of detergents, such as a cetrimonium bromide (CTAB), provides MNP encapsulation. (3-Aminopropyl)triethoxysilane (APTES) is a primary reagent for amino group surface functionalization.

Silica surfaces may be coated by polymers, drug-loaded, and modified with various address and reporter groups [125,175,179]. The preparation of hybrid drugs and NA silica nanoparticles is a well-known strategy for enhancing the efficiency of the treatment [125,180]. Combination therapies of folate-functionalized silicaMNCs loaded with VEGF shRNA and doxorubicin show a high potential for cancer treatment [18,125]. SilicaMNCs are a widely known technology for the synthesis of hybrid multifunctional smart materials [125,175,179,180]. SilicaMNCs coated with avidin and streptavidin proteins are commonly known as "magnetic beads" for specific NA capture [82,181]. The artificial biotinylated oligonucleotide forms a duplex with a targeted NA mixture. Afterward, the protein on the silicaMNCs' surface binds the biotin residue, forming a high-affinity complex. The specific NA may be isolated by a magnetic separation procedure with subsequent washing. Such avidin- or streptavidin-coated MNCs are commercially available elsewhere.

Noble metals are highly chemically inert materials used for surface protection. Related to MNPs, a noble metal such as gold protects the magnetic core against oxidation, corrosion, and aggregation and increases its biocompatibility [12,13,102,182–186]. The silver coating provides well-known antibacterial activities [186–188]. Indeed, the combination of MNPs and gold is suitable for hyperthermia, radionuclide (^{198}Au) and chemotherapeutic drug cancer treatment, tumor imaging by MRI, targeted delivery of NA and drugs, and NA detection [13,70,182–185,189,190]. The synthesis of noble metal composites can be divided into chemical and physical (Figure 6). Chemical methods are used for the gold deposition on MNPs, resulting in hybrids with chemically inert surfaces. These materials are more suitable for biomedical applications than those obtained by physical approaches. The physical method of the fabrication of noble metal MNPs involves the use of laser pulse energy to transform noble metals from macrostructures into small-sized powder.

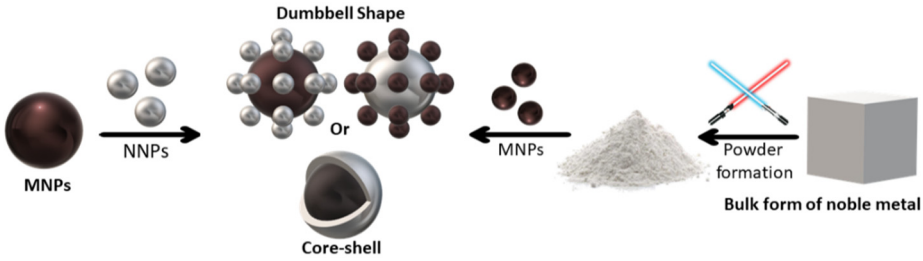

Figure 6. Noble metals–MNC synthesis. MNCs can be obtained by mixing two types of nanomaterials or by spraying noble metal powder on MNPs. Forms such as core–shell, dumbbell, flower, octahedral, star, rod, and Janus may be formed.

The optical properties with the strong adsorptive ability of a gold coating enable the utilization of AuMNCs for NA detection and delivery. These unique properties provide the fluorometric discrimination of mismatch DNA [191,192], PCR assay for CYP2C19 genotyping [193], methylated DNA detection by PCR [194], and single methyl discrimination in DNA aptamers [189]. AuMNCs may be used for NA magnetic separation [70,114,195]. However, without additional coating, AuMNCs have a relatively low capacity in comparison to PEIMNCs or silicaMNCs [114]. Recently, some research groups have reported the synthesis of polymer-coated AuMNCs, including polymers such as PEI, poly(acrylic acid), poly-l-lysine, or dextran [13,14,190,196]. Polymers may be used as an intermediate layer to improve the stability of the core for the fabrication of multilayer MNCs. The external polymer coating, such as PEI, can reduce and stabilize the Au shell [14]. Moreover, PEI's positive surface charge is suitable for the intracellular delivery of siRNA or drugs (doxorubicine) [14].

Carbon and its derivatives (Figure 7) are used for the synthesis of MNCs [43,83,197,198]. Carbon species demonstrate high intrinsic electrical conductivity, excellent stability, and extensive NA adsorption suitable for biosensor production [43,83,102,199–201]. Carbon-coated MNPs have a relatively high magnetic moment in comparison to other forms. However, carbonMNCs usually have extremely low solubility in water and colloidal stability. Furthermore, the size and shape of the nanoparticles are heterogeneous, and the synthesis control is complicated. NA interacts with the carbon surface through π stacking, as shown for carbon nanotubes, graphene oxide, and C60 fullerene [62,199,202]. However, the combination of polymer coating and carbonMNCs leads to solubility in water and stable systems for targeted drug/gene delivery [120,198,202,203].

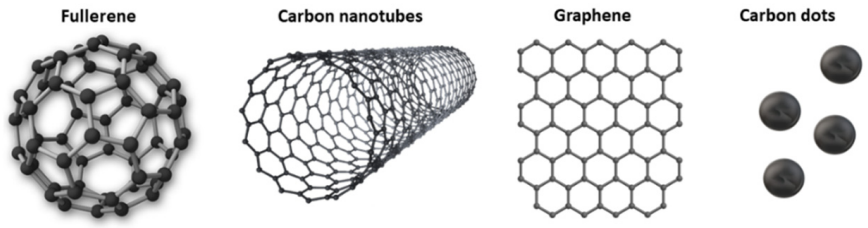

Figure 7. Carbon nanomaterial types that can be used for MNC production.

Calcium carbonate ($CaCO_3$) is a well-known mineral and a highly biocompatible material. Because of the porous structure, pH-sensitivity, and biodegradability, $CaCO_3$ nanoparticles are developed as the nanoplatform for various forms of compound isolation and drug delivery [204–209]. $CaCO_3$ is highly sensitive to acidic pH, facilitating drug release in tumor media [204–206]. $CaCO_3$ nanoparticles can bind with NA, making NA isolation and gene delivery possible [205]. For positively charged siRNA-loaded $CaCO_3$ nanoparticles, the significantly decreased proliferation of tumor cells was shown [204,205,210,211]. Recently, Fe_3O_4@$CaCO_3$ nanocomposites ($CaCO_3$MNCs) were developed [21,84,212–218]. $CaCO_3$MNCs show a high potential for ions, dyes, drug adsorption, and theranostic application [20,21,84,211–213]. PEI-coated or positively charged surfactant $CaCO_3$MNCs

provide enzyme immobilization [214], negatively charged microalgae [215], and cell adsorption [21]. Coating by positive-charged $CaCO_3$MNCs may be a possibility for NA magnetic separation and gene delivery approaches.

Metal–organic frameworks (MOFs) are hybrid materials that consist of metal ions or clusters coordinated to organic ligands forming three-dimensional structures. Recently, magnetic MOF nanocomposites (MOF-MNCs) were developed for a wide range of applications such as drug and gene delivery, NA, proteins, and other biomolecule sensors, and magnetic separation [102,203,219–222]. The combination of MOF-MNCs with aptamers or other types of NA can be utilized for the fabrication of specific biosensors and theranostic systems [203,219].

3. Biomedical Applications of Magnetic Nanocomposites

MNCs have been used for various analytical applications (biosensors, magnetic separation), drug and gene delivery, imaging, and theranostics (Figure 8). NA with MNCs display unique and bioinspired properties due to the synergistic effect. As shown before, the coating of MNCs may be inorganic, organic, bioinspired, or complex. For each coating, a specific application is possible. This section presents the primary applications of NA-based MNCs. The specific information was discussed in Section 2 concerning the type of MNCs and their application.

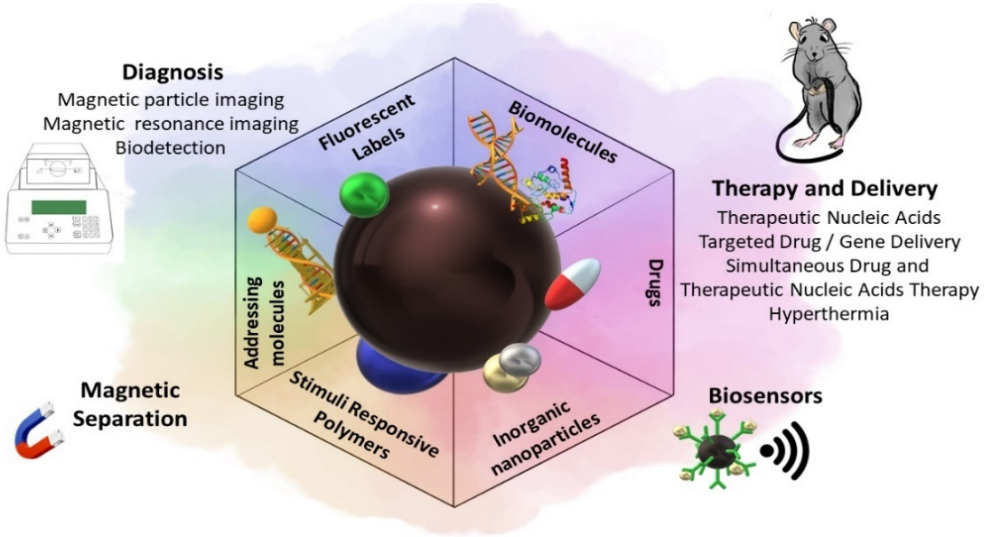

Figure 8. Diagnostics, therapy, drug and gene delivery, biosensor, and magnetic separation applications of MNCs.

3.1. Toxicity of MNCs

The toxicity of MNPs is an essential factor for future healthcare applications [31,223,224]. However, the studies on MNCs' creation and safety assessment remained largely divided. While most of the safety studies have been focused on easy nanoparticles on the cell model, the material area goes forward with various smart constructions. However, most works usually present a primary cytotoxicity test, using an MTT compound on cancer cell lines [225,226]. The MTT assay does not show the interaction with blood proteins, tissue media, and delayed toxicity of the degraded product. The cancer cells are highly adapted to ROS levels and unfavorable media conditions and have activated cell growth and survival systems. In this way, the toxicity of the MNCs is still less explored [223,224,227–229]. Recently, studies on the toxicity of MNCs on spheroids were highlighted [185,230–232]. Such three-dimensional (3D) cell aggregates can mimic the tumor microenvironment. In recent years, significant progress in the development of spheroids for use as a tumor model has been obtained. The cell viability in two-dimensional (2D) cell culture monolayers and spheroids has not shown the same results [230,232–234]. Therefore, dose predictions from

conventional cell experiments are often misleading for in vivo applications. Spheroids are a successful replacement for expensive and unethical animal experiments. However, extensive further studies are required for the stable manufacturing of various cancer spheroids and better tumor mimicking as sustainable cell growth, proliferating and non-proliferating cells, a hypoxic center, etc. [234,235].

Herein, the possible cytotoxicity and organ-specific toxicity are discussed. A high indestructibility in biological liquids or low toxicity is not required for some purposes. For example, for magnetic separation, MNCs usually do not interact with human organisms and should only have good magnetic properties and the ability to interact with a target. For in vivo studies, MNCs must be non-toxic, stable in biological liquids, and biocompatible. Unstable MNPs may be extremely toxic due to the formation of reactive oxygen species (ROS), injury to the immune system, metabolic disorders, decrease in growth rate, or changes in alterations, inflammation, ulceration, etc. (Figure 9) [44,223,224,236]. A high ROS level leads to mitochondrial membrane damage, harmful cell proliferation, modulating gene transcription, dysregulation of ion channels, RNA destruction, and DNA and lipids oxidation with a subsequent formation of a point mutation. In extreme cases, significant damage leads to cell death (Figure 9) [228,236]. The oxidative stress mechanism can be provided by the release of ferrous ions due to the instability of the MNCs, direct ROS generation on the MNCs' surface, and altering the mitochondrial function and signaling pathways [228,236].

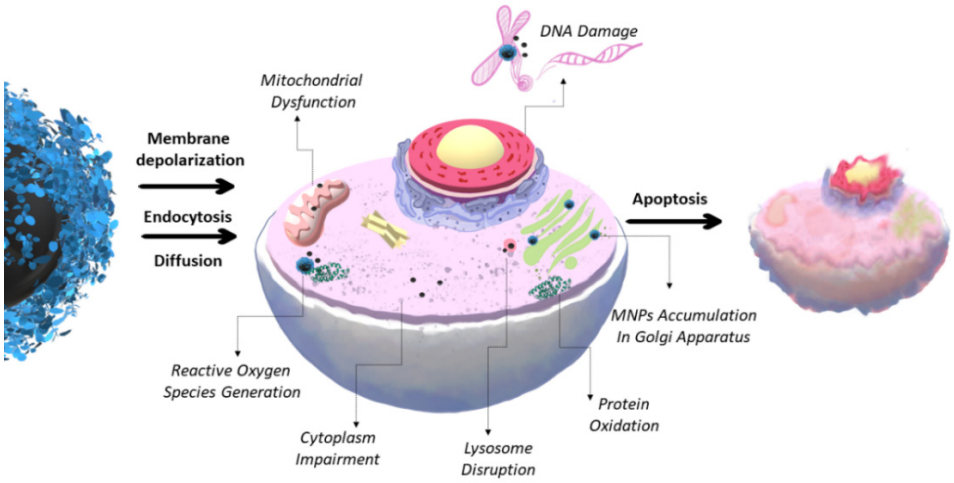

Figure 9. Possible toxicity mechanism of MNCs, leading cell processes' dysregulation and triggering cell death (necrosis or apoptosis).

The MNCs' size, shape, surface charge, coating, and surface modification highly influence biodistribution and toxicity [223]. MNCs with a size less than 10 nm are quickly removed through renal clearance. Nanocomposites greater than 180–200 nm are filtrated by the spleen. Therefore, MNCs in the range from 10 to 150 nm are the most preferred. The surfaces of nanoparticles are rapidly covered in blood by various proteins. Surface chemistry highly influences nanocomposite biodistribution. For example, albumin coating usually prevents non-specific blood interaction and liver accumulation, prolongs circulation time, and moderates particle uptake in cancer tissue [25]. The wrong coating of the nanocomposites may lead to the MNCs' destabilization, aggregation, and precipitation [29]. Inside the body, MNCs can be absorbed through interactions with proteins (e.g., protein albumin), blood components, and cells [228]. Blood compatibility is essential for any in vivo application of MNCs. Lack of stability in the blood can trigger liver accumulation with further degradation and elimination from the body. In the worst case, the co-coagulation and precipitation of MNCs and the blood component may happen, activating thrombus formation.

The MNCs' dose, initial concentration, biodistribution, and circulation time should be taken into account [42]. The nanocomposites can be distributed into various organs

and further metabolized. The overload of local MNCs may lead to high levels of free iron ion release and ROS generation in the tissue and cause aberrant cellular reactions and organ-specific toxicity [223,224,237]. The in vivo toxicity experiment is an expensive and vast work, which has greatly shut down the progress in this area [224,228]. Almost all organs are influenced by the toxic effects of MNCs. Among those are the heart, lungs, liver, kidney, and nervous and reproductive systems. For example, heart-specific toxicity provides contractile apparatus and endothelial damage, the violation of a conducting system, and ischemia [224]. The MNCs' organ-specific toxic effects may be associated with ROS generation, leading to changes in glutathione, superoxide dismutase, and coenzyme levels [224]. Afterward, the in vivo interaction of MNCs and the biological system is quite complicated and dynamic. In the future, extended toxicity studies could help to bridge the gap between in vitro research results and successful clinical trials.

3.2. Drug and Gene Delivery, Therapy, and Diagnostics (Theranostics)

Nanoparticles and MNCs became extremely popular for cancer therapy due to the possible targeted delivery. MNCs provide drug or gene delivery to the cell or tissue by an external magnetic field. Magnetic transfection, or magnetofection, is a method that uses magnetic fields to transport NA-based MNCs to target cells. Magnetofection has been adapted to various NA types, including aptamers, siRNA, miRNA, shRNA, etc. [76–78,238–240]. Such technology is a possibility to solve the drug resistance problem and the low efficiency of gene delivery through cell membranes [241]. A combination of MNCs and siRNA or antisense oligonucleotides may be successfully used instead of a chemotherapeutic drug, resulting in a therapeutic effect [76,238,242–245].

As stated above, the magnetic core of MNCs has multimodal advantages, such as possible tracking by magnetic resonance imaging (MRI) or magnetic particle imaging (MPI) and the hyperthermia effect. MRI and MPI are great non-invasive diagnostic techniques [34,43,44,148,246–249]. MRI provides a high-resolution and easy image contrast manipulation. MNPs are usually known as T_2-contrast agents, which lead to a dark zone on the MRI image. However, the previous simple MNPs were withdrawn due to the side effects. There are many successful in vitro experiments and undergoing pre-clinical animal studies for MNCs [34]. MPI is a relatively novel technology that was presented in 2005. MPI detects tracer MNCs selectively, providing the signal is observed without background with a high signal-to-noise ratio [44]. The method possesses potential for tumor, metastases, and cell detection.

The hyperthermia effect is generated by an alternating magnetic field [250,251]. In the presence of MNCs, heat appears in local regions, which damages tumor cells. The method is limited by the MNCs' quality, size, morphology, and coating. The simultaneous drug, therapeutic NA, and hyperthermia using single-MNCs is a promising anticancer strategy. Recently, various MNCs to be used for multimodal imaging and theranostics have been developed [34,40,44]. The combination of MRI, MPI, and primary used methods, such as single-photon emission computed tomography (SPECT), computed tomography (CT), positron emission tomography (PET), and optical imaging, have become known [34,40,252,253]. However, theranostic synthesis is a complicated problem, which limits progress in the area [88].

3.3. Magnetic Separation and Biosensors

Solid-phase magnetic separation is a much more efficient protocol for NA isolation than traditional approaches [62,69,71,187,254–260]. Magnetic separation is a relatively cheap, quick method for pure NA capture with a high yield. It usually requires ~10 min for NA isolation from the mixture using specific MNCs and a rack with magnets (Figure 10). However, the high cost of commercially available MNCs limits their routine applications. Moreover, the specific "bind-release" of NA MNCs is extremely rare. One of the primarily used magnetic beads has an avidin/streptavidin coating and forms a specific complex with biotin-labeled oligonucleotide [261]. Biotinylated oligonucleotide interacts with a

targeted NA. Such systems are specific. However, the high binding constant of avidin to biotin hinders the separation of NA from MNPs and their further use. Other commercially available MNCs for NA isolation are not specific and usually bind all the NA in the probe with a certain degree of purity. Such MNCs work on the ionic interaction of NA to the MNC's surface. Therefore, protein binding is possible. Finally, new cheap, high-capacity sorbents for the capture of NA are required.

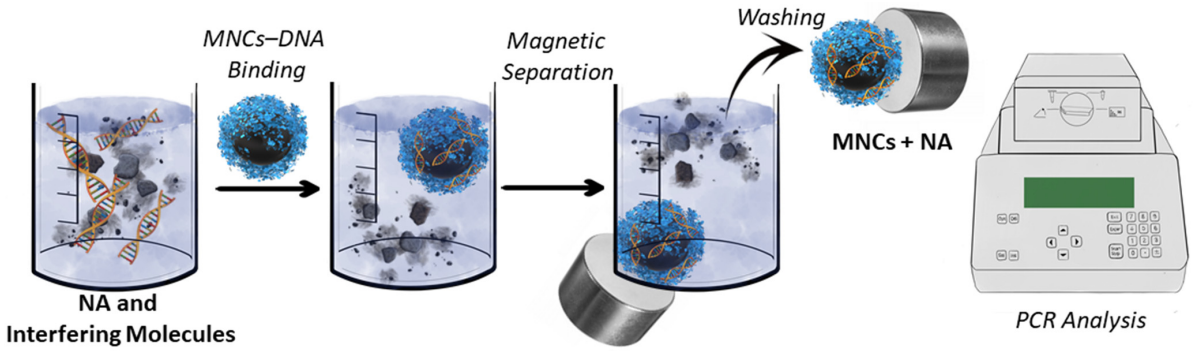

Figure 10. Basic principles of NA magnetic separation.

Recently, magnetic cell separation has become a comprehensive technology for targeted cell population separation for various applications [50]. Some MNCs for cell isolation work by using NA-based MNCs bearing aptamers. However, aptamer-based magnetic cell separation faces many obstacles, which makes it difficult to use such an approach in common practice [50]. DNA aptamers can also provide specific recognition capabilities against many targets used for magnetic separation.

Selectively sensing a single NA allows for the discovery of rich information regarding human health [71]. The separation of NA, with further analysis, is a laborious procedure that, in some cases, does not make sense. The new specific, sensitive methods are required for rapid diagnosis [49]. The surface of the specific MNCs for NA detection may be modified by NA-specific molecules, including antibodies, aptamers, NA, proteins, etc. (Figure 11). The transducer from "chemical" to "physical" signal may be electrochemical, optical, piezoelectric, etc. [49,262,263] (Figure 12). In some cases, the isolation of NA with subsequent analysis is required. This procedure may be performed using MNCs with further PCR analysis [261]. For instance, MNCs can improve the sensitivity of the PCR with an extreme detection limit. Compared to traditional PCR approaches, an MNC-based PCR shows richness and a high potential [261].

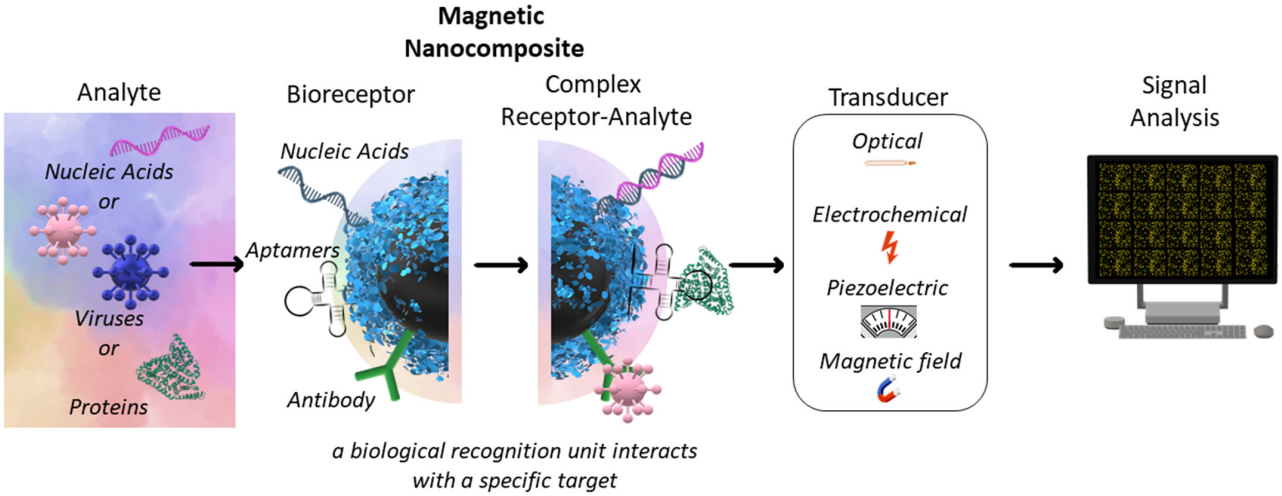

Figure 11. Basic principles of NA-based biosensors.

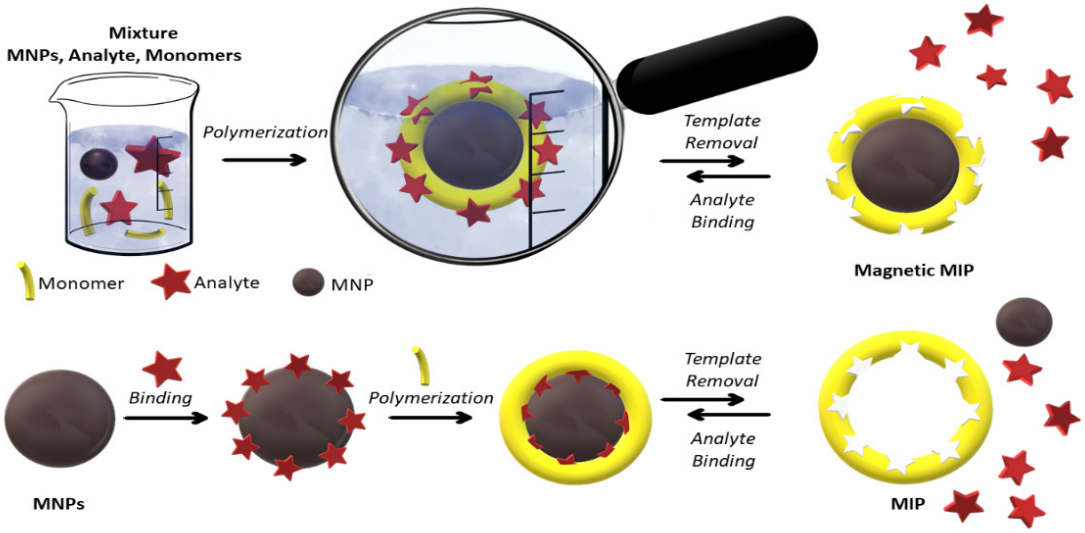

Figure 12. Schematic representation of core–shell imprinting for MMIP preparation. The analyte is a template molecule that MIP has reversibly recognized. The number of cycles is proportional to the efficiency of MIPs.

3.4. Magnetic Molecularly Imprinted Polymers

MNPs have been extensively developed for their excellent separation and extraction ability. A new innovative approach in this area is the use of MNPs with a molecularly imprinted polymer [264–266]. Molecular imprinting is a technique to create molecule (template)-specific cavities in polymer matrices (Figure 12).

The procedure is similar to the enzymes' "lock and key" model. The resulting polymer is called molecularly imprinted polymers (MIPs) [266,267]. For the magnetic core, magnetic molecularly imprinted polymers (MMIPs) have been developed [264,268]. In the structure of MIPs, some regions can specifically interact with template molecules or structurally related molecules. Recognition can occur concerning the shape, size, or due to interactions between the functionalities of the template and polymer [267]. Two types of MIPs are known [269]. Among those, the classic version involves the polymerization of functional monomers, templates, and cross-linking agents (Figure 12, top line). The second one is the change in the polymer state from liquid to solid in the presence of a template.

MIPs have many advantages, such as chemical and physical stability, easy synthesis, reusability, and cost-efficient preparation [91,266]. Polymer matrices may consist of organic or inorganic compounds capable of recognizing molecules or ions [264,266–268]. The synthesis methods of MIPs can be classified according to the format (two- or three-dimensional) and bonding type between the functional monomers and template (covalent or non-covalent) and by the nature of the functional monomers (organic or inorganic). MIPs are used in many areas, such as synthetic recognition elements, solid-phase extraction, liquid chromatography, electrochromatography, assays, drug delivery, theranostics, and biosensor production [266,267,270,271]. MIPs are primarily prepared by bulk polymerization as monoliths (3D imprinting). The first templates for MIP recognition were low molecular weight biologically active compounds such as vitamins, hormones, toxins, drugs, nucleotides, NA, and their derivatives [272–275]. However, this method has various drawbacks, such as a low amount of binding sites near the surface, inaccessible recognition sites within the polymer bulk, a wide range of particle sizes, and non-uniform morphology [276,277]. The transition to the imprinting of biomolecules (nucleic acids, peptides, and proteins) requires significant changes in the existing imprinting protocols and the emergence of new ones [266]. Obtaining MIPs for biomolecules remains a formidable challenge due to their large dimensions, low solubility and stability, complex structure, slow mass transfer, and structural flexibility in solution. The bulk polymerization is limited to the macromolecule and biomolecule imprinting, including peptides, proteins, NA, viruses, and

bacteria. Nearly 1200 research articles were published annually on MIP-based biosensors, out of which only nearly 10% included the recognition of biomacromolecules [278]. Surface molecular imprinting seems to be an alternative approach that can address some of the shortcomings of the synthesis of a primary MIP. Inorganic materials, such as silica, magnetic, gold, and silver nanoparticles, are especially widely used as a core for MIPs [279,280]. The combination of MIPs and other materials combines features yielding smart core–shell MIP structures which allow for the control of the size and distribution of the synthesis. The hybrid magnetic MIP (MMIP) has the advantages of the technology of MIPs and MNPs.

The magnetic properties of the MMIP allow for magnetic separation, imaging, hyperthermia, and selective template release [264,265,268,281–283]. MMIPs have shown a high potential in identifying a broad spectrum of analytes, from small molecule enantiomers to large proteins, NA, and macromolecules [91,264,284,285]. The possibility to automatize this process by using magnetic properties is also an interesting feature for industrialization and mass production. Nowadays, MMIPs are widely used in various fields of biomedicine, such as biosensors, drug and gene delivery, and NA isolation [267,279,285]. MMIPs show a high potential for use in cancer therapy due to targeted delivery by an external magnetic field, hyperthermia effect, and possible simultaneous drug and therapeutic NA delivery [286]. The use of MMIPs for NA-based applications is also being extensively studied [91,267,287–289]. NA can act as both templates and complex macromolecular functional monomers, which provide unique properties to the resulting MIPs [290]. Consequently, combining MIPs with NA with magnetic properties provides a new class of smart synthetic NA receptors, i.e., NA-MMIPs [287]. These materials open up new possibilities in this research area. Table 1 summarizes NA-based MNC biomedical applications.

Table 1. Some examples of nucleic acid-based biomedical applications of MNCs.

Application Area	MNCs Type	NA-Based Application	Reference
Biosensing and diagnostics	MNP@Ag-amine-modified anti-miR-155	miR-155 detection through resveratrol interaction (electrochemical label)	[291]
	MNP@Au	Ultrasensitive colorimetric and electrochemical miRNA detection	[292,293]
	MNP@graphene	Electrochemical miRNA detection	[293]
	MNP@SiO_2	DNA and RNA extraction from Hepatocellular Carcinoma, virus RNA extraction and detection by RT-PCR, Taq polymerase fixation for long-term enzyme activity for PCR	[122,261]
	MNP-oleic acid	DNA detection by PCR	[261]
	MNP-NH_2	DNA extraction from blood and detection by PCR	[122]
	MNP-COOH	DNA extraction from staphylococcus aureus bacteriophages, mRNA isolation from mammalian cells	[122]
	MNP-OH/-NH_2/-COOH	Hybrid NA separation from animal tissue samples	[294]
	MMIP	DNA detection	[287]
	MNP—rabbit antigoat immunoglobulin	Immunoglobulin (IgG) detection	[295]
Therapy and diagnostics	MNP@PEI	micro-RNA intracellular delivery for MYCN inhibition in neuroblastoma	[296]
	MNP-chitosan	Gene delivery	[297]
	MNP-Hyaluronic acid	Gene delivery	[298]
	MNP-lipids	siRNA delivery	[244]
	MNP lipoplex	Theranostics, imaging guided (MRI) delivery of NA	[298]
Magnetic separation	MNP@Ag	mRNA extraction	[63]
	MNP@Au	mRNA, dsDNA extraction	[63,114]
	MNP@graphene	dsDNA extraction	[114]
	MNP@SiO_2	DNA/RNA extraction	[62,63,114,122]
	MNP@SiO_2	NA capture from lysed white blood cells, *B. subtilis*, *E. coli*, and Rift Valley fever viruses	[299]
	MNP@SiO_2	viral NA extraction from serum	[122]
	MNP@SiO_2-organic halide	DNA extraction	[62]
	MNP@SiO_2-NH_2	DNA extraction	[62]
	MNP@polydopamine	genomic DNA extraction	[171]
	MNP-Nylon-6	RNA extraction	[19]
	MNP-Streptavidin	DNA/RNA extraction, aptamer-based cell separation	[50,299]
	MNP-CD138 (syndecan-1) antibody conjugated	Endothelial cells (HUVEC) separation	[295]
	MNP@PEI	dsDNA extraction	[114]
	MNP-thermosensitive polymer, poly(N-isopropylacrylamide-co-2-aminoethyl methacrylate)	DNA extraction	[256]
	MNP-N-isopropylacrylamide and allyl glycidyl ether, 3,5-difluoro-4-formylphenylboronic acid	*S. aureus* and *Salmonella* spp. separation	[300]

4. Conclusions and Future Prospects

The magnetic core on MNCs is a promising feature for unique properties and various applications. It endows the possibility of MRI and MPI diagnostics, magnetic separation, hyperthermia, and targeting by an external field drug/gene delivery. Combining with other types of coating is vital to develop MNCs with diversified properties. Finally, MNCs are a promising core for the creation of a new smart construction. The possible surface functionalization opens up numerous tool options, which could greatly advance the field. We believe that bioinspired MNCs have a great proven potential for cancer treatment and biosensing applications. NA-based MNCs take an important place in analytical applications, biosensing, magnetic separation, diagnostics, therapy, and theranostic areas. Recently, next-generation constructions were developed and combined with one nano platform therapeutic NA, drug, hyperthermia possibilities, imaging, and targeted delivery. Such incredible progress makes for a bright future in cancer treatment. New rapid and efficient synthesis procedures for MNCs are required for future studies. Despite the widespread advances of MNCs, nanomedicine and toxicology integration proved to be essential steps toward in vivo applications. In this regard, a collaboration of chemists, physicists, biologists, and physicians is extremely necessary for further breakthrough developments. However, new simple, cheap, and efficient toxicity models and clinical protocols need to be clearly defined. This gap in the toxicity evaluation is a great limiting factor for practical usage. Finally, NA-based MNCs will rightfully take their place among various biomedical applications. We certainly hope that the improvement in the synthesis and functions of bioinspired MNCs will afterward lead to a new era of nanomedicine.

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