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Mini-Review

Recent Advances in Metal Oxide and Phosphate Nanomaterials Radiolabeling with Medicinal Nuclides

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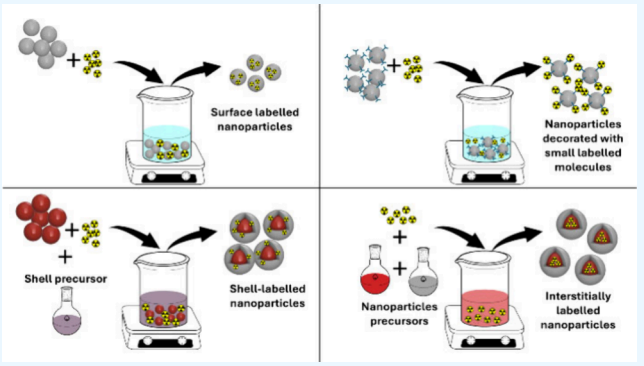
ABSTRACT: The utilization of nanomaterials in biomedical applications has surged in recent years; yet, the transition from research to practical implementation remains a great challenge. However, a promising area of research has emerged with the integration of nanomaterials with diagnostic and therapeutic radionuclides. In this Review, we elucidate the motivations behind selecting metal oxide- and phosphate-based nanomaterials in conjunction with these radionuclides, while addressing its issues and limitations. Various metal oxide- and phosphate-based nanoparticles, exhibiting low toxicity and high tolerability, have been proposed for diverse biomedical applications, ranging from bone substitutes to drug delivery systems and controlled release vectors for pharmaceuticals, including radionuclides for nuclear medicine imaging and therapy. Moreover, the potential synergistic effects of multimodal combinational therapies, integrating chemotherapeutics, immunomodulators, or hyperthermia, underscore the versatility of these nanoconstructs. Our comprehensive exploration includes the underlying principles of radiolabeling strategies, the pivotal attributes of nanomaterial platforms, and their applications. Through this perspective, we present the potential of nanotechnology-enabled nuclear medicine. Furthermore, we discuss the potential systemic and local applications of these nanoconstructs, considering their *in vitro* and *in vivo* characteristics, as well as their physicochemical properties.



INTRODUCTION

Although the number of publications on the biomedical use of nanomaterials is enormous, the number of actually used nanoformulations is relatively small.[1] This indicates that there is a rather demanding and lengthy path toward the practical utilization of nanomaterials in medicine.[2] However, a completely new area of their application has recently emerged in combination with diagnostic and therapeutic radionuclides, especially radionuclides emitting alpha particles.[3] In this Review, we highlight the motivations for choosing metal oxide and phosphate nanomaterials in conjunction with these radionuclides and address potential issues and limitations that need to be considered when deploying these nanomaterials. Several inorganic nanoparticles, for example metal oxide or phosphate, with relatively low toxicity and good tolerability were proposed as biosurrogates for biomedical applications.[4,5] They can be used e.g. as bone and teeth substitutes,[6] drug delivery systems[7] and controlled release vectors of pharmaceuticals,[8] including radionuclides intended for imaging and therapy in nuclear medicine.[9] Multimodal combinational therapies for therapeutical effect enhancement including chemotherapeutics, immunomodulators or hyperthermia were also proposed.[10−13] In this Review, we embark on a comprehensive exploration of the recent advances in metal oxide and phosphate nanomaterials (Table 1) radiolabeling with medicinal nuclides. We delve into the underlying principles of radiolabeling strategies, discuss the key attributes of nanomaterial platforms, and highlight their burgeoning applications in biomedical research and clinical practice. Through this exposition, we aim to elucidate the transformative potential of nanotechnology-enabled nuclear medicine and envision its trajectory in shaping the future of healthcare. The potential systemic and local application of such nanoconstructs is discussed, considering their *in vitro* and *in vivo* characteristics and physicochemical properties.

TITANIUM DIOXIDE NANOPARTICLES

Titanium dioxide (TiO_2) is a metal oxide that occurs naturally in nature and has been extensively studied in various fields, including medicine. TiO_2 has two common tetragonal

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Table 1. Brief Summary of Synthesis Methods, Shelf Life, Storage Conditions, and Applications of Selected Nanomaterials

Nanoparticle Type	Method of Synthesis	Shelf Life	Storage Conditions	Applications
Titanium Dioxide[14]	Sol−gel method Hydrothermal synthesis	1−2 years (depending on coating and environment)	Store at room temperature Protect from UV light and moisture Use inert atmosphere for prolonged storage	Photodynamic therapy Drug delivery Radionuclide carriers Imaging contrast agents
Iron Oxide[15,16]	Coprecipitation	1−2 years (with appropriate coating)	Store at 2−8 °C	Magnetic resonance imaging (MRI)
	Thermal decomposition		Prevent aggregation by adding surfactants	Targeted drug delivery
	Hydrothermal synthesis		Use inert atmosphere to prevent oxidation	Hyperthermia treatment Radionuclide carriers
Phosphate-Based[6]	Precipitation method	1−2 years (depending on storage and formulation)	Store at 2−8 °C, avoid freezing	Drug delivery
	Sol−gel method		Protect from moisture and high temperatures	Gene therapy
	Hydrothermal synthesis			Bone tissue engineering Radionuclide carriers

crystallographic structures, anatase and rutile, named after the two most abundant minerals. The third, rarer orthorhombic crystal structure belongs to brookite. Titanium(IV) oxide is typically produced by purifying the mineral rutile or subjecting ilmenite ($FeTiO_3$) to either a chloride or sulfate process, both of which eventually yield pure titanate. The amorphous TiO_2 can be converted into anatase or brookite through calcination, a process that occurs at approximately 400 °C.[17]

Various techniques are used to synthesize TiO_2 nanoparticles, with sol−gel and hydrothermal methods being the most commonly applied.[18] Microwave methods have also been studied recently. By modifying the synthesis parameters, such as the substrates used, solvent ratio, temperature, reaction time, and addition of different dopants, it is possible to obtain materials with specific physicochemical properties, including surface, morphology, nanoparticle size and uniformity of size distribution, crystal phase, and photoactivity. The surface of the prepared nanoparticles can be also functionalized with different organic or inorganic molecules to achieve the above properties.[17,18]

The initial studies of TiO_2 primarily examined its use in photocatalytic disinfection. TiO_2 is also commonly used as a white pigment in cosmetic products, such as sunscreens, powders, and eye shadows, due to its physicochemical properties, including a high refractive index and the ability to absorb UV radiation and reflect/scatter visible light.[19,20] Other studies involving TiO_2 have investigated the use of nanoparticles as photosensitizers in cancer treatment as well as in the photodynamic inactivation of antibiotic-resistant bacteria. TiO_2-based nanomaterials have also been explored for cancer treatment. For instance, the combination of TiO_2 with anticancer agents has been suggested as an effective platform for delivering chemotherapy drugs. Various drug release mechanisms have been investigated in this context, including pH-influenced release and irradiation-induced delivery, among others. Stimulus-triggered drug release enables more efficient and controlled delivery of bioactive molecules to tumor cells, as opposed to healthy cells.[21] Some TiO_2 nanocarriers used to deliver anticancer drugs, such as doxorubicin, have been conjugated with folic acid as a targeting agent. Folic acid is a vitamin that is involved in the synthesis of nucleic bases of DNA and is thus essential for the survival and growth of both normal and tumor cells during rapid proliferation. Folate receptors are overexpressed in the plasma membrane of tumor cells, making them attractive targets.[22]

The use of TiO_2 nanoparticles has gained increasing interest, leading to a closer monitoring of their toxicity and biodistribution in the body. Factors such as size, morphology, and surface chemistry of the nanoparticles heavily influence their toxicity. As a result, numerous studies have been conducted to evaluate the biocompatibility, biodistribution, and excretion of nanoparticles from the body. TiO_2 nanoparticles in their unmodified form have been found to accumulate mainly in the liver, spleen, and lungs after intravenous administration to rats. They are then excreted from the body primarily through renal excretion. The study subjects did not exhibit any toxic effects during the 28-day observation period after being administered doses of 7.7−9.4 mg/kg. None of the studies published to date have shown significant toxic effects of TiO_2 nanoparticles when administered intravenously. However, aggregation may significantly reduce the therapeutic effect by impairing the functionality and distribution of the nanoparticles.[23,24] To determine the biocompatibility, excretion, biodistribution, and toxicological properties of TiO_2 nanoparticles, the particles were labeled with the ^{48}V radionuclide (β^+ emitter, $T_{1/2}$ = 15.9 d).[25] This labeling allowed for tracking of the particles in the body. In some studies,[26,27] the nanoparticles were also labeled with titanium isotopes ^{44}Ti (EC decay, $T_{1/2}$ = 60 y) and ^{45}Ti (β^+ emitter, $T_{1/2}$ = 184.8 min) to enable tracking in water or the environment and detection of any impact.

Nanoparticles of TiO_2 have gained interest in the field of nuclear medicine due to their low toxicity and ability to conjugate with various substances. They are being studied as carriers of radionuclides for diagnostic or therapeutic purposes with tests conducted using radionuclides such as ^{18}F, ^{68}Ga, ^{89}Zr, ^{99m}Tc, ^{211}At, ^{223}Ra, and ^{225}Ac.

One of the most commonly used radionuclides in nuclear medicine is the technetium isotope ^{99m}Tc. In their study, Suchánková et al.[28] investigated the labeling yield of titanium dioxide with ^{99m}Tc and its stability *in vitro* in various biological matrices. The study employed two strategies for labeling TiO_2 nanoparticles: sorption of ^{99m}Tc onto the nanoparticle surface and incorporation of the radionuclide into the TiO_2 structure during nanoparticle synthesis. The results showed a high labeling yield of the formed ^{99m}Tc-TiO_2 complex, above 97%, for both labeling strategies. *In vitro* stability studies were

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conducted in saline, blood serum, and 1% and 5% albumin solutions, which suggest that some activity may be released from the carrier during 31 h, depending on the biological medium. The authors conclude that there is no significant dependence between the released activity and the labeling method of the nanoparticles. In both cases, the maximum released activity after 31 h was around 15% in the albumin solution environment, while the lowest released activity was observed in the saline.

The same research group has carried out studies on the labeling of TiO_2 nanoparticles with the α particle-emitting radionuclide ^{223}Ra. The studies discuss the stability of the prepared ^{223}Ra-TiO_2 complex as a function of the labeling method or pH. Suchánková et al.[29] also monitored short-term (up to 59 h after labeling) and long-term ($5 \times T_{1/2\ (223Ra)} = 57$ days) released activity depending on the biological environment. Four biological matrices were used in the study: saline, blood serum, 1% and 5% albumin solutions, and blood plasma. The results demonstrated the excellent stability of the ^{223}Ra-TiO_2 complex. During short-term and long-term experiments, only a maximum of 5% activity was released into the solution for surface-labeled nanoparticles, and approximately 2% activity was released for nanoparticles with incorporated radionuclide. Radium-223 is a radionuclide that decays through a cascade of alpha and beta transformations, producing daughter radionuclides that escape the original carrier. Subsequent studies[30,31] have examined the distribution of these daughter atoms, as well as the dependence of the released activity of ^{211}Pb and ^{211}Bi on nanoparticle concentration and the biological matrices. Finally, the biodistribution of the ^{223}Ra-TiO_2 complex and the released ^{211}Pb daughter was monitored in healthy mice for 24 h. The results of these studies indicate the possibility of leakage of the daughter atoms from the original carrier up to 40%, depending on the biological matrix. The study reliably demonstrated that after leakage from the original carrier into the bloodstream, the daughter ^{211}Pb can deposit at increased levels in nontarget organs such as the kidneys through biological pathways.

TiO_2 nanoparticles were tested as a carrier for ^{225}Ac, an α particle-emitting radionuclide, in a study by Cedrowska et al.[32] The nanoparticles were surface-conjugated with PEG polymer bound to substance P (SP), which has specific binding to NK1 receptors of glioma cells. The resulting TiO_2-PEG-SP product was surface labeled with ^{225}Ac and monitored for stability in saline, 0.1 M PBS solution, and cerebrospinal fluid. The cytotoxicity of the prepared complex was subsequently tested in T98G glioblastoma multiforme cells. The results indicate that the prepared composite is highly stable in the studied biological matrices, releasing no more than 5% of the initial ^{225}Ac and ^{221}Fr activity within 4 days. Cytotoxicity studies show a rapid decrease in the nonmetabolic activity of cells exposed to the prepared complex. Metabolic activity decreased as the dose and incubation time increased. The control ^{225}Ac-TiO_2-PEG NPs without the targeted biomolecule (substance P) also decreased cell viability but to a lesser extent. Therefore, the data suggest that the observed cytotoxicity was specific to NK1 receptors and was directly related to their expression level on tumor cells.

In a separate study,[33] TiO_2 was used as a stabilizing agent for SiO_2 nanoparticles labeled with ^{225}Ac. The findings of this study demonstrated greater stability of the ^{225}Ac-SiO_2@TiO_2 complex in comparison to the unsterilized ^{225}Ac-SiO_2 complex. Additionally, it was demonstrated that the modification of SiO_2 nanoparticles with a TiO_2 metal shell significantly enhances the retention of ^{225}Ac daughter decay products over the 30 day duration of the study. *In vivo* studies demonstrated improved biodistribution of the modified complexes. The ^{225}Ac-SiO_2@TiO_2 complex showed no activity in the kidney, in contrast to the ^{225}Ac-SiO_2 complex. The authors attributed this to better retention of released ^{225}Ac and its daughter products in the TiO_2 shell.

Nanoparticles of TiO_2 were also studied as a carrier for ^{211}At, which also emits alpha particles during its decay but does not cascade and emits only one α particle during its transformation. The present study proposes sorption of astatine onto TiO_2 nanoparticles modified with silver atoms due to the complex chemistry of astatine and the low stability of ^{211}At labeled bioconjugates. The labeling yields were nearly quantitative under reducing conditions but decreased to approximately 80% under oxidizing conditions. The labeled nanoparticles demonstrated exceptional stability in saline, PBS buffer, peptide solutions (0.001 M cysteine, 0.001 M glutathione), and human blood serum.[34]

In studies where positron-emitting radionuclides ^{18}F, ^{68}Ga and ^{89}Zr were attached to TiO_2 nanoparticles, the efficacy of the Cerenkov radiation and subsequent reactive oxygen species (ROS) for cancer therapy was tested.[35,36] Positron emitters have a dual role in this case. First, positron emission tomography (PET) can detect the biodistribution of nanoparticles. Second, the high energies of β^+ particles can interact with the TiO_2 surface and emit free radicals. This, in turn, can lead to cell death if the nanoparticles are targeted appropriately. These models can also be applied to other types of radionuclides and semiconductor materials. They may provide a new strategy to target and destroy tumors.

In nuclear medicine, TiO_2 material has been used as a sorbent for various radionuclides. Currently, it is used as an ^{68}Ge carrier in a ^{68}Ge/^{68}Ga generator. Additionally, it has been studied as a sorbent for ^{47}Sc and its separation from impurities produced during the preparation of this radionuclide. Furthermore, after conjugation with PEG polymer, this material served as a sorbent for the separation of ^{134}Cs and ^{60}Co radionuclides from aqueous solutions.[37−39]

Several recent studies have been carried out on TiO_2 nanoparticles, which show that TiO_2 nanoparticles are suitable carriers for cancer therapy. The studies mainly concern the labeling of various radionuclides. However, further research is needed to fully understand the material's toxicity and biodistribution before it can be widely used. Modifying the surface with different substances can enhance the physical and chemical properties. However, it is crucial to consider the impact of such modifications on the biodistribution and toxic properties of the material.

IRON OXIDE NANOPARTICLES

Nanoparticles composed of iron oxides (IONP), predominantly magnetite Fe_3O_4 and maghemite γ-Fe_2O_3, represent a multifunctional tool for use in diagnostics and therapy. Similar to titanium dioxide nanoparticles or hydroxyapatite, IONP are chemically stable, nontoxic and biocompatible. At the same time, their synthesis is simple and inexpensive. Contrary to other inorganic nanoparticles, IONPs exhibit superparamegnetism. Due to this property, they can be used to cure cancer using magnetic hyperthermia. They can be directed to tumor tissue with the help of external magnetic field, and after

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exposure to an alternating magnetic field, there is a local increase in temperature (42−46 °C), to which cancerous cells are more sensitive compared with healthy cells. At the same time, after the removal of the external magnetic field, the nanoparticles do not retain remanent magnetization, which only causes negligible aggregation.[40] Another potential of IONPs lies in their ability to influence the T_2-weighted time (spin−spin relaxation time), making them suitable for use as negative contrast agents in magnetic resonance imaging (MRI). At sizes <10 nm, they also influence T_1 relaxation time (spin−lattice relaxation time), making them analogous to commonly used contrast agents based on gadolinium complexes.[41] However, there are doubts regarding the toxicity of gadolinium-based contrast agents. In contrast, IONPs are metabolized in the body.[42] The surface of IONPs can also be modified to enhance biocompatibility by preventing phagocytosis by the reticuloendothelial system (RES) or by binding drugs and targeting molecules, which are utilized in targeted radiotherapy.[43] Currently, IONPs are clinically utilized for the treatment of iron-deficient anemia in the form of ferumoxytol preparation. A recent study demonstrated the application of this therapeutic preparation in leukemia treatment, owing to the generation of reactive oxygen species via the Fenton reaction in the presence of peroxides.[44]

Superparamagnetic iron oxide nanoparticles (SPIONs), labeled with positron emitters, have been proposed as contrast agents for hybrid PET/MRI imaging. When conjugated with an appropriate targeting ligand, they could also serve as a guide for selective imaging of the region of interest. To target folic acid receptors, magnetite nanoparticles with surface modification involving dopamine and 4-(chloroacetyl)catechol were synthesized. Folic acid was conjugated to the nanoparticle surface via the dopamine amino group. For radiolabeling, ^{18}F was used as one of the most commonly employed positron emitters. The labeling process involved converting 4-(chloroacetyl) catechol to its azide derivative, followed by a click reaction with pent-4-nyl tosylate. The tosyl group acted as the leaving group during the labeling step. An alternative method was proposed, utilizing the preparation of 5-[^{18}F]-fluoro-1-pent-4-ynyl and its subsequent click reaction with the azide group. However, due to the short half-life of ^{18}F, higher labeling yields were achieved using the first method. The ability to target folic acid receptors was confirmed in the cancerous cell line MCF-7, where the labeled conjugate exhibited greater uptake compared to labeled nanoparticles without folic acid. The authors hypothesized that the internalization of nanoparticles occurs via receptor-mediated endocytosis.[45]

Magnetite nanoparticles labeled with ^{68}Ga have been proposed for dual PET/MRI imagining of prostate cancer. In contrast to ^{18}F, ^{68}Ga could be eluted from the ^{68}Ge/^{68}Ga generator, making it more accessible. To specifically transport nanoparticles, they were conjugated with pharmacophores that have an affinity for the prostatic-specific membrane antigen (PSMA) and gastrin-releasing peptide receptor (GRPR). To achieve conjugation, the nanoparticles were modified with amino and thiol groups. Subsequently, conjugation and surface labeling with ^{68}Ga was performed at a pH of 4. The observation of the fraction of bonded pharmacophores revealed that nanoparticles bearing amino groups could bind a greater amount of the PSMA pharmacophore than nanoparticles bearing thiol groups. Additionally, amino groups bearing nanoparticles demonstrated higher radiolabeling yield. For further investigation, *in vitro* experiments were conducted using amino group modified nanoparticles. These surface labeled nanoparticles were found to be stable in human plasma, with over 80% of the bound activity retained after one hour. Even after more than 3 h, they exhibited less than 5% of red blood cell hemolysis. Experiments on PC-3 and LNCaP cell lines demonstrated specific binding of the conjugate and low values of K_d (28.27 nM for PC-3 and 11.49 nM for LNCaP). Kinetic experiments revealed rapid uptake, with the highest bound activity to cells achieved after 30−40 min.[46]

The feasibility of dual PET/MRI imagining of biotin receptor-expressing tumors with ^{68}Ga labeled magnetite nanoparticles was proposed by Gholipour et al. In the initial synthetic step, magnetite nanoparticles coated with dextran were prepared. Subsequently, vicinal hydroxyl groups were oxidized with $NaIO_4$, yielding aldehyde groups bearing nanoparticles. This was followed by the conjugation of biotin, in the form of biotin hydrazide, to form a hydrazone derivative, which was then reduced to a stable hydrazide. In the final step, the surface of the nanoparticles was modified with thiosemicarbazide to form complex with metal ions. The prepared nonactive conjugate demonstrated low hemolysis of red blood cells. Labeling with ^{68}Ga at different temperatures revealed the influence of labeling conditions on the release of ^{68}Ga from nanoparticles. While labeling yields were greater than 95% at room temperature (RT), after 2 h of incubation in blood serum, 19% of activity was released. Conversely, when nanoparticles were labeled at 60 °C, the labeling yields were also greater than 95%, but the total released activity in blood serum was only 6%. This temperature influence was attributed to the partial bonding of ^{68}Ga to surface hydroxyl groups at RT, in contrast to 60 °C, where a stable complex with thiosemicarbazide was formed. The bonding specificity of nanoparticles was demonstrated by the inhibition of ^{67}Ga-DOTA-biotin binding on the 4T1 cell line, while nanoparticles without biotin did not exhibit this property. Biodistribution of the conjugate was conducted on 4T1 Balb/C mice xenograft. Nanoparticles showed high uptake in RES organs, mainly liver and spleen. There was a 5.4% injected dose per gram (%ID/g) uptake in tumor, while blocking the receptors with non-radioactive biotin decreased this value to 1.1% ID/g. However, this value was still high enough to enable dual PET/MRI imaging.[47]

For dual single-photon emission computed tomography (SPECT)/MRI imaging, the possibility of utilizing labeled IONP with γ-emitting radionuclides exist. The most commonly used radionuclide for SPECT imaging is ^{99m}Tc. This radionuclide has been employed to synthesize interstitially labeled ultrasmall IONPs, which are promising candidates for SPECT/MRI contrast agents. The primary advantage of interstitially labeled nanoparticles is the reduced release of activity. However, in the case of ^{99m}Tc, it is challenging to achieve interstitial labeling of nanoparticles without precipitation of ^{99m}Tc in the form of an oxide. Wang et al. demonstrated a one-pot synthesis of ^{99m}Tc-labeled ultrasmall IONP with a derivatized poly(acrylic acid) zwitterionic shell. The zwitterionic shell was chosen in order to prolong blood circulation by reducing the formation of protein corona and uptake in RES. In addition to conventional IONP, which can serve as a contrast agent for T_2-weighted imaging, ultrasmall IONP demonstrated the potential to function as a T_1-weighted imaging contrast agent. Moreover, the nanoparticles exhibited no cytotoxic effects up to a concentration of 600 μg/mL on the

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4T1 cell line. *In vivo* biodistribution studies on 4T1 xenografted mice revealed a reduced uptake of nanoparticles in the RES compared to nanoparticles without the zwitterionic shell. Simultaneously, a time-dependent uptake of nanoparticles in the tumor was demonstrated, with the highest activity observed after 24 h. The safety of the nanoparticles was assessed in healthy mice, where each mouse received a dose of nanoparticles three times higher than that used for SPECT/MRI imaging. Subsequent histopathological analysis did not reveal any notable damage to organs.[48]

With a suitable choice of radionuclides, it is possible to utilize labeled IONP also in the field of therapy, eventually theranostics. For instance, IONP labeled with ^{68}Ga can be employed for PET/MRI imaging while those labeled with ^{177}Lu can be utilized for cancer therapy, rendering ^{68}Ga and ^{177}Lu an excellent theranostic pair. To harness this theranostic radionuclide pair, IONP encased in an alginic acid and polyethylene glycol shell, were synthesized. Due to the abundance of carboxylic groups on the surface of IONP, direct surface labeling was feasible. In the case of ^{68}Ga labeled nanoparticles, more than 70% of the activity was retained after 2 h in human serum at 37 °C. Similar results were achieved with ^{177}Lu labeled IONP over a period of 7 days. Additionally, IONP demonstrated a low level of hemolysis of red blood cells. Incubation of IONP with the 4T1 cancer cell line resulted in either no toxicity or low level of toxicity over 72 h at a maximum concentration of 130 μg/mL. However, incubation with ^{177}Lu labeled IONP revealed dependence of toxicity on the applied activity. Subsequent *ex vivo* biodistribution studies on healthy mice indicated rapid uptake in the major RES organs: liver, spleen, and lungs. Based on these findings, further research on intratumorally applied nanoparticles was suggested.[49]

The potential utilization of IONP as a therapeutic agent for cancer treatment via brachytherapy was elucidated by Stanković et al.[50] IONP, modified with 2,3-dimercaptosuccinic acid (DMSA), were directly labeled with ^{177}Lu with a high radiochemical yield. Additionally, the labeled IONP demonstrated low release of activity in human serum and saline solution at 37 °C, with less than 5% of ^{177}Lu being released over 144 h in both media. Biodistribution studies conducted on CT-26 grafted mice yielded promising results following intratumoral application of nanoparticles. Over a period of 11 days, more than 95% of the applied activity was retained in the tumor, while other major organs did not exhibit accumulation of the activity. However, biodistribution studies on 4T1 grafted mice revealed a retention of 72% of intratumorally applied activity in the tumor after 14 days. One of the possible explanations for this discrepancy could be the higher vascularity of 4T1 tumor. Additionally, differences in the stroma of the two tumors may also play a significant role in the transport of material in and out of the tumor. A substantial therapeutic effect was observed when labeled IONP were applied in both cancer lines grafted mice. However, due to the limited range of emitted particles in the tissue, the authors suggested the application of nanoparticles in multiple locations.

By substituting the Fe^{3+} cation in the lattice of IONP with lanthanoid ions, it is feasible to prepare interstitially labeled IONP with similar attributes as nondoped nanoparticles. For instance, doping with ^{166}Ho enables the preparation of IONP with comparable magnetic properties, which can be employed in combined radiotherapy and magnetic hyperthermia. However, the challenge with ^{166}Ho doping of IONP lies in the low *in vitro* stability of labeled nanoparticles. As a solution, it was proposed to utilize golden-covered core−shell IONP. To target the nanoparticles, they can be further conjugated via amino group with the monoclonal antibody trastuzumab, which targets the human epidermal growth factor 2 (HER2) receptor. *In vitro* stability studies demonstrated the effectiveness of the core−shell structure, with a maximum release of 13% observed compared to 40% in the case of bare IONP over a period of 72 h. Simultaneously, the gold surface enables its modification with thiol derivatives. Experiments conducted on the HER2-positive SKOV-3 cell line and the HER2-negative MDA-MB-231 cell line demonstrated the binding specificity of the trastuzumab-nanoparticles conjugate. Additionally, higher cytotoxicity was observed for the conjugated nanoparticles compared to nonconjugated nanoparticles, attributable to the presence of the monoclonal antibody.[51]

The golden shell of core−shell IONP can be labeled with some of the gold radionuclides, such as ^{198}Au. This approach enables the utilization of gold radionuclides, which have not yet been employed for radiotherapy due to their *in vivo* instability as complexes. Moreover, ^{198}Au can be produced with high yield via neutron capture on a monoisotopic ^{197}Au target.[52]

The conjugate of IONP with the monoclonal antibody trastuzumab was also employed for targeted alpha therapy combined with magnetic hyperthermia. For this purpose, Cedrowska et al.[53] incorporated the radionuclide ^{225}Ac into the core of the nanoparticles, which were subsequently modified with 3-phosphonopropionic acid. The final step of conjugation involved the creation of an activated NHS ester and subsequent reaction with trastuzumab to form a stable amide bond. The labeled nanoparticles exhibited good *in vitro* stability over 10 days in physiological solution and 1 mM PBS, with less than 2% of the parent ^{225}Ac released and less than 10% of the daughter products ^{221}Fr and ^{213}Bi. In human serum, again >98% of ^{225}Ac was retained, but only 70% of ^{221}Fr and ^{213}Bi. Measurement of the specific absorption rate demonstrated the potential of the conjugate for magnetic hyperthermia treatment. The cytotoxic effect of the conjugate was demonstrated on the SKOV-3 cell line expressing HER2 receptors, with an IC_{50} value of 2.0 ± 1.1 kBq/mL for the labeled nanoparticles compared to 66.3 ± 1.1 kBq/mL for ^{225}Ac alone. However, *ex vivo* biodistribution studies in a murine model revealed a high uptake of nanoparticles in the RES organs following intravenous administration and a low uptake in the tumor. Better results were achieved with the intratumoral administration.

Another radionuclide utilized in alpha therapy is ^{223}Ra. Due to the chemical similarity between Ra^{2+} and Ba^{2+}, hexagonal barium ferrite $BaFe_{12}O_{19}$ nanoparticles were employed as carriers for ^{223}Ra. Similar to iron oxide nanoparticles, hexagonal ferrites exhibit superparamagnetic properties and can thus be utilized similarly to IONP. Gawęda et al. prepared ^{223}Ra interstitially labeled barium ferrite nanoparticles decorated with 3-phosphonopropionic acid, which was used to conjugate the nanoparticles with the monoclonal antibody trastuzumab via NHS ester bond. *In vivo* stability studies demonstrated the nanoparticles' ability to retain the parent radionuclide and its daughter products due to back-resorption onto the nanoparticle surface. The targeting specificity of the conjugate was further confirmed on the SKOV-3 cell line, although a high degree of nonspecific binding to the cell

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surface was also demonstrated. Additionally, a high rate of nanoparticle internalization was observed, with 90.60% of nanoparticles internalized within 1 h. The high cytotoxicity against the SKOV-3 cell line suggests that these nanoparticles could be effective therapeutic agents.[54]

PHOSPHATE BASED NANOPARTICLES

One of the most studied phosphate-based materials is hydroxyapatite (HA). HA nanoparticles have garnered significant interest in the field of nuclear medicine due to their unique properties. HA, with its chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is a naturally occurring mineral found abundantly in bones and teeth, contributing to their structural integrity. This inherent biocompatibility, coupled with a low toxicity, makes HA an attractive material for biomedical applications.

A study conducted by Suchánková et al.[28] focused on 2 different approaches in radiolabeling HA nanoparticles with ^{99m}Tc: surface sorption and incorporating radionuclide into the structure of HA nanoparticles. In both cases, the radiolabeling yield was higher than 90%. *In vitro* studies were performed in physiological saline, bovine blood plasma, and serum and 1% and 5% human albumin solutions. The studies were carried out for 31 h, which is approximately 5 $T_{1/2}$ of ^{99m}Tc. For both radiolabeling strategies, the released activities were under 20% in almost all chosen medias, except for bovine blood plasma, in which case the released activity was around 60%. This study also focused on radiolabeling HA nanoparticles as a potential carrier for ^{223}Ra in the context of targeted alpha therapy (TAT). For radiolabeling HA nanoparticles with ^{223}Ra the same approach as with ^{99m}Tc was used, as well as in case of *in vitro* studies. The radiolabeling yield in both cases was higher than 95%. The *in vitro* studies were performed in short-term (59 h) and long-term (5 weeks). The short-term *in vitro* studies showed that most released activity after 59 h was around 60% in physiological saline and bovine blood plasma. This can be explained by lower stability of HA nanoparticles at lower pH or by the presence of sodium ions in physiological solution. The long-term studies showed similar results as the short-term. Most activity, about 40%, was released out of surface labeled HA nanoparticles in 1% albumin after 5 weeks post labeling and about 50% in case of intrinsic radiolabeling in 5% albumin.

Gemini-Piperini et al.[55] also studied the potential of HA nanoparticles radiolabeled with ^{223}Ra for bone cancer therapy. Nanoparticles were labeled with sorption of ^{223}Ra to the surface of the nanoparticles with the yield higher than 90% over 72 h, which showed high stability of this radiolabeling approach. *In vitro* experiments were performed on 3 different cell cultures: human fibroblast (hFB), human osteosarcoma (MG63) and human osteogenic sarcoma (SAOS-2). The cytotoxicity of radiolabeled HA nanoparticles was evaluated for 3 different activities (37, 18.5, and 4.44 kBq) for each cell line. Both osteosarcoma cell lines MG63 and SAOS-2 showed a high cytotoxicity in comparison with a healthy human primary fibroblast. More aggressive cell line MG3 showed 10% of live cells after 24-h exposure to HA nanoparticles labeled with 37 kBq ^{223}Ra, and approximately 40% of live cells with 18.5 and 4.44 kBq. In the case of SAOS-2 cells, there was a dose-dependent response. In the case of applying 37 and 18.5 kBq, about 30% of viable cells were observed, and in the case of applying 4.44 kBq, about 40% live cells.

Among other results, the authors focused on radiation damage on the nanoparticle structure due to alpha radiation. Micrographs of pure and radiolabeled HA nanoparticles were taken by electron transmission microscopy (TEM), and the crystallinity of prepared samples was investigated by selected area electron diffraction (SAED). These experiments showed no structural changes of HA nanoparticles and no change in their rod-like morphology during the process of radiolabeling. However, a decrease in crystallite size was observed, which could be explained by the alpha particles interacting with the surface of HA nanoparticles.

Other perspective therapeutic radionuclide is ^{32}P with a half-life of 14.3 days. Zhai et al.[56] focused their research on 2 different approaches to radiolabel HA nanoparticles: surface sorption and incorporating radioactive phosphorus into the phosphate group of HA. The radiolabeling yield of the surfaced labeled NPs was higher than 90%, while the yield achieved by incorporating ^{32}P into the structure of HA during its synthesis was higher than 95%. The *in vitro* studies were performed in physiological saline at different pHs (5.0, 6.8, and 7.2). The results showed almost no effect of pH on the stability of both approaches to radiolabeling. Surface labeled HA showed higher stability than ^{32}P-doped-HA up to 14 days while they were stored in saline at RT. The radiochemical purity after 14 days was above 96% for surface labeled HA nanoparticles, and about 80% for ^{32}P-doped-HA. However, the surface-labeled NPs were unstable and slowly dissolved. *In vivo* antitumor efficacy was evaluated on a 4T1 breast cancer mice model. Mice were injected with saline, doxorubicin (DOX), 3.7 MBq ^{32}P-doped-HA, 1.85 MBq ^{32}P-HA, 3.7 MBq ^{32}P-HA, 5.55 MBq ^{32}P-HA and 3.7 MBq ^{32}P-HA with DOX. Almost no change in the tumor volume was observed in mice injected with DOX and ^{32}P-doped-HA in comparison to mice injected with saline. When comparing the effect of surface labeled HA, there was almost no difference in the volume of tumors when the activity 1.85 and 3.7 MBq was administered. However, it was displayed that the tumor volume decreased in comparison to the saline, DOX and 3.7 MBq ^{32}P-doped-HA. The greatest effect was observed when the mice were injected with 3.7 MBq ^{32}P-HA in combination with DOX. In this case, the tumor growth almost stopped.

Other possible way to radiolabel nanoparticles of HA is to functionalize the surface of NPs with a molecule capable of complexing a specific radionuclide. Cipreste et al.[57] explored a way of attaching folate-MDP (medronic acid) to the surface of HA nanoparticles and then radiolabeling MDP part of the molecule with ^{64}Cu. Copper-64 is a promising β^+ and β^- radionuclide with half-life 12.7 h, which makes it a great candidate for theranostic use. 32 MBq of $^{64}CuCl_2$ was used to radiolabel folate-MDP-HA conjugate. After the supernatant liquid was separated, only 6 MBq of ^{64}Cu was bond to the conjugate, resulting in specific activity of 0.24 MBq/μg. This result suggests that the complexation route must be improved to provide higher radiochemical yields.

Other nanomaterials referenced in literature based on phosphates are calcium phosphate, calcium bisphosphonate, and zirconium phosphate.

Calcium phosphate nanoparticles with surface functionalized with the ligand DOTA were studied by Kollenda et al.[58] Uptake of the prepared nanoparticles was studied on HeLa cells, which showed that the conjugate is nontoxic and easily taken up by the cells. Radiolabeling of the functionalized nanoparticles was done with ^{68}Ga. Nanoparticles at concen-

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tration of 1.64×10^{11}/mL were labeled with 22 MBq of ^{68}Ga, which resulted in 0.79 ^{68}Ga atoms per nanoparticle. The *in vivo* experiments were performed on healthy mice and on mice transplanted with CT26 colon cancer tumor and evaluated with PET/CT imaging 1, 2, and 4 h post injection. The researchers compared 4 different application routes: intravenous (i.v.) into the tail vein, intramuscular (i.m.) into the thigh muscle, intratumoral (i.t.) into a subcutaneous tumor, and into the soft tissue (s.t.). The healthy mice were injected with 1.64×10^{11} nanoparticles with 7−10 MBq of ^{68}Ga. The intravenous application into healthy mice led to a rapid distribution via blood pool within the first minutes with high uptake in the lungs. The nanoparticles then accumulated in liver, spleen, and bone marrow. An increased uptake was observed in the kidneys and bladder at later points, which indicates a partial renal excretion. When injected i.m., almost all the activity was registered in the injection place and remained there for the entire time of study. The same effect was observed when mice transplanted with CT26 tumor were injected i.t. and in healthy mice injected s.t. This finding supports the potential use of nanoparticles in brachytherapy.

Tian et al.[59] studied the potential of calcium bisphosphonate (CaBP) nanoparticles to deplete tumor-associated macrophages (TAMs). TAMs are often related to poor prognosis after radiotherapy, and its destruction can lead to improvement of radiotherapeutic efficacy. Calcium bisphosphonate nanoparticles were surface modified with polyethylenglycol (PEG). The cytotoxicity of CaBP-PEG nanoparticles was evaluated *in vitro* on RAW264.7 murine macrophage leukemia cells. The results showed a good uptake of CaBP-PEG nanoparticles in the cells after 12 h in comparison with negative control of calcium phosphonate surface-modified with PEG. The CaBP-PEG nanoparticles were labeled with ^{99m}Tc for *in vivo* biodistribution study in mice bearing 4T1 tumors. 2, 6, and 12 h after i.v. injection of 18.5 MBq CaBP-^{99m}Tc-PEG SPECT images were done. The result showed an accumulation of nanoparticles in the tumor after 6 h. A higher signal was also observed in the liver due to clearance of nanoparticles through the RES. After 24 h, the mice were sacrificed, and *ex vivo* biodistribution was examined. The tumor uptake of CaBP-^{99m}Tc-PEG nanoparticles was determined to be 5.4% ID/g. For *in vivo* therapy experiments, the radionuclide ^{32}P was chosen. The procedure was done by anion exchange in the phosphonate by mixing prepared nanoparticles with $Na_2H^{32}PO_4$ in a weak alkaline solution. The radiolabeling yield of CaBP-PEG nanoparticles was higher than 95%. These nanoparticles showed higher cytotoxicity than the previous formulations.

Sakmár et al.[60] focused on nanoparticles of α-zirconium phosphate (ZrP) as potential carrier of alpha emitting radionuclides for TAT. In their study ZrP nanoparticles were labeled with ^{223}Ra and ^{225}Ac, with the yield of 98.5% and 98.1%, respectively. *In vitro* studies were performed in saline, bovine serum, bovine plasma, and 5% solution of albumin for 48 h in a system that allowed the recoiled daughter atoms to reabsorb to the surface of nanoparticles. The ^{223}Ra-ZrP nanoparticles were most stable in saline solution, and the lowest stability was observed in bovine plasma, where up to 14% of activity was released. In the case of ^{225}Ac-ZrP, the least activity was observed in saline. It was shown that ^{225}Ac-ZrP was less stable in all of the biological matrixes than in the case of ^{223}Ra-ZrP. Despite these results, ZrP nanoparticles showed a high potential as carrier for various alpha emitting radionuclides. However, more research in the field of optimizing the synthesis and radiolabeling is needed.

CONCLUSION AND FUTURE OUTLOOK

The utilization of various nanoparticles as carriers for radionuclides in cancer therapy displays immense potential for advancing the field of nuclear medicine. Through numerous studies, inorganic nanoparticles have demonstrated their efficacy in both diagnostic imaging and therapeutic applications, owing to their low toxicity, stability, and ability to conjugate with a multitude of substances. From surface sorption to structural incorporation, inorganic nanoparticles have been successfully labeled with a range of radionuclides, including alpha, beta, and positron emitters, enabling precise targeting and delivery to tumor sites.

Titanium dioxide nanoparticles have emerged as effective carriers for radionuclides due to their high surface area, chemical stability, and ease of surface modification. These properties allow for one of the strongest binding of radionuclides, enabling precise delivery to tumor sites. Furthermore, TiO_2 nanoparticles are known for their relatively low toxicity and good biocompatibility, which is crucial for clinical applications. However, one of the primary disadvantages is their potential in the generation of reactive oxygen species (ROS) resulting in unintended cellular damage. On the other hand, the ROS generated may enhance cancer treatment.

Iron oxide nanoparticles offer significant advantages in nuclear medicine, particularly due to their superparamagnetic properties, which facilitate their use in magnetic targeting and MRI imaging. Their surface can be easily modified to bind with a variety of radionuclides, enhancing the precision of radionuclide delivery to tumor sites. However, iron oxide nanoparticles are prone to aggregation and oxidation, which can affect their stability and the controlled release of radionuclides. Additionally, their long-term accumulation in the body raises concerns about potential toxicity, necessitating further research into their biodegradability and clearance mechanisms.

Phosphate-based nanoparticles are highly advantageous for radionuclide delivery due to their excellent biocompatibility and biodegradability. These nanoparticles can bind radionuclides and release them in a controlled manner, as the nanoparticles degrade in the biological environment. This ensures minimal long-term toxicity and makes them particularly attractive for use in sensitive tissues. However, their relatively lower mechanical and chemical stability compared to metal oxides can limit their use in applications requiring prolonged circulation and the sustained release of radionuclides. Improving their stability without compromising their biodegradability remains a key challenge.

Despite considerable progress, several challenges and opportunities lie ahead in the realm of nanomedicine. Further research is needed to comprehensively elucidate the toxicity and biodistribution profiles of oxide-based nanoparticles, particularly *in vivo*, and to ensure their safe clinical translation.[61] Additionally, exploring innovative surface modifications and synthesis techniques could enhance the physicochemical properties and functionality of inorganic nanocarriers, thereby optimizing their therapeutic efficacy and minimizing off-target effects. Moreover, investigations into the synergistic effects of combined therapies, such as radiotherapy and photodynamic therapy, hold promise for enhanced cancer treatment outcomes. As we continue to unravel the intricacies

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of oxide-based nanomedicine, interdisciplinary collaborations and translational efforts will be pivotal in harnessing its full potential to revolutionize cancer therapy and improve patient outcomes.

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