

DEVELOPMENT OF TUMOR IMAGING RADIOTRACERS USING GRAPHENE OXIDE NANO-FRAMEWORK

Urwa Muaaz¹, Natiq Hussain², Danish Ali³, Maria Khan⁴, Muhammad Muzammil Ramzan⁵,
, Misha Irshad⁶, Muhammad Abdul Haq Khan⁷, Ijaz Ali^{*8}

^{1,2}Department of Chemistry, Faculty of Physical science, Government College University Faisalabad, Faisalabad, Pakistan.

³Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan.
⁴Iqra university, Karachi, Pakistan.

^{5,7,8*}Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Government College University Faisalabad, Faisalabad, Pakistan.

⁶Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan.

^{*4}drijazali@gcuf.edu.pk

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Corresponding Author: *

Ijaz Ali

Abstract

Graphene oxide (GO) is emerging as a novel nano-framework for tumour imaging radiotracers. It can be loaded with radionuclides and surface functionalized with ligands with a high efficiency due to its large surface area and the presence of a large number of surface groups containing oxygen. The principles of the nanoradiopharmaceuticals are summarized and direct and indirect (chelator-based) radiolabeling strategies are outlined. It then elaborates the structure and properties of GO and reduced GO (rGO), and outlines the differences between covalent and non-covalent functionalisation. The expression of FAP is increased in cancer-associated fibroblasts of various solid tumors but is low in normal tissues and radiolabeled FAPIs, such as ⁶⁸Ga, ¹⁸F and ^{99m}Tc-labeled tracers, have high tumor-to-background contrast, including in low ¹⁸F-FDG avid tumors. We then briefly review radiotracers based on the carrier GO, that are labeled with ^{198/199}Au, ^{124/131}I, ¹⁸F and ^{99m}Tc. They have demonstrated fast tumor uptake, stability in vivo and several cases have demonstrated combined imaging and therapeutic function. There remains a lot to be done—pharmacokinetics and long-term toxicity and clearance of GO is not well understood, and GO-FAPI conjugates are still in their early stages and strategies to enhance biocompatibility and tumor specificity, such as PEGylation and the targeted functionalization, and describe research efforts required for advancing GO-based radiotracers for clinical use in precision oncology.

INTRODUCTION

In the last decade, significant advancements in nanotechnology have profoundly impacted the fields of disease diagnosis and therapeutics, with

particular strides in the application of functional nanomaterials for cancer molecular imaging and targeted drug delivery systems [1]. Among various nanomaterials, graphene oxide (GO) has emerged

as a versatile nano-framework due to its unique physicochemical characteristics, including a high surface-area-to-volume ratio, tunable surface chemistry, and intrinsic biocompatibility, making it an ideal candidate for the development of tumor imaging radiotracers. However, despite the promising features and initial encouraging results, substantial gaps remain in our understanding of the pharmacokinetics, biodistribution, and long-term toxicological behavior of GO-based nanomaterials, which are paramount for their clinical translation.

Nanoparticle-mediated drug delivery in oncology exploits the nanoscale size (1-100 nm) and surface malleability to facilitate precise targeting and controlled release of therapeutic agents. GO nanoparticles, functionalized with targeting ligands such as folic acid or fibroblast activation protein inhibitors (FAPi), have demonstrated enhanced tumor specificity and improved imaging contrast in preclinical models. These functional nanomaterials enable improved cellular uptake and potential to traverse biological barriers, such as the blood-brain barrier, which expands their utility in imaging complex and otherwise inaccessible tumor sites [2, 3].

Recent research developments include the synthesis of multifunctional GO-based nanoplatforms capable of simultaneous diagnostic imaging and therapeutic delivery allowing real-time monitoring of tumor response to therapy. Moreover, novel FAP-targeted radiotracers have shown promise in engaging the tumor microenvironment with exceptional imaging accuracy, potentially surpassing conventional agents in positron emission tomography (PET) [4]. Despite these advances, challenges including GO-induced oxidative stress, immunogenicity, and biological clearance mechanisms must be addressed. Studies reveal that functionalization of GO with biocompatible polymers (e.g., PEGylation) and targeting ligands can modulate these effects, enhancing safety profiles and therapeutic efficacy[5, 6].

Therefore, the development of GO-based tumor imaging radiotracers represents a cutting-edge convergence of nanotechnology and molecular imaging, with ongoing research focusing on

optimizing biocompatibility, understanding in vivo dynamics, and integrating multifunctional capabilities for precision oncology. This review aims to summarize recent progress, identify critical research gaps, and propose future directions to bridge these challenges toward clinical application.

2. Nanoparticles

The International Organization for Standardization defines a nanomaterial as any material that has at least one-dimension measuring between 1 and 100 nanometers or possesses an internal or surface structure within this nanoscale range. In addition to their size, nanoparticles can be categorised according to their form and chemical make-up, which determine their specific characteristics and applications. There are a variety of naturally occurring carbon nanomaterials that can be classified according on their chemical makeup. They are used in cancer treatment, cell imaging, and medication delivery by scientists and engineers.

The remarkable size dependence of the behaviour of nanoparticles (NPs) is largely dictated by their nanoscale size and greatly increased surface area compared to volume ratio. Some nanostructured materials are zero-dimensional (0D), while others are one-dimensional (1D), two-dimensional (2D), or three-dimensional (3D) depending on their overall shape. Polymers, carbon-based compounds, metal oxides, semiconductors, and colloidal systems make up a large portion of these materials. The surfaces of nanoparticles vary in terms of surface charge, size, hydrophobic or hydrophilic characteristics, the presence of surface coatings, and the incorporation of targeting ligands [7].

Nanoparticles, designed at the atomic or molecular level, are typically small nanospheres that can move freely within the human body [8]. These nanomaterials possess an increased surface area to volume ratio and have a high retention effect (EPR) with high permeability, active tumor targeting mediated by a ligand, controlled release of drugs. Nanoparticle-based drug delivery systems enhance the solubility of poorly water-soluble chemotherapeutic agents and extend their

circulation time within the bloodstream. Due to their nanoscale size, these particles exhibit unique structural, chemical, mechanical, magnetic, electrical, and biological properties. Nanomedicine has recently seen a surge in interest in nanoparticles due to their potential to encapsulate or bond therapeutic medicines, allowing for controlled release into specific tissues.

2.1 Types of Nanoparticles

Various organic nanoparticles including liposomes, lipid nanoparticles, polymeric nanoparticles, hydrogels, dendrimers and polymeric micelles—are extensively utilized for the delivery of drugs, genes, or siRNA, as well as for cancer therapy and diagnostic applications [9]. Graphene, graphene oxide (GO), a reduced form of graphene oxide (rGO), fullerenes, carbon nanotubes, dots, nanofibers, and graphene quantum dots are all examples of carbon-based nanomaterials. According to a study these materials have distinct physicochemical features and dimensional traits, which make them extremely adaptable for uses in areas such as energy, electronics, and biomedicine. Inorganic nanomaterials consist of metal and metal oxide nanoparticles such as iron oxide, zinc oxide, silver, gold, mesoporous silica nanoparticles, along with group II–VI core and core-shell quantum dots like CdSe and ZnS, among others.

2.2 Structure and properties of Nanoparticles

NPs are composed of multiple intricate layers, with distinct functions served by each. A functionalised surface layer exists on the topmost surface and interacts with the environment by the introduction of tiny molecules, silver ions, surfactants, the and polymers. Subsequently, there is its outer layer, which provides additional functionality like protection and changed properties. It is composed of a chemically distinct substance from the core. Central to it is the core, which is the main entity of the NP, and it provides essential properties such as optical, magnetic, or catalytic activities [10].

3. Role of nanotechnology in medicine

For centuries, humans have been looking for magical means of alleviating pain from disease and injury. Numerous scholars are certain it can be achieved largely thanks to nanotechnology in medicine. Applications utilize molecular-level built nanodevices and nanostructures for creation, repair, defense, and complete monitoring of all biological human systems [11]. Nanotechnology can revolutionize biomedical research and allow for human advancement on new paths. Prevention, diagnosis, and treatment roles of nanotechnology are established. Besides many other matters, this technology can intentionally change the body. Used nanomedicines exhibit enhanced bioavailability, decreased side effects, and enhanced uptake of therapeutics. In the medical field, nanotechnology's many traditional related qualities and attributes include: wound care, antimicrobial therapies, minimizing harm to healthy cells and nanomedicine diagnostics. The use of nanovesicles, nanoparticles, carbon nanotubes, and other nanotechnology-based concepts has further increased their efficacy and productivity in the field of medicine, where precision and intelligence are crucial on a larger scale [12].

4. Nanomedicine

Nanomedicine is an advanced field of nanotechnology aimed at developing efficient means of curing disease and delivering specific biological molecules. Biologics like peptides, therapeutic protein, and antibodies can only be given by injection. Nano-drug delivery has accelerated research on developing personalized therapy, painless injections, and a drug with greater ability to cross the blood-brain barrier (BBB) [13]. As the advancement of drug delivery mechanisms that are mediated by liposomes and encapsulation of drugs such as Doxorubicin within nanoparticles, scientists have enhanced the application of nanotechnology for medicinal purposes within this century. Lipid nanoparticles have promising results when it comes to converting man-made materials into nano-drugs. GO can be functionalized to carry anticancer drugs, enhancing their stability, cellular uptake,

and controlled release. For instance, PEGylated nano graphene oxide conjugates have been used for photothermal therapy and targeted drug delivery in cancer cells, improving therapeutic efficacy. Clinical applications of nanomedicine are enhanced by loading therapeutic protein and mRNA as nano-drugs. Recent advances in nanomedicine have enabled the use of nanoparticles such as virus-modified exosomes, extracellular vesicles, and acid-base nano-constructs for applications in immunomodulation and immunotherapy. The application of nanomedical research in clinical studies has raised safety concerns, therefore a deeper comprehension of the critical properties influencing the interactions of nanomaterials with tissues and organs is required.

5. Nanoradiopharmaceuticals

An developing class of therapeutic and diagnostic agents known as nanoradiopharmaceuticals combines radiopharmaceuticals, which are radioactive substances used in nuclear medicine for disease imaging and treatment, with nanotechnology [14]. By incorporating radionuclides into or onto nanoparticles, these nanoscale constructions improve radioactive isotope accumulation, targeting, and delivery at certain disease areas, especially cancers. Effective radionuclide loading and the potential for multifunctional platforms that combine therapeutic and diagnostic (theranostic) capabilities are made possible by the high surface area-to-volume ratio of nanomaterials [15].

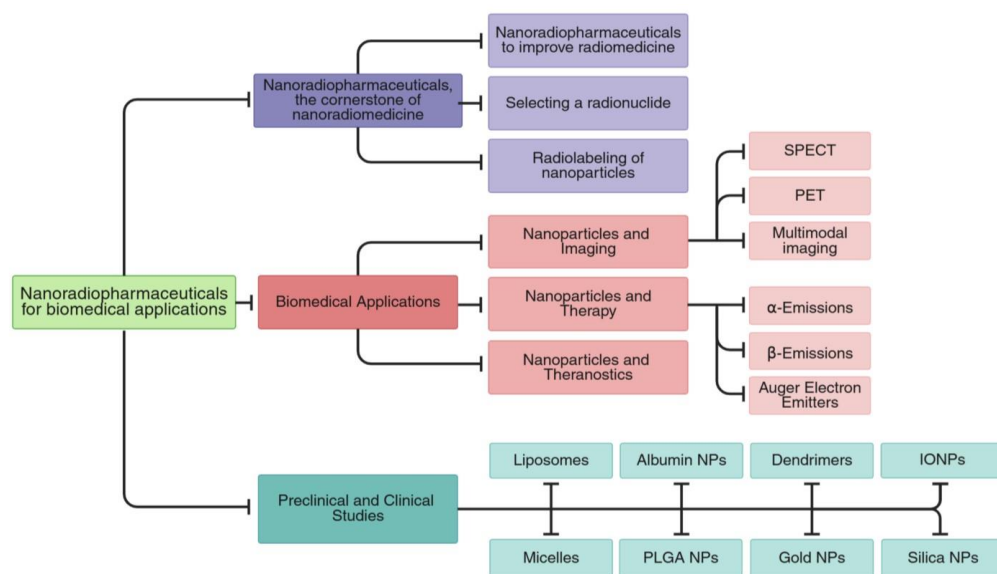


Figure 1.1: Biomedical applications of Nanoradiopharmaceuticals.

Nanoradiopharmaceuticals take advantage of the special pharmacokinetics of nanoparticles, including their extended half-life and ability to selectively bind to receptors specific to tumors by surface functionalization with targeted ligands. By increasing the concentration of radioisotopes at the tumor site while limiting radiation exposure to healthy tissues, this focused strategy may improve therapeutic efficacy and minimize adverse effects [16]. Polymeric nanoparticles, liposomes, dendrimers, and mesoporous materials are

examples of common nanocarriers. These materials are frequently tagged with radionuclides such as lutetium-177, rhenium-186, or technetium-99m.

Nanoradiopharmaceuticals are an exciting new frontier with potential for improved diagnostics and enhanced therapy due to a blend of nanotechnology and radiopharmaceutical science. Depending on the form of NPs and whether it is an organic or inorganic radioisotope, NP

radiolabelling may occur by many different methods [17].

6. Synthesis of nanoradiopharmaceuticals

6.1 Direct labeling of nanoparticles

Bioactive compounds are labeled quickly by using this approach, it is because the radioisotopes used in this method of labeling are directly attached to the desired drug. Direct radiolabeling is also made possible by the physical interaction between the nanomaterial and radionuclides without any chelating agent, such as electrostatic interactions [18]. However, because these linkages are typically weak, this approach has not been thoroughly investigated. The radiolabeling technique may also be impacted by the isotope's chemical properties. In fact, non-metallic radionuclides (such as fluorine-18, iodine-131, etc.) are typically used when labeling NPs directly [19].

6.2 Indirect labeling of nanoparticles

In this method, the radionuclide is linked to the molecule with the help of chelating agent especially bifunctional chelator. These radiopharmaceuticals consist of a radionuclide, a linker, a bioactive molecule and a bifunctional chelator. A chelating agent, or ligand system, is frequently needed for radionuclides with metallic characteristics, such as copper-64 and zirconium-89, in order to bind the radiometal ions in a stable complex. The chelator can be either linear or acyclic, like DTPA, NODAGA, DOTAFA, DOTA, TETA, NOTA etc [20].

These agents serve as molecular bridges that link a radiometal (such as technetium-99m, gallium-68, or lutetium-177) to a targeting molecule (such as a peptide, antibody, or small molecule) with high specificity for a particular biological target, such as

a tumor marker or a specific tissue receptor [21]. The molecules that can bind with nanoparticles and radionuclide and can be utilized in the radiolabeling of the nanoparticles are known as bifunctional chelators BFCs. Thus, the NPs' radiolabeling takes place "indirectly" [22].

7. Graphene Oxide (GO) Nanoparticles

GO is a nanomaterial that has been known for more than 150 years and is used in many applications [23]. It is a carbon nanomaterial synthesized through the chemical oxidation of natural graphite or carbon nanofibers using strong oxidizing agents. Historically recognized before the discovery of pristine graphene, GO serves as a primary precursor for graphene production. The material contains residual oxygen functional groups (hydroxyl, epoxy, carboxyl as shown in figure 1.2) that necessitate reduction via thermal annealing or chemical reduction processes to restore graphene-like electrical conductivity. This post-synthesis treatment removes oxygen content and partially recovers the sp²-hybridized carbon structure, bridging GO's water solubility with graphene's conductive properties for tailored applications [24].

GO possesses distinctive characteristics that enhance its performance in biomedical applications. A key advantage is its excellent solubility in aqueous and organic solvents, which simplifies processing and integration into biological systems [25]. GO demonstrates particular efficacy in therapeutic delivery, including: targeted administration of chemotherapeutic agents to cancer cells, aptamer conjugation for real-time ATP monitoring in cellular environments and non-viral gene transfer vectors for genetic engineering applications.

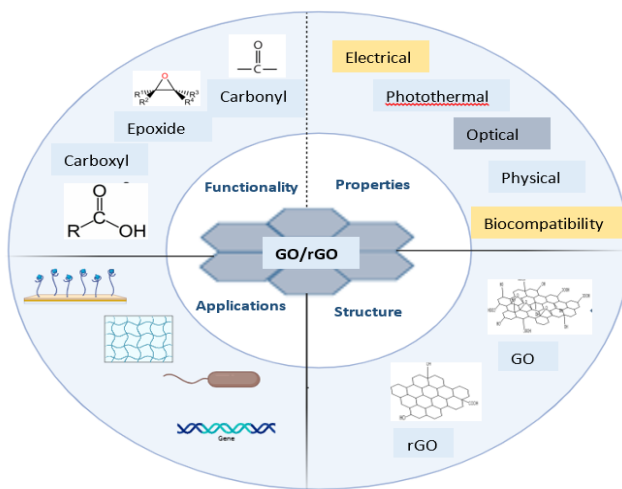


Figure 1.2: Graphical representation of biomedical advancement of GO and rGO

8. Structure and Properties of GO

One example of how nanotechnology has advanced is the use of graphene oxide, also called GO, nano in cancer research. Their size-dependent optical features and other unique properties make them particularly intriguing for medication delivery [26]. The most well-known form of graphene's derivative is graphene oxide. Graphene is a nanomaterial made of carbon that is extremely thin—just one atom thick—and has a lattice called honeycomb structure with sp^2 bonds connecting its individual carbon atoms to form a two-dimensional sheet. The structure of GO is shown in figure 1.3

Graphene's structure resembles a network of interconnected benzene rings, where hydrogen atoms are replaced by carbon atoms. Due to the

absence of oxygen-containing groups, graphene is considered hydrophobic [27]. The flat grid of sp^2 -bonded atoms in this carbon allotrope has a bond length of about 0.142 nm. With an interlayer gap of approximately 0.335 nm, graphite is formed by stacking layers of graphene. Graphene is distinguished by its diverse range of physicochemical properties, which include thermal properties, electrical conductivity, mechanical strength. Due to these attributes GO have led to its widespread use in various sectors like bio imaging, tissue engineering, gene therapy etc. Recently, graphene has been utilized in the medical sector, particularly in DNA sequencing, biosensor development, and cell differentiation and growth [28].

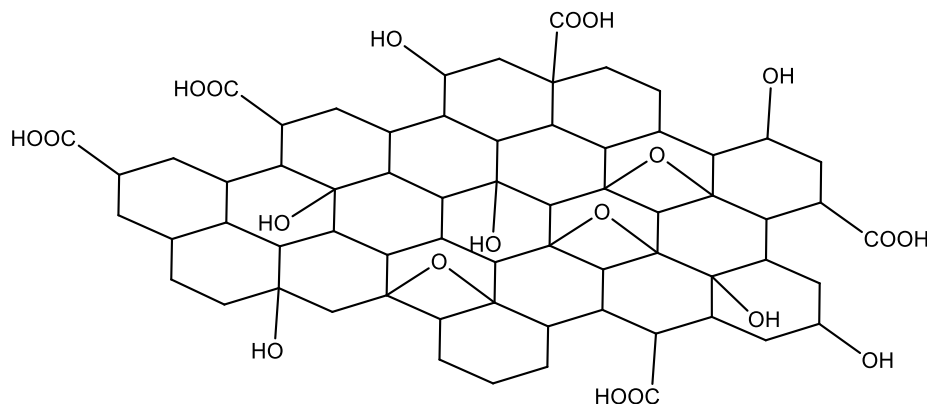


Figure 1.3: Chemical Structure of Graphene oxide

Reduced Graphene Oxide

Graphene oxide (GO) is an essential building block for rGO, which can be made via chemical or thermal reduction the handling of data. It is common practice to chemically decrease graphene oxide (GO) in order to obtain less graphene oxide (rGO). Hydrazine monohydrate, sodium borohydride, hydroquinone, hydrazine anhydrous, pyrrole, hydroxylamine, glucose, urea, and Birch reduction agents are some of the chemical reductants that have been used for this purpose. According to a study, these reductants are able to eliminate the majority of the oxygenated reactive groups and restore a portion of the sp^2 bonds made of carbon in graphene. Nevertheless, other substitutes have been explored

because of their extreme toxicity, including microorganisms, acids, alkalis, reducing agents (including oxygen), and amino ions. The elevated temperature (up full 100°C) is usually required for the reduction, and the amount of time it takes for the reaction to complete differs depending on the reducing agent. The choice of reducing agent has a substantial impact on the synthesised rGO's degree of reducing and its properties.

The reduction procedure eliminates oxygen-containing functional groups, resulting in reduced graphene oxide (rGO) with better electrical and thermal conductivity than graphene oxide (GO). The reduction process utilised can have a substantial impact on the characteristics of rGO, though.

10. Structure of rGO

Ideally, rGO should theoretically resemble a single-layer graphene structure after the oxygen groups are eliminated, although in practice, some residual oxygen content often remains as shown in figure 1.4. This partial restoration of graphene-like properties makes rGO a versatile material for applications requiring enhanced conductivity [29].

A higher percentage of sp^2 hybridised carbon atoms and the elimination of most oxygen-containing groups of functions are the principal structural differences between graphene oxide (GO) and reduced graphene oxide (rGO). The elimination of functional groups that incorporate oxygen in graphene oxide (GO) sheets leads to vacancies, as seen by an increase the ID/IG relation in the Raman spectra.

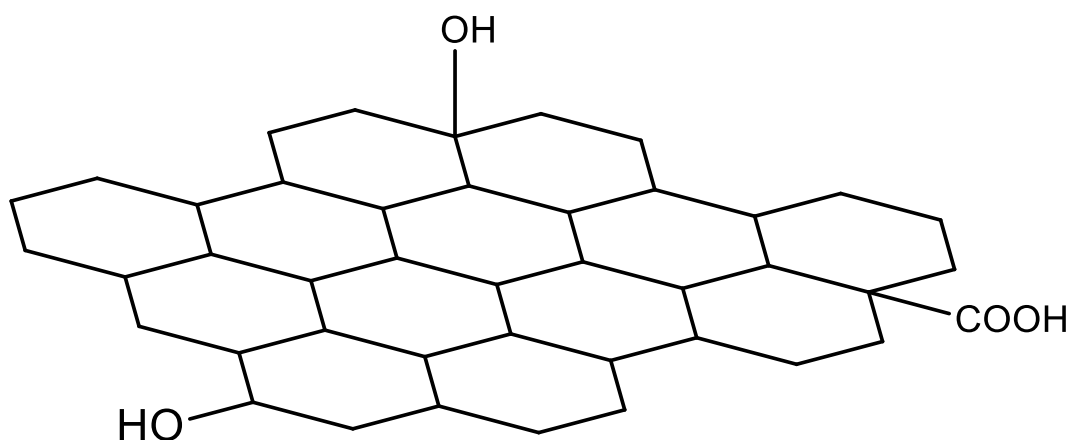


Figure 1.4: Chemical Structure of Reduced Graphene oxide(rGO)

Unfortunately, graphene's limited applicability is due to its single-component graphene material's drawbacks, such as its poor electrochemical activity, tendency to agglomerate, and difficulties in treatment and processing [30]. Graphene /

graphene oxide as functional changes are super important for all the different uses people have for them. Changing the intrinsic structure of graphene & graphene oxide is crucial for functionalisation, as seen in figure 1.5. We offer

chemical modification techniques for GO that are based on its structural functional groups that chemical bonds in this work. Graphene and graphene oxide are introduced, together with their basic structure and properties. Then, they are functionalised according to their surface structure features [31].

Toxicological studies have shown that small-sized graphene derivatives that have been appropriately surface-functionalized no longer exhibit obvious

toxicity to animal and cell tissues throughout reasonable dosage ranges, in contrast to unfunctionalized graphene or oxides of graphene GO, which have poor dispersity for physiological milieus and can cause toxic effects to biological systems. On the other hand, you can radiolabel functionalised GO or rGO with radionuclides like ^{125}I , ^{64}Cu , or ^{177}Lu , according to some other researchers [32].

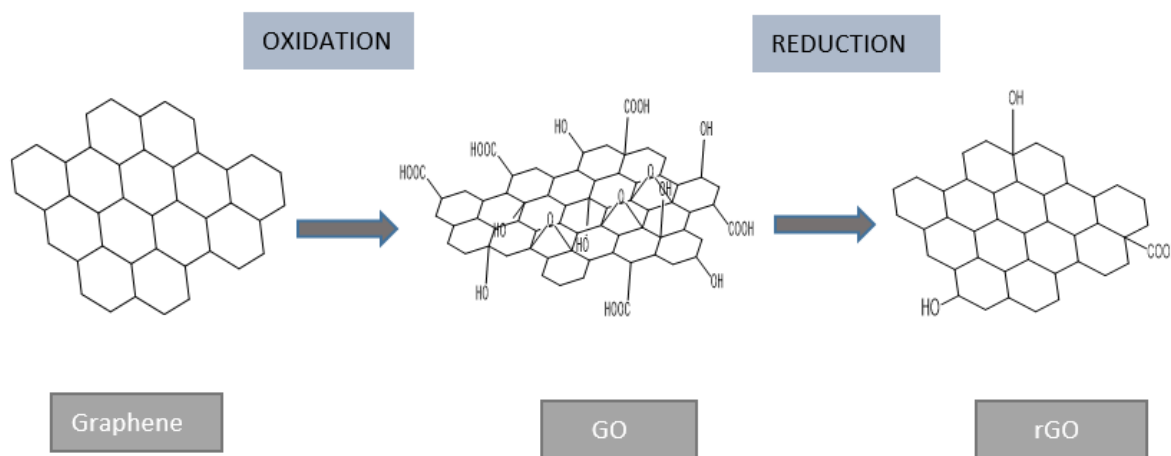


Figure 1.5: The oxidation and reduction of graphene and its structural composition

Researchers were successful to synthesize two various non-toxic nano radiopharmaceutical from GO nanoparticles having potential to selectively target cancer tissue. It was employed that the surface functionalization of GO nanoparticles with tumor imaging radiotracer enhance its tumor targeting efficiency, cellular uptake and retention in cancer cell. This preliminary study points towards promise in utilizing such a multifunctional agent for tumor imaging and targeting [33].

11. Functionalization of graphene oxide and reduced graphene oxide

Nanocarriers made of graphene are fine-tuned in terms of solubility, biocompatibility and drug-loading capacity, and releases efficiency in order to increase their functionality while decreasing their toxicity. Their biological functions and

therapeutic potential are intended to be enhanced by these enhancements. Surface functionalisation has recently been used to investigate GO and rGO. The modification the GO or rGO surfaces can be done in two ways. Primarily, covalent functionalization is typically achieved through chemical interactions with carboxylic, epoxy, and hydroxyl groups on the graphene oxide (GO) surface using various coupling agents [7]. Secondly, noncovalent functionalization of graphene oxide (GO) or reduced graphene oxide (rGO) surfaces is typically achieved through hydrophobic interactions, van der Waals forces, electrostatic attractions, and hydrogen bonding with inorganic nanoparticles and various molecules such as polymers, drugs, proteins, and small compounds [34].

11.1 Covalent bonding approach

Covalent functionalization of graphene oxide (GO) sheets involves grafting polymers or immobilizing biomolecules by targeting the chemically reactive groups present on the sheet edges (carboxylic acids) and the basal plane (epoxy and hydroxyl groups) [34]. Stable covalent surface modifications enhance the stability of immobilized proteins, enzymes, drugs, or small molecules within the system, thereby improving graphene oxide (GO) properties such as loading capacity and biocompatibility. The biocompatibility of functionalised GO has been investigated in multiple research for the purpose of chemical functionalization-based delivery of different treatments, such as synthetic medicines, proteins, antibodies, genes, and more. GO has recently demonstrated remarkable results in gene transfer applications and has significant potential as a carrier to small interfering RNAs (siRNAs).

In a work., GO was functionalized with R8 and cRGDfV peptides to create a novel nanogene delivery method into HeLa cells that may improve stability, electropositivity, transfection efficiency, cytocompatibility, and tumor inhibition [35]. Additionally, the used tris-[nitrilotris(acetic acid)] to successfully accomplish the covalent functionalization of GO. Proteins and other biomolecules labeled with oligohistidine and biotin were delivered to cells via this functionalized GO [34].

Similarly, for medication delivery, a researcher synthesized nanocarriers made of CGO modified folic acid (FA). FA was connected to the GO surface in this system and joined to polyethylene glycol (PEG). Tumor cell types that favored tumor cell death by apoptosis were used to test the nanocarrier's toxicity in vitro [36].

11.2 Non-Covalent bonding approach

Electric forces with proteins or polymers, hydrogen bonding, π - π stacker interactions, / van der Waals forces are typically involved in the noncovalent functionalisation of the graphene oxide (GO) and lower levels of graphene oxide (rGO). Thanks to these interactions, other molecules can be attached to GO or rGO without changing their covalent structure; this allows them

to keep their intrinsic features and improve their functioning. Specifically, electrostatic interactions are more prominent on GO due to its oxygen-containing groups, while van der Waals forces become more significant on rGO because of increased unfunctionalized regions [37]. Strong binding is enabled through stacks of π -electron systems, which happens when the aromatic portions of GO/rGO interact with planar macromolecules or ligands through π - π interactions. Noncovalent binding is enhanced by hydrogen bonding, which occurs mostly with molecules and polymers containing functional groups. Together, these interactions provide a versatile platform for modifying GO and rGO for applications in sensors, drug delivery, catalysis, and other fields [37]. A straightforward method for functionalizing GO or rGO with different molecules without compromising its internal structure or influencing crucial characteristics like electrical conductivity and mechanical strength following functionalization with other materials is noncovalent interaction [38].

The employing of silver (Ag) nanoparticles functionalised with inorganic nanoparticles in anticancer medication therapy was reported. The GO/rGO-Ag nanoscale composites, which shown significant GO cytotoxicity towards A549 cells, were produced by a chemical method that used vitamin C as the reducing agent. Consequently, this strategy has been proposed for the prevention and treatment of cancer [39]. The GO surface can typically be non covalently functionalized with polymers, biomolecules, and medications through absorption or wrapping, primarily through π - π interactions. PEG is the widely utilized biocompatible and nontoxic polymer for modifying the GO surface because of its continuous release properties and ease of integration with different chemotherapy drugs.

A researcher found that at acidic pH (5.8), the GO-PEG hybrid exhibited high drug loading and enhanced drug release due to the presence of two types of potential hydrogen bonding between the drug and the carrier. In contrast, at neutral pH (7.4), there were four types of hydrogen bonding interactions between the drug and carrier, which resulted in very limited drug release [7].

The layer-by-layer (LbL) technique, which involves the alternating deposition of oppositely charged polyelectrolytes onto GO sheets through electrostatic interactions for surface functionalization, has been employed in several studies to develop a new class of GO-based carriers. For example, a study utilized two naturally occurring linear polymers—positively charged chitosan (CS) and negatively charged dextran—as oppositely charged polyelectrolytes to fabricate polyelectrolyte-stabilized GO nanocomposites for drug delivery applications [40].

12. GO based Nanopharmaceuticals

According to a study, graphene oxide's unique properties make it a promising material for use in biomedical fields. To create nGO-PEG, nGO and PEG are mixed in a pH-neutral solution. The remarkable durability of the PEGylated nonproliferative radiotracer in blood serum plus cell culture settings is due to PEG's biocompatibility with outstanding membrane-forming capacity. Animal cell lines were tested for cytotoxicity, and the results showed that nGO-PEG was quite biocompatible. In light of these findings, ^{18}F -nGO-PEG is suggested as a potential radiation for imaging and CT26 tumor detection [41].

Radiolabeled diamond oxide nanoparticles are one nanoplatform that has been studied for in vivo investigations of radioisotope administration. Modifying the surface of GO nanosheets with zinc telluride quantum dots that are amino acid ligands, and Fe_3O_4 nanoparticles with magnetic characteristics for functional purposes [42]. Research has shown that [^{68}Ga] Ga-Go@ Fe_3O_4 -Cys-CdTe QDs, which were created by labelling GO@ Fe_3O_4 -cys-CdTe QDs with ^{68}Ga , have great potential as a radiolabeled imaging agent due to their high concentration in tumor locations [43].

13. Modification of GO using FAPi

The surface modification of nanoparticles is important to reduce agglomeration and enhance its efficiency for functionalization with other biomolecules. This modification of GO increases its surface area and solubility in different solvents. The oxygen-rich functional groups on the GO

surface can be directly functionalized by biological ligands, enhancing the active targeting strategy based on targeted drug delivery [44]. FAP is highly overexpressed in CAFs across cancers (e.g., breast, pancreatic, ovarian) but scarce in healthy tissues, making it a promising target for tumor imaging and therapy. The GO nanoparticles were exposed to amidation reaction to conjugate with FAPi. The carboxyl groups of rGO and the amine group of FAPi formed a covalent connection, conjugating FAPi to carboxylated graphene oxide [33].

13.1 Fibroblast activation protein (FAP)

In 1988, Wolfgang Rettig first identified fibroblast activation protein (FAP) as a cell surface antigen expressed on tumor cells in various sarcomas, reactive stromal fibroblasts involved in wound healing, and many epithelial cancers. In contrast, most normal tissues—including normal fibroblasts, non-malignant epithelial cells, and the stroma of benign epithelial tumors—do not express FAP [45]. Later, FAP was discovered to be a membrane-bound type II serine protease, which belongs to the dipeptidyl peptidase IV (DPPIV) protein family. FAP is made up of 760 amino acids, including a broad external membrane domain (734 amino acids), a small intracellular domain, and a transcellular domain [46]. The latter provides a good docking site for ligands. Because FAP has both endo- and exopeptidase capabilities, it can cleave matrix proteins to facilitate matrix remodeling. CAFs, which are present in more than 90% of epithelial malignancies to varied degrees, overexpress FAP10. For example, neoplasms with strong desmoplastic responses, like pancreatic, colorectal, and breast cancer, frequently have high levels of CAFs [47].

FAP plays a role in tissue remodeling and influences processes such as tumor development, angiogenesis, and metastasis. FAP is found in the tumor stroma rather than on tumor cells, making FAP-based imaging valuable for understanding a tumor's structure and metabolic activity [48].

Solid tumors typically form organized masses containing two interconnected compartments: cancerous cells and supportive stroma. Tumors require stroma composed of connective tissue,

fibroblasts, extracellular matrix, immune cells, and blood vessels to grow beyond 1–2 mm. Initially tumor-suppressive, stromal components evolve during malignancy, fostering cancer progression through interactions that drive growth, invasion, and metastasis. A key stromal feature is the abundance of cancer-associated fibroblasts (CAFs), activated by cellular and environmental cues. CAFs exhibit diverse phenotypes marked by surface biomarkers, including fibroblast activation protein (FAP), a protease linked to fibrosis, ECM remodeling, and tumor-promoting processes like immunosuppression [49].

FAP is highly overexpressed in CAFs across cancers (e.g., breast, pancreatic, ovarian) but scarce in healthy tissues, making it a promising agent for tumor targeting. Researchers are developing pan-cancer theranostic radiotracers that focus on FAP+ CAFs rather than cancer cells directly [50]. Recent advances include FAP-targeting radiopharmaceuticals derived from antibodies, inhibitors (e.g., UAMC1110), and peptides, which show preclinical and clinical potential or diagnostics and treatment. FAP inhibitor, stands out as a promising agent for the development of FAP-targeted radiopharmaceuticals due to its high potency and selectivity [51].

13.2 Fibroblast activation protein Inhibitor (FAPi)

“FAP protein domain can be bound by a tiny molecule called the fibroblast activation protein inhibitor (FAPi). The use of FAPi to identify and attack tumors with elevated FAP expression is therefore growing. In PET/CT imaging, FAPi can be used with the radiotracer gallium-68 (^{68}Ga) and the chelating agent 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) [52]. In cancer diagnosis, PET/CT imaging yields encouraging results, especially for the most prevalent gynecologic cancers. The FAPi PET/CT test doesn't require fasting or rest time and can be completed in ten to one hour. Consequently, FAPi PET/CT has a higher patient throughput than ^{18}F -FDG PET/CT [53].

In PET/CT imaging, ^{68}Ga -FAPi is also utilized as a radiotracer. The uptake of ^{68}Ga -FAPi is low in healthy tissue and high in a variety of malignancies, including those that have low ^{18}F -FDG affinity. ^{68}Ga -FAPi can help distinguish between normal and malignant tissue since it has a greater tumor-to-background ratio (TBR) than ^{18}F -FDG PET/CT [54]. However, FAPi can also be used along with beta-emitting isotopes like yttrium-90 (^{90}Y) or lutetium-177 (^{177}Lu) to treat cancers that have a strong fibroblastic activity, such pancreatic and breast cancer [55].

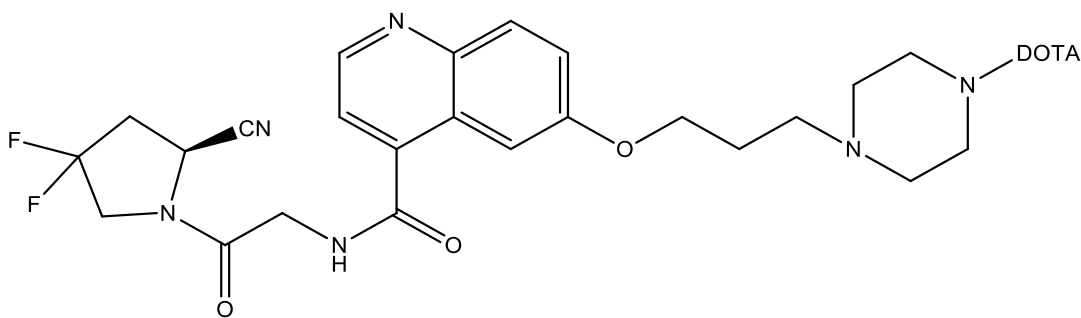


Figure 1.6: Chemical Structure of FAPi-04

14. Tumor Imaging Radiotracers

When it comes to cancer diagnosis, staging, therapy monitoring, and treatment, radiolabeled molecules are crucial. These molecules, which are made up of different radioligands, are injected into the body and release different kinds of

radiation, allowing for efficient treatment and progress tracking in real time.

Imaging is essential for diagnosing, determining the extent of breast tumors, and assessing therapy response. Both 2D imaging, like scintigraphy, and 3D imaging, such PET or SPECT scans (Positron Emission Tomography or Single-Photon Emission

Computed Tomography), are used in nuclear medicine [56]. While radioactive metals are less appropriate for tiny molecules, radioactive halogens and carbon are frequently used to label a variety of radiopharmaceuticals. By conjugating metal chelators like HYNIC, DTPA, NOTA, and DOTA, radioactive metals, are frequently used to label larger molecules like peptides and antibodies. This discrepancy results from the radioactivity type (β^+ or γ) that is employed, which dictates scanner designs. Whereas SPECT use a planar detector or a ring of detectors to gather individual gamma rays, PET uses a ring of detectors that enable quasi-simultaneous detection of the photons released during positron annihilation by two separate detectors [57].

The majority of other oncologic radiotracers currently in use are specifically targeted at the weak spots of FDG. These include, first and foremost, gamma-imaging agents like iodine-123 for well-differentiated thyroid cancer and [^{123}I]MIBG for pheochromocytoma as well as the various new PET agents that have emerged recently, like [^{68}Ga]DOTATATE (DOTA-octreotate) and [^{68}Ga]DOTATOC (edotreotide), which target somatostatin receptors and detect neuroendocrine tumors, and PSMA-targeted tracers like [^{68}Ga]gozetotide, [^{18}F]piflufolastat, and [^{18}F]flutofolastat, which detect prostate cancer more sensitively than previous agents like [^{18}F]fluciclovine or [^{11}C]choline [58].

14.1 ^{177}Lu -Radionuclide

The Lutetium-177 radionuclide has recently attracted a lot of attention and demonstrated great promise for use in many therapeutic procedures in the scientific, clinical, and commercial communities. Although it was late to the party, ^{177}Lu has established itself as a frontrunner in targeted radiation treatments (TRT) and fulfilled all of its potential. The use of ^{177}Lu in in vivo radiation treatments has been rapidly expanding, and it may soon become the radionuclide of choice for targeted radiation therapy (TRT). More and more people are interested in using ^{177}Lu in targeted mechanical therapies because of the remarkable breakthroughs in molecular / cell

biology, such as the usage of peptides the objective cell surface receptors.

Beta particles with no more than energy ($E_{\beta(\text{max})}$) percent 0.497 MeV and an estimated half-life most 6.65 days are emitted by lutetium-177 as it decays to the stable baseline state of ^{177}Hf mercury in 76% of occurrences. Furthermore, highly excited states - ^{177}Hf are decayed to in 9.7 percent of events with a maximum energy of 0.384 MeV and in 12% of events with a maximum energy of 0.176 MeV. According to a study, these excited states, which are situated 0.24967 and 0.32132 MeV above the surface. state, respectively, de-excite to the baseline state through photon emission.

Broad use of ^{177}Lu therapeutic chemicals has not only sped up the development of TRT but also led to the extension of these therapeutic approaches, and the utilisation of ^{177}Lu in nuclear healthcare procedures has been significantly increasing. Consequently, ^{177}Lu is an established and rapidly expanding tool in targeted radionuclide therapy, and its usage has unveiled a plethora of therapeutic radiopharmaceuticals labelled with the radioactive isotope for the treatment of a wide variety of diseases.

Properties of ^{177}Lu

Astonishing perspectives and widespread use of ^{177}Lu lead in TRT can be attributed mainly to the following causes.

- ^{177}Lu is an ideal radioisotope for targeting small volumes, including tumour cells at cavity surfaces or micrometastatic disease, because to its 670 μm mean penetration number of β^- atoms in soft tissue. With a typical tumour size of beneath three millimetres in diameter, lutetium-177 can effectively destroy metastatic lesions and tiny tumours with little damage to the healthy tissue around it. As an added bonus, it works well for targeting relatively small regions with cytotoxic radiation.
- Dosimetry can be done before and during treatment using low-energy gamma photon emission. The same radiolabeled material used for therapy can also be utilised to image biodistribution and excretion kinetics. Important for both diagnostic and therapeutic purposes, this

quality is required for the development of "theranostic" agents for "personalised" medicine, which may target specific cells in injured tissues to administer therapy.

- The emitted radiation of low-energy gamma photons also moderate-energy beta β -particles, and result in a relatively low radiation dosage, offers the possibility of controlling relatively high ^{177}Lu activity amounts during the manufacture and formulation of radiopharmaceuticals and during patient administration.

- Due to its unique +3 oxidation state, lutetium is not affected by reduction-oxidation conflicts in solution biology and may build nine coordination complexes with relative ease. Thus, this capability enables the radiolabeling of various molecular carriers with the specific therapy-needed qualities. These carriers can be small molecules, peptides, proteins, or antibodies. Radiolabeling peptides and protein molecules with a bifunctional chelating substances (BFCA) attached via a chemically resistant covalent bond is possible with $\text{Lu}+3$ due to its chemical characteristics.

- The 6.65-day half-life of ^{177}Lu means that more involved procedures for radiolabeling and purifying ^{177}Lu -labeled radiopharmaceuticals, including quality control and delivery, may need longer time periods. A therapeutic radionuclide with a prolonged half-life, such as ^{177}Lu , is ideal for radiolabeling antibodies of slow targeting kinetics.

- The relatively long 6.65-day bodily half-life of ^{177}Lu provides significant logistical advantages for export to far sites from the reactor manufacturing plant and radionuclide-processing facilities, in addition to minimising decay loss that may happen during transit and distribution to consumers.

Numerous ^{177}Lu radiopharmaceuticals have been effectively created and assessed. Radioimmunotherapy, bone pain palliation radiation synovectomy, and peptide receptor radionuclide therapy are among the in vivo uses of important ^{177}Lu radiopharmaceuticals for various therapeutic procedures.[59]. The number of ^{177}Lu -labeled radiopharmaceuticals under review in preclinical research or product development phases is continuously growing; these could be

utilized to assess radionuclide therapy in vivo in humans [60].

14.2 Gallium (^{68}Ga) Radionuclide

The contribution of gallium-68 (^{68}Ga) to the expansion of clinical research and routine positron emission tomography (PET) has been substantial, particularly over the past two decades [61]. Several factors have driven this progress, including generator-based production of ^{68}Ga , the commercial availability of $^{68}\text{Ge}/^{68}\text{Ga}$ generators, the versatility and robustness of its labeling chemistry, and its relevance to personalized medicine and radiotheranostics [62].

A broad range of molecules has been successfully radiolabeled with ^{68}Ga , including small organic compounds, biological macromolecules, and nano- and microparticles. The resulting imaging agents have shown encouraging performance in both preclinical and clinical settings. In particular, ^{68}Ga has been used to label ligands targeting disease-associated proteins, including receptors, enzymes, and antigens; small effector or hapten molecules for pre-targeted imaging; and compounds designed to visualize general biological processes such as cell proliferation, apoptosis, hypoxia, glycolysis, and angiogenesis [63].

Although ^{68}Ga -labeled radiopharmaceuticals are most widely applied in oncological diagnostics, their utility has also been demonstrated in imaging myocardial perfusion, pulmonary ventilation and perfusion, inflammation, and infection. An emerging area of interest is the non-invasive assessment of transplanted tissue and beta-cell survival in diabetes mellitus [64].

The generator-based availability of ^{68}Ga and its suitability for kit-based radiopharmaceutical preparation make it operationally comparable to $^{99\text{m}}\text{Tc}$. However, PET with ^{68}Ga offers additional advantages, including higher sensitivity, improved spatial resolution, quantitative imaging, and the ability to perform dynamic studies. Furthermore, because several therapeutic radionuclides share coordination chemistry with Ga(III) , ^{68}Ga facilitates the development of radiotheranostic pairs in which the same targeting vector can be labeled with a diagnostic radionuclide for

pretherapeutic imaging or with a therapeutic radionuclide for treatment [65].

Numerous ^{68}Ga -labeling strategies have been developed, enabling selection of an approach according to the intended biological application, required radiochemical performance, and local production logistics. Clinical production can be broadly classified into three categories: manual production under good manufacturing practice (GMP) conditions, automated GMP manufacturing, and kit-based preparation.

14.3 Technetium-99m radionuclide

Technetium-99m ($^{99\text{m}}\text{Tc}$) serves as a highly effective radiotracer for gamma nuclear imaging, functioning both as a biomolecule-labeled diagnostic agent and as an inert radionuclide. $^{99\text{m}}\text{Tc}$ is the metastable daughter radionuclide of molybdenum-99 (^{99}Mo) and can be generated through either fission or non-fission processes. This radionuclide exhibits an 89% abundance for gamma photon emissions at an energy of 140 keV during isomeric transition. Its radioactive half-life of 6.04 hours renders it particularly suitable for radiochemical and radionuclide testing, as it facilitates minimal tissue attenuation from the time of administration to the acquisition phase thereby enhancing biological half-life [66]. Technetium 99mTc, is a transition metal with atomic number 43 and mass number 99. It decays by isomeric transition, emitting a 140-keV gamma photon with a physical half-life of approximately 6 hours, properties that are well suited to single-photon emission computed tomography (SPECT) imaging [67].

The chemistry of technetium is notably diverse. Technetium can exist in eight oxidation states, ranging from -1 to $+7$, reflecting its position among the transition metals and its capacity to form coordinate covalent bonds with a wide variety of ligands. In aqueous solution, the most stable chemical form is the pertechnetate anion TcO_4^- , where technetium is in the $+7$ oxidation state.

Although pertechnetate is chemically stable, it has limited ability to form stable complexes directly with biological targeting molecules. Therefore, radiopharmaceutical preparation generally

requires reduction of Tc(VII) to a lower oxidation state—most commonly Tc(I) , Tc(III) , Tc(IV) , or Tc(V) in the presence of an appropriate chelator or ligand. Stannous chloride is the most commonly used reducing agent in kit-based formulations; it reduces pertechnetate while the ligand stabilizes the reduced technetium core and prevents formation of colloidal technetium dioxide [68].

Among the accessible oxidation states, Tc(V) has been particularly important in radiopharmaceutical chemistry. Many clinically established $^{99\text{m}}\text{Tc}$ agents contain oxo-technetium cores, including $[\text{TcO}]^{3+}$, $\text{trans-}[\text{TcO}_2]^+$, and $[\text{TcN}]^{2+}$. For example, $^{99\text{m}}\text{Tc}$ -sestamibi contains Tc(I) , whereas $^{99\text{m}}\text{Tc}$ -tetrofosmin contains a Tc(V) dioxo core; both illustrate how ligand design can stabilize different oxidation states and produce tracers with distinct biological behavior [69].

15. Radiolabeled FAPI based radiotracers

15.1 Gallium 68 (^{68}Ga)-labeled FAPI

PET imaging uses fibroblast activation protein inhibitors (FAPIs), which target cancer-associated fibroblasts, a group of cells in the tumor surrounding the stroma. Gallium 68 (^{68}Ga)-labeled FAPIs have the best imaging characteristics among the FAP-based radiotracers that have been created thus far in terms of a high detection rate in a range of malignancies, especially in situations that are thought to be difficult for traditional fluorine 18 (^{18}F) fluorodeoxyglucose (FDG) PET [70]. Fibroblasts are activated not just in the surrounding tissue of the tumor but also in benign circumstances such as ischemia, inflammation, or wound healing. As a result, FAPI PET imaging may also be able to show a number of frequent benign disease processes linked to high rates of morbidity. An oncologic imaging technique called gallium 68-labeled fibroblast activation protein inhibitor PET targets fibroblast activation protein, which is widely expressed in the stroma of epithelial malignancies [4].

- One radiotracer that can assist in identifying minor primary or metastatic lesions in vital organs such as the brain, liver, pancreas, and gastrointestinal tract is gallium ^{68}Ga -labeled

fibroblast activation protein (FAP) inhibitor (FAPI) for PET imaging [71].

- ^{68}Ga -FAPI PET is particularly helpful for tumors that have a strong desmoplastic reaction, like pancreatic, breast, and colon malignancies, as well as peritoneal carcinomatosis, which exhibits crisp picture contrast and high lesion uptake.
- The most popular ^{68}Ga -labeled FAPIs (such as ^{68}Ga -FAPI-04 and FAPI-74) and advanced FAP-targeting peptides are distinguished by their ability to accumulate tracer in target lesions quickly and steadily.
- ^{68}Ga -FAPI PET can also be used to visualize inflammation (such as rheumatoid arthritis, Crohn's disease, atherosclerosis, and myocardial ischemia), fibrosis (liver, kidney, and lung), and wound healing.
- The molecular structure's DOTA chelator enables the coupling of the FAPI molecules with therapeutic emitters like lutetium 177 and yttrium 90 for theranostic applications.

The synthesis of ^{68}Ga -labeled FAPIs with specific binding to FAP, which demonstrated a quick and nearly total internalization of the ligand-receptor complex, was initially accomplished by Lindner et al. A favorable equivalent dosage of roughly 3–4 mSv for 200 MBq of both radiopharmaceuticals was found by subsequent dosimetric study comparing ^{68}Ga -FAPI-02 and -04 in patients. This is comparable to ^{18}F -FDG or ^{68}Ga DOTA-(Tyr3)-octreotate (DOTATATE) [72]. Tumor-to-background ratios (TBRs) were further improved with increased image contrast, particularly for ^{68}Ga -FAPI-21 and -46. ^{68}Ga -FAPI-46 in particular demonstrated a rising TBR over time, indicating

that this tracer is a promising tool for upcoming theranostic applications [73].

15.2 $^{99\text{m}}\text{Tc}$ labeled FAPI

Numerous methods for $^{99\text{m}}\text{Tc}$ labeling of FAPI-based molecules have been studied. In particular, [$^{99\text{m}}\text{Tc}$]Tc-FAPI tracers have been produced using the mono-oxo, tricarbonyl, isonitrile, and HYNIC approaches, and they have been evaluated in both in vitro and animal models [74]. Compared to other radionuclides, technetium-99m ($^{99\text{m}}\text{Tc}$), a transition metal, has a major disadvantage in its ability to form bonds with molecules that are physiologically active. In particular, $^{99\text{m}}\text{Tc}$ cannot substitute a hydrogen atom (such as ^{18}F and iodine-123 (^{123}I)) or a carbon atom (such as carbon-11 (^{11}C)) in a targeted molecule.

The incorporation of a $^{99\text{m}}\text{Tc}$ atom into the molecular structure of a biologically active molecule requires the application of coordination chemistry principles, which go beyond the straightforward idea of labeling, which is defined as the replacement of an atom in the biomolecule with the radionuclide of interest [75]. All things considered, these labeling strategies have shown excellent binding and great efficiency. In both in vitro and animal model experiments, the resulting [$^{99\text{m}}\text{Tc}$]Tc-FAPI tracers have demonstrated great specificity for FAP-positive cells and xenografts, respectively. Most [$^{99\text{m}}\text{Tc}$]Tc-FAPI tracers, however, have shown varying degrees of lipophilicity, which results in preferred excretion via the hepatobiliary pathway and undesired binding to lipoproteins [76].

Table 2.1: FAPI based radiotracers

FAPI based radiotracers	Radiotracers	Diagnostic purpose	Target cancer type	Reference
^{68}Ga -FAPI-04	^{68}Ga	PET/CT imaging of tumor stroma; detection of primary, nodal, and metastatic lesions	Breast, lung, pancreatic, sarcoma, cholangiocarcinoma,	[77]
^{18}F -FAPI-74	^{18}F	PET imaging with larger batch production		[78]

		capability; improved image quality and lower cost		
^{90}Y or ^{177}Lu -labeled FAPI	^{90}Y , ^{177}Lu	Radioligand therapy (theranostic applications) targeting FAP-expressing cancer-associated fibroblasts	Various cancers expressing FAP in tumor stroma; under clinical investigation	[79]
FAPI-02, FAPI-46	^{68}Ga , ^{18}F	Diagnostic PET imaging with varying pharmacokinetics and tumor uptake profiles	Broad range including sarcomas, gastrointestinal, breast, lung, and head and neck cancers	[80]

In order to improve pharmacokinetic characteristics and get a more favorable biodistribution, especially in the abdominal area, attempts have been made to synthesize more hydrophilic FAPI-based molecules. When compared to other imaging modalities, SPECT imaging with [$^{99\text{m}}\text{Tc}$]Tc-FAPI has shown comparable or better diagnostic performance, producing encouraging outcomes in patients with gastrointestinal malignancies. Subjects with gliomas, lung cancer, breast cancer, and cervical cancer have also shown good results [81]. [$^{99\text{m}}\text{Tc}$]Tc-FAPI-based imaging has been effectively used in myocardial and idiopathic pulmonary fibrosis in addition to oncological applications. Because they specifically target FAP on CAFs, FAPI-labeled tracers are becoming more potent tools for cancer imaging, offering a novel method for diagnosis, staging, treatment monitoring, and even theranostic applications. A list of FAPI based radiotracers are given below in Table 2.1

The limitations in the use of ^{68}Ga -FAPI PET/CT have prompted other researchers to focus on the potential utility of radiotracers [$^{99\text{m}}\text{Tc}$] Tc-FAPI to conduct Computed Tomography through Single Photon Emission (SPECT). Technetium-99m ($^{99\text{m}}\text{Tc}$) radioisotope is a remarkable icon in nuclear medicine by virtue of its physical characteristics. $^{99\text{m}}\text{Tc}$ with the ability to emit 140 keV of gamma radiation and half-life of approximately six hours, is ideal in sophisticated detection-based imaging [82]. The $^{99\text{m}}\text{Tc}$

SPECT/CT is more practical and relatively cheaper than the PET scan therefore an incentive towards employing the SPECT/CT when there is no convenient PET scan available. Also, labeled $^{99\text{m}}\text{Tc}$ FAPIs have versatile and detailed imaging used in the clinic. The other aspect is that Tc- $^{99\text{m}}$ has a high level of detail and low amount of radiation transmitted to the patient making it perfectly suitable to diagnostic imaging. Labeled fibroblast activation protein inhibitors (FAPIs) labeled with $^{99\text{m}}\text{Tc}$ have opened up a wide range of possibilities in the area of oncological and fibrotic diagnosis due to the ability to selectively target tumor microenvironment and treatment response [83]. In the literature, the development and validation of a new radiotracer, [$^{99\text{m}}\text{Tc}$] Tc((R)-1-((6-hydrazinyl)nicotinoyl)-D-alanyl) pyrrolidin-2-yl) boronic acid ([$^{99\text{m}}\text{Tc}$]Tc-iFAP) that targets SPECT imaging of the tumor microenvironment are discussed. Another fact that the text appreciates is the relevance of SPECT imaging owing to the greater abundance of SPECT cameras as opposed to PET cameras

16. GO based radiotracers

16.1 Gold based graphene oxide

For quick in vivo targeting and SPECT imaging (high purity germanium-spectrometry) of tumors, graphene oxide (GO) sheets functionalized by aminopropylsilyl groups (8.0 weight percent) were labeled by $^{198,199}\text{Au}$ nanoparticle radioisotopes (obtained by reduction of HAuCl_4 in sodium

citrate solution followed by thermal neutron irradiation). The physicochemical characteristics of the amino-functionalized GO sheets tagged with $^{198,199}\text{AuNPs}$ ($^{198,199}\text{Au}$ @AF-GO) were determined to be sufficiently stable in organic phases, such as human serum, to be dependably employed in bioapplications using the immediate thin layer chromatography method. Rats with fibrosarcoma tumors were used to study the in vivo biodistribution of the $^{198,199}\text{Au}$ @AF-GO composite at different post-injection times [54].

With a tumor to muscle uptake ratio of 167 following a 4-hour intravenous injection, the $^{198,199}\text{Au}$ @AF-GO nanostructure demonstrated both rapid and high tumor uptake, leading to effective tumor targeting and imaging. At the same time, the kidneys (as well as the urinary system) quickly excrete the $^{198,199}\text{Au}$ @AF-GO throughout the body due to its low lipophilicity (~ 24 hours). Since $^{198,199}\text{Au}$ radioisotopes have a short half-life, $^{198,199}\text{Au}$ @AF-GO, which has good tumor targeting and imaging capabilities and quickly leaves the body, can be considered one of the most promising and successful nanomaterials for cancer diagnosis and treatment based on nanotechnology [84].

16.2 ^{124}I labeled GO-1-COSAN

Graphene oxide is covalently bonded to monoiodinated boron-cluster derivatives to create a carbon-based hybrid nanocomposite. A new boron-rich cobaltabis (dicarbollide) precursor with one iodide group joined to one of the cluster's boron atoms (I-COSAN) was used to produce this GO-I-COSAN. It is intended to be later labeled with radioactive ^{124}I for use in positron emission tomography (PET). Studies of GO-I-COSAN's in vitro cytotoxicity using HeLa cells at varying concentrations for up to 48 hours demonstrated that the nanomaterial's cytotoxicity was negligible, with cell mortality being less than 10%. Surprisingly, transmission electron imaging demonstrated that the nanomaterial had been internalized by cells and accumulated in the cytoplasm without altering the size or shape of the cells.

The results of the in vitro investigations were also supported by in vivo tests employing

Caenorhabditis elegans, which demonstrated that GO-I-COSAN could be consumed by the worms with negligible toxicity and no discernible harm. In order to create the radioactive nanocomposite GO- ^{124}I -I-COSAN, which was promptly injected into mice, I-COSAN was radioisotopically labeled utilizing a palladium-catalyzed isotope exchange process with Na^{124}I and functionalized onto GO. Excellent in vivo stability of the new nanomaterial was demonstrated by PET scans taken at various intervals. Even after extended periods, no action was seen in the stomach or thyroid, demonstrating that the iodide did not separate from the substance. The biodistribution profile of GO- ^{124}I -I-COSAN was excellent; it was mostly concentrated in the liver and to a lesser extent in the lung, had a lengthy residence period on blood, and was gradually eliminated through the gastrointestinal tract. Notably, this material's high boron content opens the door to theranostics by providing traceable boron delivery for boron neutron capture therapy [85].

16.3 ^{131}I Labeled GO

Applications of nano-graphene and its derivatives in cancer theranostics have garnered significant interest in biomedicine. In order to create ^{131}I -RGO-PEG for nuclear imaging-guided combination radiation and photothermal therapy of cancer, we produce ^{131}I labeled, polyethylene glycol (PEG) coated reduced nano-graphene oxide (RGO). ^{131}I -RGO-PEG shows better cellular absorption than free ^{131}I , which results in increased radiotherapeutic efficacy against cancer cells. Gamma imaging shows that ^{131}I -RGO-PEG efficiently accumulates in tumors following intravenous injection. Due to the radioionization effect, ^{131}I can release high-energy X-rays that can cause cancer to be killed, but RGO has a strong near-infrared (NIR) absorbance and may effectively cause photothermal heating of the tumor when exposed to NIR light. The animal tumor model studies demonstrate that cancers can be effectively eliminated by combining photothermal therapy with radiation, both of which are administered by the same agent, ^{131}I -RGO-PEG. Additionally, toxicology investigations show that mice do not experience any significant

harm from ^{131}I -RGO-PEG at the treatment dose. This research shows the enormous potential of combining photothermal therapy and nuclear medicine as a unique therapeutic approach to

achieve synergistic efficacy in the treatment of cancer. Here is the list of Graphene oxide based radiopharmaceuticals in table 2.2

Table 2.2: Graphene oxide based radiopharmaceuticals

GO based radiopharmaceuticals	Radiotracers	Diagnostic purpose	Target cell type	Reference
$^{99\text{m}}\text{Tc}$ -labeled Carboxylated Graphene Oxide (NGO-COOH)	$^{99\text{m}}\text{Tc}$	SPECT imaging and targeted radiotheragnostic therapy	Solid tumors (breast cancer model tested)	[86]
PEG-coated Reduced Graphene Oxide (rGO)	^{131}I	Combined Photothermal therapy (PTT) and internal radiation therapy; imaging-guided therapy	Various cancers (general tumor models)	[87]
GO-Folic acid	$^{99\text{m}}\text{Tc}$, ^{68}Ga , ^{18}F	Targeted drug delivery, diagnostic imaging, and therapy	Breast cancer, gliomas, cervical cancer	[88]

Conclusion:

Graphene oxide (GO)-based nanoplateforms are promising platforms for the combination of the high sensitivity of nuclear imaging and physicochemical versatility of nanomaterials. The surface chemistry of their modification can be achieved by covalent or non-covalent attachment of targeting ligands, chelators and biocompatible polymers, which is suitable for the development of multi-functional imaging and therapeutic agents. In fact, the preclinical research using radiolabeled GO and rGO has shown effective tumor accumulation, good stability in biological environments and potential of combined imaging-guided photothermal and radiotherapy, as illustrated by ^{131}I -rGO-PEG.

In parallel, fibroblast activation protein (FAP)-targeted radiotracers, particularly ^{68}Ga -FAPi, have established the clinical relevance of imaging the tumor stroma. By targeting cancer-associated fibroblasts, ^{68}Ga -FAPi PET can provide high lesion-to-background contrast and detect lesions that may be difficult to visualize with conventional ^{18}F -FDG PET, particularly in desmoplastic or low-glycolytic tumors.

However, radiotracers with GO properties are still in the preclinical development stage. Optimization of pharmacokinetics, biodistribution, long-term biosafety, manufacturing reproducibility, and regulatory compliance details are to be optimized for their translation to clinical practice. Thus, it is premature to consider GO-based radiotracers as clinically validated imaging agents and they should be considered as promising nanoplateforms for preclinical studies.

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