Review Article

Application of Cyclopentapeptide Cyclic (Arginine-Glycine-Aspartate-Phenylalanine-Lysine) in Nano-Drug Delivery Systems

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Targeting the alpha-v beta-3 integrin receptor holds promise for active targeting in cancer therapy and imaging diagnostics. Cyclic (arginine-glycine-aspartate-phenylalanine-lysine), an arginylglycylaspartic acid tumor-targeting peptide, has shown efficient selective binding to the alpha-v beta-3 integrin receptor, making it a highly effective ligand. Its potential for active targeting in drug delivery has garnered significant attention, leading to extensive research and development efforts. This paper provides a comprehensive review of the application of the cyclic (arginine-glycine-aspartate-phenylalanine-lysine) in nanoparticle-mediated drug delivery systems, aiming to serve as a valuable reference for the expedited development of active targeting formulations. This article explores the utilization of cyclic (arginine-glycine-aspartate-phenylalanine-lysine) as a targeting ligand in these systems and discusses its role, either as the sole ligand or in combination with others, in enhancing the precision of drug delivery to specific lesion sites, with a primary focus on tumors. This paper describes the current status of cyclic (arginine-glycine-aspartate-phenylalanine-lysine)-modified nanoparticles in various carrier materials for targeted therapy, molecular imaging, cytotoxic peptide delivery, photosensitizer delivery, and modified biomaterials. The strategic manipulation of drug distribution and pharmacokinetic properties aims to optimize therapeutic outcomes while minimizing systemic side effects, showcasing the potential of cyclic (arginine-glycine-aspartate-phenylalanine-lysine)-modified nanoparticle systems in advancing precision medicine.

Key words: Cyclic (arginine-glycine-aspartate-phenylalanine-lysine), alpha-v beta-3 integrin receptor, targeted therapy, drug delivery system, nanomedicine

The application of nanotechnology in medicine provides new opportunities for the diagnosis, treatment, and prevention of diseases, holding significant potential for advancing healthcare[1-3]. Nanomedicines are therapeutic or diagnostic agents designed and developed at the nanoscale, capable of traversing external barriers, various compartments, and cell membranes through biological transport processes, thereby achieving targeting at the cellular or subcellular level. The physicochemical properties of Nanoparticles (NPs), including size, shape, surface charge, and the binding of active ligands, influence their effects within the organism[4,5]. Due to their large surface area, unique structural characteristics, and prolonged circulation time compared to small molecules in the bloodstream, NPs have become a focal point in research aimed at optimizing personalized medicine and therapies[6,7]. The main advantages of NP drug delivery include targeting specific cells, protecting drugs from premature degradation, enhancing the solubility of poorly soluble drugs, and achieving sustained and controlled drug, thereby reducing side effects, improving therapeutic efficacy, and safety[8,9]. Targeted formulations are divided into active and

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passive targeting. Passive targeting formulations accumulate at specific sites through Enhanced Permeation and Retention (EPR) effect, while active targeting formulations utilize NPs modified with affinity ligands to bind to biological targets, promoting the internalization of NP drugs and further enhancing targeting effects[10,11].

Integrins are transmembrane proteins composed of Alpha (α) and Beta (β) subunits that play a crucial role in cell adhesion and signal transduction. αvβ3 integrin, as an important receptor, interacts with arginylglycylaspartic acid (RGD) peptides and serves as a significant therapeutic target[12,13]. The RGD sequence (Arg-Gly-Asp) was initially discovered by Ruoslahti *et al*., in the 1970's and later recognized as the smallest natural ligand binding to αvβ3 or other integrin receptors[14]. NP drug delivery systems often select RGD peptides as ligands targeting αvβ3 integrin, improving their binding characteristics through cyclization reactions to enhance stability[15,16]. Cyclotides, as a synthetic molecular family, possess advantages such as small size, high affinity, good biocompatibility, high stability, and low immunogenicity[17]. Therefore, this paper primarily reviews the application of the cyclotide cyclic (arginine-glycine-aspartate-phenylalanine-lysine) (cRGDfK) in NP drug delivery systems.

cRGDfK is a peptide with a cyclic pentameric structure, composed of arginine, glycine, aspartic acid, D-phenylalanine, and lysine, linked by a head-to-tail amide bond. As an RGD tumor-targeting peptide, it can bind to the αvβ3 integrin receptor, serving as a highly efficient and selective inhibitor for this receptor. For cancer therapy and imaging, αv-RGD integrin inhibitor drugs are considered promising for active targeted NP drug delivery, showing considerable potential in this regard[18,19]. Currently, these drugs have reached the late stages of clinical trials for cancer indications, but active targeting formulations have not yet successfully entered the market[20].

This paper aims to introduce the application of cRGDfK peptide in various carrier materials, involving lipid-based systems, organic polymers, inorganic systems, and other nanoscale drug delivery systems. Additionally, it categorizes the functional aspects of its application, including targeted therapy, molecular imaging, and delivery of cytotoxic peptides, photosensitizer effects, and modification of biomaterials, showed in fig. 1. The paper systematically summarizes the current status and prospects of cRGDfK peptide in nanoscale drug delivery systems, providing important references and guidance for researchers. Moreover, by categorizing its application in different carriers and functional aspects, it helps researchers better understand the performance of this peptide in various environments, offering new insights and possibilities for its application in the field of drug delivery. Ultimately, this contributes to the advancement application of active targeted formulations, offering more choices and possibilities for the treatment of diseases such as cancer.

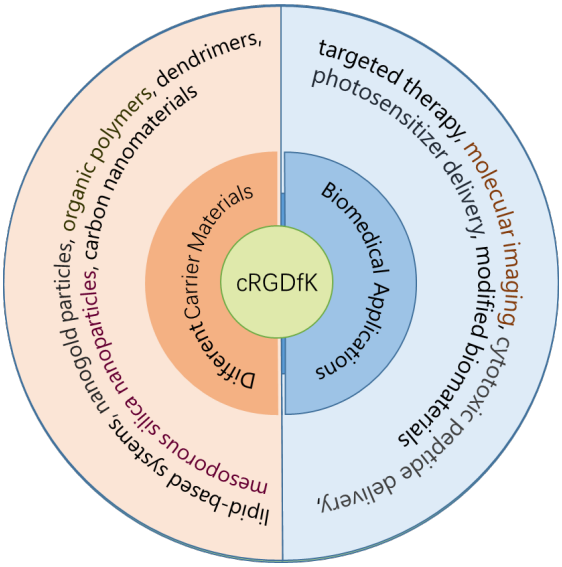

Fig. 1: Application of cyclopentapeptide cRGDfK in delivery systems

APPLICATION OF DRUG DELIVERY SYSTEMS IN DIFFERENT CARRIER MATERIALS:

The ligand cRGDfK has been incorporated into various nanomaterials, including lipid-based systems, nanogold particles, organic polymers, dendrimers, mesoporous silica NPs, and carbon nanomaterials, for targeted therapy.

Lipid-based systems modified with cRGDfK:

Currently, lipid-based systems mainly comprise liposomes, micelles, emulsions, and solid lipid NPs. Liposomes consist of phospholipid bilayers, exhibiting nanoscale particle sizes, a structure similar to biological membranes, and excellent biocompatibility. One of the biological functions of functionalized NPs is biomimetic cell membrane technology, making biomimetic nanosystems more suitable for drug delivery *in vivo*[21]. Doxil, the first Food and Drug Administration (FDA) approved nanodrug (approved in 1995 for the treatment of Kaposi's sarcoma and marketed in Europe as Caelyx® in 1997), encapsulates Doxorubicin (DOX) within liposomes[22]. Liposomes are relatively stable, with their aqueous phase capable of containing hydrophilic drugs and their phospholipid bilayers capable of localizing lipophilic drugs. Furthermore, their surface modification properties expand the application of liposomes to targeted and environmentally responsive drug delivery systems[23].

Sun *et al*.[24] developed a novel targeted liposomal system for the anticancer drug DOX by modifying the functional Disteroyl Phosphatidylethanolamine-Polyethylene Glycol (DSPE-PEG2000) with cyclic peptide cRGDfK. This liposomal system enhances drug accumulation and uptake in cancer tissues, directly killing cancer cells, offering potential for the treatment of invasive breast cancer.

Organic polymer systems modified with cRGDfK:

Polymer NPs commonly exist in the forms of nanocapsules (enclosed by a polymer membrane or shell) and nanospheres (solid matrix systems). Within these categories, NPs further include polymer vesicles, micelles, and dendrimers. Polymer vesicles, artificial vesicles with membranes made of amphiphilic block copolymers, resemble liposomes and often exhibit local responsiveness, superior stability, and drug-loading capacity, making them effective carriers for delivering therapeutic agents into the cytoplasm[25,26]. A significant paper published by Langer *et al*.[27] in 1994 demonstrated that amphiphilic block copolymers with PEG significantly enhance the controlled release and circulation half-life of polymer NPs. Due to their biocompatibility, biodegradability, and flexibility in chemistry and technology, widely used polymeric materials for controlling drug release include Poly (Lactic-co-Glycolic Acid) (PLGA), Polycaprolactone (PCL), PEG, and Polydimethylsiloxane (PDMS). Polymer micelles, typical responsive block copolymers, self-assemble into nanospheres with a hydrophilic core and hydrophobic shell, protecting water-soluble drug cargoes and improving circulation time. Dendrimers are hyperbranched polymers with a complex three-dimensional structure, allowing precise control over their mass, size, shape, and surface chemistry. The active functional groups on the external surface of dendrimers can conjugate with biomolecules or imaging agents, while drugs can be loaded internally[28].

Kulhari *et al*.[28] investigated a novel targeted drug delivery platform for ovarian cancer by synthesizing Gemcitabine (GEM) hydrochloride-loaded-PLGA NPs. As the integrin receptor αvβ3 is overexpressed in malignant ovarian cells, cRGDfK was chosen as the targeting ligand. The NPs were functionalized with cRGDfK to selectively deliver anticancer drugs to ovarian tumors, forming cRGDfK-GEM-PLGA NPs.

Li *et al*.[29] designed micelles (cRGDfK-PEG-PLA/PEG-PLA/DTX) loaded with Docetaxel (DTX) and characterized and evaluated them using HeLa cells. The study showed that targeted micelles containing cRGDfK were more effectively absorbed by HeLa cells compared to non-targeted micelles, significantly improving cytotoxicity. The cell inhibition rate of targeted micelles increased by 20 % after 24 h. These results suggest that targeted micelles may serve as a promising anticancer drug delivery system.

Kong *et al*.[30] prepared a novel multifunctional dendritic carrier by integrating a long hydrophobic C12 alkyl chain, PEG chain, and the cRGDfK ligand. This dendritic molecule tightly encapsulated the hydrophobic anticancer drug 10-Hydroxycamptothecin (10-HCPT) through simple complexation and selectively targeted

cancer cells overexpressing the αvβ3 integrin receptor through high affinity interactions. As the drug remains inactive before internalization, this carrier can reduce issues such as drug leakage in circulation and off-target effects on normal tissues.

Inorganic NP systems modified with cRGDfK:

Inorganic NPs are often synthesized from inorganic particles and biodegradable polycations. They include metals, metal oxides, carbon materials, NPs Superparamagnetic Iron Oxide Nps (SPION), and mesoporous silica NPs, among others, possessing characteristics such as uniform porosity, ease of functionalization, good biocompatibility, high surface area, large pore volume, and biodegradability[31]. Metal NPs typically consist of a metal core and a functional material shell, including magnetic NPs and Gold NPs (GNPs), which confer targeting, controllability, and imaging capabilities to certain delivery materials. However, the limited degradation of metal NPs *in vivo* and the safety concerns they raise restrict their clinical applications. Inorganic non-metal NPs are obtained by hybridizing inorganic materials with functional molecules, including carbon and silicon materials. The biocompatibility of inorganic non-metal NPs is superior to that of metal NPs, although the biodegradability of inorganic non-metal NPs still needs improvement.

Enferadi *et al*.[32] utilized cRGDfK-modified ultrasmall GNPs as radiosensitizers in mouse ALTS1C1 glioma cell lines, investigating the uptake, toxicity, and radiosensitivity of GNP-PEG-cRGDfKs in ALTS1C1 cells under proton, kilovolt, and megavolt photon irradiation. Results showed accumulation of GNPs in the cytoplasm without significant cytotoxicity at moderate concentrations. Therefore, ultrasmall GNP-PEG-cRGDfK could be considered as a radiosensitizer.

Graf *et al*.[33] demonstrated that PLGA-PEG NPs encapsulating cisplatin prodrug modified with cyclic pentapeptide cRGDfK could target the αvβ3 integrin on cancer cells. RGDfK-targeted Pt(IV)-encapsulated NPs exhibited stronger cytotoxicity than conventional doses of cisplatin *in vitro* models of prostate and breast cancer epithelial cells. Compared to cisplatin, RGDfK-targeted PLGA-PEG NPs were more effective and tolerable in a xenograft model of human breast cancer.

Two or more nanostructures:

NPs containing two or more nanostructures may simultaneously utilize organic and inorganic materials to achieve functionality, which may encompass a combination of organic and inorganic materials to achieve specific functionalities while mitigating adverse reactions. For instance, Lipid-Polymer hybrid NPs (LPNs) integrate both organic lipids and synthetic polymers, offering a versatile platform for drug delivery and biomedical applications. LPNs represent a sophisticated approach to drug delivery systems, leveraging the advantages of both lipid-based and polymer-based NPs. By combining lipids and polymers, LPNs can achieve desirable characteristics such as enhanced drug encapsulation efficiency, controlled drug release kinetics, improved stability, and biocompatibility.

The organic components, typically lipids such as phospholipids or triglycerides, provide LPNs with a biocompatible and biodegradable matrix that mimics biological membranes. This lipid layer can encapsulate hydrophobic drugs within its core, shielding them from degradation and facilitating their transport through cellular membranes. On the other hand, the inclusion of synthetic polymers, such as PEG or PLGA, enhances the stability and structural integrity of LPNs. These polymers can form a protective shell around the lipid core, preventing premature drug release and minimizing interactions with the biological environment, thus reducing the risk of immunogenicity and other adverse reactions. Moreover, the hybrid nature of LPNs allows for the incorporation of targeting ligands or functional moieties onto their surface, enabling targeted drug delivery to specific cells or tissues. This targeted delivery approach enhances therapeutic efficacy while minimizing off-target effects, thereby improving the overall safety profile of the NP system.

Agrawal *et al*.[34] investigated Folate (F)-modified Polymer-Lipid hybrid NPs (PLNs) encapsulating Paclitaxel (PtxR) modified with cyclic cRGDfK (PtxR-FPLNs) for glioma targeting. The prepared NPs were believed to target glioma cells rich in integrins. Experimental results indicated that the developed dual-targeting nanoscale formulation could effectively penetrate the blood-brain barrier and deliver more drugs to brain tumors, thereby achieving better therapeutic outcomes.

BIOMEDICAL APPLICATIONS OF cRGDFK

Targeted therapy

Systemic toxicity remains a significant concern associated with many conventional chemotherapy agents. To address this challenge, the cRGDfK peptide, which specifically targets the αvβ3 integrin receptor frequently overexpressed in tumor cells, offers a promising strategy for mitigating adverse effects in cancer treatment protocols. Through its targeted delivery mechanism, cRGDfK can be effectively utilized in conjunction with other therapeutic agents to enhance treatment efficacy while minimizing systemic toxicity. This approach holds promise for optimizing cancer therapy regimens by precisely targeting malignant cells while sparing healthy tissues from detrimental side effects.

Kulhari *et al*.[35] synthesized cRGDfK peptide-conjugated succinyl-PEG succinate ester NPs for targeted delivery of DTX to enhance its therapeutic potential. The peptide-conjugated DTX loaded nanospheres exhibited small particle size, narrow distribution, controlled drug release, and high physicochemical stability. In DU 145 human prostate cancer cells and Human Umbilical Vein Endothelial Cells (HUVEC), a comparative analysis of cytotoxicity, cellular uptake, apoptosis, and anti-angiogenesis highlighted the critical role of the cRGDfK peptide in enhancing the drug delivery performance of nanospheres. Deepak *et al*.[36] optimized Nanostructured Lipid Carriers (NLCs) containing gefitinib (GF-NLC) for the treatment of Hepatocellular Carcinoma (HCC). The NLCs were modified with cRGDfK pentapeptide to enhance targeting, binding to the αvβ3 integrin receptor overexpressed on HCC cells. cRGDfK@GF-NLC demonstrated significantly higher cytotoxicity, growth inhibition, and cellular internalization. Bio-distribution studies revealed enhanced accumulation at the tumor site without causing organ toxicity. These findings suggest that cRGDfK-GF-NLC is an efficient targeted drug carrier, specifically targeting HCC cells and improving the outcomes of liver cancer treatment.

Applications in molecular imaging:

Angiogenesis is a pivotal process in the progression and metastasis of solid tumors, marked by the upregulation of integrins on the surfaces of endothelial cells. The utilization of radiolabeled cRGDfK peptide, which selectively integrins involved in angiogenesis, holds significant potential for molecular imaging of tumors[37]. The cRGDfK peptide, coupled with radiolabeling, facilitates precise visualization and characterization of tumor neovascularization, offering insights into tumor growth, progression, and response to therapy. By exploiting the specificity of cRGDfK towards integrins implicated in angiogenesis, molecular imaging techniques such as Positron Emission Tomography (PET) or Single-Photon Emission Computed Tomography (SPECT) can accurately delineate tumor vasculature and assess the extent of angiogenic activity within the tumor microenvironment. Furthermore, the use of radiolabeled cRGDfK peptide for molecular imaging not only enables early detection and diagnosis of tumors but also assists in treatment planning and monitoring therapeutic responses. Real-time assessment of angiogenesis through molecular imaging helps clinicians make informed decisions about treatment strategies and evaluate the efficacy of anti-angiogenic therapies.

Lobeek *et al*.[38] validated the feasibility of 68Ga-RGDfK for PET/Computed Tomography (PET/CT) imaging in observing angiogenesis in patients with Oral Squamous Cell Carcinoma (OSCC). All patients exhibited tumor accumulation of 68Ga-RGDfK. The results demonstrated that 68Ga-RGDfK PET/CT imaging of αvβ3 integrin expression in OSCC patients is feasible, offering new possibilities for using angiogenesis as a marker of the tumor microenvironment in head and neck squamous cell carcinoma. Zhou *et al*.[39] developed a targeted imaging agent based on cyclodextrin for safe and effective cancer molecular Magnetic Resonance Imaging (MRI). Using Polyhedral Oligomeric Silsesquioxane (POSS) NPs as carrier molecules, they incorporated a gadolinium-based diamond alkane-modified Gd (III) contrast agent, cRGDfK peptide, and the fluorescent probe Cy5 as ligands. The targeted NPs cRGDfK-POSS-βCD-(Tetraazacyclododecane Tetraacetic Acid (DOTA)-Gd) specifically bound to αvβ3 integrins on malignant 4T1 breast tumors and exhibited a noticeable fluorescent signal in tumor tissues. Histological analysis confirmed its specificity and effectiveness against αvβ3 integrins. This targeted imaging agent shows strong potential for specific cancer molecular MRI and fluorescence imaging. Pathak *et al*.[40] investigated the impact of

SPION encapsulated in poly(butyl cyanoacrylate) Microbubbles (MB) with a polymeric shell on angiogenesis in 4T1 mouse breast cancer. SPION-MB, functionalized with biotin-linked streptavidin and RGDfK, confirmed the specificity of RGD-SPION-MB binding to the αvβ3 integrin receptor. This study suggests that RGD-SPION-MB can serve as a molecular Magnetic Resonance/ Ultrasound (MR/US) imaging agent, capable of assessing αvβ3 integrin expression in malignant tumor neovasculature. Wu *et al*.[41] proposed a Near-Infrared II (NIR-II) fluorescence imaging-guided activatable molecular phototherapy platform (IR-FEP-RGD-S-S-S-Fc) for active tumor targeting, imaging, and Sulfur dioxide (SO_2)-enhanced combination therapy. The cRGDfK peptide segment successfully targeted tumors, facilitating NP internalization. The high concentration of Glutathione (GSH) in the tumor microenvironment triggered the separation of the fluorescent molecule from ferrocene *via* GSH-sensitive trisulfide bonds, enabling NIR-II fluorescence imaging.

Delivery of cytotoxic peptides:

The cyclic pentapeptide cRGDfK exhibits a remarkable capability to selectively transport and release functional peptides into various cell lines *in vitro*, inducing specific cellular responses such as mitochondrial depolarization, apoptosis, and cell death. This unique property of cRGDfK holds significant implications for targeted drug delivery and therapeutic interventions. By harnessing the specificity of cRGDfK for integrin receptors overexpressed on certain cell types, functional peptides can be precisely delivered to their intracellular targets, eliciting specific biological effects. The ability of cRGDfK to induce mitochondrial depolarization, apoptosis, and cell death underscores its potential as a versatile tool for modulating cellular processes and combating diseases characterized by dysregulated cell survival and proliferation.

Dufort *et al*.[42] designed a molecular raft, RAFT-cRGDfK, covalently linked to the pro-apoptotic peptide KLA through an unstable bridge, forming RAFT-RGD-KLA. To increase the concentration of active peptides reaching target cells, they encapsulated and protected RAFT-RGD-KLA in stealth liposomes or nanovesicles. Upon collapse of the envelope within the tumor, the targeted delivery system released near tumor cell surfaces, where RAFT-RGD effectively mediated the internalization and delivery of KLA. This study provides evidence that RAFT-RGD can deliver peptides intracellularly. The anti-tumor peptides can induce cell death in tumor cells in an RGD-dependent manner, thereby reducing non-specific cytotoxic effects that may arise from the use of cationic cytotoxic peptides.

Combination with photosensitizers:

By subjecting tumor sites to radiation, there is a notable upregulation of specific integrins. Subsequently, utilizing the cRGDfK peptide as a ligand offers a strategic approach for precisely targeting therapeutic drugs to the irradiated tumor site. The increased expression of integrins in response to radiation creates an opportunity for targeted therapeutic intervention. By specifically targeting upregulated integrins, cRGDfK serves as an effective ligand to guide and deliver therapeutic drugs selectively to the irradiated tumor microenvironment. This targeted drug delivery system holds promise for enhancing the effectiveness of radiation therapy while minimizing off-target effects on healthy tissues. The combination of irradiation-induced integrin upregulation and the use of cRGDfK as a ligand offers a strategic approach for localized drug delivery to tumor sites following radiation therapy. This targeted delivery system has the potential to optimize therapeutic outcomes by concentrating the effects precisely within the irradiated tumor microenvironment. Kadem *et al*.[43] achieved rapid, reversible light-switchable cell adhesion by incorporating cRGDfK-azo-benzene into the surface of PEG. Azo-benzene can be biologically functionalized with active molecules, such as the cRGDfK peptide, which binds to members of the integrin cell adhesion receptor family. This biologically functionalized azo-benzene surface enabled rapid, reversible light-switchable cell adhesion. Chen *et al*.[44] developed NPs composed of a photosensitizer in the aqueous core, a hydrogen peroxide enzyme, a polymer shell containing a black hole quencher, and functionalized with the tumor-targeting ligand cRGDfK. Once the NPs are selectively absorbed by tumor cells rich in αvβ3 integrins, hydrogen peroxide penetrates the shell and is catalyzed by the enzyme to generate oxygen (O_2). This process leads to shell rupture and the release of the photosensitizer. Under irradiation

conditions, the released photosensitizer, in the presence of O_2 induces the formation of cytotoxic singlet O_2, thereby killing cancer cells. Silk Fibroin (SF) protein is a natural biopolymer with excellent biocompatibility and biodegradability. Mao *et al*.[45] conjugated the cyclic pentapeptide cRGDfk and the photosensitizer Chlorin e6 (Ce6) to SF peptides, preparing 5-Fluorouracil (5-FU)-loaded SF-based NPs. They studied the Photodynamic Therapy (PDT) potential and active targeting properties against MGC-803 cells overexpressing αvβ3 integrin receptors. The results demonstrated that the SF-based multifunctional NPs, combined with PDT, induced high levels of Reactive Oxygen Species (ROS) and led to cell death in gastric cancer MGC-803 cells.

Promoting cell adhesion to biomaterials:

cRGDfK, with its specific targeting of integrin αvβ3-prominently expressed in osteoblasts and crucial for bone tissue development-presents a promising approach for enhancing the integration of implants with bone tissue. By immobilizing the RGDfK sequence on the surface of titanium or titanium alloy implants, osteoblast adhesion to the implant surface can be enhanced. This facilitates implant-bone integration and increases the success rate of the implants. The interaction between cRGDfK and integrin αvβ3 acts as a molecular bridge, promoting the attachment of osteoblasts to the implant surface. This enhanced adhesion promotes osteoblast proliferation and differentiation, ultimately leading to improved osseointegration, where the implant becomes securely anchored within the surrounding bone tissue. The ability to enhance implant-bone integration is particularly significant in orthopedic and dental applications, where the success of implants depends heavily on their stability and durability within the host tissue. By leveraging the specific affinity of cRGDfK for integrin αvβ3, implant surfaces can be modified to enhance their biocompatibility and promote favorable interactions with surrounding bone, ultimately improving the long-term performance and longevity of implants. Seemann *et al*.[46] covalently bound the biologically active cyclic peptide cDfK to titanium used in biomedicine through PEG linkers of varying lengths. This resulted in chemically modified titanium plates with enhanced bone-inducing properties, showing potential for applications in dental and orthopedic implants[47].

CONSTRUCTION OF cRGDFK-MODIFIED NP

cRGDfK-modified NP drug delivery systems, whether utilizing cRGDfK as a sole targeting ligand or co-modifying NPs with other ligands, are designed to enhance the precision of drug delivery to specific lesion sites, particularly tumors. These systems strategically manipulate the distribution and pharmacokinetic properties of drugs *in vivo*, aiming to optimize therapeutic outcomes. When combined with other ligands, cRGDfK contributes to a multifaceted targeting strategy, further refining the NPs ability to navigate the complex *in vivo* environment. The cooperative action of these ligands enhances the system's adaptability and responsiveness to the unique characteristics of the target tissue, maximizing the potential for effective drug delivery (Table 1).

TABLE 1: COMPOSITION AND FUNCTION OF NPs MODIFIED WITH CRGDFK AS A LIGAND (PARTIAL)

Carrier type	Loaded drug	Function or target cells	Other ligands	Reference
SPIONs	Streptomycin and DOX	Magnetic/laser-assisted cascade drug delivery against human non-small cell lung cancer A549 cells	Biotin	[48]
Peptide carrier containing 1-3,4-dihydroxyphenylalanine (DOPA)	Titanium coating	Titanium endothelialization and scaffold coating	Peptide SIKVAV	[49]
Liposomes	DOX	Inhibition of glioma growth	Peptide-22	[50]
Poly (Lactic Acid)-PEG (PLA-PEG) micelles	Hydrophobic photosensitizer and quencher	Hydrophobic photosensitizer and quencher	Photosensitizer	[51]

Short-chain PEG	Conjugated polymer poly(9,9-dihexylfluorene-thiophene-2,1,3-benzothiadiazole) (PFBD)	Novel bioimaging probe targeting membrane integrin αvβ3 receptor on HT-29 adenocarcinoma cells	None	[52]
	111Indium (111In)	Micro-SPECT imaging, tracer	DOTA	[53]
	Peptide TTR1	Functional fibers targeting cell adhesion and spreading	None	[54]
Poly(l-lysine) dendrimers with cubic silquioxane cores+PEG	DOX and siRNA	Targeting glioblastoma U87 cells	None	[55]
PCL-Poly(Phosphoethanolamine (PEEP)+maleimide-PEG-PCL micelles	PtxR	Effective chemotherapy for Glioblastoma Multiforme (GBM)	Transferrin (Tf)	[56]
porous Silicon NPs (pSiNPs)	Camptothecin	Enhanced cytotoxicity toward a C4-2B prostate carcinoma cell line	Antifouling poly-N-(2-hydroxypropyl) acrylamide (polyHPAm)	[57]

LIMITATIONS

cRGDfK, a relatively simple and structurally stable ligand that can effectively target the αvβ3 integrin receptor, thereby enhancing the tolerance of cytotoxic chemotherapy drugs in the body and increasing drug accumulation in tumors, making it an ideal targeting ligand. Through multi-ligand-modified drug-loaded NPs, the specificity of NPs for tumor cells can be further improved, leading to enhanced therapeutic effects. These studies demonstrate the potential of cRGDfK-modified nanomaterials in cancer therapy and provide a basis for designing personalized treatment regimens. However, it is important to note that excessive modification with functional groups may lead to increased side effects and complicate industrial manufacturing processes. Therefore, careful consideration is required when selecting personalized treatment options.

Clinically relevant imaging agents can be synthesized by conjugating radioisotopes such as ^{99}mTc, ^{111}In, $_{68}$Ga, and the therapeutic radioisotope 90Y with cRGDfK to form copolymers of new monomer derivatives containing these isotopes. These copolymers can serve as imaging agents or as multifunctional platforms combining imaging and drug delivery capabilities[47-57]. While targeted radiotherapy offers certain advantages in tumor treatment, a significant challenge remains in avoiding non-specific accumulation of therapeutic radioisotopes in non-target organs such as the liver, spleen, and kidneys. The increase in particle size of cRGDfK-modified NPs may impact their intracellular trafficking pathways and internalization efficiency. Indeed, NPs of varying sizes and physicochemical properties have been demonstrated to exhibit different modes of cellular uptake and internalization efficiency[58-60]. Thus, the selection of targeting concentration for NPs must be carefully considered. Some studies suggest that larger-sized NPs may encounter more barriers during internalization, such as reduced contact area with the cell membrane, leading to lower internalization efficiency. Conversely, smaller-sized NPs may be more readily taken up by cells, as they more closely resemble the handling of particulate matter in the cell's natural environment. Therefore, when designing nanodelivery systems, the selection of targeting concentration must be carefully considered. This involves evaluating factors such as NP size, surface modifications, drug loading capacity, and the physiological characteristics and internalization pathways of target cells to achieve optimal therapeutic effects. This comprehensive analysis is crucial for ensuring the effectiveness and safety of nanodelivery systems, and it provides essential guidance for subsequent *in vivo* and clinical studies.

CONCLUSION

This review provides a comprehensive discussion on the application of cRGDfK-modified nanomaterials in targeted therapy. These materials include liposomes, GNPs, organic polymers, dendrimers, mesoporous silica NPs, and carbon

nanomaterials. This modification allows drugs to be delivered specifically to tumor cells, enhancing therapeutic efficacy while reducing side effects. cRGDfK, as a targeted drug delivery system for cancer, holds promising prospects for future applications. Currently in advanced clinical trials, it shows significant potential in the field of cancer treatment. Despite this progress, active targeting formulations, including those using cRGDfK, have yet to successfully reach the market.

Future applications of cRGDfK are expected to expand across various cancer types. It may be integrated into combination therapies with chemotherapy, immunotherapy, or radiation to synergistically enhance treatment effectiveness. As personalized medicine advances, tailored treatment plans based on patient-specific factors such as genotype, phenotype, and tumor characteristics could optimize the use of cRGDfK, further improving treatment outcomes. Additionally, ongoing scientific advancements may lead to the development and market introduction of more cRGDfK-like drugs, offering new therapeutic options for cancer patients.

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