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REVIEW

# Applications of Biomolecular Nanostructures for Anti-Angiogenic Theranostics

Kevin Kent Vincent Canlas, Hansoo Park

School of Integrative Engineering, Chung-Ang University, Seoul, 06974, Korea

Correspondence: Hansoo Park, Email heyshoo@cau.ac.kr



**Abstract:** Angiogenesis is a physiological process of forming new blood vessels that has pathological importance in seemingly unrelated illnesses like cancer, diabetes, and various inflammatory diseases. Treatment targeting angiogenesis has shown promise for these types of diseases, but current anti-angiogenic agents have critical limitations in delivery and side-effects. This necessitates exploration of alternative approaches like biomolecule-based drugs. Proteins, lipids, and oligonucleotides have recently become popular in biomedicine, specifically as biocompatible components of therapeutic drugs. Their excellent bioavailability and potential bioactive and immunogenic properties make them prime candidates for drug discovery or drug delivery systems. Lipid-based liposomes have become standard vehicles for targeted nanoparticle (NP) delivery, while protein and nucleotide NPs show promise for environment-sensitive delivery as smart NPs. Their therapeutic applications have initially been hampered by short circulation times and difficulty of fabrication but recent developments in nanofabrication and NP engineering have found ways to circumvent these disadvantages, vastly improving the practicality of biomolecular NPs. In this review, we are going to briefly discuss how biomolecule-based NPs have improved anti-angiogenesis-based therapy.

**Keywords:** anti-angiogenesis, peptides, lipids, oligonucleotides

## Angiogenesis and Its Pathogenesis

Blood vessel growth or angiogenesis is a finely tuned process of recruiting endothelial tissue in areas where blood flow is needed. This is one of the most important processes in embryonic development, which continues throughout the human lifespan as an essential part of homeostasis.[1] In adults, angiogenesis is activated to either replenish old tissues or to repair injuries.[2] However, this intricate biological mechanism can also lead to a spectrum of diseases when dysregulated. The tightly controlled balance between pro-angiogenic and anti-angiogenic factors can be disrupted, resulting in the excessive growth of blood vessels, which in turn contributes to the development and progression of numerous pathological conditions. An abnormal rate of blood vessel formation is triggered which can lead to or aggravate disease symptoms including diabetic retinopathy, psoriasis and cancer.[3] In cancer, blood vessels are purposefully recruited by tumor cells to increase nutrient absorption[4] as well as enable the cells to spread and metastasize[5] as shown in Figure 1.

Inflammation is a fundamental response of the immune system to harmful stimuli.[6] While it plays a critical role in defending the body against infections and injuries, its dysregulation can lead to a spectrum of inflammatory diseases.[7] Emerging evidence suggests that angiogenesis is intricately involved in the pathogenesis and progression of many inflammatory disorders. Inflammation involves the recruitment of immune cells, release of pro-inflammatory cytokines, and activation of various signaling pathways.[8] In response to these inflammatory cues, angiogenesis can be initiated to provide the growing tissue with oxygen and nutrients, facilitating immune cell migration and tissue repair. This physiological interaction between inflammation and angiogenesis serves to restore tissue homeostasis after injury or infection. However, when inflammation becomes chronic or dysregulated, the balance between pro- and anti-inflammatory signals can be disrupted.[9] This sustained inflammatory state can trigger excessive angiogenesis, contributing to the development and perpetuation of various inflammatory diseases as shown in Figure 2.

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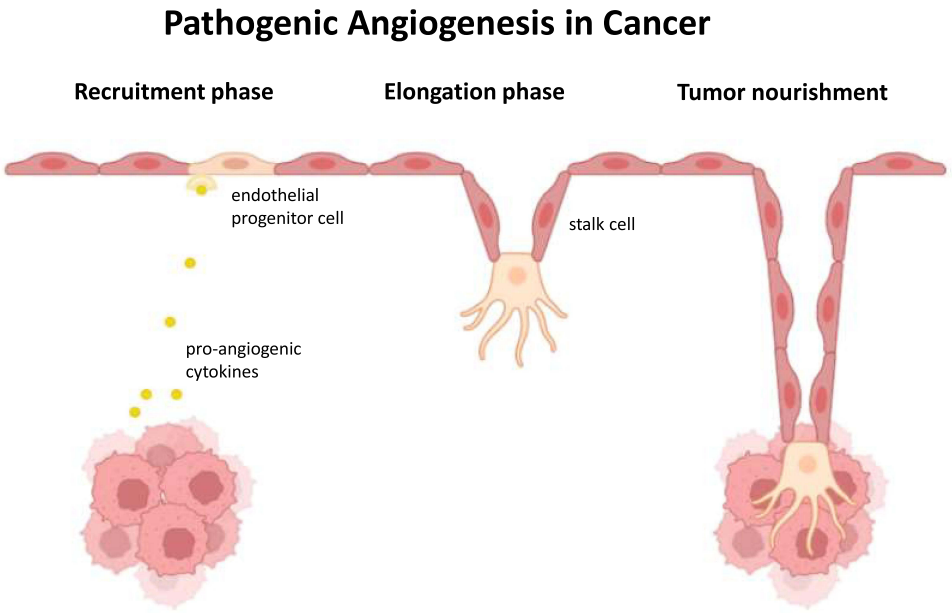


**Figure 1** Stages in progression of blood vessel recruitment in cancer. Created with Biorender.com.

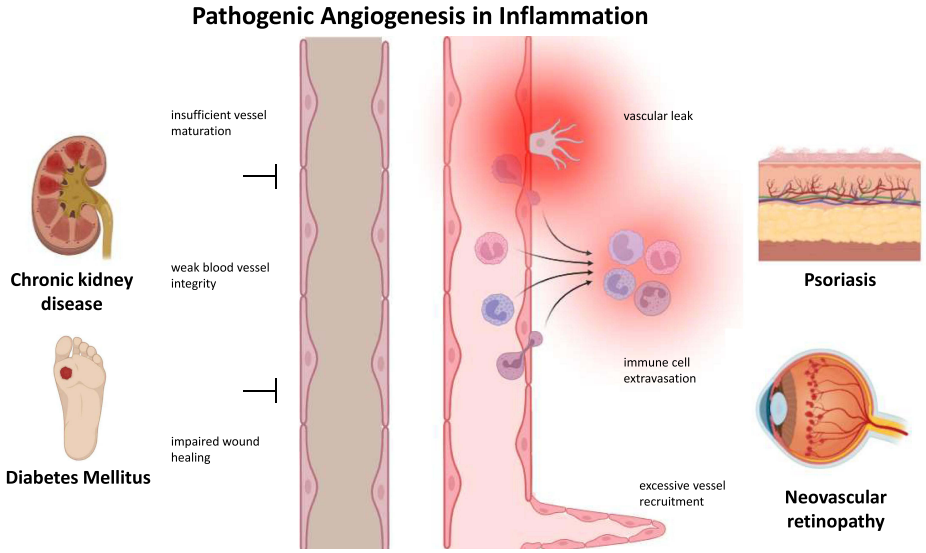


**Figure 2** Examples of inflammation in pathogenic angiogenesis-related diseases. Created with Biorender.com.

Another disease that is affected by angiogenesis is diabetes. Chronic hyperglycemia, a hallmark of diabetes, leads to endothelial dysfunction and impaired angiogenic responses.[10] This dysregulation of angiogenesis contributes significantly to the pathogenesis of diabetic complications such as diabetic retinopathy, nephropathy, and impaired wound healing. This tends to be different depending on the affected organ, however. For instance, diabetes triggers dilation of retinal blood vessels that over time leads to inflammation and abnormal vessel recruitment.[11] Aberrant capillaries can form a mass that blocks the retina,[12] and they tend to be more fragile than normal blood vessels such that they can rupture and cause a hemorrhage. Other parts of the body, on the other hand, experience inhibited angiogenesis, leading to impaired wound healing.[13] Psoriasis and several other inflammatory skin diseases have also been found to be exacerbated by aberrant angiogenesis.[9] Inflammatory cytokines trigger increased expression of angiogenic factors which lead to structural changes in the dermal vasculature and making them permeable.[14] This variety of angiogenesis-related diseases has spawned interest in researching treatment strategies that could hopefully be applicable to these types of diseases.

## Strategies for Angiogenesis Regulation and Treatment

Angiogenesis-related diseases have fundamental differences from each other which would seem to make them unrelated. Diabetic retinopathy is centered around chronic inflammation which results in abnormal blood vessel recruitment, whereas cancer is actively inducing angiogenesis to sustain its growth and establish the foundation for invasion and spreading. Pathways related to angiogenesis are interconnected, however, which makes anti-angiogenesis highly effective for treatment. Therapeutic and diagnostic (theranostic) approaches on dealing with these diseases can be divided into four groups: receptor blocking, cytokine interception, direct blood vessel apoptosis, and pinpointed imaging and diagnosis (Figure 3).

### Receptor Blocking

The discovery of angiogenesis's pivotal role in tumor growth and metastasis opened doors to innovative therapeutic approaches. Traditional cancer treatments like chemotherapy and radiation therapy often have limitations due to their non-specific nature and potential for severe side effects.[2] Targeting angiogenesis receptors offers a more focused strategy to inhibit tumor growth by directly disrupting the blood supply that nourishes the tumor cells.[1]

Researchers have developed a range of drugs that target angiogenesis receptors, such as VEGF receptors (VEGFRs) and FGF receptors (FGFRs).[15] These inhibitors disrupt the angiogenic signaling cascades, preventing endothelial cell proliferation and migration, thereby inhibiting new blood vessel formation.[16] Some well-known angiogenesis receptor inhibitors include bevacizumab, a monoclonal antibody targeting VEGF, and lenvatinib, a multi-kinase inhibitor with anti-angiogenic effects through its action on various receptors, including FGFRs.[17] The success of angiogenesis receptor inhibitors has been most evident in the field of oncology. Bevacizumab, for instance, has been approved for the treatment of various cancers, including colorectal, lung, and kidney cancer.[18] These inhibitors can complement traditional treatments or serve as part of combination therapies, enhancing overall treatment efficacy. Moreover, angiogenesis receptor inhibitors have the potential to reduce tumor size, alleviate symptoms, and extend patient survival.[3]

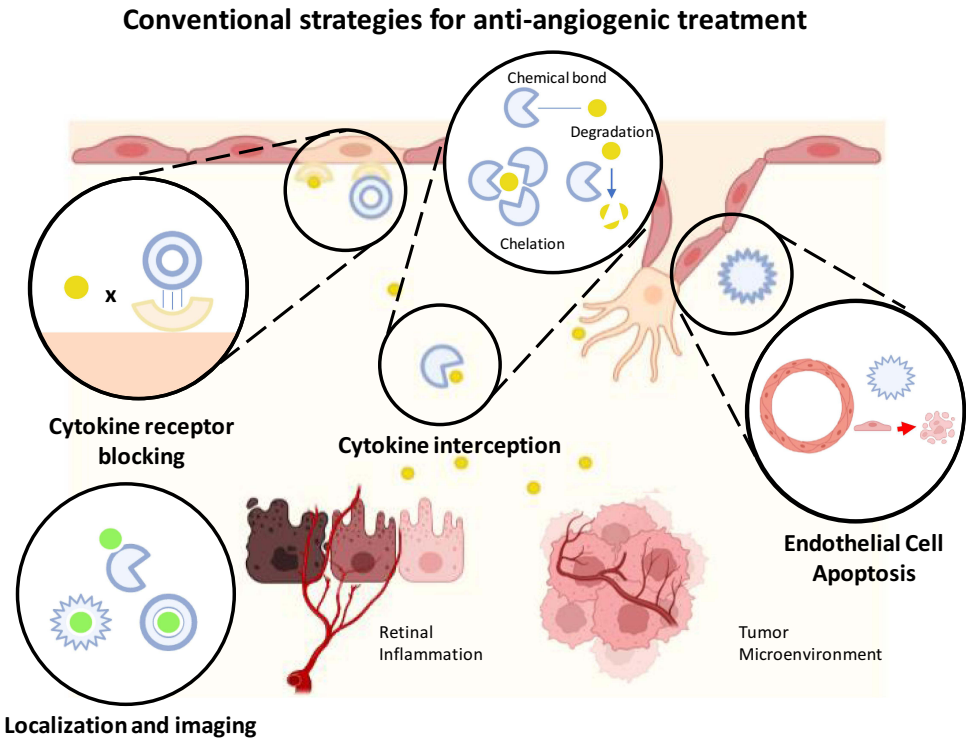


**Figure 3** Treatment strategies for angiogenesis-related diseases. Created with Biorender.com.

## Cytokine Interception

Cytokines are small proteins that serve as signaling molecules, orchestrating various cellular processes including inflammation, immune responses, and angiogenesis.[19] In the context of angiogenesis, cytokines play a critical role in promoting or inhibiting the formation of new blood vessels. Pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) stimulate the growth and migration of endothelial cells, which are the building blocks of blood vessels.[20] Conversely, anti-angiogenic cytokines like endostatin and angiostatin counteract these pro-angiogenic signals, maintaining vascular homeostasis. The dysregulation of cytokine signaling contributes to the pathological angiogenesis observed in many diseases.[21] Targeting cytokines involved in angiogenic pathways offers a promising approach to disrupt these processes at a molecular level. By neutralizing pro-angiogenic cytokines or enhancing the activity of anti-angiogenic cytokines, researchers aim to restore the balance between angiogenesis and vessel regression, thereby inhibiting the growth and spread of diseases like cancer.

Several strategies have been explored for cytokine targeting in anti-angiogenic therapy. Monoclonal antibodies have been designed to specifically bind to pro-angiogenic cytokines like VEGF, preventing them from binding to their receptors and initiating angiogenic signaling.[22] Additionally, small molecule inhibitors have been developed to interfere with the downstream signaling pathways activated by these cytokines. Gene therapy approaches involving the delivery of genes encoding anti-angiogenic cytokines have also shown potential in preclinical studies. Cytokine-targeted anti-angiogenic therapies have gained momentum in the clinical setting. Bevacizumab, a monoclonal antibody targeting VEGF, has been approved for the treatment of various cancers.[23] However, challenges such as resistance to therapy, unpredictable side effects, and limited effectiveness in certain patients still need to be addressed. Moreover, the intricate interplay between cytokines and other signaling molecules adds complexity to the design of effective therapies.

## Blood Vessel Apoptosis

Induced blood vessel apoptosis is used in cancer therapy to prevent tumors from receiving nutrients that would sustain its growth. In retinopathy, the abnormal blood vessels that develop in response to the underlying disease are a major contributor to vision problems.[7] Current treatment strategies include laser therapy and drug injections, which aim to slow the progression of the disease.[24] Laser therapy for retinopathy, known as photocoagulation, is a procedure that utilizes high-intensity laser beams to selectively target and coagulate abnormal blood vessels in the retina. There are two primary types of laser therapy commonly used in the management of retinopathy: focal laser photocoagulation and panretinal photocoagulation (PRP).[11] In focal laser therapy, precise laser spots are applied to seal leaking blood vessels or destroy abnormal ones. This approach is frequently employed for treating diabetic macular edema, preventing further fluid leakage and swelling in the macula. Also known as scatter laser therapy, PRP involves applying numerous laser spots across the peripheral retina. It is used in the treatment of proliferative diabetic retinopathy to reduce the abnormal blood vessels' oxygen demand, effectively shrinking them. The goal of laser therapy is to slow or halt the progression of retinopathy, which effectively means multiple treatments that become progressively less effective over time. Also, not all patients may be candidates for laser therapy, and some individuals may experience temporary or permanent side effects such as reduced night vision or visual field defects.

## Imaging and Diagnosis

Angiogenesis holds significant diagnostic potential as it is intricately linked to various diseases, ranging from cancer to cardiovascular disorders.[25] Tumors, for instance, require new blood vessels to sustain their growth and metastasis,[26] while certain ocular diseases involve abnormal angiogenesis that contributes to vision impairment.[7] By detecting and quantifying angiogenesis, clinicians can gain valuable insights into disease progression, response to therapy, and overall patient prognosis.[2] Advanced imaging modalities such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET), and ultrasound can provide detailed anatomical and functional information about blood vessel formation. Contrast agents can be used to visualize angiogenic blood vessels. Angiogenesis-related biomarkers, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and various

angiogenic receptors, are assessed in blood or tissue samples. High levels of these biomarkers can indicate increased angiogenesis. Biopsies of tissues, particularly in cancer cases, can also reveal angiogenic characteristics through histological examination. Abnormal vessel patterns, high microvessel density, or specific molecular markers can signal pathological angiogenesis.

## Current versus Emerging Anti-Angiogenic Drugs

Over the past few decades, there has been remarkable progress in the development of anti-angiogenic therapies, and current anti-angiogenesis therapies primarily focus on targeting key signaling pathways involved in angiogenesis. These have become integral components of treatment regimens, demonstrating efficacy in inhibiting tumor angiogenesis as well as suppressing abnormal blood vessel growth in the retina of patients with ocular retinopathy. Despite the success of current anti-angiogenic therapies, several challenges persist. These include the development of resistance, off-target effects, dosing complexities, and the high cost of treatment. To address these limitations and further optimize anti-angiogenic therapy, researchers are exploring emerging strategies that leverage advances in the emerging field of nanotechnology. These novel approaches aim to overcome resistance mechanisms, and enhance treatment efficacy while minimizing off-target effects.

### Standard Anti-Angiogenic Compound Drugs

The current standard for anti-angiogenesis therapies involves chemical drugs that directly target the growth factors involved in the signaling pathway for endothelial cell proliferation and recruitment. Clinical studies have shown that anti-angiogenic therapies can lead to significant improvements in progression-free survival and overall survival in patients with certain types of cancer, including colorectal, lung, renal, and liver cancers. By disrupting the tumor's blood supply, these drugs effectively deprive cancer cells of oxygen and nutrients, thereby inhibiting tumor growth, metastasis, and angiogenic switching. In addition to their role in oncology, anti-angiogenic therapies have also proven effective in the treatment of ocular disorders, particularly neovascular age-related macular degeneration (AMD) and diabetic retinopathy. Angiogenic inhibitors administered via intravitreal injection have been shown to suppress abnormal blood vessel growth in the retina, leading to improvements in visual acuity and preventing vision loss in many patients. Moreover, anti-angiogenic therapies have demonstrated efficacy beyond cancer and ocular diseases, with potential applications in conditions such as psoriasis, rheumatoid arthritis, and endometriosis.

One of the more famous examples of these compounds include VEGF inhibitors like sorafenib,[27] sunitinib,[28] and pazopanib[29] which are commonly used to target tumor angiogenesis, either as monotherapy or in combination with other anticancer agents. There are also angiopoietin inhibitors such as trebananib[30] and nesvacumab[31] that target the angiopoietin-Tie pathway, which regulates blood vessel maturation and stability.

One significant challenge with current anti-angiogenesis therapies is achieving target specificity. Targeted growth factors like VEGF and angiopoietin are also involved in physiological angiogenesis, which would make direct inhibition negatively impact normal tissue. Non-specific inhibition of angiogenesis can lead to adverse effects, including impaired wound healing, hypertension, proteinuria, and increased risk of thromboembolic events. Another major limitation of current anti-angiogenesis therapies is the development of resistance over time. Tumors can adapt to prolonged treatment by activating alternative pro-angiogenic pathways or acquiring genetic mutations that render them less sensitive to anti-angiogenic agents. This phenomenon not only compromises the efficacy of therapy but also poses challenges for long-term disease management. Moreover, resistance mechanisms may vary among patients, making it difficult to predict and overcome resistance in clinical practice. The dosing and administration of anti-angiogenesis drugs present additional challenges. Many of these agents are administered intravenously, requiring frequent hospital visits and increasing the burden on patients. Achieving optimal dosing is crucial for maximizing therapeutic efficacy while minimizing toxicity, but determining the appropriate dose can be complicated by factors such as inter-patient variability in drug metabolism, renal function, and the presence of co-morbidities. Additionally, the cost of anti-angiogenesis therapies can be prohibitive, limiting access for some patients and healthcare systems.

## Emerging Biomolecule-Based Drugs

The emerging field of nanotechnology has opened up new avenues in medical research and treatment, including drug synthesis. In the context of angiogenesis, nanoparticles have garnered significant attention as potential tools for innovative treatment strategies.[2] They hold promise for modulating angiogenesis through targeted delivery of therapeutic agents and direct interactions with signaling pathways, offering novel ways to manage angiogenesis-related disorders. Nanoparticle synthesis was certainly not a new concept, as nanometer-sized materials have been used for a considerably long time, represented by the infamous carbon nanotubes and other carbon-based NPs.[32] Since then, NPs have been created from various materials including inorganic metal, ceramic, carbon-based, and polymer-based NPs. However, current improvements are increasingly geared towards biomedical use, and have resulted in the emergence of the new class of biomolecule-based NPs (Figure 4). Biomolecules are major groups of compounds that make up living cells and are essential for cellular function. Lipids, nucleotides, and proteins are examples of biomolecules that have been developed for NP production. Liposomes can successfully compartmentalize and solubilize bulky drug compounds. Liposomes can successfully compartmentalize and solubilize bulky drug compounds.[33] DNA nanocages are able to diffuse through cell membranes easily. Peptide aptamers can bind certain surface ligands on specific tissues for targeted drug delivery. There has been a growing interest in the development of these nanoparticles due to their inherent biological properties.

Lipids are mainly involved in the maintenance of the cell membrane, where a different type of lipid known as phospholipids forms a semi-permeable lipid bilayer that regulates material transfer to and from the cell.[34] This mechanism has since been used to introduce molecules into the cell, using liposomes which are either artificially synthesized or naturally obtained and modified. One way of synthesizing liposomes is the top-down approach which is done via breakdown of existing cultures of cells and extruding the cell membranes over nanopore-sized filters.[35] The bottom-up approach, on the other hand, involves phospholipids along with the desired load can be assembled into vesicles ready for delivery.[36] Besides mechanical techniques, chemical methods can also be done to create liposomes such as using detergents that aid in micelle formation like detergent removal method.[37]

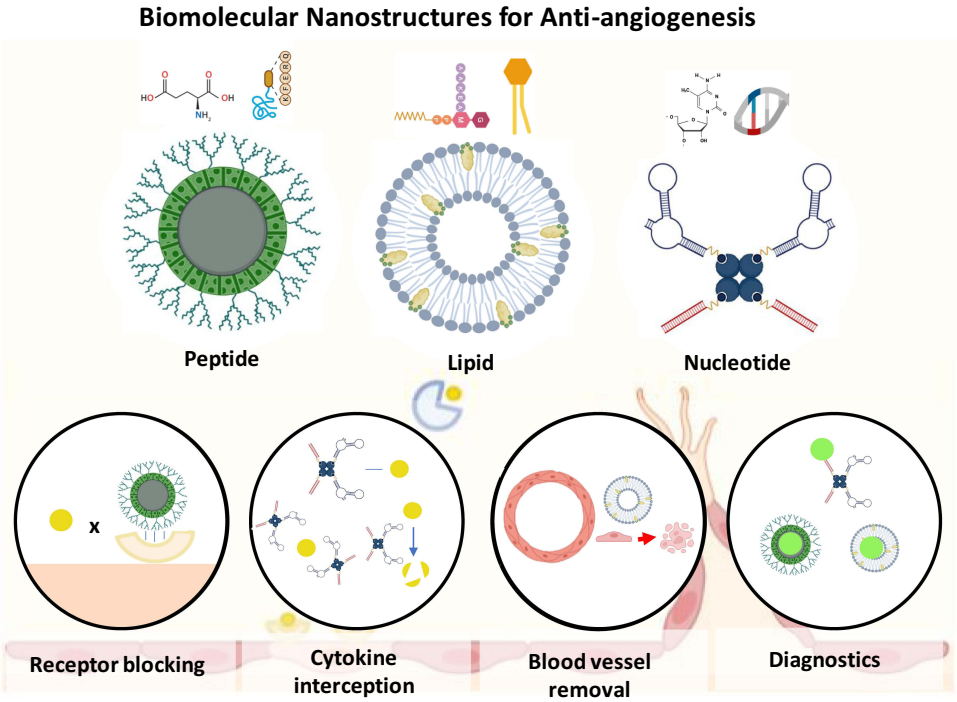


**Figure 4** Anti-angiogenic nanostructures developed using biomolecules. Created with Biorender.com.

Proteins are one of the most complex naturally occurring macromolecules, with almost infinite variations in structure and a wide range of biological functions. These molecules are made up of a string of amino acids (AA), which have carboxyl and amine backbones and the variable R-group that defines this class of biomolecules.[38] Proteins do most of the biological functions of the cell, from maintaining cell structure and overall homeostasis to inducing a variety of cellular changes like cell division or apoptosis.[39] Proteins are naturally produced by living systems, and modifications can be introduced in the sequence itself to change both the structure and biological properties of the protein itself through recombinant DNA.[40] Individual amino acid building blocks can also be synthesized from scratch depending on their R functional group, after which native chemical ligation (NCL) can be done to produce the final sequence of the protein.[41] Another way of producing peptide NPs is through the dendrimer chain growth, which starts from a core peptide either preformed and dissolved in solution or anchored to a catalyzing scaffold platform and sequential injection of the desired amino acid eventually produces branches that surround the core and produce its spherical dendritic structure.[42]

Nucleic acids in biological systems come in two very well-known forms: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).[43] There are 4 nitrogenous bases and that interact in pairs such that when two strands have the exact complementary sequence of each other they can form a stable double-stranded structure through annealing.[44] The double-helix structure makes each strand relatively rigid and stable, while purine-pyrimidine base complementarity opens up different variations in shape and chemical composition.[45] This provides an opportunity to create a nanoparticle that can have various conformations, such as the case of DNA nanocages.[46] Oligonucleotides are cycled to produce specific structures such as cyclic single strands that would join interconnecting "tiles" of nucleic acids which would eventually grow into the NP.

## Advantages of Biomolecular Nanoparticles

The key feature of biomolecules is their natural biocompatibility and bioactivity.[47] Many biomolecules are derived from natural sources or synthesized to mimic biological structures, making them well-tolerated by the body and suitable for therapeutic applications. Biomolecules, such as antibodies, enzymes, and nucleic acids, can be highly specific in targeting their intended molecular targets. This specificity allows for precise therapeutic interventions with minimal off-target effects, reducing the risk of adverse reactions and improving treatment outcomes. Biomolecules exhibit a wide range of functions and can be engineered or modified to perform specific tasks in therapy. Biomolecules can be made responsive to different environmental stimuli such as pH, enzyme, redox potential, temperature and light. This facilitates oral delivery of hormones or peptides and reduce degradation before the site of action.[48] In combination with nanoparticle technology, they were initially applied therapeutically in the design of cancer therapies,[49] but a wider range of applications have been and continue to be explored both in the lab[50] and clinic.[51]

Biomolecules can be synthesized as nanoparticles (NP) to overcome internal and external barriers in ensuring effective drug delivery by conjugating nanoparticles with helper components. In drug delivery applications, nanoparticle systems possess high biodegradability,[52] nontoxicity, and prolonged circulation.[53] Nanoparticles for drug delivery have revolutionized the field of medicine by offering a powerful platform for precise and controlled drug administration.[54] The advantages of NPs, such as high drug-loading capacity, adjustable physiochemical properties, and flexibility to modification, make them appropriate for encapsulating anti-cancer drugs, which alter their solubility, stability, and in vivo behavior.[55] Their nanometer-scale size is also beneficial in drug delivery, as it enables direct transfer of the bioactive agent to cells through diffusion or endocytosis. These minuscule carriers enable targeted therapy and ensure that therapeutic agents are delivered directly to specific tissues, cells, or organs, while sparing healthy tissues.[56] This targeted approach not only enhances the therapeutic efficacy of drugs but also minimizes side effects, reducing the burden on patients.[57] Nanoparticles also improve drug stability, protecting pharmaceutical compounds from degradation and enhancing their bioavailability.[58] By controlling the release of drugs, these carriers can provide sustained and controlled delivery, reducing the need for frequent dosing and improving patient compliance.[59] Moreover, nanoparticles are highly adaptable, offering solutions for challenging problems, such as enhancing the solubility of poorly water-soluble drugs.[60] Synthetic NPs can be tailored to have desirable characteristics such as prolonged circulation half-life, improved drug encapsulation, and sustained or triggered drug release that allow for more effective delivery of therapeutic agents to

desired sites of action.[61] Their versatility makes them valuable tools in diverse applications, including cancer therapy,[62] gene therapy,[63] vaccines,[64] and treatment for neurological disorders.[65]

## Impact of Biomolecular Nanostructures in Anti-Angiogenesis

Peptides, liposomes, and nucleotides have been utilized in anti-angiogenic treatment methods, either as components of a drug delivery system or as main agents of anti-angiogenesis. Biomolecules can be highly versatile and effective in combating angiogenesis-related diseases, as is highlighted in Table 1 and further discussed in subsequent chapters.

### Lipids Excel in Anti-Angiogenic Drug Delivery

One of the most promising aspects of biomolecular nanoparticles in angiogenesis treatment is their ability to serve as efficient carriers for therapeutic agents. By encapsulating drugs or biomolecules within nanoparticles, researchers can achieve targeted delivery to specific tissues, minimizing off-target effects and enhancing treatment efficacy. Nanoparticles can be engineered to release their cargo in response to specific triggers, such as pH or enzymatic activity, ensuring that the therapeutic payload is released precisely where needed. This approach is particularly valuable in anti-angiogenic therapy, where localized delivery is essential to inhibit abnormal blood vessel growth. In a proof-of-concept study, researchers created synthetic micelles composed of phospholipids conjugated with PEG. When incorporated into the exosome, the nanoparticle spans the entire vesicle with the PEG domain protruding from the exosome surface. PEG can then be functionalized with the ligand of choice (in this case, EGFR-specific ligand nanobodies) to target EGFR-bearing cells and potentially deliver anti-angiogenic agents.[66] A different approach was used in a study that aimed to improve the cell-penetrating capability of a viral protein envelope. In that study, the liposome-like viral envelope was conjugated with a maghemite nanoparticle whose surface was coated by either protamine sulfate (PS) or heparin.[78] The cationic PS was able to deliver the NPs across the cell membrane more effectively in vitro, but heparin was better when used for in vivo mouse experiments. This means that PS NPs are suitable for transfection purposes, whereas heparin is a candidate for actual gene therapy studies. The use of cell membrane-derived liposomes has also been studied due to their homologous targeting capability. Since these liposomes are derived from specific types of cells, they will also be home to their source cell type during circulation. This opens the possibility of targeting complex tissue types such as cardiac cells and cancer cells. In one study, PLGA nanoparticles have been coated with membranes from cardiac stem cells to create a cell-mimicking microparticle (CMMP) which could be delivered to cardiac tissue to induce

**Table 1** Nanostructure Systems Currently Available for Anti-Angiogenesis

| Biomolecule | Nanostructure | Disease | Reference |
|---|---|---|---|
| Lipid | EGFR-ligand-PEG-coated liposome | Cancer | [66] |
| | Heparin-liposome-maghemite NP | Cancer | [67] |
| | PLGA-coated CMV liposome | Cardiovascular disease | [68] |
| | Mannose/ligand-coated liposome | Cancer | [69] |
| Protein | RGD-coated exosome | Cancer | [70] |
| | pH sensitive poly-His-NP | Cancer | [71] |
| | Poly-glutamic acid conjugate | Psoriasis | [72] |
| | Albumin NP | Cancer | [73] |
| Nucleic acid | DNA nanocage | Diabetic Retinopathy | [74] |
| | Tetrahedral DNA | Cancer | [75] |
| | Antisense ssRNA | Cancer | [76] |
| | Short-hairpin RNA | Diabetic Retinopathy | [77] |

regeneration.[68] In another study, liposomes derived from modified lipids were used to target tumor cells and tumor-associated macrophages together.[69] Mannosylated lipids together with an anti-PDL1 nanobody were used to assemble the dual-targeting liposome which was then loaded with the drugs rapamycin and regorafenib. This takes care of both tumor angiogenesis and the tumor microenvironment.

## Peptide Engineering Enhances Anti-Angiogenic Treatment

Peptide aptamers and nucleotide NPs are mutable and can easily be engineered to bind to their protein and gene targets subsequently, which makes them suitable as drug candidates themselves. These properties can also be matched with other biomaterials to create even better NPs with combined properties that could be used in specific applications. For example, target-specific detection has been performed by coating the nanoparticle with biomolecules that bind to specific surface molecules of certain cell types. In this manner, an apoptosis-detecting nanoparticle can be created, using SPIONs coated with a modified peptide, which can bind to phosphatidylserine.[67] The peptide contains Zn (II) di-2-picolylamine (a compound that alone can bind to phosphatidylserine) and the unnatural amino acid, diaminopropionic acid, to create an Annexin V mimic with a multivalent binding capability. The SPION can then be imaged using magnetic resonance imaging even in vivo. Homing capabilities have also been explored, as researchers attempted to functionalize NPs with signal peptides that would attach to specific surface receptors. One study incorporated the iRGD peptide on an exosome surface, enabling it to deliver the anti-cancer drug doxorubicin exclusively to tumor cells.[70] Another study used a different peptide sequence, they called a "tumor-penetrating peptide" that was conjugated to a pH-sensitive poly-histidine-based nanoparticle to create a tumor-specific delivery system. Through the EPR effect, the INGR peptide can facilitate tumor cell uptake to lead the nanoparticle to the tumor site. Once inside, the change in pH due to the tumor microenvironment will trigger a phase transition that releases the drug into the cytoplasm.[71] In a similar manner, human serum albumin (HSA) nanoparticles were used to prevent clearance while still reaching the tumor target. By varying the HSA density and several synthesis parameters macrophage uptake and liver clearance was minimized.[73] Phase-transition delivery has also been explored on poly-amine NPs, whose drug release kinetics upon pH change has been thoroughly investigated.[79] A modified polypeptide was also used to deliver a corticosteroid in topical treatments for the autoimmune disease psoriasis.[72] Poly-glutamic acid (PGA) was conjugated to the drug to enhance its bioavailability and improve its pharmacological activity.

## Nucleotides Provide Innovative Anti-Angiogenic Applications

The linear structure of DNA can be used to construct three-dimensional polygon structures ranging from tetrahedrons to icosahedrons.[46] These cages can load compounds, bind substrates, and even house other types of NPs inside. Gold nanoparticles have been successfully loaded into cubic DNA origami container, which can be manipulated by adding different amounts of the DNA scaffold.[80] There have also been studies on reconfiguring DNA cages in real time, where addition of a "fuel" strand can change the volume and surface porosity of the nanocage.[81] DNA NPs have also been used to deliver antibiotics in the cornea. Multiple uracil-based nucleotides were used to assemble a particle that could then bind either fluorophores or antibiotics tethered onto aptamers. They have been shown to adhere to the corneal epithelium where they can release the drug.[74] Tetrahedral DNA nanocage has also been shown to deliver the anti-angiogenic drug pegaptanib in vitro.[75] Single-stranded nucleic acids are conventionally used for gene silencing and can be incorporated into different NPs. In one study, antisense oligonucleotides were tethered to gold NPs and tested for their efficiency in knockdown experiments.[76] The researchers found successful downregulation of the target EGFP gene in the mouse endothelial cell line C166. They also observed that the oligonucleotides could persist even in the presence of DNAses, which extends their circulation time and make them ideal for therapeutic applications. Short hairpin RNA (shRNA) has been used to silence c-Met expression that drives angiogenesis in diabetic retinopathy.[77] They observe an increase in expression of the circular RNA-MET during neovascularization, and thus applied shRNA inhibition to see if it will suppress retinopathy. The shRNA not only directly interfered with circ-Met through binding, but it was also successful in suppressing pathological angiogenesis in vivo.

## Summary and Outlook

The combination of nanotechnology and medicine has unlocked a new era of targeted therapeutics, exemplified by nano drugs targeting blood vessels. By leveraging the precision of nano drug design and the unique characteristics of angiogenic blood vessels, researchers are advancing treatment strategies for a wide range of diseases from cancer to inflammatory illnesses like diabetes. Although biomolecule-based delivery systems and NPs in general are enjoying their popularity in the biomedical field nowadays, there are still several issues that should be addressed which would help improve NP usability. The first of this is the inefficient biodistribution of biomolecular NPs. Base biomolecule NPs that do not have targeting ligands rely on blood circulation and the EPR effect to reach their target tissue, which significantly decreases the effect of the drug.[82] And even in the presence of homing signals, their high biocompatibility can lead to cell uptake by unintended targets which might negatively affect these tissues while unnecessarily decreasing the number of NPs still in circulation. Natural drug filter systems like the liver and kidneys also contribute to rapid clearance of these NPs. This could be remedied by using biomaterial polymers like polyethylene glycol (PEG) as stealth ligands,[83] although this could prove to be chemically and sterically daunting to attach to biomolecular NPs.

As technology advances, challenges are being addressed, and nano drugs are poised to transform the landscape of medicine, offering new hope for patients and clinicians in the fight against angiogenesis-related disorders. Beyond drug delivery, nanoparticles can have multifunctional roles in angiogenesis treatment. They can be engineered to carry both imaging agents and therapeutic payloads, allowing for real-time monitoring of treatment efficacy. Additionally, nanoparticles can be designed to interact with angiogenesis-related signaling pathways directly. For example, nanoparticles coated with ligands that bind to angiogenic receptors can inhibit the receptor activation and downstream signaling, providing a unique mechanism to disrupt angiogenesis. And as the field of nanomedicine continues to advance, nanoparticles for drug delivery hold the promise of transforming the way we approach medical treatments, offering a path to more effective and personalized healthcare solutions.

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## Disclosure

The authors declare that there are no conflicts of interest with regards to this work.

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