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The Principles and Applications of Avidin-Based Nanoparticles in Drug Delivery and Diagnosis

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Abstract

Avidin-biotin interaction is one of the strongest non-covalent interactions in the nature. Avidin and its analogues have therefore been extensively utilized as probes and affinity matrices for a wide variety of applications in biochemical assays, diagnosis, affinity purification, and drug delivery. Recently, there has been a growing interest in exploring this non-covalent interaction in nanoscale drug delivery systems for pharmaceutical agents, including small molecules, proteins, vaccines, monoclonal antibodies, and nucleic acids. Particularly, the ease of fabrication without losing the chemical and biological properties of the coupled moieties makes the avidin-biotin system a versatile platform for nanotechnology. In addition, avidin-based nanoparticles have been investigated as diagnosis systems for various tumors and surface antigens. In this review, we will highlight the various fabrication principles and biomedical applications of avidin-based nanoparticles in drug delivery and diagnosis. The structures and biochemical properties of avidin, biotin and their respective analogues will also be discussed.

Graphical abstract

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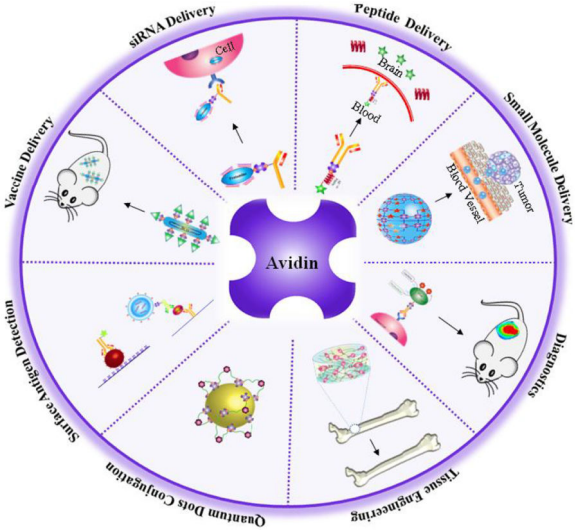

Keywords

Nanotechnology; Avidin; Neutravidin; Streptavidin; Non-covalent interaction; Drug Delivery; Imaging; Diagnosis

1. Introduction

Nanotechnology holds the greatest potential and promise for biomedical research. Significant progress has been achieved in nanotechnology across a wide spectrum of fields from applied physics to biotechnology to medicine. Naturally occurring interactions are the crux for such innovations and have been explored for various nanoscale applications to achieve uncountable scientific goals. As one of the strongest non-covalent interactions in the nature, the avidin-biotin interaction has been utilized in nanoscale drug delivery systems for pharmaceutical agents, including small molecules, proteins, vaccines, monoclonal antibodies, and nucleic acids (Figure 1).

Avidin is a basic tetrameric glycoprotein composed of four identical subunits, each binds to biotin with high specificity and affinity (K_d ~10^{-15} M). Avidin is originally derived from the eggs of aves, reptiles and amphibians. Avidin-biotin interaction is considered one of the most specific and stable non-covalent interactions, which is about 10^3 to 10^6 times higher than an antigen-antibody interaction.[1] Several genetically and chemically engineered avidin and its analogues have been studied to enhance our knowledge about the functional and structural characteristics of avidins, which may lead to more successful applications.[2] The biggest advantage of this system is its high affinity interaction, which is robust and stable against manipulation, proteolytic enzymes, temperature, pH, harsh organic reagents, and other denaturing reagents. [3–6] Therefore, the avidin-biotin interaction serves as a great tool in the biomedical and nanotechnological applications. On the other hand, biotin-based conjugates are easy to synthesize and have less impact on the activity of the biomolecules.

Compared to other covalent and non-covalent interactions, the avidin-biotin system provides enormous advantages such as amplification of weak signals, efficient operation, highly stability and enables the use of highly diluted primary antibodies. Therefore, avidin has been a very versatile modality in the field of biotechnology, especially biochemical assays and affinity purification, over four decades. Tremendous efforts have also been converged to utilize the inherent properties of avidin in biotechnology medicines, and some of them have been evaluated in clinical studies. Recently, the avidin-biotin technology underwent a renaissance in nanoscale drug delivery and diagnostics. Targeting ligands or imaging agents can be easily coupled to nanocarriers via the avidin-biotin linkage. For example, PMA hydrogel capsule functionalized with biotin forms a stable nanocomplex with avidin-coupled antibodies and improved its cellular uptake in cancer cells.[7] Liposomes modified with biotinylated polyethylene glycol can attract a layer of neutravidin on the surface to resist nonspecific binding to serum proteins, thus leading to prolonged circulation time.[8] Microbubbles coupled with RGD peptide via avidin-biotin linkage were developed for the detection of Hep-2 related tumor angiogenesis.[9] Neutravidin conjugated superparamagnetic iron oxide nanoparticle has also been explored as an imaging agent for rhodopsin degeneration.[10] More recently, the avidin-based nanotechnology has found its applications in tissue engineering and cellular regeneration.[11, 12] In one such study, an avidin-biotin system was used to improve osteoblast-like cell adhesion to a highly porous calcium phosphate glass scaffold for bone tissue engineering.[12]

The aim of this review is to highlight the unprecedented advantages of avidin and its analogues in nanotechnology. We will critically evaluate a wide variety of applications that have been recently explored for drug delivery and diagnosis. It is our hope that this review will serve as a one-stop reference for investigators who are interested in exploring the avidin-based nanotechnology in their fields.

2. Biochemical Insights of Avidin, Biotin and Analogues

2.1 Structure and physical-chemical properties of avidin

Avidin is a basic (pI ~10), highly stable, tetrameric glycoprotein (molecular weight 66–69 kDa) that contains terminal N-acetyl glucosamine and mannose moieties.[13] Each of the four subunits contains 128 amino acids and binds to biotin with high specificity and affinity (K_d ~10^{-15} M).[14, 15] Each subunit is composed of eight antiparallel β-strands that form a β-barrel, whose wide end binds to biotin.[16] The avidin-biotin interaction is approximately 10^3 to 10^6 times higher than an antibody-antigen interaction. However, avidin may have a high degree of nonspecific binding *in vivo* due to its basic pI and glycosylation. Rigorous efforts have been made to study the structural properties of avidin using x-ray analysis of its 3D structure for the purpose of improving its stability and functional properties.[17, 18] Investigators have successfully generated several chimeric avidin analogues with better thermal stability and resistance toward proteolytic enzymes. [18, 19]

On the other hand, the strong interaction between avidin and biotin may pose a limitation in releasing the tagged biomolecules from the biotin or avidin. Reversibility of the avidin-biotin interaction can be achieved by addition of a highly concentrated biotin solution. Researchers have also developed biotin analogues that have slightly low affinity toward

avidin in comparison to biotin. For example, desthiobiotin can be easily released from avidin by addition of a moderately concentrated biotin solution.[20] Another method is to insert a cleavable linker, such as a stimuli-responsive linker, between biomolecules and the biotin or avidin. It is noteworthy to mention that chemical modification of the biomolecules may compromise their activity.[21]

2.2 Avidin analogues

Despite its enormous advantages and wide applicability, avidin has several limitations including non-specific binding and possible immunogenicity. To circumvent these limitations, tremendous efforts have been devoted to discovering and engineering superior variants of avidin by genetic modification or finding a completely new source, e.g., a different species.

The most widely used analogue of avidin is streptavidin. Derived from *Streptomyces avidinii,* streptavidin is a ~56 kDa non-glycosylated tetrameric protein that binds to four biotins with a K_d of ~010^{-14} M.[22] Homologs of streptavidin have been discovered from other species, including fungus, bacteria, chickens and frogs.[23] Similar to avidin, streptavidin is also resistant to denaturing agents, temperature, pH and proteolytic enzymes. Despite having a tertiary/quaternary structure and amino acid arrangement similar to those of avidin, streptavidin only shows a moderate sequence homology level of ~30% sequence identity and 40% similarity with avidin.[24, 25] Moreover, streptavidin is non-glycosylated and has a slightly acidic pI of ~ 5-6.[26, 27] Due to its different physical-chemical properties, streptavidin shows an *in vivo* tissue distribution and clearance profile very different from those of avidin.[27] Furthermore, streptavidin protects the biotinyl esters from hydrolysis, whereas avidin augments this hydrolysis.[24] A variety of genetically engineered streptavidins such as *Strep*-Tactin have been developed to exploit the outstanding specificity of the genetically encodable peptide *Strep*-tag II. [28] Strep-tag II has been used for protein purification and detection [29] as well as in numerous *in-vivo* applications.[30–32]

Neutravidin is another commonly used avidin analogue. It is the deglycosylated derivative of avidin and has a molecular weight of ~60 KDa. In the absence of the carbohydrate moieties, the pI of neutravidin is only slightly acidic (~6.3), which prevents its nonspecific binding to cell surfaces and proteins.[33, 34] As a result, neutravidin can be coated on the surface of quantum nanorods to stabilize them and prevent aggregation.[35] In addition, neutravidin has been utilized as a bridge between biotinylated moieties and biotin-coated surfaces for the detection of protein-specific antibodies.[36] The physical-chemical properties of avidin, neutravidin, and streptavidin are summarized in Table 1.

Bradavidin II is a relatively new avidin analogue that was isolated from *Bradyrhizobium japonicum*, a nitrogen-fixing bacteria found in the root nodules of the soybean plant.[37] Bradavidin II shows a moderate amino acid similarity with avidin (38%) and streptavidin (32%), but exhibits the same biotin binding affinity as avidin. Compared to streptavidin, bradavidin II could be a better choice for therapeutic applications because it is less immunogenic.

Most of the avidin analogues have a similar tetrameric structure, which is beneficial to high amplification of a desired signal. On the other hand, the tetrameric assembly may affect the accuracy of binding quantitation due to the uncertainty of the precise binding stoichiometry and possible crosslinking. As a result, avidin analogues, such as hoefavidin and rhizavidin, which have dimeric arrangement, are of interest. [38] [39] Their tertiary topologies remain the same as avidin.

Other recombinant or naturally occurring avidin include Tamavidin 2 (*Pleurotus cornucopiae*)[40], Shwanavidin (*Shewanella denitrificans*)[41], Switchavidin (chicken avidin mutant)[42], Zebavidin (Zebrafish)[43].

2.3 Biotin and analogues

Biotin is a vitamin also known as vitamin H, vitamin B7 or co-enzyme R. Biotin is composed of a tetrahydrothiophene ring fused to a tetrahydroimidizalone (ureido) ring. It plays a key role in cell signaling and acts as a cellular growth promoter. Biotin receptor (sodium-dependent multivitamin transporter and high-affinity biotin transporter) is widely expressed in nearly all living cells. Moreover, its expression in dividing cancer cells is higher than in normal cells, making biotin a potential targeting moiety for cancer therapeutics.[44] Extensive effort has therefore been made to develop biotin-based platforms for tumor targeting and diagnosis.[45]

The functional groups of biotin have been chemically modified to synthesize biotin analogues, such as iminobiotin, ethylbiotin, desthiobiotin, biotin-carbamate, and biotin-carbonate for various applications.[46] Chemical modification of biotin may affect its affinity towards avidin. For example, iminobiotin shows pH-dependent K_d values. Its K_d is 3.5×10^{-11} M at basic pH but lower than 10^{-3} M at acidic pH (3–4).[47]

Apart from biotin, strep-tag (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) is a novel peptide that shows high affinity (K_d 2.7×10^{-4} M) to streptavidin.[48] It has been used in a variety of nanotechnology and biotechnology applications [49, 50]. Recombinant proteins of interest can be easily fused to strep-tag for protein purification and other applications. Researchers have also discovered other variants of strep-tags, strep-tag I and strep-tag II, with higher affinities. The strep-tag I (Ac-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and strep-tag II (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) peptides bind to streptavidin with a K_d of 37×10^{-6}M and 72×10^{-6}M, respectively.[51]

3. Applications in nanoscale delivery systems

3.1 Nucleic acid delivery

Nucleic acids, including plasmid DNA, siRNA, miRNA, aptamers, and oligonucleotides have been extensively explored as therapeutic agents for a wide variety of diseases, and some of them have been applied clinically. Their negative charge and poor stability are the two major hurdles that prevent nucleic acids from reaching their full potential as therapeutics. Although viral vectors are generally effective for nucleic acid delivery, virus-associated safety concerns make non-viral systems better candidates for most therapeutic applications. Numerous non-viral platforms, especially nanoscale delivery systems including

liposomes, peptide nanocomplex, polymer-based nanoparticles, and inorganic nanoparticles, have been rigorously developed for nucleic acid delivery over the past two decades because of their small size and capacity to incorporate multiple components.

Nano-formulations for the delivery of siRNA face tremendous challenges, including aggregation, short systemic half-life and low cellular uptake.[52–54] Protein-based delivery can efficiently counter such shortcomings. For the delivery of siRNA-like negatively charged molecules, cationic proteins, peptides or polymers are recommended for use. These counter-ions produce a high likelihood of nanocomplex aggregation. In a related study, neutravidin was utilized to target podocytes for delivery of siRNA by conjugation of a monovalent IgG (mIgG) to neutravidin through a sulfhydryl group (Figure 2A). Here, instead of the biotinyation of the siRNA, protamine (a cationic protein) was linked to biotin that was coupled to the neutravidin tag on the mIgG. Cationic protamine therefore condenses multiple siRNA molecules and deliver the complex to the target podocytes. Within 30 min of incubation, the IgG with siRNA was detectable in the cytoplasm. This delivery system showed a tremendous reduction (greater than 80%) in the expression of the target protein (p57/Kip2) compared to the control. [55]

In addition to their use as Trojan horses, viral proteins have also been utilized to target nucleic acids to cells. A viral domain that specifically targets a particular cell type is coupled to the avidin moiety via a covalent bond, and the avidin molecule acts as a carrier for the delivery of the nucleic acids. Leisi et al. developed one such delivery system, in which they utilized the VP1u domain of parovirus B19 conjugated to neutravidin to specifically deliver biotinylated DNA or fluorophores to the targeted erythroid cells that were undergoing differentiation around the proerythroblast stage. For this study, maleimide-activated neutravidin was used because it can attach to 5-6 VP1u in a manner that excludes the four biotin-binding sites. Neutravidin is very versatile and retains its activity after minor chemical modifications. The results demonstrated that this VP1u-neutravidin nanocarrier is highly efficient and has excellent therapeutic and diagnostic applications in a wide variety of blood-associated conditions, including hematological ailments such as thalassemia or leukemia. [56]

Similarly, streptavidin has been employed to target the delivery of siRNA for the treatment of liver fibrosis. The tetravalent structure of avidin makes this protein a very efficient tool for the delivery of biotinylated siRNA by producing stable nanocomplexes. These nanocomplexes have not only shown tremendously efficient uptake but rapid silencing of the target gene as well. As shown in Figure 2B, the biotinylated siRNA and biotinylated cholesterol anchored to the streptavidin backbone provide efficient silencing of the PCBP2 gene for liver fibrosis treatment.[57, 58] Streptavidin-biotin technology has also been utilized for the delivery of Kv1.3 siRNAs in the treatment of autoimmune disorders. Biotinylated polyethylene glycol and cholesterol were functionalized with biotinylated-CD45RO antibodies through the use of streptavidin to provide highly specific targeting and uptake of nanoparticles by Memory T cells, which had the consequence of decreasing the calcium ion influx, thus producing a therapeutic effect. [59] Several other simple and efficient strategies for nucleic acid delivery have recently been reported for the targeted delivery of nucleic acids.

Delivery of siRNA to normal cells is relatively easy compared to the process in stem cells. Biocompatibility is a major concern because incompatibility could otherwise cause a fatal immunogenic response. [60, 61] Synthetic vectors pose various limitations for the transfection of undifferentiated human embryonic stem cells (hESC) with nucleic acids. Huang and coworkers developed a light responsive siRNA delivery system using hollow gold nanoshells (HGN) in which the HIV-derived TAT peptide was utilized to deliver the siRNA into the hESCs under the influence of biocompatible infra-red radiation (~800nm). The direct conjugation of the TAT peptide to the siRNA resulted in aggregation due to surface charge. As depicted in Figure 2C, the TAT and siRNA were coupled to different binding sites of the streptavidin molecule, which kept them separated. This structure prevents electrostatic contact, which inhibit particle aggregation.[62]

Another novel method of delivering nucleic acids is the avidin-nucleic acid nanoassembly (ANANAS). Here, the nucleic acid acts as a central unit onto which several avidin molecules are nucleated. This assembly forms a toroidal structure, which is further complexed with biotinylated polyethylene glycol. [63] Due to the excellent pharmacokinetic properties of avidin, after *in vivo* administration, fluorescently labeled ANANAS nanoparticles showed substantial subcellular internalization in the mucosal vasculature. This enabled the localization of nanoparticles at the target site, whereas no accumulation was observed in healthy tissues. ANANAS shows tremendously promising characteristics, including easy preparation, no immunogenicity, and excellent pharmacokinetic properties, which make it an outstanding translational therapeutic and diagnostic tool.[64, 65]

3.2 Protein and Peptide Delivery

Protein and peptide delivery has generated considerable interest in the past two decades on the basis of its potentially important applications in targeted therapy. Therapeutic peptides, enzymes and recombinant proteins are among the highest revenue-generating products among all the pharmaceutical products offered across the globe.[66] However, the macromolecular drugs face substantial delivery challenges including slow or low permeability across biological membranes and low target-specific biodistribution. [67] The greatest hurdle in the delivery of peptides and proteins is their encapsulation. Special consideration must be given to the chemical and physical properties of the biologics before contemplating the nanocarrier. Because proteins are prone to structural distortion that may lead to the loss of biological activity[68], special care is needed in proteins modification to minimize adversely affecting the activity of these molecules.

Due to the minimal modification required by the biotinylation of a protein molecule, avidins have been employed by several researchers as a carrier for peptide delivery, such as cell penetrating peptides (CPP) [69, 70] and Tat peptide [71]. The biotinylated peptide sequences are complexed with avidin to reduce the chance of aggregation. Additionally, covalent modifications of peptides may induce conformational changes that can interfere with their ability to be translocated. However, biotinylation acts as a spacer that reduces the constraints on a peptide mandated by the carrier.[70] The four biotin-binding sites on avidin can be exploited for the delivery of different peptide sequences for specific roles such as targeting a ligand, as depicted in Figure 2B. In one such study, a tumor-targeting peptide (bio-CREKA)

and an arginine-rich peptide (Bio-R8) were used simultaneously to deliver the p53 tumor suppressor gene. Here, avidin allowed the use of the two biotinylated peptides, where the Bio-R8 moiety promoted the internalization of the nanocomplex and bio-CREKA provided for specific binding to the receptors on MCF-7 cells. [72]

The internalization of a protein by a target cell involves overcoming a series of barriers. Investigators have developed fusion proteins that include avidin or streptavidin to enhance the uptake of small synthetic molecules mediated by receptors. The presence of the streptavidin in these fusion proteins triggers internalization and thus overcomes the delivery barriers. [73] The avidin modification not only provides a very useful carrier for peptide delivery but substantially promotes the cellular internalization. [74] Several parameters including particle aggregation, size and surface charge play vital roles in cellular internalization. Rational modification of the surfaces of the nanoparticles with avidin, as reported by Steinbach et al, produced a higher uptake than that of the unmodified nanoparticles. In contrast to the DSPE-PEG nanoparticles Av-MPG-NPs showed a higher uptake due to their surface charges, which resulted in a smaller size and a higher ligand density. These properties have a combined impact on the internalization kinetics that resulted in the higher internalization of the Av-MPG-NPs. [75]

Peptide sequences such as cell-penetrating peptides (CPP) hold considerable potential for the cellular internalization of molecules that are difficult to transfect. However, CPP-based delivery to colorectal cancer (CRC) cell lines or metastatic cancer cells is even more challenging. Streptavidin-like proteins have been used as a model protein carrier for the delivery of several CPP that can promote the internalization of macromolecular drugs, such as DNA or siRNA. Cell-penetrating sequences such as transportan-10 (TP-10) and transportan (TP) have recently been evaluated by Wierzbicki et al. Biotinylated analogues of TP and TP-10 have demonstrated an endocytosis-independent and highly efficient delivery of siRNA when complexed with streptavidin in HCT116 (metastatic CRC model) and HT29 (early stage CRC model) cell lines and resulted in a high silencing activity of SASH-1 mRNA. [76] It can clearly be inferred that streptavidin provides a substantial advantage for cellular and tissue internalization in addition to its use as a dynamic stoichiometric support (back-bone) for nanocomplexes.

3.3 Vaccine delivery

In recent years, vaccine development has generated much interest. Rigorous efforts have been made to reduce the time between the discovery of vaccine candidates and their clinical development. The greatest challenge involves the production of antigens in an appropriate quantity to induce an optimal immune response in body.[77] Avidin also provides the advantage of varying and adjusting surface chimeric proteins on bacteria to induce a higher immunogenic response toward the vaccine. Furthermore, biotinylation of the bacterial surface proteins has no effect on the phenotypic characteristics of the bacteria or the exogenous properties of the protein, all of which make avidin a very applicable moiety for vaccine development. [78] In addition to all of the other advantages, deglycosylated monovalent avidin substantially reduces the chances that the vaccines will aggregate. [78]

The stability of vaccines is the most important aspect of vaccine development. Much effort is being directed toward improving the stability and efficiency of the development of vaccines for a range of conditions, from HIV to dengue. One issue is that the addition of protein molecules that may help to stabilize a vaccine may compromise its activity. Solutions have recently been devised to express an avidin fusion protein along with the target protein for stable and efficient antigen expression. In one such study, shown in Figure 2D, Bacillus Calmette-Guerin (BCG) bacteria were biotinylated, and the surface of the bacteria was decorated with a monovalent avidin fusion protein. This technique produced BCG vaccines that are more responsive to T cells, and which were reproducible and highly stable for an extended period of time after freeze drying.[78] Another research group developed a technique to employ avidin in the development of a Lassa fever vaccine that undergoes self-assembly. Here, MtbHSP70-avidin proteins were coupled to biotinylated peptides from the Lassa GP1 and GP2 proteins, which are naturally immunogenic. This self-assembling vaccine showed tremendous stability and optimal immunogenicity. [79]

The vaccine research and development process also faces the challenge of the extended time required to reach an effective *in vivo* immunogen concentration. Investigators have reported efforts to minimize the long time required for the B cell-specific antigen detection and the slow onset of the immune response. This method involves the sequential staining of biotinylated antigenic gonadotropin-releasing hormone (GnRH)-like peptides with streptavidin or neutravidin. This process increases the ligand avidity, and the `single epitope multiple staining' principle permits the rapid detection of the B cells after immunization. [80]

In addition to binding to the biotinylated antigen, streptavidin is also used as a potent immunostimulant in cases of less immunogenic antigen-based cancer vaccines. Currently, cancer vaccine research utilizes “self” tumor antigens, which are only weakly immunogenic. These antigens are not much different from other cells in patient's body, hence, the patient becomes tolerant very rapidly. To circumvent such problems, immunostimulants of bacterial origin, such as streptavidin, have been used. Moreover, streptavidin has also been demonstrated to have a high affinity for surface-bound tumor proteins. The RYDS sequence of streptavidin assists in cell adhesion through the RGD cell adhesion domain. [81] These two qualities have led to the use of streptavidin for the delivery of cancer vaccines. The vaccine-streptavidin combination produced 6 times higher reactivity than the vaccines alone. Streptavidin bound to biotinylated soluble tumor proteins have successfully produced tumor reduction and remission in a 9L glioma rat model and in canine patients. [82]

Vaccine development requires the vaccine immunogen to be presented at a high density with a uniform orientation through which it can attain ideal epitope spacing and mimic the multivalent epitope of a virus. [83, 84] Controlling the orientation and density of the coupled antigen by chemical conjugation poses a tremendous challenge. Additionally, the incorporation of complex antigens onto virus-like particles (VLPs) compromises their assembly and thus the activity. Thrane et al designed a genetically modified HPV16 L1 VLP (Human papilloma virus 16 L1 virus-like particle) to overcome these challenges. The insertion of AviTagTM (Avidin tag) in the L1 coding sequence of HPV16 L1 VLP permits it

to be biotinylated specifically. This, in turn, can be utilized to fuse the antigen with monovalent streptavidin without disrupting the assembly of VLPs and their activity. [85]

The use of avidin in recombinant vectors has increased the possibility of delivering various classes of biotinylated antigens. Vectors that target dendritic cells using a single chain antibody (scFv) fused with a streptavidin core is an example of this approach. Dendritic cells play an essential function in the management of antigen-specific immunogenicity. Antigenic vaccines that can specifically target the dendritic cells to activate the required immunogenic response are being developed and tested clinically. [86, 87] However, the optimal immune response was not observed following either *in vivo* or *ex vivo* stimulation. A receptor-targeting approach has been developed to use dendritic cells receptor-specific ligands that can efficiently deliver antigens to the dendritic cells. [88] Investigators have developed a single chain antibody (scFv) fused with a streptavidin core that targets the DEC-205 receptor of dendritic cells. This streptavidin core and biotinylated antigen subsequently form a complex and delivers the antigens to the target dendritic cells. [89]

3.4 Monoclonal Antibody (MAb) Delivery

Avidin-biotin technology has made it possible for a monoclonal antibody to carry a payload to the vital target sites. Avidin-coupling or fusion proteins not only make it easier to formulate the delivery carrier but help to increase the uptake at a target site. Avidin provides a greater advantage in the delivery of drugs via monoclonal antibodies. Avidin-fusion proteins coupled to monoclonal antibodies by genetic engineering have shown excellent target specificity. Molecular Trojan horse and avidin-biotin technologies have been recently exploited to delivery biologics across the blood brain barrier (BBB) via transferrin receptors (TfR). Specifically, TfR-MAb functions as a ferry to transport the biologics into the brain through the BBB TfR. [90] Chemically cross-linking avidin with TfR-MAb is one strategy that has been applied to deliver a peptide across the BBB.[91] Unfortunately, the specificity of monoclonal antibodies is compromised when they are chemically modified. However, an IgG-Avidin fusion protein expressed in Chinese hamster ovary (CHO) cells retained the specificity of the MAb. Similarly, avidin-chimeric-TfR-MAb fusion protein was also expressed and engineered in biotin-depleted CHO cells.[92] Using the same methodology, the amyloid plaques that accumulate in the brain and result in Alzheimer's disease have also been targeted for imaging and diagnosis using an TfR-MAb-avidin fusion protein. As depicted in Figure 2E, radiolabeled $A\beta^{1-40}$ was attached to the TfR-MAb-avidin fusion protein. This complete conjugate was used to ferry the radiopharmaceutical across the BBB. [93]

Antibodies are also extensively used in radioimmunotherapy, which requires a highly specific interacting molecule for the binding of the effector to the pre-targeting molecule. [94] To achieve a high specificity of the effector for the pre-targeting molecule, avidin-biotin technology has been recently implemented. In contrast, a fusion protein approach using the avidin-biotin system is very effective and produces a superior homogeneity. However, this approach is highly time-consuming and requires extensive efforts and optimization.[95] A pretargeted ^{211}At-radioimmunotherapy developed by Frost et al consists of an avidin-monoclonal antibody that has a high affinity towards radiolabeled and biotinylated poly-L-

lysine conjugates. This radioimmunotherapy delivery system is highly efficient, less time-consuming and demonstrates excellent tumor specificity.[96] Compared with conventional radioimmunotherapy, the avidin-conjugated monoclonal antibody produced a higher uptake of radiopharmaceuticals. [97]

Patrick Ng et al also developed an avidin-antibody fusion protein (Av-anti-rat TfR IgG3) that is capable of efficient delivery of various molecules into cancer cells. In this approach, the CH3 region of a human lgG3 (rat transferrin receptor-specific) is genetically fused with avidin. Generally, the fusion protein approach requires the chemical conjugation of specific protein components with various applications. This results in decreases in the activities of the respective components, and its development is very difficult. In contrast, the avidin-Mab construct is a universal construct that eliminates the need to use a specific protein for a particular application. Ng and coworkers demonstrated that the Av-anti-rat TfR IgG3 possesses an ability to deliver various biotinylated molecules and has a strong pro-apoptotic activity against the T cell lymphoma cell line Y3-Ag1.2.3 as well as the rat C58 (NT) D. 1.G.OVAR (malignant cancer cells), while a non-recombinant anti-rat TfR IgG3 did not show any activity.[98] They further showed that this recombinant avidin system may induce a high antitumor activity *in vivo* by delivering the biotinylated agents into the cancer cells. [98]

Molecular Trojan horses are also a type of genetically engineered MAb that has recently become the most promising tool for the delivery of macro-molecules across biological membranes such as the BBB. The only caveat of this fusion technology is that the protein cannot be fused with oligonucleotides. However, the avidin-biotin technology enables the targeting of siRNA and peptides *in vivo* in association with fusion protein technology.[99] Merging the Trojan horse technology and the avidin-biotin technology has also allowed a highly specific receptor-mediated means to deliver siRNA using Mab, which have extensive stability *in vivo*. In a similar study, human insulin receptors (HIR) were targeted using HIRMAb that were conjugated with streptavidin (HIRMAb-SA) and subsequently bound to a 3′-biotinyl-siRNA. The delivery of luciferase siRNA with HIR-MAb-SA caused 90% silencing of the luciferase gene. In comparison, avidin, unconjugated streptavidin, or the HIR-MAb alone showed negligible effect.[100]

Non-convalent avidin-biotin interactions are very helpful for the surface decoration of nanoparticles with antibodies. The use of avidin-biotin to conjugate the MAbs to the nano-carrier surface helps to retain the MAb's function. Hajdu et al biotinylated antibodies to CD45RO, a cell surface marker for memory T cells. These biotinylated MAbs were used to functionalize the surfaces of nanoparticles made from pegylated/biotinylated phosphoethanolamine and cholesterol through the use of streptavidin. The resulting CD45RO-functionalized nanoparticles showed highly efficient targeting to memory T cells for potential therapy for autoimmunity (Figure 2F).[59]

3.5 Small molecule delivery

Nanotechnology has provided many ways to efficiently deliver chemotherapeutic drugs to the site of action. Efficient delivery of small molecules requires high specificity to the target site which can only be achieved by the attachment of receptor-specific ligands.[101] Avidin

provides an excellent bridge for diagnostically relevant ligands that target specific receptors.[75] These ligands may or may not be functionally modified with stimulus-responsive cleavable linkage for delivery of these small molecules. Mesoporous silica nanoparticles capped with avidin functionalized with an MMP9 (matrix metalloproteinase 9) specific cleavable linker is one such example.[102] Because MMP9 is over-expressed in the areas of lung tumors that are beginning to metastasize, it allows the controlled release of the drug at the site of metastasis.[103] The mesoporous silica nanoparticles mentioned above are constituted with tunable pores and volume for higher drug loading. The outer surfaces of these mesoporous silica nanoparticles can be functionalized with certain molecules that do not interfere with the pore morphology and integrity. In one such example, the mesoporous silica nanoparticles were tightly capped with avidin molecules, and the pores were blocked by biotin, which prevented the release of the payload (cisplatin) from the core.[102] Thus, the avidin-biotin served as a guard on the surface of the nanoparticles to regulate the release of drug.

The avidin-biotin system has also been widely used as a pretargeting strategy.[104, 105] Additionally, biotin and avidin have been reported to target and accumulate, respectively, in tumors.[106–109] These properties make them an excellent choice for a tumor-targeting formulation strategy. High avidin accumulation in the liver after treatment, as well as the properties mentioned above, have been exploited to target hepatic carcinoma. Recently, chitosan nanoparticles modified with biotin and avidin were designed in which the mannose sugar and acetyl glucosamine of avidin were used to target the liver. Moreover, avidin allows the addition of multiple ligands on the surface to achieve highly specific targeting. These authors demonstrated that the avidin bound to the biotin on the surface of the nanoparticles was responsible for the higher liver accumulation of nanoparticles and thus enhanced the anticancer activity.[110]

Biotin labeling of the surface of the carrier dramatically increases the possibility of surface functionalization through avidin-modified moieties. This technique has been applied to couple liposomes to the surface of microbubbles. Microbubble-liposome therapeutic carriers for which ultrasound is used to target the therapy toward breast cancer has recently a field of extensive research. Yan et al proposed this novel form of therapy in which they used avidin for the surface conjugation of paclitaxel-loaded liposomes on the microbubbles. Ultrasound exposure caused the drug payload to be efficiently delivered to the target site.[111]

In another approach (Figure 2G), hyaluronic acid (HA)-based micro hydrogels were prepared by exploiting the avidin-biotin technology. Here, neutravidin-biotin was used to link micro-hydrogel containing doxorubicin to the HA molecule to function as a switch. Avidin-biotin has also been exploited by several researchers for the hydrogel grafting.[112, 113] These HA-biotin- neutravidin micro-hydrogels show fine porosity in conjunction with micro-beads. Upon addition of excess biotin, the HA microhydrogel-encapsulated drug disassembles to rapidly release additional doxorubicin. In this application, biotin acts as the triggering agent or a switch to release the drug at the target site.[114]

We have described the advantage of avidin in the tumor-specific delivery of drug molecules. Delivery across the BBB and other complex biological barriers is even more challenging

than metastasized malignant tumor cells. To address this issue, investigators have devised several approaches including the use of apolipoproteins in the design of nanoparticles that mimic natural particles.[115] Low-density lipoprotein receptor-specific apolipoprotein E (Apo-E) target the endothelial cells of the BBB very efficiently. However, chemical conjugation and other modifications of Apo-E reduce its function substantially. Avidin-biotin technology serves as a convenient tool to attach these Apo-E molecules to a drug delivery carrier without compromising their activity. Here, Apo-E was biotinylated and used to functionalize avidin-conjugated solid lipid nanoparticles. The avidin-biotin system is thus useful for the strategic functionalization of nanocarriers for highly efficient delivery of therapeutic small molecule drugs.[116]

Stimulus-responsive technology for drug development, controlled release and targeted therapy has been extensively harnessed by research groups across the globe. In this context, the switchable release of avidin from biotin is one of the most simplistic, intriguing and innovative strategies. Imino-biotin is a type of biotin which has avidin-binding properties similar to those of natural biotin. Imino-biotin is coupled to the nano-carrier, where it retains its structure and its noncovalent bond with avidin at physiological pH. Upon protonation under lower physiological pH conditions, its affinity for avidin is decreased, and it releases the drug attached to it. This switch-release technology using a modified biotin is highly efficient in comparison to the disulfide linkages.[117, 118]

4. Applications of Nanoscale Avidin Systems in Diagnosis and Biotechnology

4.1 Surface Antigen Detection

The interaction of avidin with biotin provides an excellent platform for the development of various assay systems. Although antibody-based assay systems are highly specific, chemical conjugation with fluorescent dyes or chemiluminescent compounds may have some impacts on the antibody specificity. Quantum dots have proven to be a highly efficient tool compared to the traditional antibody-fluorescent dye-based assays. Quantum dots provide several advantages such as high signal strength, high photo stability and high quantum yield for the detection of proteins with an enhanced signal amplification.[119] Protein quantification using quantum dots in elemental mass spectrometry (ICP-MS) based immunoassays has also proven to be very effective and efficient. Quantum Dots possess outstanding photoluminescent properties, which makes them very important and novel as an antigen-detection system. Quantum dots have a tremendous capacity to improve bioanalytical applications that use bio-labeling and bioimaging methods. To fully exploit the functions of quantum dots, it is necessary to establish strict control for their synthesis and surface modification. The noncovalent interaction between biotin and streptavidin serves as the best tool for the surface modification of the quantum dots and permits the development of selective assay systems without compromising their target-binding specificity. In a similar development, an immunoassay platform based on streptavidin-conjugated quantum dots, which are used to bind to a biotinylated antibody and can be detected using ICP-MS, has been designed for amplified protein quantitation. This system enables the quantitation of protein in samples with concentrations as low as 50 ng/mL.[120] Neutravidin bioconjugated

to highly luminescent quantum dots has also been developed for the detection of the tyrosine kinase B (Trk-B) receptors present on the neurons of the hippocampus. Here, neutravidin was specifically used instead of avidin or streptavidin because the presence of the lysine allows the amide to bind to the carboxylic groups on the quantum dots. The quantum dot-neutravidin bound to the biotinylated anti-TrkB antibody specifically detected the distribution of the TrkB receptors. The quantum dot-neutravidin conjugated to the biotin-TrkB serve as an excellent tool for long-term observations of the trafficking of the fluorescence signals during live imaging of neurons. [121] Quantum dots have also been employed for detecting and tracking enzymes in vivo. As demonstrated in Figure 3, several hydrolase enzymes have been conjugated to quantum dots through the avidin-biotin interaction. It was found that the catalytic activity was retained by the quantum dot-conjugated enzymes compared to the free biotinylated enzymes. The avidin-biotin interaction in this application was critical because it is a robust interaction with a high resistance toward wide range of pH. The avidin–biotin system has ubiquitously been proven to be the best method to functionalize the luminescent quantum dots-enzyme conjugates compared with the traditional approaches. [122]

Non-invasive bio-medical imaging techniques such as magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) and positron emission tomography (PET) can evaluate and diagnose the progression of numerous diseases through the use of surface antigen detection. Among these techniques, MRI is considered to be the technique of choice for imaging soft tissues.[123] Iron oxide nanoparticles are widely utilized as the MRI contrast agent. However, iron oxide nanoparticles need to be stabilized with low-molecular-weight high-affinity dispersants, [124] and polymers such as poly ethylene glycol are used to stabilize them against oxidization. In one such study, iron oxide nanoparticles were stabilized with poly-ethylene glycol coated with neutravidin for the immobilization of the biotinylated VCAM-1 (vascular cell adhesion molecule 1) antibodies. VCAM-1 bearing neutravidin-coated nanoparticles showed outstanding affinity and specificity toward the VCAM chimeras. Neutravidin also provides flexibility in varying the ligand density on the nanoparticles, which makes it an excellent agent for the detection of antigens *in-vivo*.[125, 126]

Quantification of a specific antigen at extremely low levels and over a wide range is a very important capability in the field of life science.[127, 128] For dynamic sensitivity over a wide range of antigen concertation, immuno-PCR (IPCR) is a system that utilizes antibodies and DNA conjugates. However, this method has several disadvantages, such as challenging preparation, purification, and a low DNA-to-antibody ratio that results in a low sensitivity and high non-specific background signals. To address these issues, as depicted in Figure 4, modified liposomes with an encapsulated reporter DNA have been designed. The surface of the liposomes is conjugated to a biotin-PEG (polyethylene glycol) phospholipid that acts as a detection agent. Microplate wells on which the target antigen specific antibody is immobilized capture the antigen. A biotin-labeled secondary antibody with a neutravidin bridge binds to the antigen. Biotin-coated liposomes bind to the neutravidin-bridge and anchor the reporter DNA-loaded liposomes. Upon binding to the target antigen, the liposome bursts to release the reporter DNA, which permits the target protein to be quantified using real-time PCR.[129]

Although sandwich ELISA-based assays are well known and widely applied [130, 131], a new assay approach that harnesses the streptavidin-biotin interaction and exhibits a 10-fold higher sensitivity has been recently developed.[132] In addition, this approach reduces the cost by 20-fold in comparison to RT-PCR. In this assay, named NLFOA (nuclease-linked fluorescence oligonucleotide assay), nanoparticles coupled with several streptavidin molecules interacts specifically with the biotinylated TurboNuclease for detection of the HIV-1 p24 antigen. The NLFOA provides a highly sensitive assay system for the clinical diagnosis of HIV or other infectious diseases.[132]

4.2 Imaging and diagnosis

Imaging technology has been extensively evolved in recent years. Its biggest application is the diagnostics and detection of biological markers for specific diseases, such as cancer. Several radiolabeled antibodies are being extensively studied or are now undergoing clinical trials for the imaging and diagnosis of cancer. [133, 134] However, this method poses several disadvantages that prevents its broad clinical application. These include slower tumor diffusion and delayed clearance kinetics that lead to a decrease in the tumor-to-nontumor ratio. To address these shortcomings, the concept of pretargeting has been proposed. For pretargeting, avidin and streptavidin can be conjugated to either monoclonal antibody or radioisotope. The extremely high binding affinity, rapid blood clearance and high tumor uptake of biotin and avidin make them highly reliable and essentially the best candidate for pretargeting imaging technology.[94] Generally, avidin is used to clear the circulating biotinylated antibodies from the systemic circulation, but for the pretargeting strategy streptavidin or neutravidin are extensively used. Interestingly, it has been found that the fluorescently labeled or radiolabeled streptavidin showed a higher uptake in the tumors compared to the normal tissues than that of the radiolabeled antibody alone. [135–137]

For diagnostic purposes, the expression of a specific antigen on a particular cell type can be harnessed and subsequently detected with favorable techniques.[138, 139] Similarly, the types of antigens or markers present on cancer cells reveal the degrees of malignancy, invasion, neovascularization, and metastasis. Fluorescently tagged antibodies specific for these cell-specific markers are used for efficient detection via imaging or other diagnostic tools. Because avidin is very stable to the chemical modification, it serves as a good candidate for conjugation purposes. Maleimide activated avidin was utilized to modify a MAb which is specific for the embryonic cancer delta-like protein (Dlk-1). A fluorescent bioluminescent protein (FBP) was designed by the conjugation of biotinylated Cypridina luciferase (CLu) to a far-red derivative of fluorescent indocyanine (Figure 5). The FBP was subsequently coupled to the Dlk-1-specific MAb to identify the embryonic cancer antigen Dlk-1 via the avidin-biotin interaction.[140]

Theranostic modalities that can simultaneously diagnose and treat a condition have driven the development of calcium phosphosilicate nanoparticles (CPNP). CPNP is another type of vehicle that is used for various imaging and therapeutic applications in biological systems. [141, 142] These particles function by accumulating in the solid tumors via enhanced permeation and retention (EPR) effect. Conjugating these nanoparticles to fluorescent probes has proven to be highly effective. Barth et al utilized avidin to engineer a novel

CPNP that was conjugated to biotinylated diferric transferrin (human holotransferrin), biotinylated anti-CD71 antibody (transferrin receptor specific), and biotinylated pentagastrin using an avidin-biotin coupling. These avidin-CPNP-based theranostic materials were shown to specifically target, diagnose and treat breast cancer and other rapidly dividing and transferrin-expressing cells. [143]

Another widely accepted theranostic application is radioimmunotherapy, which employs streptavidin for the coupling strategy. Direct radionuclide labeling of MAb has been conventionally used for disease control. However, complete eradication is still a challenge because the tumor to normal cell localization ratio is extremely low. Malignant plasma cells are highly radiosensitive, and the management of plasmacytomas and multiple myeloma is possible with the help of radioimmunotherapy combined with streptavidin and biotin technology. Streptavidin-coupled antibodies are highly selective for the tumor tissues or malignant cells. Once the streptavidin-coupled antibodies accumulate at the target site, a biotinylated radioactive small molecule is administered that specifically binds to the streptavidin that has accumulated in the malignant cells or tumor site. Green et al developed such a streptavidin-biotin pretargeted radioimmunotherapy system that targets the CD38 antigen for the delivery of a radionuclide for the eradication of multiple myeloma. Here, anti-CD38 was conjugated to streptavidin, and ^{90}Yttrium was bound to biotin for pretargetting.[144, 145] The ^{90}Y–DOTA–biotin treatment produced the maximum survival rate in treated mice. [145]

Another diagnostic modality that has received significant interest due to its unique properties and characteristics (Figure 6) is nanoparticle clusters. However, their structurally and morphologically controlled development is highly challenging. For successful size-controlled construction of nanoparticle clusters, Ryu et al proposed a method using a DNA-binding zinc finger protein that utilizes the avidin-biotin system. Direct chemical conjugation of metal ions (Zn) to DNA limits their further ability to derivatize the nanoparticle surfaces. To circumvent these challenges, DNA was conjugated to biotinylated zinc finger protein and subsequently incubated with neutravidin-conjugated nanoparticles. These nanoparticle clusters showed not only the successful development of a size-controlled construct but also increased spin–spin relaxivity three-fold compared to conventional contrast agents such as Feridex.[146]

PET scanning is also a highly sensitive and extensively used technique for the quantitative imaging of tumor specific markers. Hypoxic inducible factor 1 (HIF-1) plays a key role in the progression of malignant tumor and radiotherapy resistance. The oxygen-dependent degradation domain (ODD) of HIF-1α has therefore been used for the development of tumor imaging and therapeutic agents. HIF-1-positive tumor cells are one type of the targets that have been most quantitatively diagnosed with the help of noninvasive imaging techniques such as PET.[147] A protein transduction domain (PTD) coupled to the oxygen-dependent degradation domain and to monovalent streptavidin was used to produce the fusion protein PTD-ODD-streptavidin (POS) because of the outstanding *in-vivo* stability of biotin-streptavidin. Streptavidin modification reduces the degradation of POS. The POS conjugated with radioactive ^{123}I-IBB specifically target the HIF-1 exclusive regions in tumors.[148] Another streptavidin fusion protein was developed by the same group to deliver the

radiolabeled biotin derivative synthesized as 4-^{18}F-fluorobenzoyl-norbiotinamide (^{18}F-FBB). It was concluded that the areas showing ^{18}F-FBB localization corresponds to the HIF-1α positive areas.[149]

Streptavidin-based imaging modalities have negligible effect on the pharmacokinetics of their parent drug, which makes them ideal for the application in diagnosis. A streptavidin-based nanoparticle has been developed as multimodal imaging agents for the fluorescence and nuclear imaging and detection of tumors in a mouse model. Liang et al designed a biotin-binding streptavidin-based nanocomplex that was coupled to a biotinylated anti-Her2 antibody. The tumor targeting was achieved using a biotinylated anti-Her2 (Herceptin antibody), and diagnostic imaging was performed using a biotinylated DOTA-chelator labeled with ^{111}In and a biotinylated Cy5.5 fluorophore. Both radiolabeled and fluorophore-labeled streptavidin nanoparticles complexes have proved to be very efficient and promising tools for Her2-positive tumor imaging.[150]

4.3 Tissue engineering

Skin grafts are considered the most common method used for the clinical repair of skin defects.[151] However, contraction of the grafts, rejection due to immunity and graft dysfunction are a few shortcomings of the grafting technique. Tissue engineering is one promising alternative to overcome the deficiencies of the graft techniques This alternative strategy involves the fabrication of a scaffold that mimics the natural extracellular matrix (ECM) of the target.[152] Avidin is immobilized on biomaterial surfaces, and the cell membranes are conjugated with biotin. The extraordinary affinity of these two molecules mediates the efficient attachment of the cells to the biomaterials. Natural cellular adhesion occurs by the formation of integrin-mediated bonds between integrin in the cell membrane and adhesion proteins on matrix.[153] Investigators have demonstrated that that avidin-biotin binding system was found to be superior to the integrin-serum protein system with respect to the cell adhesion strength.[154] Recently, the avidin-biotin binding system (ABBS) was used to fabricate PLCL/Pluronic nanofiber matrix for skin care application. [155]

Silk biomaterials with attached bioactive molecules are extensively used for drug delivery and tissue engineering. Covalent coupling has a very large negative impact on the bioactivity of the biomolecules due to the amine group reactivity. Non-covalent methods of coupling such as avidin-biotin are therefore recommended. The versatility and simplicity of the avidin-biotin system made it possible to immobilize various moieties, such as growth factors and gelators for tissue regeneration. [156]

New bone ingrowth at a bone defect site is also the most important alternative to simple bone replacement, which prevents the possibility of bone rejection by immune system. Investigators have reported the application of avidin and biotin-based systems to attach cells to the surfaces of nonporous 2D and 3D biodegradable scaffolds.

Streptavidin has been used with grafting materials, such as Mylar™ and Teflon-AF™, for adhesion and proliferation of endothelial cells. In one such study, human umbilical vein endothelial cells (HUVEC) were biotinylated and incubated with streptavidin to immobilize

grafting materials. The streptavidin-coupled HUVEC showed significant spreading in comparison to uncoupled HUVEC.[157] In a similar study, ABBS was utilized in the calcium phosphate glass scaffold for bone tissue adhesion. The scaffold immobilized with avidin showed substantially higher cell attachment. ABBS has a major advantage over other scaffolds because it does not inhibit cell proliferation. Scaffold functionalized with ABBS can improve its adhesion with osteoblast-like cells. It was concluded that the ABBS scaffold helps in proliferation after efficient attachment of the osteoblast like cells. [12]

In addition to a prominent scaffold for cell adhesion, the biggest prerequisite for tissue engineering is to imitate the local bio-microenvironment and bio-ceramic matrix to provide a 3D support that enables the growth of bone tissue.[158] Biologically active molecule, such as protein and peptide, decorated on the surface of these bioceramics can improve bone regeneration.[159] However, there are still few challenges for tissue engineering: (i) A strong interaction between proteins and the surface of scaffold; (ii) No change in the native structure and biological activity of the proteins; and (iii) Site-directed immobilization. Baeza et al utilized the avidin-biotin technology to address these challenges in tissue engineering. [160] Another research group developed biotinylated nanofibrous hydrogels for efficient cell adhesion in a 3D matrix of C2 based gelators made from 1, 4-benzyldacarboxamide. As shown in Figure 7, avidin modified cells were used to adhere on the biotinylated gelator-based 3D matrix. This system has shown to produce a universal 3D matrix system where avidin modified cells freely proliferate without any sign of denaturation. Moreover, the density of the cells in the matrix can be manipulated by varying the biotin-gelator amount in the matrix. [161]

3D matrices used in tissue regeneration are required to be biodegradable. Repair and regeneration of a bone defect with the help of a biodegradable polymer-based scaffolds are often used along with bone-inducing factors and/or osteogenic cells. In addition to the biocompatibility and appropriate mechanical strength for weight bearing, scaffold for bone regeneration must have interconnected porous structure for tissue in-growth which can support the regeneration.[162–164] BMP-2 and BMP-7 (bone morphogenetic protein) are two main osteoinductive factors which have the potential to induce differentiation of osteocytes, followed by mineralization and bone regeneration.[165, 166] Encapsulation of these growth factors on to polymeric scaffolds using various solvents and electric field for fabrication may result in the loss of biological activity.[167] To overcome such challenges, streptavidin was employed for binding biotin-BMP2 and biotin-SAP (self-assembling peptides). Furthermore, with the help of streptavidin, the degree of BMP2 and biotinylated peptide can be controlled. Intra scaffold retention of BMP2 was also increased by tethering of BMP2 which further prolongs its half-life as well.[168]

A group of scientists also focused on the localized 3D differentiation of BM-MSCs (Bone marrow-derived mesenchymal stem cells) by orthogonal matrix-immobilization of BMP-2. A recombinant Glutamine-streptavidin linker peptide, a bio-mimetic scaffold, was genetically engineered. It was covalently coupled to the TG–PEG (Trans-glutamase) hydrogels. Controlled presentation of recombinant rhBMP-2 with streptavidin coupled PEG hydrogels provides the consequent 3D-localized osteogenic differentiation of BM-MSCs. [169]

5. Conclusion

As one of the strongest non-covalent interactions in the nature, the avidin-biotin interaction has evolved to become a very versatile platform for a great variety of applications in biotechnology and nanotechnology. At a constant pace, new analogues of avidin are being developed with better efficiency and physical-chemical properties to serve specific purposes. As simplistic this system is in its fundamental principle, avidin-biotin system is as strong and applicable. [23] Extensive use of this system in a wide variety of fields, such as drug delivery, antigen targeting, diagnostics, and tissue engineering is the proof of its unparalleled advantages.

Noncovalent interactions including hydrogen bonding, hydrophobic interaction, van der Waals interaction, and ionic interaction have also been utilized for nanoscale drug delivery systems. These interactions are stronger when present collectively but are very weak individually. By comparison, the biotin-avidin interaction employs multiple hydrogen and hydrophobic interactions, leading to an extremely high affinity.[170] On the other hand, the use of ionic interactions to load drug may cause problems in the formulation development. For instance, drug molecules with $–NH_2$ may interact with –COOH to form large aggregates.[171] Ionic interaction can also be influenced by pH, which may affect the stability of nanocarriers in acidic tumor microenvironment.[172, 173] Loading of drug to nanoparticles using hydrophobic interaction may result in particle aggregation, which will affect the biodistribution of nanoparticles in the body. [174, 175] By contrast, nanocarriers made from the biotin-avidin interaction have a uniform size-distribution and quite stable against enzymes, temperature, pH and harsh organic regents.[57, 94]

Tetravalent structure of avidin and its analogues provides a great advantage in the nanoparticle design, development and delivery. It provides a strong backbone for the biotinylated ligand and drug molecules with four biotin binding sites per avidin molecule. This characteristics provides the flexibility in loading drugs and ligands to nanoparticles. [176] Biotin and avidin are also readily available with various functional groups for chemical conjugations. Moreover, the chemical conjugation on biotin or avidin avoids direct modification of the active biomolecule, thus maintaining their activity.

The application of avidin-biotin system is more than just coupling biotinylated molecule to avidin-conjugated moieties. Properties such as higher relative tumor accumulation, immune modulation and easy genetic engineering makes them highly advantageous for a variety of applications in nanotechnology. Particularly in the pretargeting field, the avidin-biotin system is a leap ahead of the conventional radiolabeled antibody approach. Despite of multiple advantages of this system in biotechnology, further research is required to understand the immuno-toxicity of the avidin and its variants. In this review, we have compiled the most recent reports that have utilized this system in various fields of nanotechnology.

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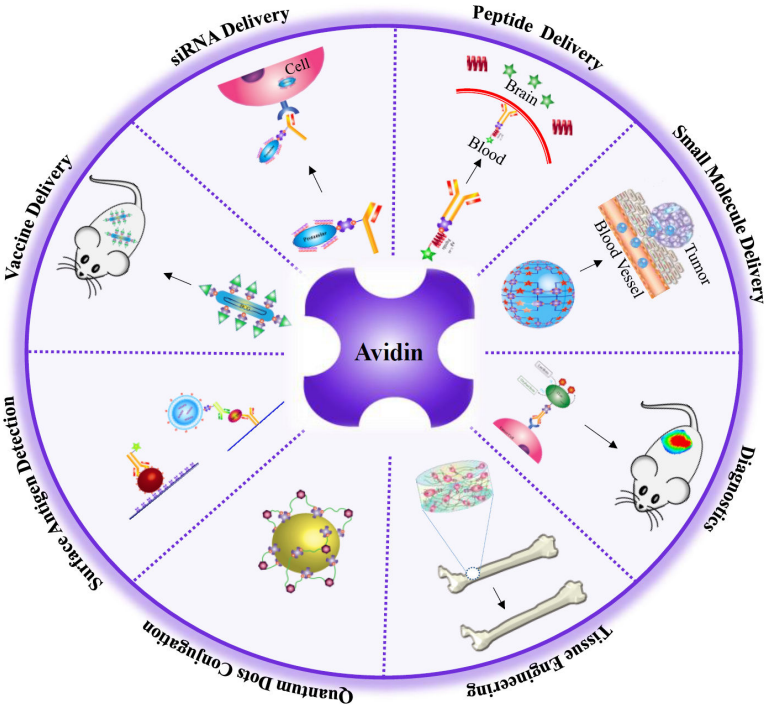

Figure 1.
Avidin-based nanoscale systems in various applications.

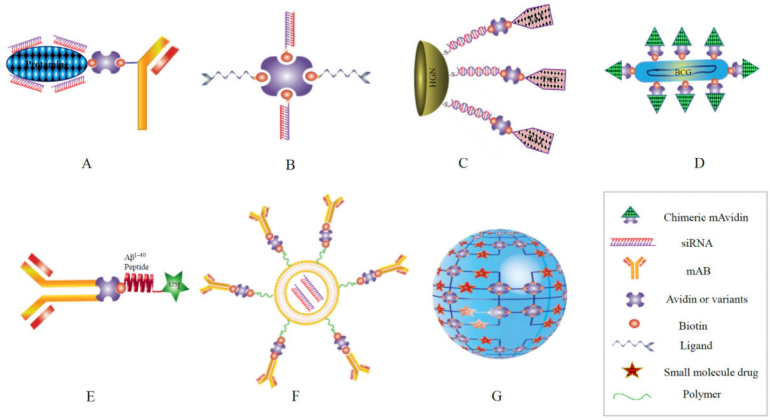

Figure 2. Various fabrication mechanisms of avidin-based nanoscale drug delivery systems

(A) Monovalent anti-podocyte antibody is coupled to protamine through the biotin-neutravidin linkage. Cationic protamine therefor condenses multiple siRNA molecules and deliver the complex to the target podocytes. (B) Biotinylated siRNA and biotinylated ligand are anchored to the same streptavidin backbone. (C) Plasmonic hollow gold nanoshells (HGN) are decorated with siRNA molecules via thiol bond. The other end of the siRNA is biotinylated and coupled to biotinylated TAT peptide through streptavidin. Upon exposure to near infrared (NIR) light (~800 nm), the siRNAs are released from the nanoshells due to gold-thiol dissociation. (D) Bacillus Calmette-Guerin (BCG) bacteria are biotinylated and coupled to a chimeric fusion protein composed of a monovalent avidin (mAvidin) and an antigen. The antigen coated BCG is then inoculated into animals to deliver antigens and subsequently induce specific T cell responses. (E) Radiolabeled $A\beta^{1\text{-}40}$ is biotinylated and coupled to the TfR-MAb-avidin fusion protein. This complex can deliver the radiopharmaceutical across the blood brain barrier. (F) Liposome is functionalized with biotinylated PEG, which is coupled to a biotinylated antibody through streptavidin. (G) Neutravidin-biotin is used to link microhydrogel containing doxorubicin and biotinylated hyaluronic acid.

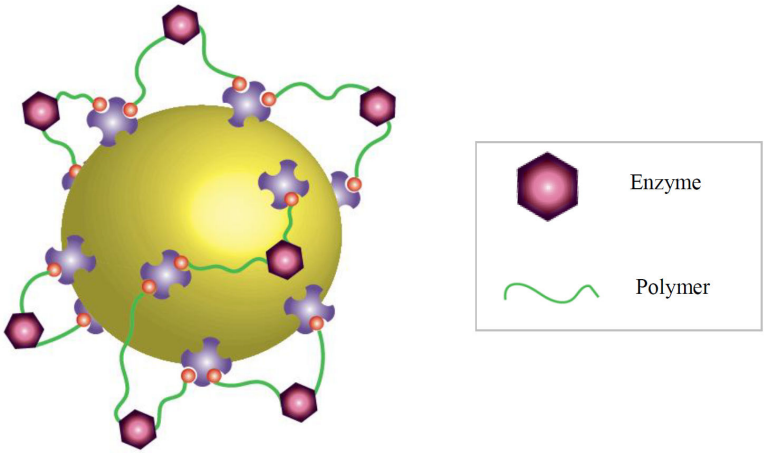

Figure 3. Streptavidin coated Quantum dots modified with biotinylated enzyme

Streptavidin is linked to the carboxylic groups on the surface of the quantum dots. The streptavidin conjugated quantum dots are coupled to biotinylated enzyme via the avidin-biotin interaction for in-vivo tracking of the enzyme activity *in vivo* tracking of antigens like enzyme.

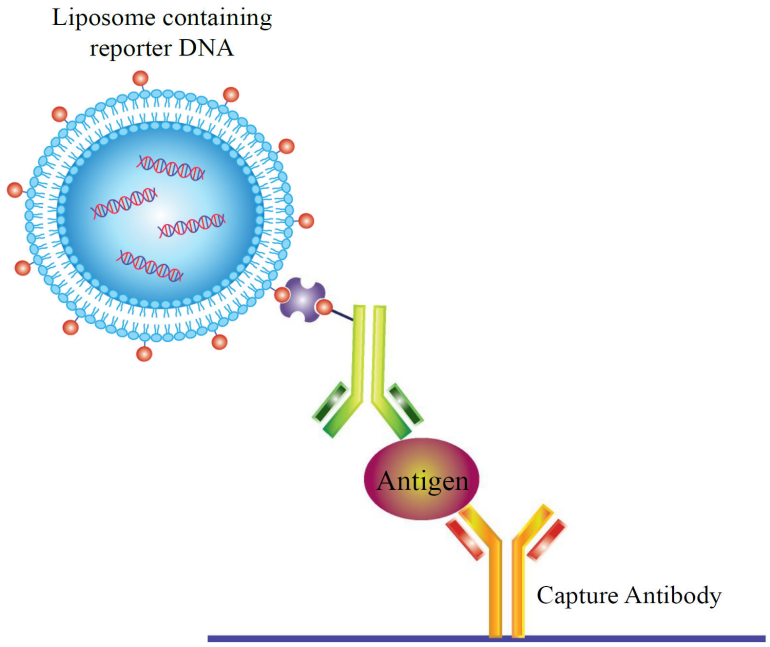

Figure 4. Avidin-based immunoliposome-PCR

Liposomes encapsulating reporter DNA are conjugated to a biotin-PEG (polyethylene glycol) phospholipid that acts as a detection agent. A biotin-labeled secondary antibody with a neutravidin bridge binds to the antigen. Biotin-coated liposomes bind to the neutravidin-bridge and anchor the reporter DNA-loaded liposomes. Upon binding to the target antigen, the liposome bursts to release the reporter DNA, which permits the target protein to be quantified using real-time PCR.

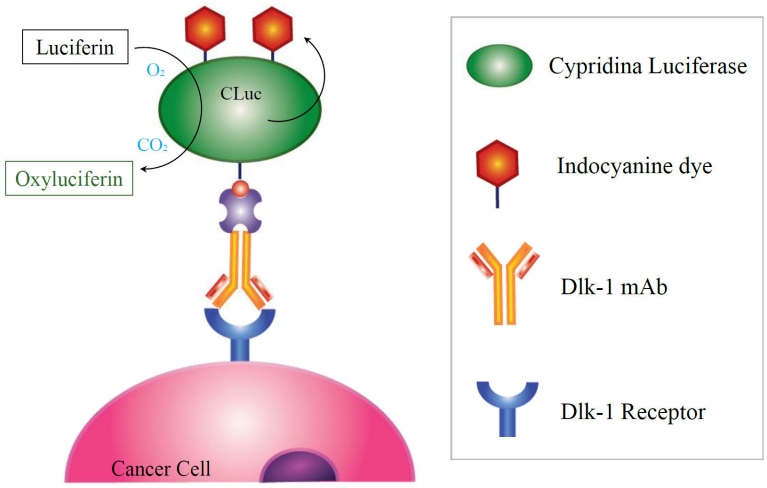

Figure 5. Avidin-based fluorescent bioluminescent protein probe for cancer imaging

A fluorescent bioluminescent protein (FBP) is designed by the conjugation of biotinylated Cypridina luciferase (CLu) to a far-red derivative of fluorescent indocyanine. These FBP is subsequently coupled to the Dlk-1-specific MAb to identify the embryonic cancer antigen Dlk-1 via the avidin-biotin interaction.

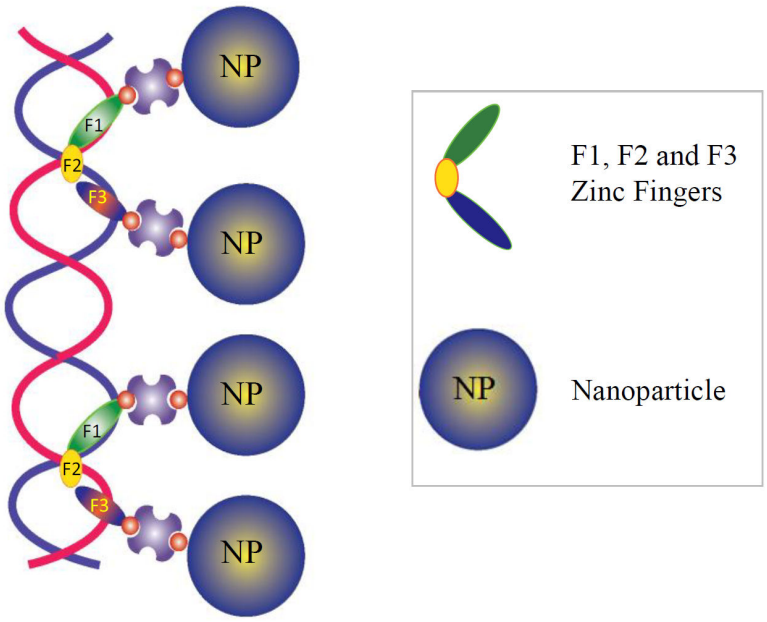

Figure 6. Neutravidin-based nanoparticle clusters for diagnosis and imaging

Biotinylated zinc finger protein is coupled to avidin conjugated nanoparticles. The zinc finger proteins (F1, F2, F3) bind to the DNA template. These zinc fingers and DNA templates are highly sequence specific which helps in the size-controlled construction of NPC for application in diagnosis and imaging.

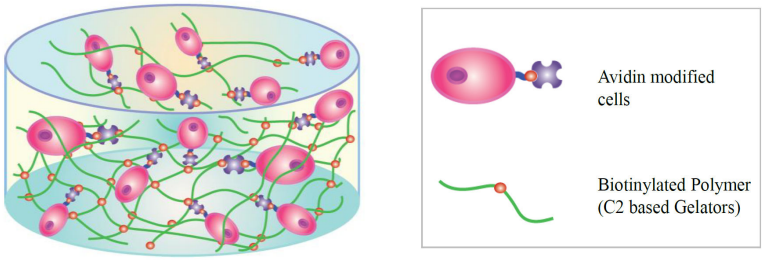

Figure 7. Avidin-based 3D nanofibrous hydrogels for tissue regeneration

MC3T3 osteoblastic, EAhy926 human endothelial, and SMMC-7721 human hepatoma cells were biotinylated with an EZ Links Sulfo-NHS-LC-LC-Biotin. Using avidin as a bridge, the modified cells were in situ encapsulated in a 3d hydrogel system made of biotinylated and nonbiotinylated 1,4-benzyldicarboxamide (C2) based supramolecular gelator.

Table: 1

Physical-chemical properties of Avidin and derivatives

Properties	Avidin	Neutravidin	Streptavidin	Bradavidin II
Origin	Chicken egg	Derivative of Avidin	*Streptomyces avidinii*	*Bradyrhizobium japonicum*
Molecular Weight (KDa)	67	60	53	58.4
Iso-electric Point	10	6.5	5.3–6.5	9.6
Specificity	++	++++	+++	+++
Biotin Binding site	4	4	4	4
Applications	Imaging, Peptide Delivery Mab-Fusion protein Delivery, Vaccine Development, Pre-targeting	Imaging, Pre-targeting, siRNA Delivery, Drug Delivery, Antigen detection	Immunological Assays, Nucleic acid Delivery, Vaccine Development, Mab-Fusion Protein Delivery	Affinity purification