INTRODUCTION

Nanotechnology is a multidisciplinary field which can be defined as the science and engineering involved in the design, synthesis, and application of materials in one-billionth of a meter scale of nanometre. Growth in the field of nanotechnology or bio-nanotechnology has opened several possibilities in medical sciences especially in drug delivery system. Nanoparticle is small object which act as a whole unit with respect to its transport and properties. They are of great scientific interest in drug delivery system because of their promising nature in promoting the efficacy of drug. Nanoparticles are the colloidal particles ranging in size from 10 to 1000 nm in diameter. They are formulated using macromolecular materials in which the therapeutic agent is dissolved, entrapped, absorbed, encapsulated or chemically coupled/attached.

The development of nanoparticle-based drug delivery systems is rapidly growing due to their great therapeutic potential. Various types of materials including polymers, lipids, polysaccharides, and proteins have been explored as drug delivery carriers. The selection of nanoparticle materials is dependent on many factors including (a) the size of nanoparticles needed, (b) inherent properties of the drug such as aqueous solubility and stability, (c) drug release profile desired, (d) surface charge and hydrophobicity of nanoparticles, (e) biocompatibility and biodegradability of nanomaterials, and (f) antigenicity and toxicity of the product. Biopolymer-based nanoparticles including protein nanoparticles have gained considerable interest in recent years due to their many desirable properties such as low toxicity and biodegradability. They are actively being developed for both pharmaceutical and nutraceutical delivery.

The major goal in designing the nanoparticles as drug delivery system are to control particle size, surface characteristics properties, and release of drug agent to achieve the site specific action at therapeutically optimal rate. Nanotechnology has the tremendous potential to make a contribution in the field of medical science by contributing in detection, imaging, prevention and treatment of diseases. The product of bio-nanotechnology are expected to revolutionize modern medical science.

PEPTIDE NANOPARTICLES

Introduction

Peptides are emerging as the new class of biomaterials due to their unique chemical, physical, and biological properties. Peptide based nanoparticles can be of special choice of research scientist as peptides are biocompatible, easy to design and synthesis, less immunogenic, and offer easy surface functionalization properties for targeted delivery. Due to defined primary structure of proteins possibilities for covalent drug attachment and surface alteration is wide.

The major advantages of using protein based nanoparticles in delivery system are as follows:

- Ease to synthesis in large amounts, as the structural and functional nature of protein is well understood and can be modified, if required.
- Being peptidic in nature, expected to be highly biocompatible and safe for human use.
- Possess natural tendency for cell penetration and targeting.
- Peptide offer structural smartness in nanostructures due to their ability to respond to external parameters.
- Due to ease in surface functionalization with different targeting ligands effective drug delivery can be achieved.
Nanoparticles can be used to deliver more than one bioactive molecules with diverse functionalities with multiple epitopes for effective therapy.

Level of functionality in nanoassemblies can be enhanced by the use of non-natural amino acids or functional moieties in the peptide sequence.

Thus, peptides and proteins are the building blocks for making self-assembled nanosystems because of their great deal in folding and stability engineering making them good for potential biological applications.

**TYPES OF PROTEINS USED FOR PREPARATION OF PROTEIN NANOPARTICLES:**

Proteins are the building blocks of natural molecules which possess unique properties making them a potential candidate to be used in medical science. Peptide nanoparticles are successfully derived from water soluble proteins (e.g., bovine and human serum albumin), insoluble proteins (e.g., Zein and Gliadin). Preparation of protein nanoparticles has been successfully reported from daily consumed soy and milk proteins.

**Gelatin**

Gelatin is one of the protein materials used for production of nanoparticles. The major fact of using gelatin as nanomaterial is that it is biodegradable, non-toxic, easy to crosslink and to modify chemically thus possessing immense potential to be used for preparation of drug delivery system [Jahanshahi et al., 2008]. The high content of amino acids glycine, proline, and alanine, is its key characteristic feature. It also has many ionizable sites for conjugation or modifications. The release of drug from gelatin nanoparticles is highly dependent on degree of crosslinking, such crosslinking is obtained by addition of chemical crosslinking agent glutaraldehyde improving the integrity and performance [Jameela et al., 1995]. These properties make gelatin a promising carrier system for drug delivery.

**Albumin**

Albumin is the major macromolecular carrier protein and many endogenous molecules and drugs bind to albumin, as it is obtained in variety like BSA (bovine serum albumin), ovalbumin and human serum albumin (HSA). This protein is freely soluble in water and diluted salt solution. High solubility of albumin at pH 7.4 makes it an attractive carrier capable of accommodating a wide variety of drugs [Kartz et al., 2008]. Albumin nanoparticles are biodegradable and easy to synthesis in different sizes. It also carry advantage that ligands can be easily attached by covalent linkage. The macromolecular carrier albumin can release drug by protease digestion.

**Elastin**

Elastin is an essential component in connective tissues, formed through lysine-mediated crosslinking of its precursor tropoelastin. Alpha-elastin and elastin-like ploypeptides are the two types of elastin derived polypeptides used in drug delivery system.

**Gliadin**

Gliadin is extracted from the gluten of wheat, which exhibits bioadhesive property and has been widely explored for oral and topical drug delivery applications. It is used for nanoparticle preparation because of its biocompatibility, biodegradability, and natural origin. This protein is rich in neutral and lipophilic amino acid residues. It enhances both humoral and cellular responses [Nakaoka et al., 1995].

**Zein**

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Zein is a class of prolamine protein found in maize. It is one of the best understood plant protein. It is widely used in coating or encapsulation of drugs. It is a GRAS polymer approved by FDA for human application. Nanoparticles from zein proteins have been prepared, and in vitro release of coumarin was reported using zein nanoparticles (Podaralla et al., 2010).

**WHEY PROTEINS**

Whey protein concentrates (WPC) and whey protein isolates (WPI) are industrially produced as food protein ingredients. The use of whey protein and specifically BLG (whey protein hydrogels (Y.Qiu et al., 2001). BLG is highly efficient in ability to bind hydrophobic constituents, thus making it suitable candidate for preparation of drug delivery carrier for lipophilic compounds.

**PREPARATION OF PEPTIDE NANOPARTICLES:**

The Methods of Preparation of protein nanoparticles is based on balancing the attractive and repulsive forces in the protein. The crucial property for the formation of protein nanoparticles depends on increasing protein unfolding and decreasing the intramolecular hydrophobic interactions (Gunasekaran et al., 2006). During particle formation, the protein undergoes conformational changes depending on its composition, concentration, cross-linking, and preparation conditions such as pH, ionic strength, and type of solvent. Usually, surfactants are required to stabilize the nanoparticles of water-insoluble proteins such as gliadin. The basic methods for preparation of peptide nanoparticles are as follows:

**EMULSIFICATION METHOD**

In this method, an aqueous solution of the protein is emulsified in oil by using high speed homogenizer or ultrasonic shear and the nanoparticles are formed at the W/O interface. Phosphatidylcholine and Span 80 are added as stabilizers to produce nanoparticles (L.Yang et al., 2007). The oil phase is then removed using an organic solvent, thus forming nanoscopic proteinaceous particles where the size of the internal phase determines the ultimate size of particles. The emulsion-based method has been used to prepare a variety of protein nanoparticles including albumin and whey protein nanoparticles.

![Figure 3.1: Preparation of protein nanoparticles by emulsion/solvent extraction method, (Courtesy: Warangkana et al., 2014)](image)

*For example:* The process of preparation of albumin nanoparticles with emulsification method involves, formation of aqueous solution from albumin into emulsion at room temperature. Later by mechanical homogenization with high speed, homogenous
emulsion is obtained, which will be added to a high volume of pre-heated oil drop by drop. This process will result in formation of nanoparticles.

**Disadvantages of emulsion method:**

- Need for applying organic solvents for removal of oily residues.
- Need of surfactants for emulsion stabilization.

**DESOLVATION / COACERVATION METHOD**

In this method, particles in aqueous will formed by coacervation process and later on will be stabilized by cross linking agent. Coacervation or desolvation is the method based on the differential solubility of proteins in solvents. The process of desolvation reduces the solubility of the protein leading to phase separation. precipitation or coacervation of the protein is achieved by addition of desolvating agent. After the formation of the nanoparticles, they are crosslinked by agents such as glutaraldehyde and glyoxal [Coester et al., 2000]. Increase in antisolvent/solvent ratio decreases the particle size due to rapid diffusion of the solvent into antisolvent phase, which limits the growth of particles. The effects of several factors on formation of nanoparticles have been studied, especially with albumin nanoparticles.

![Diagram](image)

**Figure 3.2:** Preparation of protein nanoparticles by desolvation method, (Courtesy: Warangkana et al., 2014)

Drugs can be loaded into the protein nanoparticles by surface adsorption or by entrapping the drugs in the particles during preparatory phase.

**BIOCONJUGATION OF PEPTIDES TO THE NANOPARTICLES**

The goal for the bioconjugation is to uniformly display the peptides on the Nanoparticle surface with their active regions all clearly extended away and available for activity.

**Four general schemes commonly used for the conjugation of peptides to nanoparticle materials are as follows:**

(A) Electrostatic interactions use opposite charges on the surface of the NP and the peptide to mediate charge-charge-based NP-peptide assembly [Rozenzhak et al., 2005].

(B) Direct interaction involves certain peptide motifs that can bind to/coordinate with the NP surface with high affinity. Examples include the interaction of free thiols with the surface of Au-NPs and the high-affinity coordination of polyhistidine tracts with NPs (e.g., QDs) with Zn2+-bearing surfaces (Pinaud et al., 2004).
(C) Secondary interactions utilize specific ligand-receptor interactions and are almost completely exemplified by the biotin–streptavidin interacting pair. The incorporation of the biotin moiety at the peptide’s terminus can mediate directional assembly of the peptide with the nanoparticle (Invitrogen corp 2010).

(D) Covalent attachment linkages utilize classical bioconjugation chemistry such as EDC-based coupling of amines to carboxyls and NHS- and maleimide-mediated conjugation to amines and thiols. Bt: Biotinylate; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; NHS: N-hydroxysuccinimide (Hermanson et al., 2008).

APPLICATIONS

PEPTIDES IN THERAPEUTICS DELIVERY OF NANOPARTICLES:

Most peptide nanostructures are based on relatively longer peptides and concerns about their stability under in vivo conditions is perhaps the main reason for most drug delivery studies being limited to in vitro conditions. Researchers involved in the development of therapeutics have understood, that the fundamental part of drug development is the drug delivery system. Drug delivery system should be designed in order to improve the stability, absorption, and the therapeutic concentration of the drug within the targeted and long term release of the drug at the targeted sites.

The most commonly used peptides that have been employed for the cellular delivery of Nanoparticles and their therapeutic applications are as follows:

TAT PEPTIDE

The first CPPs (cell penetrating peptides) to be described was TAT peptide. It is peptide sequence of 11-mer (YGRKKRRQRRR) bearing positively charged residues i.e. 2 lysine residues, 6 arginines which are determinants of cellular uptake initiating peptide interaction with negatively charged cell surface (Vives E et al., 2003). The uptake of TAT and TAT-like peptides and associated cargos have been proposed with number of mechanisms. In many cases, the TAT peptide and its derivatives have been implied for therapeutics applications by delivering a variety of nanoparticles on cellular level.

APPLICATIONS IN DRUG DELIVERY:

- TAT peptides have been used effectively for cellular targeting of drug carriers and their associated cargos.
- pH-responsive drug-carrying vehicles to acidic solid tumors was targeted by TAT in a ‘smart’ micelle-based system, described by (Sethuraman et al., 2008). In-vitro testing of the pH sensitivity was done in MCF-7 breast cancer cells, where at pH 6.6 cellular entry and localization within nucleus was effective while at pH 7.4 observation showed no uptake of drug vehicle (Liu et al., 2009).
- Liposome drug vehicle mediated by TAT peptide was exposed by a pH responsive drug delivery Nanoparticle using TAT peptide (Liu et al., 2009) demonstrated the imaging of injury site by utilizing nanoparticles, PEGylated liposome nanoparticles was formulated loaded with an iron and were conjugated to TAT and shown to cross blood-spinal cord barrier (BSCB) in a rat model (Liu Y et al., 2000).
- TAT peptide was able to import 90 nm beads into the nuclei of digitonin-permeabilized cells, suggesting that its interaction with the nuclear envelope follows a mechanism different from that of NLS (Nitin et al., 2009).
- A peptide issued from the Bcl2 homology domain 4 (BH4) or Bcl2/Bclxl protein associated with Tat can regulate apoptosis and induce cytoprotection in vivo (Sugioka et al., 2003).
- Survivin mutants associated with Tat facilitate apoptosis in cancer cells (Wadia et al., 2004).
- TAT -Bclxl protein can be delivered into mouse brain to decrease neuronal cell death in the area of ischaemic damages (Cao et al., 2002).
Tat-δV-1 peptide, a selective inhibitor of PKCγ has been reported to attenuate heart ischaemia (Bright et al., 2004).

TAT peptide has also been used for the delivery of modular antigen molecules useful for treatment of allergy and vaccine production (Rhyner et al., 2007).

**RGD PEPTIDE**

The first peptide domain to be identified as cell recognition sequence in the extracellular matrix protein fibronectin was the short RGD peptide domain (Engel J et al., 1981). Later on this sequence was found in many more extracellular matrix proteins like osteopontin, collagen, vitronectin, laminin and fibrinogen. The interaction between the cell surface receptors containing alpha and beta subunits combine in a pairwise manner to bind to different extracellular matrix proteins. The cell-substratum and cell-cell interaction is mediated by binding of integrins to extracellular matrix proteins is involved in regulations of cellular homeostasis functions as motility, differentiation, cell growth and angiogenesis (Hood JD et al., 2002). Thus, implication of RGD – ligating interins has been seen in various pathological conditions from the platelet response to variety of cancers (Desigrosellier et al., 2010). RGD-functionalized nanoparticles targeting integrins has been used in various multimodal combination of gene therapy, drug delivery, diagnostic imaging, implants and tissue engineering.

**APPLICATIONS IN DRUG DELIVERY**

- Pharmacokinetics may be improved when RGD peptides are attached to nanoparticles, for example increasing their half-life (Montet X et al., 2006).
- Used to deliver doxorubicin and paclitaxel via PLGA-nanoparticles thus exemplifies it as the effective delivery vehicles for chemotherapeutic agents (Murphy et al., 2008).
- Degree of successful drug delivery was observed in treated animals, increasing the survival and anti-metastatic activity.
- When compared with systemic delivery, lower drug-induced weight loss was observed for nanoparticle-delivered drug.
- Improved tumor suppression capability was reported by use of protease-cleavable RGD peptide (iRGD) as exemplified by use of Abraxane, a nanoparticle version of paraxel has been targeted to tumors in vivo (Sughara KN et al., 2009).
- RGD-CH-NP loaded with siRNA significantly increased selective intratumoral delivery in orthotopic animal models of ovarian cancer (Han HD et al., 2010).
- A cell chip composed of ITO, gold nanoparticles (GNP) and RGD-MAP-C peptide composites was fabricated to enhance the electrochemical signals and proliferation of undifferentiated human neural stem cells (HB1.F3) (Tae hyung kim et al., 2013)
- Genetic incorporation of RGD peptides on the coat protein of TMV particles and the resulting mutant virus could enhance the adhesion of BMSCs and accelerate the stem cell differentiation. (PongKwan Sitasuwan et al., 2014)

**PEP-I PEPTIDE**

It is a synthetic peptide rationally designed into a single peptide moiety capable of cellular entry incorporating three different functional domains. It is a well-known cell penetrating peptide that transports full length proteins to the nucleus of the cell. It is also a short amphipathic peptide consists of hydrophobic tryptophan-rich domain and a hydrophilic lysine-rich domain separated by a spacer. For efficient cellular uptake and peptide stability, the synthesized peptide is modified by acetylated at is N-terminus and at c-terminal it bears a cysteamide group (Simeoni F et al., 2003). Pep-1 localizes rapidly to the nucleus of
the cell via an endocytosis-independent internalization process, demonstrated by the efficient uptake of Pep-1-cargo complexes at 4°C (Morris MC et al., 2007).

APPLICATIONS OF IN DRUG DELIVERY

- An et al., demonstrated the ability of the therapeutic fusion protein to cross the BBB, by taking the purified recombinant of Pep-1-HSP-27 fusion protein in bacteria conferring protection against cell death induced by oxidative stress[An et al., 2008].
- In rat model of spinal cord injury, the decreased levels of intracellular reactive oxygen species(ROS) and inhibition of pro-apoptotic enzymes caspase-9 and caspase-3 was demonstrated by using the Pep-1 fusion protein (Yune et al., 2008)
- Pep-3 peptide, a variant of Pep-1, was successfully applied to the delivery of PNA and analogs targeting the cell cycle regulatory protein cyclin B1 in vitro and in vivo (Morris et al., 2004b2007b).
- Pep-1 strategy was also applied to the evaluation of the antitumoural activity of peptide inhibitors of protein kinases or to repair a defective step in a cellular signalling pathway in vivo (Gros et al., 2006; Morris et al., 2008).
- Pep-1 technology has also been demonstrated to be a potent strategy to deliver therapeutic proteins in vivo and across the blood brain barrier (Gallo et al., 2002; Aoshiba et al., 2003; Gallo, 2003; Maron et al., 2005; Gros et al., 2006).
- Pep-1 strategy has been applied in vivo to the delivery of proteins into the lungs of mice to produce alveolar wall apoptosis or to correct defects in protein kinase A function (Aoshiba et al., 2003; Maron et al., 2005).

NEUROPEPTIDE

Allostatin , Ast 1 (APSQAQRLYGFGL) a member of a family of 13 neuropeptides found in insects has been used to deliver Nps to mammalian cells. Their whose receptors are highly homologous to somatostatin and galanin receptors. (Lenz et al., 2000).

Applications:
- Biju et al. identified the peptide’s ability to mediate the rapid uptake of commercial streptavidinconjugated QDs after its assembly onto the QD via an avidin–biotin interaction linkage .
- In NIH 3T3 and A431 cells the QDs were endocytosed and localized primarily within the endosomal compartments

ORGANELLE TARGETING PEPTIDE

- Peptide NPs are used to deliver NP to a particular subcellular location or organelle with materials being directed to either the nucleus or the mitochondria.

Application
- The mitochondrial targeting peptide derived from cytochrome C oxidase
- (MSVLTPLLLRLGTSVESARRLPVPRAKIHWC) (Hosino et al.,2004)

RABIES VIRUS-DERIVED PEPTIDE

- These peptide conjugated NPs can prove ot be promising agent for treatment of neurological diseases
- a 29-amino acid peptide RVG29( TYIWMPPRPFGTPCDFTNSRGKRASNG) derived from the rabies virus glycoprotein (RVG) has been used for the transvascular delivery of a small interfering RNA (siRNA) to the brain (Kumar et al., 2007). This peptide bound with high specificity to the acetylcholine receptors (AchR) expressed on neuronal cells,
RVG peptide conjugated to PEGylated PAMAM dendrimers that were subsequently self-assembled with plasmid DNA encoding create PEG/PAMAM/DNA NPs (Liu 2009). Upon intravenous injection, these NPs concentrated in the mouse brain within 80 min.

<table>
<thead>
<tr>
<th>Compartment upon initial uptake</th>
<th>PEPTIDE</th>
<th>SEQUENCE</th>
<th>CARGO TYPES/NANOPARTICLES</th>
<th>Targeted cells</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT</td>
<td>YGRKKRRQRRR</td>
<td>Gold particles, Ph-sensitive liposomes, Iron-loaded liposomes, Iron oxide NPs, Quantum dots</td>
<td>Human embryonic kidney, African monkey kidney, Hela and murine mesenchymal stem cells, MCF-7</td>
<td>Human breast cancer cells, U-87 MG astrocytoma cells, mouse neural progenitor cells</td>
<td>In-vivo tracking and recovery of progenitor cells, diagnosis and targeted therapy of diseases, delivery of CGRP transgene in treatment of cerebral vasospasm, act as membrane shuttle</td>
</tr>
<tr>
<td>RGD</td>
<td>Arg-Gly-Asp</td>
<td>Iron oxide NPs, Chitosan NPs, PLGA NPs, Gold particles, Viral nanoparticles</td>
<td>Mouse xenografts, mouse tumor vasculature, endothelial cells, neuronal</td>
<td>Tumor targeted delivery system for siRNA, Targeting of tumor endothelium, targeted Doxorubicin delivery, enhance electrochemical</td>
<td></td>
</tr>
</tbody>
</table>
Table 1: Representative nanoparticles delivered into cells by peptide

<table>
<thead>
<tr>
<th>Peptide Type</th>
<th>Peptide Sequence</th>
<th>Nanoparticles/Delivery Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organelle-specific peptide</td>
<td>(MSVLTPLLRLGTGSARRLPVRAKH)</td>
<td>Quantum dots (20–50) Vero cells Sub cellular labelling, microchondria</td>
</tr>
<tr>
<td>Neuropeptide (Aβ1)</td>
<td>APSGAQRLYGFGL</td>
<td>Quantum dots (15) Fibroblasts (NIH 3T3 and A431 Cellular labeling)</td>
</tr>
<tr>
<td>Rabies virus-derived peptide</td>
<td>TYIWMPENPRPGTCIFTNSGKRA</td>
<td>siRNA Brain capillary endothelial cells (in vivo)</td>
</tr>
<tr>
<td></td>
<td>SNG)</td>
<td>PAMAM-PEG/plasmid DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in vivo gene silencing (in vivo gene expression)</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The multidisciplinary field of nanotechnology is expected to bring a vast change in pharmaceuticals fundamental system of drug delivery. There is a great need to identify nanoparticle material that are safe and effective in delivering therapeutic agents to the target sites. Protein nanoparticles are the highly promising materials for the construction of nanostructures. When Drug delivery system through nanomaterial is considered, the key parameter is the size of the nanoparticle, which influence
degradation, uptake and clearance from the body. Furthermore a close collaboration between those working in drug delivery and particle production is necessary for the exchange of concepts, methods and how to contribute in field of health care.

Abbreviations: NP: Nanoparticle; PAMAM: Poly(amido amine); PEG: Polyethylene glycol; PEI: Polythelenimine; PL

REFERENCES


43. Tae-Hyung Kim et al., “ITO/gold nanoparticle/RGD peptide composites to enhance electrochemical signals and proliferation of human neural stem cells”, Nanomedicine, April 2013 vol:9