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Review Article

IMMUNONANOPARTICLES A NOVEL APPROACH FOR DRUG DELIVERY

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ABSTRACT

Immunoglobulin, commonly known as antibody are natural biological milieu produced from vertebrates, act as therapeutic entities which get initiated with the development of monoclonal antibodies. Using the antibodies as delivery conduits for targeted site, a number of immunotherapeutic agents prevail as the marketed products. At the same time, nano-carriers are one of the versatile, self-engineered and potentially promising, site-specific commercialized tool trending in the 21st century. In this overview, a jot down of antibody with pros and cons as well as innovativeness of nanoparticles and nanotechnology has been discussed with a limelight on immunoglobulin and its conjugation with nano-carrier for central nervous system (CNS) delivery system. Since CNS delivery system mainly lacked by the circumvention of blood–brain barrier (BBB), a highly protected and secured region where the entry of foreign particles are strictly restricted due to the self-controlling security of the highly tactful brain, a need of new approach is a vital issue to overcome all the restrictions. Conjugation of antibody with nano-carrier representing, the immunonanoparticles are new and innovative tool showing efficacy exerting desired biological effects accessing through the BBB. The detail whereabouts of the new tools have been discussed in the different form of antibody conjugated nano-architectures with their ease in treating diseases circumventing BBB with *in vivo* characterization. Thus, various immune nano-architectures have been discussed with their potential effect, method of preparation and limitation creating an image of new nano-biological mimetic, effective for site specific delivery with effective biological behavior, and safety profile useful for commercialization economically.

Keywords: Immunonanoparticles, Immunotherapeutics, Biological mimetic.

INTRODUCTION

With the course of time, the nanotechnology mediated therapeutic drug delivery has been the emerging trend with a perceptive of overcoming the major difficulties in administering drug to the targeted site. The disease perpetuates to be the major impact on human well-being affecting a large population. In the last few years, mortality has decreased due to the advancement of therapeutic and diagnostic advancement still effective therapy is yet to prosecute. Even though there are pros and cons in any drug delivery system, nano-carriers tends to have more efficacy in terms of therapeutic value with rationality in active as well as passive circumvention encountering any biological barriers. The nominal toxic effect is the additional advantage resulting in new hope to cure the diseases.

On the other side, antibody conciliated therapeutic drug delivery is also potential targeting approach since it being the naturally occurred in vertebrates. More specifically the monoclonal antibodies are used in therapeutic uses, having clinically diagnostic report for many years, but now-a-day, these tools are being licensed for therapy and treatment purpose. These tools have reported to be the promising cure to the specific diseases and often as adjunct to standard treatment yet not without side effects [1].

However, the nano-carriers being multifaceted, regardless of their size, have the capability to conjugate with ligands, immune elements, drugs as well as other complements improving the therapeutic and diagnostic properties. Hence, the conjugation of antibody with nanoparticles combines therapeutic effects having rationality over the other drug delivery systems. Thus, immunonanoparticle emerges as a promising tool for CNS drug delivery. Immunoglobulin, i.e., antibody when gets conjugated with nanoparticles, the hybrid artefact shows a versatility having biocompatible, long shelf life exhibiting high differential target efficacy. This hybrid one has specific and selective recognition site offering specific physiochemical and physical properties having great impact in treatment. Although this tool is in the development phase, in the next coming years, this will one of the promising deliveries to cure most of the devastating diseases. This review demonstrates the innovativeness of artefact drug delivery system and its designing including the biasedness and rationality of this tool over another delivery system. Furthermore, the preparation methodology and clinical aspects have been discussed keeping in mind the societal and economic benefits for the purpose of commercialization.

ANTIBODY

Antibody (anti-foreign bodies) also known as immunoglobulin is one of the most important constituents of specific defense mechanism in vertebrate animals, particularly in jawed vertebrates.

- It is a "Y" shaped structure.
- It is synthesized by host β lymphocytes as matured in bone marrow.
- Induction occurs to immunogenic molecules when introduced in the host's lymphoid system.
- It is basically a protein, more specifically a glycoprotein or globulin. It is present in tissue fluid or serum in blood or lymph produced by plasma cells secreted by white blood cells (W. B. C.), more specifically β cells or β - lymphocytes of all vertebrates in response to an immunogen (antigen) [2,3] and bind specifically to induce their formation (Fig. 2).
- It is a bi-functional molecule containing specific neutralizing substance or antitoxins.
- Since serum components being separated electrophoretically, the heterogeneity of the immunoglobulin is being observed in α, β and γ fraction.
- Most the antibody molecules are present in γ fraction, hence referred as γ - globulin.
- Act as foreign antigen (generating antibodies) when come in contact with foreign infectious microbes.
- Tip of each antibody contains a structure analogous to lock, known as paratope which is specific to a structure analogous to key on antigen known as epitope (antigenic determinant).

- The paratope of an antibody and epitope of an antigen combine together to tag a microbe and protect the immune system.

Antigen - It is defined as the foreign substance that elicits am immune response. Any antigen which produces an adaptive response after injecting into vertebrates and it is termed as immunogen. This immunogen is designed so that antibodies generate specific proteins [2,4].

The factors on the basis of which an antibody binds with an antigen are as follows:

I. During infection, the pathogen should express the antigen
II. On pathogen surface, either the antigen is secreted or found
III. In all strains of the pathogen, expression of antigen is must
IV. A host immune response should be elicited by antigen, and
V. The survival of pathogen is only due to antigen, at least while it is in the host (Fig. 1).

Structure of antibody

I. Chemical nature - protein/polypeptide/globulin.
II. All antibody molecules composed same basic four polypeptide chains having a symmetrical molecular structure comprised of two identical halves with the antigen binding sites formed between the ends of the heavy and light chains on both sides.
    - Two identical heavy chains.
    - (H-Chains) - 55-70κD [5] also called longer chains.
    - Two identical light chains.
    - (L-Chains) - 24-25κD [5] also called as shorter chains.
III. Bonding between chains - disulphide bond (s-s) and non-covalent interactions [2].
    - The two heavy chains are connected together by [3-6] disulfide bond (s-s) and non-covalent interactions.
    - Each of the light chains is attached to the heavy chains by the [3-6] disulfide bonds (s-s).
    - Intracatenary di-sulfur bridges are also present along with di-sulfur bridges which give stability to the molecule [2,6].
IV. Each of the heavy chain and the light chain is a repeating homologous unit of amino acids forming a globular motif.
V. The heavy chains vary in molecular weight that determines the isotypes and designated by α (alpha), δ (delta), ε (epsilon), γ (gamma), μ (mu), respectively, as per classes of immunoglobulin.
    The light chains having a constant molecular weight are those having two types designated by κ (kappa) and λ (lambda) present in the ratio of 2:1 in human serum, out of which only one type is found in each antibody.
    N.B.: Immunoglobulin serotype is determined basically by the type of heavy chain present. Variations of heavy chains are observed in different isotypes or classes of antibody or immunoglobulin having distinct structures and antigenic property (Fig. 3).
VI. Heavy chain: - It being fissures into domains of approx. same size, but the number vary between 5 isotypes of heavy chains. Although in two domains, it has been divided consisting of (a) constant region and (b) variable region.
    a. Constant region - It is the constant region being similar for same isotypes, designated by C_H. Although there are three or four constant domains depending on the isotypes - C_H1, C_H2, C_H3, CH4 [2,7].
    b. Variable region - It is the variable domain showing considerable sequence of variation. It is abbreviated by V_H (Fig. 4).
    c. Hinge region - The area between C_H1 and C_H2 of heavy chain having a variation of inter-chain di-sulfide bonds.
VII. Light chain: It is consist of two distinct domains of 110 amino acids approx. The two distinct domains consist of (a) constant region and (b) variable region.
    a. Constant region - It is the identical one end to all membrane of the same isotype. It is designated by C_L.
    b. Variable region - It is of considerable sequence of variation designated by V_L.
    Variation of antibody is found mostly in these variable regions of both the light chains and heavy chains where antigen binding function takes place. Difference in sequence is largely confined in three short stretches known as hypervariable region or complementary determining region (CDRs). More specifically three CDRs in V_L domain and three CDRs in V_H domain [1,2,7,8]. These regions are structurally separated by FRW (framework), highly conserved sequences [1,2,7,8].
    The hypervariable region, also known as recognition sites, has unique amino acid sequence varying with antigen specificities having varying degree of strength because antibody make complementary contact with antigen. Thus, this CDRs is responsible for the amino acid sequence to which an antigen is recognized by a given antibody.
VIII. Paratope: It is the antigen combining site, a tertiary structure formed by the combinatorial of VL and VH situated at the amino (N) terminal [5]. Each antibody has two paratopes, which is highly specific.
IX. Epitope: The antigenic determinant where an antibody binds. It is a polypeptide chain usually of 5-8 amino acids long on surface protein, exist in solution or its native form [4]. Depending on epitope, an antibody recognizes and may bind only in fragments or denatured segments of protein. It may also have the ability to bind with native protein.
X. Each antigen binding region of antibody is highly specific and varies with classes and isotypes since each antibody has a distinct specificity (Fig. 6).

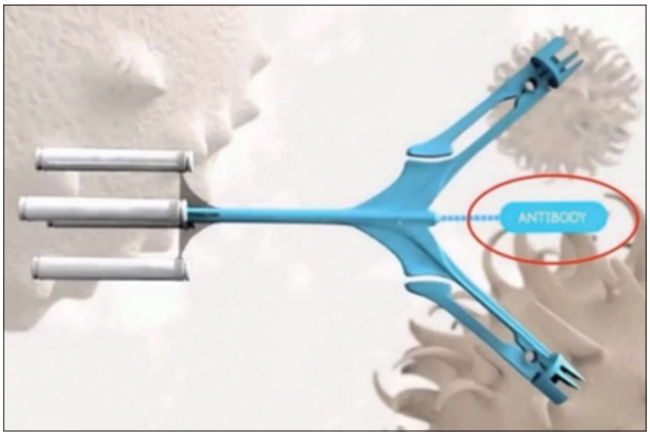


Fig. 1: Antibody [49]

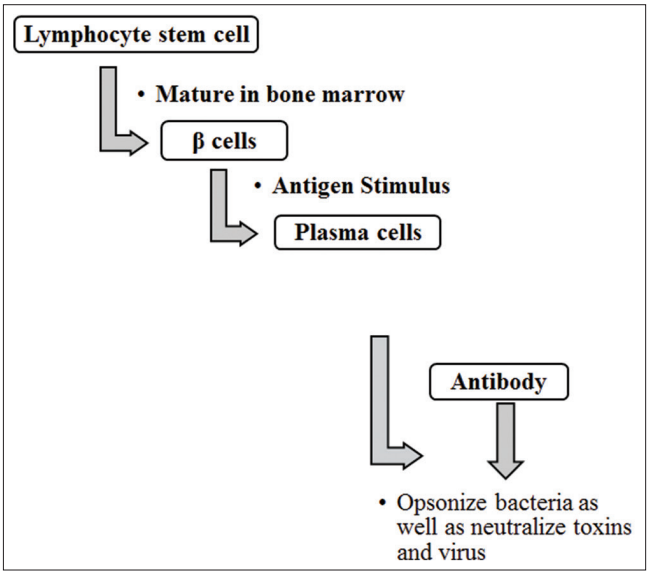


Fig. 2: Schematic description of synthesis of antibody

XI. Structurally antibody consist of two end terminals;

(a) amino - (N) - terminal: It is terminal end of V_H and V_L where the paratope is present - the antigen recognizing site. Since the light chain and heavy chain are identical, each antibody has identical paratope.

(b) Carboxyl - (C) - terminal: It is the biological activity constant terminal of both the heavy chains and the light chains (C_L and C_H), same for all members of same class and isotypes (Fig. 7).

XII. Ig is being cleaved by number of enzymes namely papain, pepsin, etc. resulting in cleavage at distinct points generating peptide fragments. The bi-functionality of immunoglobulin molecule is due to (a) two identical domains for antigen recognition known as Fab fragment and (b) two identical domains with effector function known as Fc fragment.

a. Fab region - Two identical fragment antigen binding region or antigen recognition domain containing paratope.

b. Fc region - Fragment crystallisable domain, the backbone structure similar for all antibodies including the isotypes. This consists of two identical domain with effector function including secondary biological action such as phagocytosis and activating complement (Fig. 3).

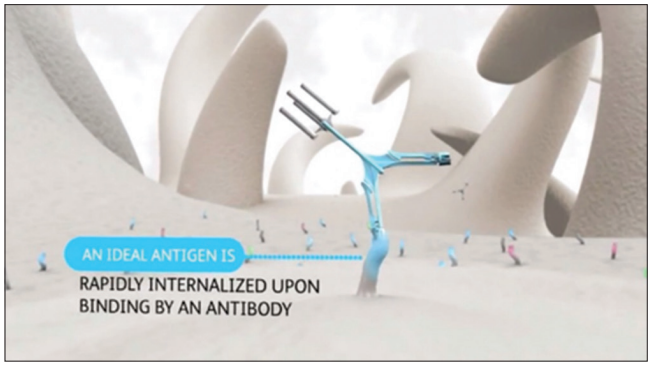


Fig. 3: Recognition site [49]

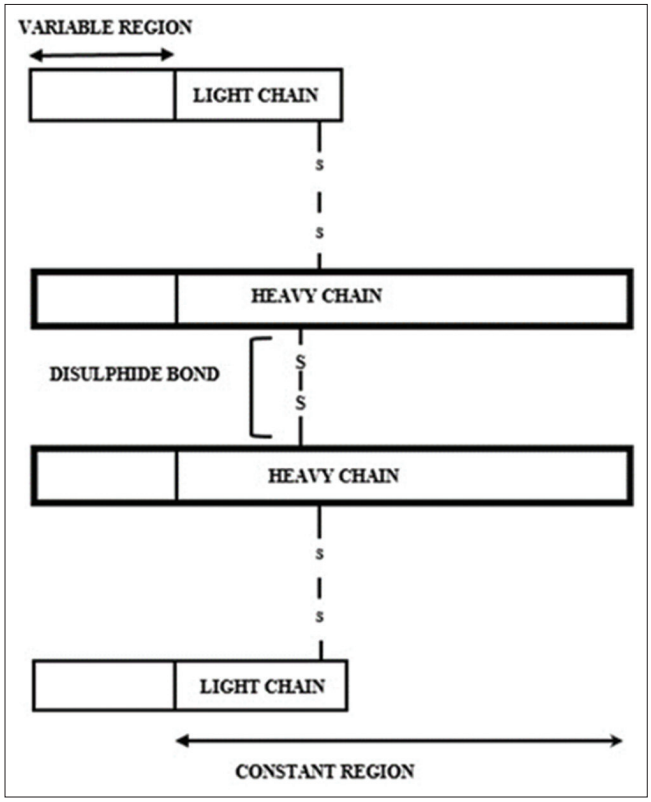


Fig. 4: Basic structure of antibody

Basically, enzymes like papain when cleaved an Ig, produces two univalent antigen-binding fragments - Fab and a portion of Fc having C_H2 and C_H3 heavy chain domains. Pepsin enzyme also undergoes cleavage in Igs, but mostly in IgG isotype inducing a divalent antigen binding fragments (Fig. 5).

Although there is difference between various isotypes of antibodies, *yet al*l antibodies are of same fundamental structure with Fab portion having the antigen recognition paratope present in the amino - (N) terminal chain and Fc region performs the activities protecting the host- effector functions present in the carboxyl - (C) - terminal chain.

Classification of antibody

There are basically five classes or isotypes depending upon the heavy chains of antibody generated in vertebrate animals:

- IgA: Immunoglobulin alpha (α)
- IgD: Immunoglobulin delta (δ)
- IgE: Immunoglobulin epsilon (ε)
- IgG: Immunoglobulin gama (γ)
- IgM: Immunoglobulin mu (μ)

Above mentioned naturally occurring classes vary in size, charge carbohydrate content and amino acid composition (Fig. 8). Within these

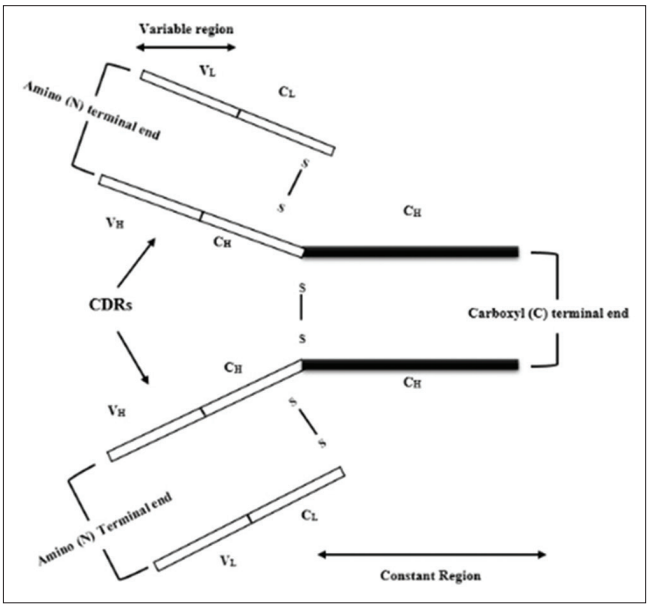


Fig. 5: Complementary determining regions

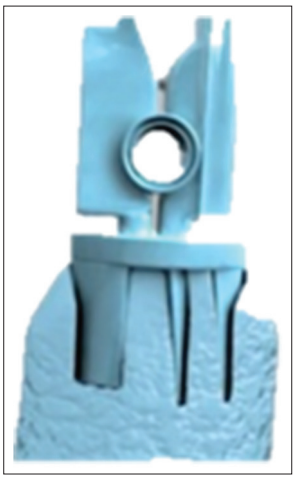


Fig. 6: Binding site [49]

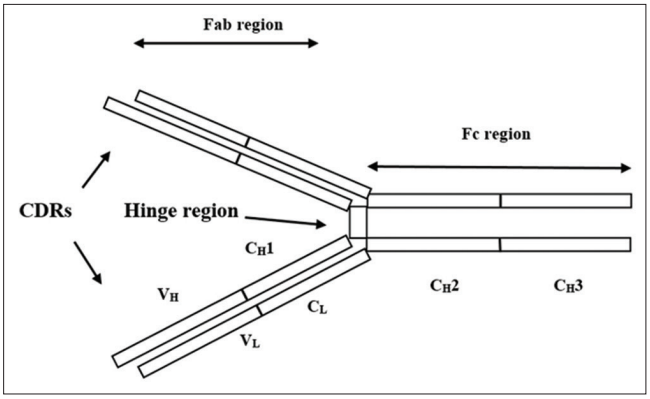


Fig. 7: Cleaved regions

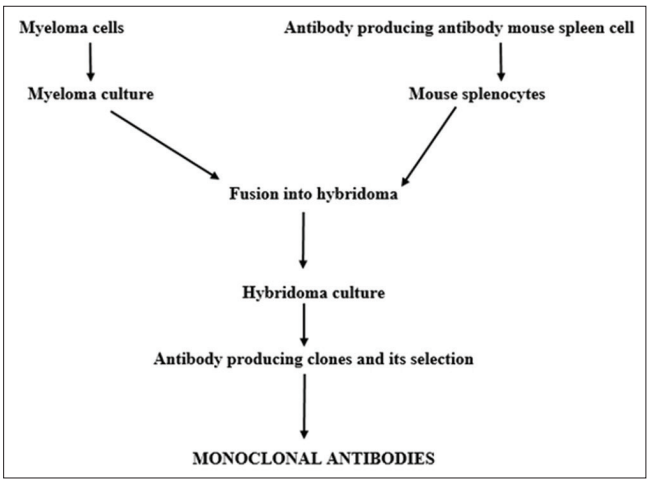


Fig. 8: Schematic diagram for preparation of monoclonal antibody

classes, there are certain classes having subclasses varying slightly in structure and functions which can be distinctly separated from each other serologically [2].

Mode of action

B lymphocytes act as membrane receptor of Igs, which get activated by mediated antigens such as virus, bacteria, parasites, etc., resulting in β lymphocytes to become plasma cells along with T lymphocytes secreting antibodies releasing from cells having a variety of effector functions [2,10].

Function of antibody

The vital functions of antibody which they performed are as follows:

a. Restriction of pathogens and toxins with neutralization of foreign harmful particles.
b. Antibodies particularly have the capability of complement activation by activating the proteolytic cascade affecting the pathogens.
c. Even opsonization and phagocytosis of pathogens is being facilitated by antibody, acting on their receptor.
d. Cytolytic functions even are achieved by binding the Fc potion of the antibody to the target cells.
e. Antibody also acts as ADCC, NK and macrophage lyse cells.
f. The naturally produced Ig act on parasites and allergic conditions protecting mucosa even secretion occur with breast milk.

Complications of antibody in therapeutic use

The immunoglobulin is a part of immune system occurring naturally in the body of human beings as well as animals. In normal condition, actually, the antibodies act on specific antigens such as germ or any foreign particles, recognizing the binding site (epitope) protecting the inbuilt immune system showing specific functions helpful for human

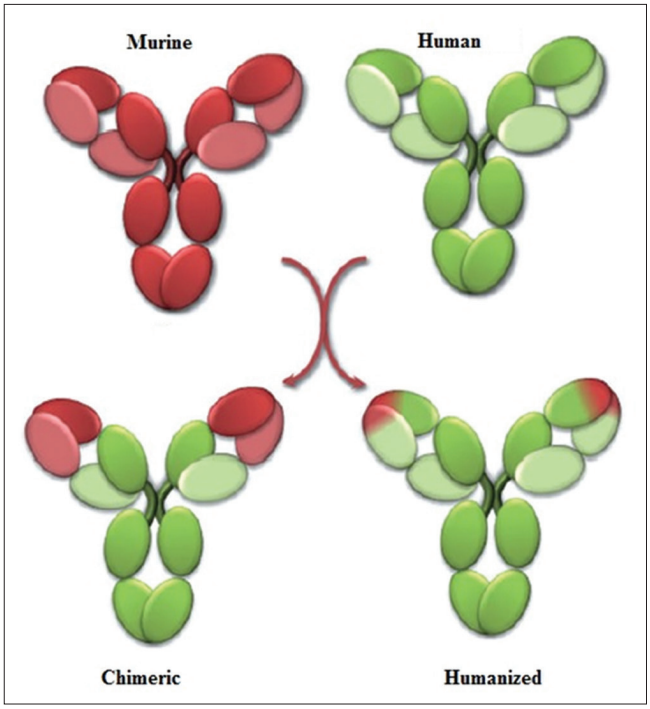


Fig. 9: Chimeric Abs and humanized Abs [50]

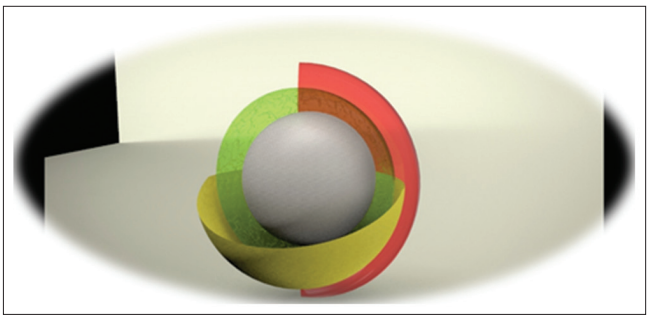


Fig. 10: Nanoparticle

antibody [11]. Even the antibodies have the affinity to attract other individuals such as receptors, various types of proteins exist on the normal cells as well as particular molecules on cancer cells using them as a trigger for other body molecules. Hence, these Abs are very versatile having multifaceted potential for human therapeutic purpose such as binding with cytotoxic agent resulting in destruction of particular cancer cells not the normal cells [12,13].

However, the complication arises here is that [12,13];

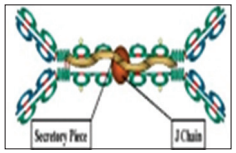
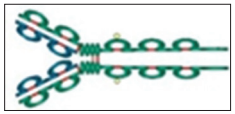
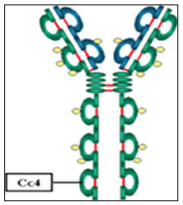
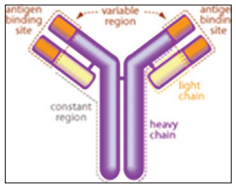
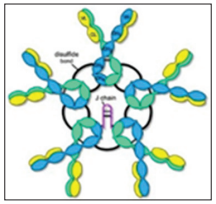
a. The immune system produces a huge quantity of antibodies having a wide span of effector as well as binding region - which must be specific.
b. The response that is being passed toward any antigen from any simplest antibody is polyclonal, i.e., derived from more than one clone.
c. Normal cells are replicative senescence, whereas cancer cells have the capability for indefinite proliferation. So during isolation of a single antibody-secreting cell, due to limited growth potential somatic cells will die after a limit of generation production (Fig. 9).

Thus to overcome this problem, the emergence of monoclonal antibody came into existence [9].

MONOCLONAL ANTIBODY

Monoclonal antibody is single cloned specific antibody obtained from cloned B cell. It came into emergence in treatment around 1986 when

Table 1: Physiochemical properties of antibody

Property	IgA	IgD	IgE	IgG	IgM
Heavy chain	A	Δ	ε	Γ	μ
Light chain	κ/λ	κ/λ	κ/λ	κ/λ	κ/λ
Nature	Monomer/dimer	-	-	-	Pentamer
Sub classes	IgA1 and IgA2	-	-	IgG1, IgG2, IgG3, IgG4	-
Functions	Mucosal protection	-	Act on allergic conditions and parasites	Facilitate phagocytosis of pathogens Activate proteolytic cascade	Activate proteolytic cascade
Image					
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Reference	Arruebo M, *et al.* Antibody-conjugated nanoparticles for biomedical applications. Nanomaterials 2009, Article ID 439389: 24	Arruebo M, *et al.* Antibody-conjugated nanoparticles for biomedical applications. Nanomaterials 2009, Article ID 439389: 24	Arruebo M, *et al.* Antibody-conjugated nanoparticles for biomedical applications. Nanomaterials 2009, Article ID 439389: 24	Arruebo M, *et al.* Antibody-conjugated nanoparticles for biomedical applications. Nanomaterials 2009, Article ID 439389: 24	Arruebo M, *et al.* Antibody-conjugated nanoparticles for biomedical applications. Nanomaterials 2009, Article ID 439389: 24

first monoclonal antibody was approved by U.S. F.D.A. having notable therapeutic effect for chronic disease treatment [2,14].

Antibody administered drug carrier was discovered, when Cesar Milstein and George Kohler, in 1975 [2], had formulated hybrid cells or hydrobomas by amalgamating antibody which is produced from B cells (antibody producing B cells) with tumoral cells. The produced hybrid cells have the capability to produce huge number of isolated specific type antibody. These single cell procured antibody split into like cells. Thus, the antibody initiated from them is monoclonal antibody. Thus, this valuable discovery led to the prestigious award of Nobel Prize in Physiology or Medicine in 1984 to the above-mentioned scientists [2].

In preparation of monoclonal antibody in clinical practice [1,2], mostly the fusion is being made between lymphoma cells with that of B cells of immunized mice. This type of antibody are called murine antibody since B cells of immunized mice is present. Murine antibody may be defined as antibody having both the types of structural chain originated from mouse origin, having pre-stem -o- in its INN [15]. However, the murine antibodies may show toxic effect particularly allergy [1] as well as decrease in therapeutic efficacy since of mouse origin. Thus by recombinant technology, less effective immunogenic antibody (mAbs) is prepared. Chimeric antibody and humanized antibody are the products of this technology with therapeutic potentials. Chimeric antibody is defined as the engineered antibody having chimeric types of both the chain containing (V-D-J-REGION) - the variable foreign domain linked up with the constant region (C - region) of human identity, having pre-stem -xi- in its INN for identification [15]. At the same time, humanized antibody is identified by pre-stem -zu- in its INN, having both chains types of human origin [15]. These are used against transcription factors, pathogenic agents, hormones as well as in identification as well as diagnostic technique like enzyme-linked immunosorbent assays (ELISA), etc.

Monoclonal antibodies for therapeutic purpose [46]
In the regimen of therapeutic purpose, mAbs in its initial phases faces severe technical difficulties but its advantageous properties over conventional ones make it eligible for therapeutic purpose. It is classified into first generation monoclonal antibody and second generation monoclonal antibody.

First generation monoclonal antibody [1,2]
This type of antibody is basically of mouse origin. More specifically these are murine antibodies which though beneficial over conventional one but the problems occurred due to this mAbs are:

a. Allergic conditions
b. Less therapeutic efficacy
c. Low short life
d. HAMA and HARA production affects the patient's therapy
e. Interaction of murine antibody with human antibody leads to limitations
f. Desired targeting is getting blocked
g. All the above leads to useless therapy.

This leads to the second generation of monoclonal antibody.

Second generation monoclonal antibody [2]
To avoid the HAMA or HARA production, this is being produced fully from human antibodies. Although having technical difficulties, using traditional method human monoclonal antibody could not be obtained so in 1980 recombinant technology technique was invented used in production second generation antibody [2,16], also known as recombinant antibody. These antibodies are produced by gene codifying immortalization.

NANOPARTICLES AND NANOTECHNOLOGY

Drug delivery system has been the most innovative area for each and every researcher since it has a range of limitations. It is considered to be the most fascinating domain as it attracts the limelight of commercialization with economic benefit. The only restriction of this interdisciplinary field is delivery of a drug, releasing bioactive agent to its specific site at specific rate for therapeutic action. In today's scenario, site-specific drug delivery is a bottleneck which only can be deal with effective modernized drug delivery system over the conventional drug delivery. The rationality in designing new drugs according to the classes is very vital for improvement in clinical effectiveness, novelty, patient compliance as well as extension of product life cycle than those of the old once. In the last few years, among several drug delivery technologies, the utilization of nano-metric application of drug is at hike in terms of effectiveness and potentiality. Without changing the active pharmaceutical ingredient (A.P.I), the use of nanoparticle allows to change the pharmacokinetic property of any drug having high surface area to volume ratio as well as ability to interact the cell surface biomolecules absorbing the drugs to diffuse through the body. Thus, the idea of nano-medicine came into being which helps in flourishing numerous strategies to administer combination of various conventional drugs, proteins, vaccines, colloidal, nucleotides, etc., circumventing any barrier encapsulated optimally in spite of shape and sizes. Hence, the rise of nanotechnology has a valuable impact on drug delivery domain and also be envisioned as the future tool in therapeutic and diagnosis having socioeconomic benefit.

A number of commercialized as well as under clinical trials nanocarriers have been jotted to overview its contribution in treating diseases (Fig. 10).

NEED OF ANTIBODY TO CONJUGATE WITH NANOPARTICLES

In todays' market, already, there is commercialization of various antibodies as well as several nano-carriers due to their distinct specification jotted down (Table 1).

Advantages as well aspects of using antibody conjugated nanoparticles.

This conjugated innovative drug delivery strategy is very versatile as well as multifunctional with specificity still commercialization is yet to prosecute. The potential and flexile of this tool are summarized as follows:

- Functionalization of NPs with antibody by covalent conjugation for specific targeted tissues.
- NPs having large surface area to volume ratio allows antibody to be attached with specific sites increasing the binding affinity through multivalent functionalization.
- Rate of cell penetration increases showing nominal off target side effects.
- Desirable properties can be engineered in NPs with combing effect of Abs by using particular vehicle help in encapsulation of chemotherapeutic agents.
- Furthermore, this combination helps to reach specific tissue and cells permitting the circumvention of biological barriers as well as physiological conditions.
- This engineered delivery is very much specific and has a versatile function as choreography of antibody structure can be tailored (Fig. 12).
- Finally, one of the main mediators of multidrug resistance P-glycoprotein (P-gp) cannot identify this engineered delivery which internalized with the help of receptor-mediated endocytosis, accumulating in cells. Thus, this consequence helps to gear up the intercellular drug concentration (Fig. 13).

Thus, above points favors the development of potentially beneficial new tool on specific site active drug targeting by conjugation of functionalized antibody specifically human mAbs with nanoparticles resulting in therapy of serious diseases. This gives rises to a new type of tool for site-specific drug delivery system known as Immunonanopaticles.

IMMUNONANOPARTICLES

Immunonanoparticles - A new site-specific drug delivery strategy derived by conjugating functionalized immunoglobulin (antibody) with

engineered nanoparticle. It is a very new approach or tool for targeted drug delivery having a wide range of advantage as well as minimal toxicity.

Since this review is based on CNS delivery, so CNS delivery based approach of immunonanoparticle has been discussed with preparation methodology as well as beneficial aspects (Fig. 11).

Blood brain barrier (BBB)

Central nervous system (CNS) delivery is mainly based on BBB circumvention [17,18]. It is a dynamic interface which separates the brain from the circulatory system [17,19]. It protects the CNS from potentially harmful chemicals while regulating transport of essential molecules and maintaining a stable environment [17,20]. The barrier is formed by highly specialized endothelial cells that lined up the brain capillaries [17,18,20]. It transduces signals from the vascular system and from the brain [17,21]. A complex integrity among a variety of cells and extracellular matrix of BBB [17,22] in capillaries is the dependent factor of structural and functional factors of BBB, being located at converge of cells at brain capillary. The brain microvessel endothelial cells (BMECs) [17,23] in BBB, have characteristic morphological features which includes Gulati *et al*. [17], Roney *et al*. [23], Chivukula and Chhabria [24]:

- The presence of tight junctions between the cells.
- The absence of fenestrations.

A diminished pinocytic activity that supports to restrict the passage of compounds from the blood into the extracellular environment of the brain [17,25,26].

Thus BMECs, due to tight junction plays a significant trans-endothelial electrical resistance resulting in impeding the penetration therapeutic potential agents such as antibodies, peptides proteins, etc. Another feature of the BBB is the presence of P-gp in the luminal plasma membrane of BMEC which prevent the intracellular accumulation of an extensive variety of chemotherapeutic agents and hydrophobic compounds since being an ATP-dependent efflux pump [17,27].

The BBB forms both a physical and electrostatic barrier having homeostatic defense mechanism of the body to limit brain permeation of therapeutics [17,28]. Being a highly complex structure protecting from injuries and diseases particularly against pathogens and toxins, it is a complex barrier with proper regulations and ability to screen the biochemical, physicochemical, and structural features of solutes. It only allows passage of only selective molecules into the brain parenchyma, since a compact regulating ion manoeuvre of a limited number of small molecules and of less number of macromolecules from the blood to the brain.

However, the BBB also significantly precludes the drug delivery to the brain, preventing numerous therapies of neurological disorders such as brain cancer, epilepsy, Alzheimer's disease, and schizophrenia. Thus, to gain entry into brain, the drugs have to overcome a set of menacing factors. The only method of crossing the BBB is passive diffusion. Thus, circumvention of BBB is initial and vital point for drug delivery tool [17,27-29].

Approaches to circumvent BBB [17]

Preference is given to localized and controlled delivery of drugs at their desired site of action because it reduces toxicity and increases treatment efficiency. The brain is a complex organ protected by barriers controlling homeostasis and endogenous compounds into the brain. This complex interplay with tight regulation of brain cell access is important for neurons' survival because they do not have a significant capacity to regenerate; at the same time, they prevent therapeutic compounds, small and large, from evading the brain. Overall, sundry strategies viz. disruption of BBB, chemical modification, molecular antibody technology and various carrier systems are being formulated to enhance access of drugs to the brain parenchyma at therapeutically meaningful concentrations to effectively manage disease and distribution of drugs into brain. The approaches include:

BOON OF IMMUNONANOPARTICLES OVER OTHER DRUG DELIVERY STRATEGY

In CNS, due to poor access of therapeutics, effective non-invasive treatment of neurological diseases has become a limited. Due to the presence of BBB as well as blood - cerebrospinal fluid barrier, a majority of drugs and biological milieu unable to circumvent readily. Hence, a vital issue is the effectiveness of the brain targeting tool, since the majority of drugs and biological milieus such as recombinant proteins, peptides, monoclonal antibodies, small-interfering RNA, and gene therapeutics have a large molecular weight that fails to permeate into brain parenchyma [17,27]. Among the approaches discussed above, conjugation of biological milieu particularly antibody with nanoparticle, known as immunonanoparticles [2], have provided promising solutions to this problem. Although having several established carriers from conventional to nanocarriers, effective transport across *in vivo* models for chronic diseases reducing neurotoxicity has shown a successful performance in case of immunonanoparticles with increasing drug-trafficking performance and specificity for brain tissue using novel targeting moieties, improving their BBB permeability by endocytosis or transcytosis.

LITERATURE REVIEW

Nanoparticle is have been used extensively in delivering drug to specific site using various living and non-living agents likely gold, silver, etc., even various biologically active agents. However, antibody or immunoglobulin linked nanoparticle is found to be most promising and advantageous delivery system. Although for effective delivery, necessity of optimization and construction related to proper target binding maintaining several variables are to be taken into concern.

From a historic point of view till today, with the advancement of technology, there have been several methods which have been reported for the preparation of immunonanoparticle. Based on the type of utility, researchers have prepared different types of nanoparticles from liposomes to engineered nano-carriers. Some of them are listed below.

Immunoliposomes [28]

From around 90's, evidence shows that there has been the use liposome conjugated with biological milieu for site specific delivery substantially. The liposomal delivery in conjugation with antibody is one of the noteworthy delivery systems in which targeting the affected site by liposomes with the help antibody has been studied in which it has been adopted to be very promising. It took a long time span to prompted liposomal drug carrier concept having wide potentially therapeutic agents due to its dual character: Effective with various biological agents while in *in vivo* delivery of drug and at the same time shows efficacy as membrane research experimental tool.

Advantages of liposomal carrier:

- Liposomes are basically lipid in nature which is present widely in the body.
- It has assembled structure where drug can easily put up in both the phase as per solubility whether lipid or aqueous.
- It satisfies as per needs of delivery with respect to technology as well as *in vivo* optimization.
- Even evidence are there showing that it helps to reduce toxicity of many antitumor drugs as well as antifungal drugs.
- It is also effective against intracellular infections like leishmaniasis when as anti-parasitic drugs act. Even helps in immune system acting as artificial vaccines.

Since this review aims on the CNS delivery particularly BBB circumvention, so usefulness of liposomal delivery with biological milieu along with its preparation methodology is being discussed in detail for effective therapeutics and potentiality to the diseases cure.

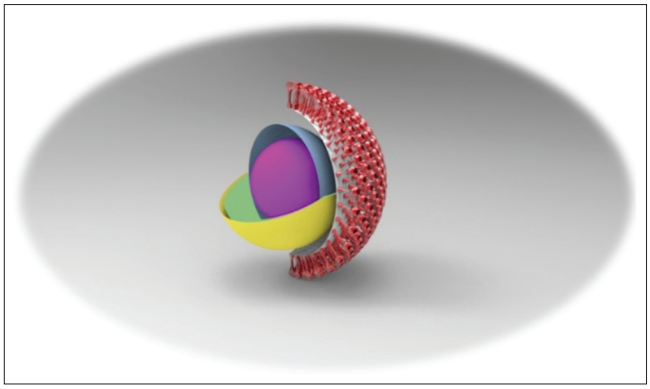


Fig. 11: Immunonanoparticle

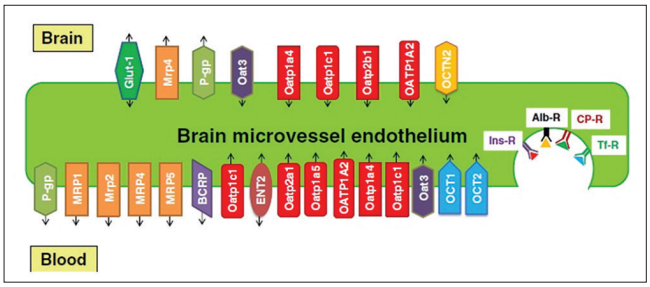


Fig. 12: Blood brain barrier containing endothelial cells [27]

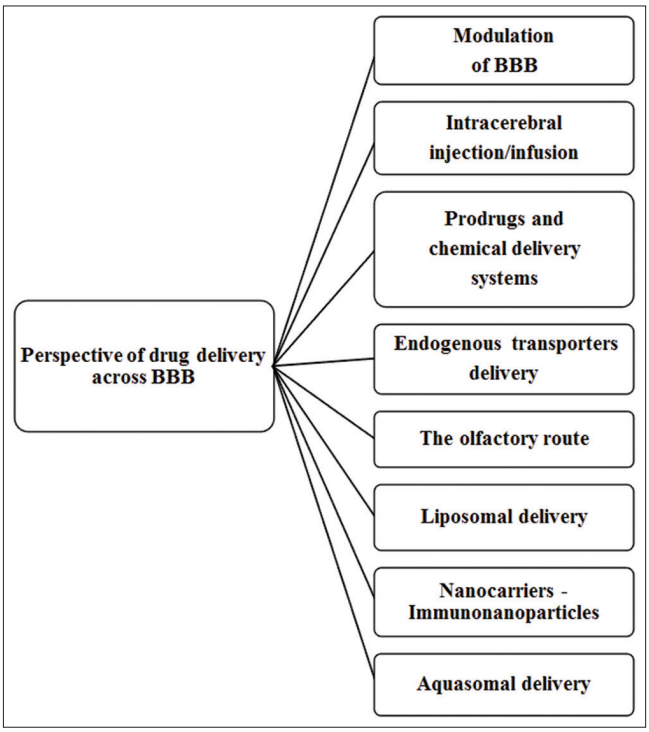


Fig. 13: Approaches for drug delivery in BBB

Conjugating the liposomes with biological milieu particularly antibody or mABs is summarized to be most promised when carried out in systemic *in vivo* model. Initially, the immunoliposomes are being constructed having optimized and suitable *in vivo* target delivery with effective retention factor.

Method of preparation [29]

The fundamentals followed for the preparation of immunoliposomes were as follows:

- Antibody coupling with hydrophobic anchor covalently: Hydrophobic anchor refers to the receptor present in liposomes which are conjugated with antibody by two ways.

N.B. The above process would vary according to the preparation of immunoliposomes.

The Hydrophobic anchor used in both the coupling was N-glutaryl-phosphatidylethanolamine (NGPE). Synthesis of the anchoring is discussed below.

Fig. 1 shows the carboxyl group of NGPE was first activated using hydrophilic carbodiimide-1-ethyl-3(3-diethylaminopropyl) carbodiimide EDC. N-hydroxysulfosuccinimide (Sulfo-NHS) was used during EDC activated carboxylic derivative (o-acylisourea). This was converted to nonisomerizable activated carboxylic derivative (N-hydroxysulfosuccinimide esters). The antibody molecules were attached after that using the amino group reacting with activated carboxylic groups of NGPE forming a stable covalent amide bond at pH 7.5 in between 0-4°C.

- Immunoliposome preparation was developed in two different strategies having difference in antibody covalent coupling with liposomes. These two methodologies are discussed below according to their usefulness.

Detergent analysis method

In this method using detergent namely octyglucoside, reconstruction of membrane protein was developed. NGPE conjugation was done with antibody in the presence of detergent leading to mixing with lipid-detergent mixture subsequently incorporation of antibody by removal of detergent with the help of dialysis. In $CHCl_3$, NGPE was dissolved and

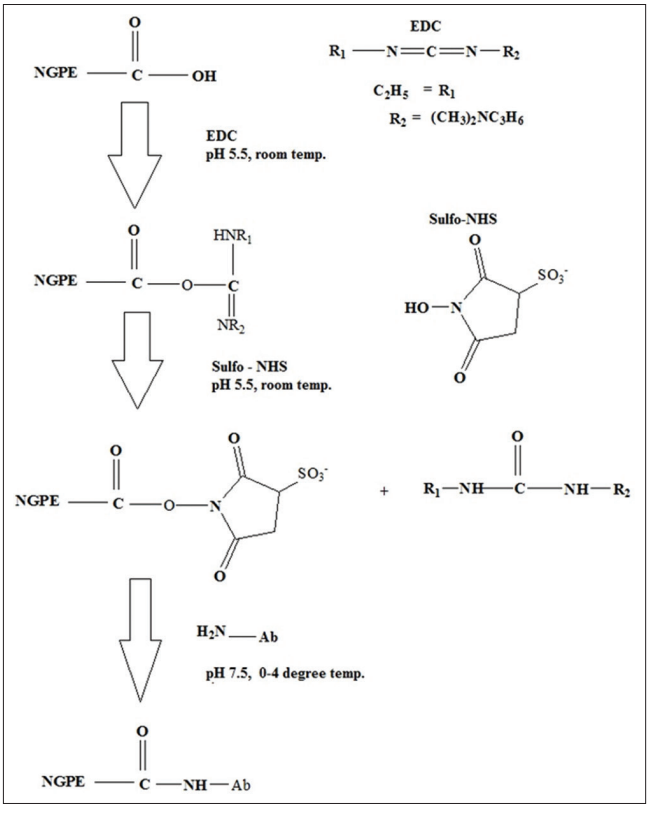


Fig. 14: Coupling reaction of N-glutaryl-phosphatidylethanolamine with antibody in presence of water-soluble carbodiimide and N - hydroxysulfosuccinimide [29]

dried under N_2 with vacuum desiccator. Solubilizations of dried NGPE with octylglucoside in 2 - [N-morpholino] ethanesulfonic acid (MES) buffer having pH 5.5 was done, from which 200 nmol was taken out and (10 µmol of water) EDC and sulfo-NHS (4-µmol in water) were added.

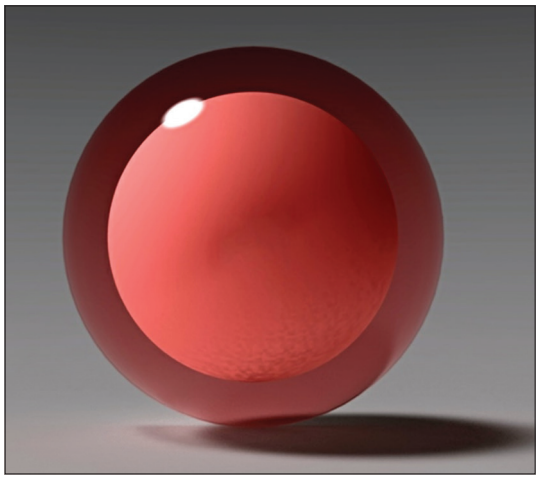


Fig. 15: Aquasomes

Further incubation was done at room temperature for 10 minutes and neutralized with 100 mM HEPES buffer at pH 7.5 and 1N NaoH at pH 7.5. Then, antibody was added having molar ratio of antibody to NGPE = 1:14. The mixture resulted was incubated for 8 hrs at 4°C with gentle stirring.

In liposome preparation - thin film of solvent free lipid mixture containing [11] Indium chelated to diethylenetriamine pentaacetic acid distearyl amide complex was used as a lipid marker with octylglucoside phosphate buffered saline (PBS) at pH 7.5 having a ration of lipid and octylglucoside of 1:5. The prepared solution was mixed vigorously with NGPE conjugated antibody and dialysis was performed to remove the detergent at pH 7.5 against PBS at 4°C for 12 to 18 hrs. Finally, the immunoliposomes were prepared having 200-300 nm diameter in average. The unbound antibody was removed by chromatography. Around 35% to 50% of antibody was incorporated in liposomes.

Advantages

No involvement of organic solvents and sonication.
Possibility of entrapment of macromolecules like proteins.
Favorable for lipid compositions.

Disadvantages

Low entrapment efficacy for hydrophilic solutes.
Need a prolong dialysis to remove detergent.

Reverse-phase-evaporation method

In this method, unilamellar and multilamellar liposomes containing NGPE was used by extrusion. The liposomal NGPE containing carboxylic groups was activated and by co-incubation antibody molecules are conjugated.

Liposomal preparations were performed by a thin film of solvent-free lipid mixture containing 10 mole% NGPE and [11] In-DTPA-SA dissolving in ether in 50 ml RBF (Fig. 14). A MES buffer was prepared having pH 5.5 which was added in 1:4 ratio of aqueous to organic phase undergoing sonication for 10 minutes in bath sonicator preparing homogeneous emulsions (Fig. 15). The organic solvent was removed by evaporation under reduced pressure at room temperature. The resulted REV was extruded in which EDC and sulfo-NHS was added in MES buffer having pH 5.5 and the mixture was incubated in room temperature for 10 minutes. Finally by adjusting the pH, the antibody of [25] in-labeled was mixed and incubated at 4 degree centigrade for 4 hrs with gentle stirring resulting in the formation of immunoliposomes. Through chromatography, unbounded antibody was removed. About 50% of antibody got coupled with liposomes in this method.

Advantages

Relatively high efficacy of entrapment with hydrophilic solutes.

Disadvantages

Might not be suitable for lipophilic solutes due to the use of organic solvents and sonication step.
Interference of coupling reactions might occur due to the occurrence of coupling at the surface of liposomes.
Interference of coupling might also occur due to certain lipid or amphipathic molecules with long side chains due to their steric hindrance effects.

Characterization [29]

Optimization of in vivo immunoliposomes targeting

The efficacy of the immunoliposomes depends independently on two categories namely:

a. Antibody-antigen interaction - It included the antibody specificity, affinity, antigen concentration on target sites and antigen accessibility to antibody (Table 7).
b. Liposomes' characteristics - It included the use of monoclonal antibody of rat IgG_{2a} (MoAb 34A), which got bind specifically to a glycoprotein antigen (gp112) expressed in high concentration of luminal surface of the capillary endothelial cells of mouse lungs.

In detergent analysis method, there was a typical biodistribution pattern of intravenously injected MoAb 34A-bearing liposomes. It gained direct access and bind effectively in lungs, while liposomes without antibody did not bind. The immuno-specific interaction showed that the binding of immunoliposomes at lungs were perfect blocking the preinjected free MoAb 34A.

The lipid composition and the antibody density had revealed the efficient targeting of immunoliposomes, judging the two parameters - binding and the retention factors at the target sites. The accumulation of immunoliposomes in target sites was determined by the two kinetically competing process namely: Binding to target site and Uptake by reticuloendothelial system (RES). The rate of target binding is directly proportional to the antibody density of the immunoliposomes. At the same time, RES uptake is also proportional to the liposomal lipid composition used. Consequently, the reduced in affinity in RES, increased the high antibody density in immunoliposomes resulting in potentially fruitful delivery.

Therapeutic potential of immunoliposomes

About 34 A-liposomes showed high efficacy in binding at target site with prolonged retention. The lung was the exclusive target, at which 70% to 75% of the injected dose accumulated under optimal conditions within 15 minutes of the administration. Furthermore, this organ-specific tool was expected to be released from the bound state and diffused a short distance to reach the targeted cells.

Future perspectives

From this, it can be concluded that that immunoliposomes showed highly efficient target binding and prolonged retention at target sites resulting in high performance effective delivery tool useful for future treatments.

Antibody conjugated gold nanoparticles [30]

Here, the synthesis of pendant carboxylic acid and alcohol functional groups with gold nanoparticles having 10 nm diameter was discussed which was conjugated with antibody to *Escherichia coli* O157:H7 and characterization using transmission electron microscopy (TEM) and infrared spectroscopy were performed. Using EDC coupling chemistry, anti-*E. coli* O157:H7 was reacted with gold nano-carriers and characterization was performed with X-ray photoelectron spectroscopy (XPS) with demonstration of binding antibody-gold conjugates to *E. coli* O157:H7 using TEM.

Method of preparation [30]

Materials

Hydrogen tetrachloroaurate (III) hydrate, 11-mercapto-1-undecanol, 16-mercaptohexadecanoic acid, N-hydroxysuccinimide (98%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), PBS, anhydrous toluene, isopropanol, anhydrous acetone, goat anti-*E. coli* O157:H7.

The procedure followed is summarized below. The enteropathogen [30,31-33] *E. coli* O157:H7 was detected by ELISAs since being selective and sensitive [30,34]. The amplification of DNA from a bacterial cell in 1 hr [30,34] was a PCR-based method though PCR has few useful detecting biological samples [30,35]. The fluorescent bioconjugated silica nanoparticles, highly sensitive fluroscent marker was developed for the detection of *E. coli* O157:H7 [30,36].

Anchoring of antibody with conjugated-gold nanoparticles (Au NP) [3]

N. B.: EDC = 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide hydrochloride. NHS = N-hydroxysuccinimide.

This synthesis is very much useful for detection of pathogens. Fig. 2 shows the synthesis procedure of the gold nanoparticles having 10 nm diameter with pendant carboxylic acid and alcohol functional groups using a colloidal synthetic method by gold ions reduction in the presence of a mixture of carboxylic acid and alcohol terminated alkanethiols. Solution of 0.25 g 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 0.25 g N-hydroxysuccinimide in 5 mL 0.01 PBS having pH 7.4 was added 100 mg of Au nanoparticles with pendant carboxylic acid and alcohol functional groups, and 2 mg goat anti-*E. coli* O157:H7 [30,36] with stirring of suspension for 0.5 hrs at room temperature. The obtained product was collected by centrifugation, washed twice with 0.01 PBS, and once with ethanol and then characterized using XPS, measured with a Surface Science Instrument SSX-100.

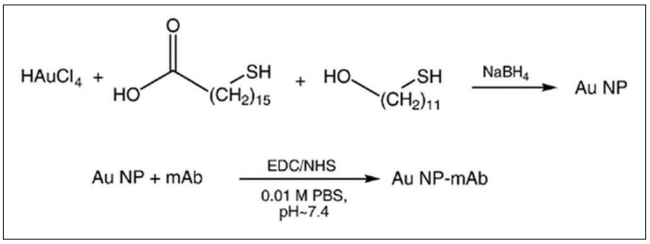
Anchoring of antibody (anti-*E. coli*) to the carboxylic acid via 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was performed.

Synthesis of gold nanoparticles with bifunctional alkanethiols

The synthesis of Au nanoparticles was performed having functionalized with 11-mercapto-1-undecanol and 16-mercaptohexadecanoic acid [30,37]. In a solution containing $HAuCl_4 \bullet 3H_2O$ of 410 mg with water of 6 mL was added 200 mL of 2.7 mmol 11-mercapto-1-undecanol and 0.3 mmol 16-mercaptohexadecanoic acid ethanol solution. Cooling of the solution up to 0°C, 20 ml of a freshly prepared aqueous solution containing 380 mg of $NaBH_4$ was added with vigorous stirring drop by drop. A dark brown solution was formed which contain Au nanoparticles capped alkanethiol having pendant alcohol and carboxylic acid functional groups stirred for 3 hrs. After the stirring material was allowed to precipitate to the bottom of the flask. The resulted particles were washed two times dispersing them in 100 mL of 80% ethanol followed by centrifugation and decantation. Finally washed with 100 mL of ethanol containing ~50 µL of 1 M HCl solution. For 10 hours, the washed material was dried under vacuum. Antibody conjugation was also synthesized using alternate method, synthesizing gold nanoparticles [30,38], 4 nm gold nanoparticles functionalized with 11-mercapto-1-undecanol and 16-mercaptohexadecanoic acid.

Binding of antibody-gold nanoparticles to *E. coli*

In binding of antibody-gold nanoparticles with anti-*E. coli* O157:H7, the conjugated Au nano-carrier was suspended in a 0.01 M PBS (pH 7.4) solution containing *E. coli* O157: H7 bacteria near their stationary phase. By the adding of 1.0 mL of 4% paraformaldehyde, the bacteria was fixed in the solution after 30 minutes with a drop of resulting solution placing on formvar/carbon-coated copper grid. Drying was performed, and TEM images of the sample were collected using an FEI Tecnai T12 S/TEM instrument. Finally, TEM images of suspended *E. coli*, the Au-antibody conjugate and Au nanoparticles in the presence of *E. coli* was collected. Thus, the binding of the antibody-gold conjugates to *E. coli* O157:H7 was investigated using TEM.



Characterization [30]

Characteristics of gold nanoparticles with bifunctional alkanethiols

The TEM of the material was obtained with an FEI Tecnai T-12 S/TEM operating at 120 KeV and the Fourier transformed infrared (FT-IR) spectrum was obtained on a Nicolet IR200 FT-IR spectrometer. By using an alternative synthetic method to gold nanoparticles, 4 nm gold nanoparticles functionalized with 11- mercapto-1-undecanol and 16-mercaptohexadecanoic acid were also synthesized for antibody conjugation.

Binding of antibody-gold nanoparticles to *E. coli*

TEM images of the sample were collected using an FEI Tecnai T12 S/TEM instrument. As controls, TEM images of suspended *E. coli*, the Au-antibody conjugate, and Au nanoparticles in the presence of *E. coli* were also collected.

The TEM image of the gold nanoparticle, showed well-dispersed nanoparticles of ~10 nm diameters. The FT-IR spectrum showed peaks at various ranges corresponding to different stretching and bending modes of the alkanethiols. Peaks at 2987 and 2901 cm^{-1} were assigned to C–H stretching of the alkyl groups of the alkanethiol and the peak at 1407 to the C–H deformation of the alkyl group. The peak at 1635 cm^{-1} had been assigned to C=O stretching of the carboxylic group. Furthermore, a peak at 1394 cm^{-1} corresponding to O–H bending was observed. A peak at 1067 cm^{-1}, which corresponded to C–OH stretching of the alcohol terminated groups, was also present. The peak at 1330 cm^{-1} was characteristic of the out-of-plane O–H bending mode in an intermolecular hydrogen-bonding structure. The peak at 3416 cm^{-1} was due to O–H stretching. The presence of the antibody on the nanoparticles was demonstrated by XPS. A peak at ~398, which corresponded to nitrogen present in the sample from antibody [30], was observed. XPS had been shown to be important for the characterization of Au substrates decorated by S-layer protein layers with immobilized camel antibody, and for the confirmation of F(ab') and F(ab) binding onto Au surfaces [30]. The binding of the Au-antibody conjugate to *E. coli* was studied using TEM. A single isolate *E. coli* bacterium which had the expected rod-like shape with lengths and widths of ~1500 and ~500 nm, respectively was determined. Addition of the Au-antibody conjugate to a suspension containing *E. coli* resulted in the binding of the conjugate to the surface of the cell through antibody-antigen recognition. The appearance of at least three *E. coli* cells, each having attached smaller high-density material had been observed. Under higher magnification, individual Au-antibody conjugates of ~15 nm, attached to the surface of the cell, could be identified.

Future perspectives

The synthesis of the gold nanoparticles containing terminal carboxylic acid and alcohol groups conjugating with anti-*E. coli* antibody were synthesized. Reaction of $HAuCl_4 \bullet 3H_2O$ with 11-mercapto-1-undecanol and 16-mercaptohexadecanoic acid results in gold nanomaterials having 10 nm in diameter with pendent carboxylic acid and alcohol functional groups was demonstrated by infrared spectroscopy and TEM. XPS - XPS analysis summed up that antibody had been conjugated to the gold nanomaterial, which was visualized when incubated in PBS undergone at pH 7.4 the antibody-conjugated gold nanoparticles binding to *E. coli*. Thus, a successful delivery tool synthesis had been observed useful for the treatment for fatal diseases as well as in future cure.

Antibody conjugated magnetic nanoparticles [39]
In this study, BRCAA1 monoclonal antibody was prepared conjugated with fluorescent magnetic nanoparticles (FMNPs) having 50 nm in diameter. The final product was characterized by TEM and photoluminescence spectrometry.

Method of preparation
Anti-BRCAA1 monoclonal Antibodies was prepared by purifying fusion protein BRCAA1. BALB/c female mice, 4-6 weeks old was used for the experiment. By intraperitoneal injection with 50 μg of purified BRCAA1 protein the mice were immunized, emulsifying with an equal volume of Freund's complete adjuvant. Using incomplete adjuvant every two weeks, three injections were again administered. The spleen cells of the mice were prepared and fused with the Sp 2/0 mouse myeloma cell line three days after the last injections. Finally, ELISA test confirmed that the solid phase was coated with the recombinant BRCAA1 protein (2 μg/mL) used for the immunization. The monoclonal antibodies were bind with coated BRCAA1 protein. Positive colonies were subcloned by limiting dilution twice. From mice, ascetic fluids were harvested primed with a 0.5 mL intraperitoneal injection of pristane and injected to 106 hybridoma cells. Purification of mAbs was done using a protein G-Sepharose. Finally, ELISA methods were used to determine the antibody titers [39,40]. Finally, anti-BRCAA1 monoclonal antibodies were prepared for the experimental purpose.

Preparation and surface functionalization of FMNPs
By coprecipitation of ferrous and ferric ion solutions (1:2 molar ratio) [39,41-44], the preparation of Fe_3O_4 nanoparticles was performed. 5 m mol of $CdCl_2$ was dissolved in 110 ml of water, and 12 m mol of TGA were added under stirring, followed by pH adjustments up to 11 by addition of 1 M NaOH solution dropwise. In a three-necked flask the mixed solution was placed deaerated by N2 bubbling for 30 minutes. by continuous stirring, 2.5 m mol of oxygen-free NaHTe solution was injected into flask, which was freshly prepared from tellurium powder and NaBH4 having molar rate of 1:2 at 0°C in water. The product was of size 3 nm diameter in the resulting solution nm diameter product emitted with a maximum around 630 nm. In the above way, CdTe nanocrystals were synthesized. Using the reverse micro-emulsion approach, FMNPs were synthesized. Functionalization of the surface functional group of FMNPs as carboxyl group was undergone first, then coupling of FMNPs with the BRCAA1 was performed. A solution was prepared using 95 mL ethanol and 2 mL 3-aminopropyltriethoxysilane (APS) solution and allowed to react at room temperature for a day. Being washed with deionized water for 3 times, the separation of aminosilane-modified FMNPs were performed by permanent magnet. A mixed solution of re-dispersed FMNPs-NH2 in 100 mL dimethylformamide added excessive succinic anhydride was made to react for 24 hrs at room temperature. Again separation of carboxyl - modified FMNPs was performed by magnet and washed with deionized water 3 times.

Preparation and characterization of BRCAA1 antibody conjugated FMNPs
Stable anti-BRCAA1-FMNPs conjugation [38,43,44] was obtained by two processes. In 2 mL pH-7 PBS buffer 1.5 mg FMNPs-COOH solution was dispersed with sonication for 10 minutes. In pH 6.0 MES buffer, mixing of 1 mL of fresh 400 mM EDC and 100 mM NHSS was done with rotation was performed at room temperature for 15 minutes. The resulted solution was separated by magnetic field. Then in the resulted solution, 1 mg/mL BRCAA1 monoclonal antibody were added with stirring in dark place for 2 hrs. The residual reaction was separated by magnetic field to get the free BRCAA1. With 1 mL PBS buffer three, the remaining solid was washed times. Fnally, in BRCAA1-FMNPs conjugation, 1 mL 0.05% Tween-20/PBS was added. At 4°C, the final bio-conjugation was kept which when used should get diluted with PBS/0.05% Tween-20. By using nano-drop device, the quantification of coupling rate of BRCAA1 antibody with FMNPs-COOH was determined. The measurement of the total concentration of BRCAA1 antibody was calculated. After performing the coupling reaction, the measurement of BRCAA1 antibody concentration in residual reaction mixture was noted. It was calculated according to the equation of coupling rate: Coupling (%) = (1- concentration of BRCAA1 antibody in residual reaction mixture/Total concentration of BRCAA1 antibody) ×100. Finally, the characterization of the prepared product was performed to analyze the efficacy.

Characterization
Characterization of anti-BRCAA1 monoclonal antibody was obtained having two positive clone cell lines S-200-5 and S-335-5, their titters were different finally we selected the anti-BRCAA1 monoclonal antibody from S-200-5 cell line as the first antibody to stain gastric cancer tissues and control tissues. It was found BRCAA1 protein exhibited overexpression in 64% gastric cancer tissues, no expression in normal control gastric mucous tissues; there existed statistical difference between two groups ($p<0.01$). This result suggested that BRCAA1 antigen might be selected as the potential target for most gastric cancer, if as-prepared nano-probes may recognize 64% patients with early gastric cancer, it will be very useful for diagnosis and therapy of clinical gastric cancer patients.

Characterization of BRCAA1 - FMNPs
FMNPs were composed of silica-wrapped CdTe and magnetic nanoparticles; their size was 50 nm or so in diameter. After FMNPs were conjugated with anti-BRCAA1 antibody, as-prepared nanoprobes' photoluminescence (PL) intensity was lower than that of FMNPs, exhibiting left-shift of 40 nm, which was due to decrease of the polarization rate of the surrounding molecules and resulting in the decrease of stokes displacement, finally resulting in a blue shift in the emission spectra. Similarly, magnetic intensity of as-prepared nano-probes was also lower than FMNPs. Since surface functionalization of FMNPs was very key to conjugate anti-BRCAA1 antibody with FMNPs via covalent bond, different functional groups of FMNPs have different zeta potential values. FMNPs had negative Si-O-group, their zeta-potential value was −34.05 mV, the FMNPs with amino group had positive zeta-potential value of 24.80 mV, FMNPs with carboxyl group had negative zeta potential value of −30.50 mV. It was observed that carboxyl groups on the surface of FMNPs conjugated with anti-BRCAA1 antibody easier than amino groups on the surface of FMNPs. The average coupling rate of anti-BRCAA1 antibody with FMNPs-COOH was 80.28%. Targeting ability of as-prepared nanoprobes *in vitro* were observed by fluorescence microscope and calculated by flow cytometry (FACS)-calibur flow cytometer. FMNPs randomly dispersed in the inner of the cytoplasm, and anti-BRCAA1-FMNPs nano-probes existed around the nucleolus. Both FMNPs and prepared BRCAA1-FMNPs nano-probes had the ability to enter into the cytoplasm of MGC803 cells after 4 hrs incubation with MGC803 cells; FMNPs could label 25.23% MGC803 cells, the remain 74.77% cells could not be labeled. 45.92% MGC803 cells could be labeled by the BRCAA1-FMNPs nanoprobes. When FMNPs and anti-BRCAA1-FMNPs, nanoprobes were respectively incubated nucleolus. Both FMNPs and prepared BRCAA1-FMNPs nanoprobes could enter into the cytoplasm of MGC803 cells after 4 hrs incubation with MGC803 cells, FMNPs could label 25.23% MGC803 cells, the remain 74.77% cells could not be labeled. 45.92% MGC803 cells could be labeled by the BRCAA1-FMNPs nano-probes when FMNPs and anti- BRCAA1-FMNPs nanoprobes were, respectively, incubated *in vivo* tumor tissues [39]. In this study, it was designed and prepared a novel imaging probe, which was composed of silicon-wrapped quantum dots and magnetic nanoparticles with the aim of enhancing their biocompatibility. The prepared silicon-wrapped quantum dots and magnetic nanoparticles were very stable having own strong fluorescent signals and magnetic intensity. Using the strong fluorescent signals of as-prepared nano-probes, successfully obtained the fluorescent images of *in vivo* gastric cancer tissues with 5 mm in diameter in nude mice model.

Future perspectives
Successful preparation of BRCAA1 monoclonal antibody was performed exhibiting prepared very low toxicity with a good targeting efficacy. The result depicted no damage of obvious organ damage. The high-performance BRCAA1 monoclonal antibody-conjugated FMNPs showed

Table 2: Monoclonal antibodies for therapeutic use

Drug	Trade name	Company	Application	Adminstration	Clinical trial	Approval	Year	Reference
Abciximab	ReoPro	Centocor B. V.	Chimeric 7E3 directed against platelet glycoprotein IIb/IIIa	-	-	FDA	1994	[2,51]
Adalimumab	Humira	Abbott laboratories	Human anti TNF-α. Rheumatoid arthritis	-	-	FDA EMEA	2002 2003	[2,51]
Arcitumomab	CEA-scan	Immunomedics Inc.	Mouse Ig Fragment-Tecnecio1. Directed against carcinoembrionic antigen	-	-	FDA EMEA Retired	1996 1996 2005	[2,51]
Alemtuzumab	Campath	Millennium/ilex partners LP	Humanized Ig anti CD52; chronic lymphocytic leukaemia	-	-	EMEA FDA	2001 2001	[2,51]
Basiliximab	Simulect	Novartis pharmaceutical corporation	Chimeric anti-IL-2α (CD25). To avoid renal transplant	-	-	FDA EMEA	1998 1999	[2,51]
Bevacizumab	Avastin	Genentech Inc.	Humanized anti VEG; tumours	-		FDA EMEA	2004 2005	[2,51]
Capromab pendetide	ProstaScint	Cytogen corporation	Mouse Ab-Indio2. Detection of prostate tumour	-	-	FDA	1996	[2,51]
Centrolizumab pegol	Cimzia	UCB Pharma	Humanized Fab anti TNF-α. Morbus Chron, rheumatoid arthritis	-	-	FDA	2008	[2,51]
Cetuximab	Erbitux	Bristol-Nyers squibb/Merck KgaA	IgG1 directed against EGFR; colorectal tumour	-	-	FDA	2004	[2,51]
Daclizumab	Zenapax	Hoffman-LA Roche Inc.	Humanized IgG1 anti-IL-2α. To avoid transplant rejection	-	-	FDA EMEA	1997 1999	[2,51]
Eculizumab	Soliris	Alexion	Humanized anti CD59, Paroxysmmal nocturnal hemoglobinuria	-	-	FDA EMEA	2007 2007	[2,51]
Efalizumab	Raptiva	Genentech Inc./Roche	Huamnized Ig anti-CD11a	-	-	FDA	2003	[2,51]
Gemtuzumab ozogamicin	Mylotarg	Wyeth averst	Huamnized Ig anti CD33; acute myeloid leukaemia	-	-	FDA	2000	[2,51]
Golimumab	Simponi	Centocor (Johnson and Johnson)	Human anti TNF-α, Rheumatoid psoriatic arthritis, ctiveAnkylosing spondylitis	-	-	FDA	2009	[2,51]
Ibritumomab tiuxetan	Zevalin	IDEC Pharmaceuticals Corp.	Mouse Ig-Itrio 90 anti CD20. Non-Hodgkin lymphoma	-	-	FDA EMEA	2002 2004	[2,51]
Imciromab-pentetate	Myoscint	Centocor (Johnson and Johnson)	Mouse Fab-Indio2, directed against human heart miosin	-	-	FDA	1996	[2,51]
Muromomab	Orthoclone OKT3	Ortho Biotech, Inc. (Johnson and Johnson)	Mouse IgG2a anti-CD3, Transplantation rejection	-	-	FDA EMEA	1986 1987	[2,51]
Natalizumab	Tysabri	Biogen idec	Huamnized anti CD49d. Multiple sclerosis, Chron's diseases	-	-	FDA EMEA	2006 2006	[2,51]
Nofetumomab	Verluma	Boehringer Ingelheim Pharma KG	Mouse Fab IgG2b directed against glycoprotein 40kD (expressed in several tumours). Conjugated to tecnecio1	-	-	FDA	1996	[2,51]
Omalizumab	Xolair	Genentech Inc./Roche	Humanized IgE. Severe asthma	-	-	FDA EMEA	2003 2005	[2,51]
Palivizaumab	Sinagis	Medimmune Inc.	Huamnized IgG1; respiratory syncitial virus	-	-	FDA EMEA	1998 1999	[2,51]
Panibizumab	Lucentis	Genetech Inc.	Huamanized anti VEGF-A. Wet Macular degeneration	-	-	FDA EMEA	2006 2007	[2,51]

(*Contd...*)

Table 2: *Continued*...

Drug	Trade name	Company	Application	Adminstration	Clinical trial	Approval	Year	Reference
Panimumab	Vectibix	Amgen	Human anti EFGR (epidermal growth factor receptor). Metastatic colorectal carcinoma	-	-	FDA	2006	[2,51]
Remicade	Infliximab	Centocor (Johnson & Johnson)	Chimeric anti TNF-α. Rheumatoid arthritis, Crohn's disease	-	-	FDA EMEA	1998 2002	[2,51]
Rituximab	Rituxan	Genentech Inc.	Chimeric Ig antiCD20 for Non-Hodgkin lymphomas	-	-	FDA EMEA	1997 1998	[2,51]
Tositumomab	Bexxar	GlaxoSmithKline	Murine Ig-Iodo2 anti CD20. Non-Hodgkin lymphoma	-	-	FDA	2003	[2,51]
Trastuzumab	Herceptin®	Genentech Inc.	Humanized IgG anti –HER2; breast cancer	-	-	FDA EMEA	1998 2000	[2,51]

a high efficacy in targeting organ having positive result which would be helpful for future delivery system. BRCAA1 antigen is only over-expressed in 64% or so of gastric cancer tissues from clinical surgery patients, it also confirmed that BRCAA1 antigen is over-expressed in some gastric cancer cell lines such as MKN-1, MKN-74, SGC-7901, KATO-III and MGC803 [39]. Here used MGC803 cells to prepare nude mice model loaded with gastric cancer, and successfully observed that as-prepared nano-probes preferentially accumulated in tumor tissues compared with normal control tissues, and as the post-injection time increased. Also observed that injected nano-probes in the whole body exhibited the time-dependent clearance and the fluorescent signals gradually decreased as the time elapsed due to the liver-cholecyst excretion system and kidney clearness of as-prepared nano-probes. Several reports showed that kidney only clear nanoparticles with 5 nm in diameter, in this study, it was observed that as-prepared nanoprobes with 50 nm in diameter also could be cleared within 12 hrs. This concrete mechanism might under way. Nano-probe biosafety also questioned as an important problem, which decided the application prospect of as-prepared nanoprobes. The results fully showed that as-prepared nano-probes did not damage important organs including liver, kidney, heart, lung, etc., also did not exhibit long-term staying in important organs, which highly suggested that as-prepared nanoprobes own good biocompatibility, and had great potential in applications such as dual model imaging and selective therapy of early gastric cancer.

Antibody conjugated poly (lactic acid-co-L-lysine) nanoparticles [45]

In this study, sensitivity, accuracy and specificity of epidermal growth factor receptor monoclonal antibody (EGFRmAb) modified poly (lactic acid-co-L-lysine) nanoparticles (PLA-PLL-EGFRmAb) NPs delivery system was examined over EGFR positive cancer cells. An *in vivo* investigation was performed to a newly prepared PLA-PLL-EGFR mAb NPs to understand the cellular cytotoxicity, cellular uptake, and the targeted effect for epatocellular carcinoma of PLA-PLL-EGFRmAb NPs. *In vitro* proof showed that PLA-PLL-EGFRmAb NPs could bind to hepatocellular carcinoma cells effectively. Hence, an *in vivo* model was prepared to summarize the efficacy of the delivery tool.

Method of preparation [45]

Materials

Ne-(carbonylbenzoxy)-L-lysine and D-alanine, L.L-lactide, N, N-diisopropylethylamine, EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide), N-hydroxysuccinimide (NHS), Rhodamine B (RB), other reagents (analytical grade) and EGFR monoclonal antibody (EGFRmAb).

The EGFRmAb was prepared [45,47]. The copolymers PLA-PLL (poly (lactic acid-co-L-lysine) was synthesizd [45,48].

By ring-opening polymerization of monomer L,L-lactide with stannous octoate as initiator; III to prepare diblock copolymer PLA-PLL by deprotected the copolymer, PLA-PLL(Z) in HBr/HoAc solution 3-(Ne-benzoxycarbonyl-L-lysine)-6-L-methyl-2,5-morpholinedione; II was prepared to diblock copolymer poly(lactic acid-co-(Z)-L-lysine) (PLA-PLL(Z) [45].

Methods for preparation of NPs

As per reference of Liu *et al*. [45], Duan *et al*., [49] preparation of RB loaded PLA-PLL NPs (RB-PLA-PLL) were performed. With an ultrasonic processor, emulsification of 20 ll of a10 mg/ml RB solution with 200 ll mixture of methylene dichloride and acetone (3:2, v/v) containing 5 mg of polymers (PLA-PLL) was prepared. In 2 ml aqueous solution of pluronic F-68 (1%, w/v), the prepared emulsion was poured with sonication for stirring as well as subsequently stirred at room temperature for 4 hrs to evaporate the organic phase. By centrifugation at 14,000 rpm for 30 minutes resultant NPs were purified and obtained.

Same procedure for the PLA-PLL NPs was prepared according to the above-mentioned method only omitting the RB [18]. Finally, size and z potential of NPs were measured.

Table 3: Liposomal carrier for therapeutics uses

Drug	Trade name	Company	Application	Adminstration	Clinical trial	Approval	Year	Reference
Amphotericin B	Abelcet	Enzon	Fungal infections	Intravenous	-	-	-	[2,51]
Amphotericin B	AmBisome	Gilead Sciences	Fungal and protozoal infections	Intravenous	-	-	-	[2,51]
Cytrabine	DepoCyt	SkyePharma	Malignant tymphomatous meningitis	Intrathecal	-	-	-	[2,51]
Daunorubicin	DaunoXome	Gilead sciences	HIV-related Kaposi's sarcoma	Intravenous	-	-	-	[2,51]
Doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer	Intravenous	-	-	-	[2,51]
IRIV vaccine	Epaxal	Berna Biotech	Hepatitis A	Intramuscular	-	-	-	[2,51]
IRIV vaccine	Iflexal V	Berna Biotech	Influenza	Intra muscular	-	-	-	[2,51]
Morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia	Epidural	-	-	-	[2,51]
Verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis	Intravenous	-	-	-	[2,51]
PEG doxorubicin	Doxil/Caelyx	Ortho Biotech Schering-Plough	HIV-related Kaposi's sarcoma metastatic breast cancer, metastatic ovarian cancer	Intra muscular	-	-	-	[2,51]
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy	Topical	-	-	-	[2,51]
Annamycin	L-Annamycin	Callisto	Acute lymphocytic leukemia acute myeloid leukemia	Intravenous	Phase-I	-	-	[2,51]
Cisplatin	SLIT Cisplatin	Transave	Progressive osteogenic sarcoma metastatic to the lung	Aerosol	Phase-II	-	-	[2,51]
Doxorubicin	Sarcodoxome	GP-Pharm	Soft tissue sarcoma	Intravenous	Phase-I/II	-	-	[2,51]
Fentanyl	AeroLEF	Delex therapeutics	Postoperative analgesic	Aerosol	Phase-II	-	-	[2,51]
Lurtotecan	OSI-211	OSI Pharmaceuticals	Ovarian cancer	Intravenous	Phase-II	-	-	[2,51]
Vincristine	Onco TCS	Inex, Enzon	Non-Hodgkin's lymphoma	Intravenous	Phase-II/III	-	-	[2,51]

Method of conjugation of EGFRmAb to NPs

Coupling strategy was performed for EGFRmAb conjugation between EGFRmAb and RB-PLA-PLL or PLAPLL NPs having EDC and NHS as the coupling agent. The procedure followed by incubation of 50 ll of 50 mg/ml EDC and 50 ll of 50 mg/ml NHS for 4 hrs at room temperature with gentle stirring with 1 ml of RB-PLA-PLL or PLA-PLL NPs suspension (5 mg/ml, pH 7.1). In the NPs suspension, the EGFRmAb was added and incubated for 4 hrs. After the conjugation, the centrifugation of the EGFRmAb modified RB-PLA-PLL (RB-PLAPLL-EGFRmAb) or PLA-PLL NPs (PLA-PLL-EGFRmAb) was performed for purification at 14000 rpm 9 30 minute twice and redispersed in PBS. Finally, washing of RB-PLA-PLL-EGFRmAb or PLA-PLL-EGFRmAb NPs with PBS was performed several times and kept at 4-degree centigrade in PBS.

Cellular uptake of NPs [45]

The experiment was performed by up taking RB encapsulated in NPs. 6-well plates were seeded with SMMC-7721 cells and incubated for 24 hrs. About 80 % confluence of cell culture, RB-PLA-PLL and RB-PLA-PLLEGFRmAb NPs were added at equivalent RB per well with simultaneous incubation with cells for 2 hrs at 37°C. 3 times washing was performed with PBS with fixation with 4% p-formaldehyde (PFA) for 20 minutes.

Cellular binding of NPs [45]

As discussed earlier, SMMC - 7721 cells were cultured in 6 well plates which were used for investigating the binding ability of NPs. After that, the cells were treated with RB-PLA-PLL, RB-PLA-PLL-EGFRmAb and 50-fold moles of free EGFRmAb. RB-PLA-PLL-EGFRmAb NPs for 2 hrs at 37°C with PBS washing twice. Finally, the residual was trypsinized and harvested and washed and resuspended in PBS.

Targeting of NPs

RB was encapsulated in NPs - The prepared product RB, RB-PLA-PLL, RB-PLAPLL-EGFRmAb NPs were injected into tumor-bearing mice through the tail vein and administered for targeted delivery.

Characterization

Physicochemical characterization of NPs

RB-PLA-PLL NPs were prepared using emulsion-solvent evaporation method. Orthogonal design was applied to optimize the preparation technology on the basis of the single factor evaluation. The optimal conditions for preparation of NPs were as follows: 25 mg/ml and 10 mg/ml were the concentration of PLA-PLL and RB, respectively. The methylene dichloride/acetone ratio was 3:2 (v/v), the concentration of pluronic F-68 was 1%, and the volume ratio of O/W was 1/10 (v/v). The size of RB-PLA-PLL-EGFRmAb NPs increases 21 nm as compared with RB-PLA-PLL NPs, presumably owing to the presence of antibody on the NPs surface. The mean zeta potential of RB-PLA-PLL NPs was 11.2±4.1 mV, whereas the zeta potential of RB-PLA-PLL-EGFRmAb NPs is 14.0±7.2 mV. It further demonstrated that the increase in zeta potential might be attributed to the presence of antibody on the NPs surface.

Table 4: Polymeric carrier for therapeutics uses

Drug	Trade name	Company	Application	Adminstration	Clinical trial	Approval	Year	Reference
L-Glutamic acid, L-alanine, L-lysine, and L-tyrosine copolymer	Copaxone	TEVA Pharmaceuticals	Multiple sclerosis	Subcutaneous	-	-	-	[2,51]
Methoxy-PEG-poly (D, L-lactide)	Genexol-PM	Samyang	Metastatic breast cancer	Intravenous	-	-	-	[2,51]
PEG-Adenosine deaminase	Adagen	Enzon	Severe combined immunodeficiency disease associated with ADA deficiency	Intramuscular	-	-	-	[2,51]
PEG-anti-VEGF aptamer	Macugen	OSI Pharmaceuticals	Age-related mascular degeneration	Intravitreous	-	-	-	[2,51]
PEG-α-interferon 2a	Pegasys	Nektar, Hoffmann-La Roche	Hepatitis B, Hepatitis C	Subcutaneous	-	-	-	[2,51]
PEG-GCSF	Neulasta	Amgen	Neutropenia associated with cancer chemotherapy	Subcutaneous	-	-	-	[2,51]
PEG-HGF	Somavert	Nektar, Pfizer	Acromegaly	Subcutaneous	-	-	-	[2,51]
PEG-L-asparaginase	Oncaspar	Enzon	Acute lymphoblastic leukemia	Intravenous intramuscular	-	-		[2,51]
Poly (allylamine hydrochloride)	Renagel	Genzyme	End-stage renal desease	Oral	-	-	-	[2,51]
HPMA copolymer-DACH platinate	ProLindac	Access pharmaceuticals	Ovarian cancer	Intravenous	Phase-II	-	-	[2,51]
L-leucine, L-glutamate copolymer, and insulin	Basulin	Flamel technologies	Type-I diabetes	Subcutaneous	Phase-II	-	-	[2,51]
PEG-anti TNF-α antibody fragment	Cimzia	Nektar	Rheumatoid arthritis and Crohn's disease	Subcutaneous	Phase-III	-	-	[2,51]
PEG-arginine deaminase	Hepacid	Phoenix	Hepatocellular carcinoma	Intravenous	Phase-I/II	-	-	[2,51]
PEG-camptothecin	Prothecan	Enzon	Various cancers	Intravenous	Phase-I/II	-	-	[2,51]
PEG-naloxol	NKTR-118	Nektar	Opioid-induced constipation	Oral	Phase-I	-	-	[2,51]
PEG-uricase	Puricase	Phoenix	Hyperuricemia from gout	Intravenous	Phase-III	-	-	[2,51]
Pluronic block-copolymer doxorubicin	SP1049C	Supratech pharma	Esophageal carcinoma	Intravenous	Phase-II	-	-	[2,51]
Polycyclodextrin camptothecin	IT-101	Insert therapeutics	Metastatic solid tumors	Intravenous	Phase-I	-	-	[2,51]
Polyglutamate camptothecin	CT-2106	Cell therapeutics	Colorectal and Ovarian cancer	Intravenous	Phase-I/II	-	-	[2,51]
Polyglutamate paclitaxel	Xyotax	Cell therapeutics	Non-small-cell lung cancer, Ovarian cancer	Intravenous	Phase-III	-	-	[2,51]
Poly (isohexylcyanoacrylate) doxorubicin	Transdrug	BIOALLIANCE pharma	Hepatocellular carcinoma	Intra-arterial	Phase-I/II	-	-	[2,51]

Particle size and zeta potential of NPs

RB-PLA-PLL NPs RB-PLA-PLL-EGFRmAb Nps
Particle size (nm) 126±20.4 147±26.2
Zeta potential (mV) 11.2±4.1 14.0±7.2
Data were expressed as the mean ± SD (n=6) that PLA-PLL-EGFRmAb NPs may be used as a target delivery carrier.

Binding and internalization of antibody modified NPs

FACS was used to observe the binding ability of RB-PLA-PLL and RB-PLA-PLL-EGFRmAb NPs to tumor SMMC-7721. As shown in Fig. 2, a little fluorescence was detected in cells incubated with RB-PLA-PLL NPs, whereas much higher fluorescence intensity was found in cells treated with RB-PLA-PLL-EGFRmAb NPs, which could be caused by only a little RB-PLA-PLL NPs that were internalized into the cells by endocytosis or phagocytosis. While in the case of RB-PLA-PLL-EGFRmAb NPs, the ligand-receptor recognition could help to increase the internalization of NPs. Confocal microscopy was used to observe the intracellular distribution of the uptaken NPs. After incubation with either RB-PLA-PLL or RB-PLA-PLL-EGFRmAb NPs, the fluorescence was mainly localized in cytoplasm. Fluorescence intensity of cells treated with RB-PLA-PLLEGFRmAb NPs was much higher than that of those cells treated with RB-PLA-PLL NPs, which was consistent with above result of cellular binding. To demonstrate the specificity of the interaction of RB-PLA-PLL-EGFRmAb NPs with the cells, a competition

Table 5: Miscellaneous nano-carrier for therapeutics uses

Drug	Trade name	Company	Application	Adminstration	Clinical trial	Approval	Year	Reference
Albumin-bound paclitaxel	Abraxane	Abraxis BioScience	Metastaltic breast cancer	Intravenous	-	-	-	[51,52]
Nanocrystalline aprepitant	Emend	Elan, Merk	Antiemetic	Oral	-	-	-	[51,52]
Nanocrystalline fenofibrate	Elan, Abbott	Antihyperlipidimic	Oral	Oral	-	-	-	[51,52]
Nanocrystalline sirolimous	Rapamune	Elan, WyethPharmaceuticals	Immunosuppressant	Oral	-	-	-	[51,52]
Calcium phosphate nanoparticle vaccine adjuvant	BioVant	BioSante	Vaccine adjuvant	Subcutaneous	Phase-I	-	-	[51,52]
Nanocrystalline paliperidone palmitate	Paliperidone palmitate	Elan, Johnson and Johnson	Schizophrenia	Intramuscular	Phase-III	-	-	[51,52]
Nanocrystalline 2-methoxyestradiol	Panzem NCD	Elan, EntreMed	Various cancers	Oral	Phase-II	-	-	[51,52]
Nanoemulsion-based therapy	NB-001	NanoBio	Herpes labialis	Topical	Phase-II	-	-	[51,52]
Nanoemulsion-based therapy	NB-002	NanoBio	Onychomycosis	Topical	Phase-I/II	-	-	[51,52]
Paclitaxel nanoparticles in porous hydrophilic matrix	Al-850	Acusphere	Solid tumors	Intravenous	Phase-I	-	-	[51,52]
Poly-L- lysine dendrimer	VivaGel	Starpharma	Antimicrobial protection from genital herpes and HIV infection	Topical	Phase-I	-	-	[51,52]
Propofol IDD-D	Propofol IDD-D	SkyePharma	Anesthetic	Intravenous	Phase-III	-	-	[51,52]

Table 6: Distinct specification of antibody and nanoparticle

Antibody	Nanoparticle
Specific nano-sized biological entity	Multifunctional intriguing tool with active potential
Essential component of immune system	Specific physio-chemical properties and biomimetic features
Distinct properties vary with isotypes	Constant physical entity regardless of size
Specific recognition and binding domain	Controlled drug release
Naturally occurring in vertebrates	Specific site drug delivery
Glycoprotein in nature biosynthesized from β –lymphocytes	Able to carry drug pay load
Coupling with specific antigen elicit distinct immunogenic response	High chemical and biological stability
Each isotypes shows distinct and definite immune response	Feasibility with hydrophobic and hydrophilic pharmaceuticals
Feasibility in binding with receptor	Administered by a variety of route including parental, oral and inhalation
Very less side effects since naturally produce	Minimal side effects
No question of toxicity	Nominal toxicity

assay was performed using free EGFRmAb. The binding of RB-PLA-PLL-EGFRmAb NPs to SMMC-7721 cells was inhibited by EGFRmAb, which demonstrated the RB-PLA-PLL-EGFRmAb NPs was internalized by ligand-mediated approaches.

***In vivo* ligand-mediated target delivery**

Fluorescence dye RB was encapsulated in NPs so as to investigate the efficacy of target delivery. The tumor fluorescence intensity of mice injected with RB and RB-PLA-PLL NPs were gradually decreased. In sharp contrast, the tumor fluorescence intensity of mice treated with RB-PLA-PLLEGFRmAb NPs did not decrease significantly with time post-injection. At 24 hrs of post-injection, the tumor fluorescence intensity of mice treated with RB had decreased significantly as compared with mice treated with RB-PLA-PLL or RB-PLA-PLL EGFRmAb NPs. At 48 hrs of post-injection, the differences of tumor fluorescent intensity of mice treated with RB, RB-PLA-PLL, and RB-PLA-PLL-EGFRmAb NPs were more significant. It was clear that for RB-PLA-PLLEGFRmAb NP-treated mice, the fluorescent intensity of tumor was relatively stronger and could lasted longer when compared with the RB or RB-PLA-PLL NPs-treated mice. The main reason resulting in the difference of fluorescence intensity between RB-PLA-PLL and RB-PLA-PLL-EGFRmAb NPs was the difference in tumor cells uptake to NPs. RB was quickly showed up in the kidney

Table 7: Conjugation of Antibody covalently with hydrophobic anchor

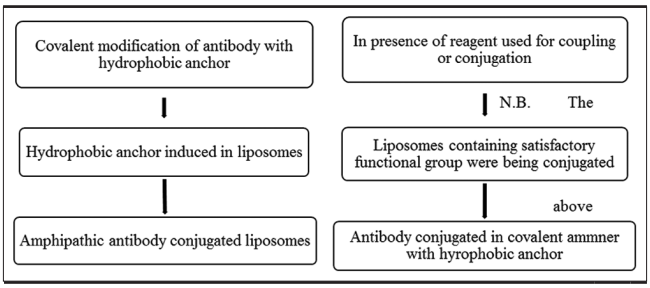


Table 8: Immunonanoparticle for therapeutics uses

Drug	Trade name	Company	Application	Adminstration	Clinical trial	Approval	Year	Reference
Antibody-enzyme-conjugated nanoparticles (immunoenzymosomes)	-	-	Ovarian cancer antibody-directed enzyme prodrug therapy	-	Preclinical development	-	-	[51,53]
Biotinylated antibody-conjugated polymeric micelles	-	-	Brain targeting daunomycin	-	Preclinical development	-	-	[51,53]
Anti-HER2 antibody targeted gold/silicon nanoparticles	-	-	Metastatic breast cancer nanoshell-assisted infrared photothermal therepy	-	Preclinical development	-	-	[51,53]
Immuno-PEG-liposomes (100)	-	-	Metastatic stomach cancer doxorubicin	-	Phase-I	-	-	[51,53]
Immunoliposomes (110-150)	-	-	Doxorubicin platinum based drugs, vinblastin, vincristin, topotecan, paclitaxel	-	*In* vivo	-	-	[51,53]
Immunotoxins, Immunopolymers, and fusion proteins (3-15)	-	-	Various types of cancer, various drugs, toxins	-	Phase-I-III	-	-	[51,53]

and bladder region and then eliminated. Therefore, the accumulation in tumor tissue was lower. As compared with RB, the RB-PLA-PLL NPs passively target to tumor by enhanced permeability and retention effect (EPR) and showed more accumulation in tumor. This was mainly because that the NPs, generally controlled under 200 nm, can help to leak preferably throughout the tumor vasculature. The RB-PLA-PLL-EGFRmAb NPs could actively target to tumor via the ligand-receptor recognition, which helped the further enhance delivery of NPs.

Future perspectives

Biological milieu mediated *in vivo* targeted delivery resulted to be accurate carrier increasing the efficacy to cure the disease state having a distinct capacity as a carrier with binding moieties. The efficient PLA-PLL-EGFRmAb NPs could target to tumors showing better targeting affinity compared to PLA-PLL NPs. Thus, better targeting of PLA-PLL-EGFRmAb attributed a better result.

Carbohydrate modified ultrafine ceramic nanoparticles for allergen immunotherapy [55]

Aquasomes are basically three layered self-assembled peptide/protein nano-carrier which helps to deliver enzymes, antigens, structural proteins, etc., to the desired site. This nano-delivery - a niche as protein, peptide carrier, are composed of:

a. Solid central Nanocrystalline core - the initial core giving stable structural form
b. Polyhydroxy oligomers – the first carbohydrate coating over the initial one protecting from dehydration as well as stabilizes the A.P.I.
c. Biochemically active molecules (A.P.I.), which get absorbed over the first coating – the drug.

The conformational integrity of A.P.I. molecules with the carbohydrate coating and the core is due to the non-covalent bonds, van der Waal's forces as well as ionic bonds [54] which make this delivery system potentially successful in delivering insulin, antigens, hemoglobin even serratiopeptidase - an oral enzyme delivery, etc., clinically proving to be a reliable approach. The ceramic core which is the foundation of the nano-delivery system is having a carbohydrate modified non-covalentl surface to get a sugar ball. This gets absorbed to the therapeutic agent helping to resolve serious disease belonging to the class of protein and peptides.

Method of preparation [55]

Materials

The (NH4)2•HPO4, Ca (NO3)2•4H2O, trehalose, aluminum hydroxide gel (Alhydrogel®), Protein assay kit and the reagents used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and bicinchoninic acid (BCA), bovine serum albumin, horseradish peroxidase conjugated goat antimouse IgG, IgG1, IgG2a and IgE antibodies, cytokine kits, and distilled deionized water.

Preparation of hydroxyapatite (HA) core

By self-precipitation method HA core was prepared [55,56]. 0.5 M With vigorous stirring (NH4) 2HPO4 was added quickly to 0.5 M Ca (NO3)2•4H2O ethanol solution. By adding concentrated NH4OH solution drop wise the pH of both the solution were kept at 10. Mixture was stirred vigorously for 1 hr with mechanical stirrer and overnight drying at 40°C in oven. Washing the precipitated HA particles with ethanol and then with distilled deionized water was performed. Lastly, ceramic particle suspension was again dried overnight at 40°C overnight. The KBr sample disk was prepared using 1% (w/w) of HA powder and was compressed and dried at 100°C.

Preparation of OVA adsorbed aquasomes

Aquasomes were prepared according to the references [55,57]. A brief view; HA weighing of about 15 mg was initially suspended in 30 ml of 29.2 mM trehalose solution in a 50 ml clean beaker by vigorous stirring. The formed suspension was sonicated for 10 minutes at a frequency of approximately 20 kHz at 25°C using a probe sonicator. The dispersion was clarified by centrifugation at 10,000 rpm for 20 minutes at 4°C. The remaining pellets were then discarded, and the mixture was lyophilized overnight at a condenser temperature of −82°C and pressure of <10-1 mbar. Unadsorbed trehalose was removed by centrifugation against 20 ml of 25 mM phosphate buffer (pH 7.4). 10 mg of sugar-coated particles was dispersed in OVA solution (8 mg/ml in PBS pH 7.4) and kept at 4°C overnight. OVA adsorbed aquasomes were washed 3 times with de-ionized water by using centrifugation at 10,000 rpm for 30 minutes and stored at 4°C until used.

***In vivo* immunological response**

Female BALB/c mice (age 7-9 weeks; weight 15-20 g) were used in all experiments. Animals were housed in climate (23±2°C; RH: 60%) and photoperiod (12 hrs light-dark cycles) controlled animal quarters at the animal house and resource facility in group of six (n=6). They were fed standard rodent pellet and had free access to drinking water. After one week of acclimatization with the environment, immunization protocols were followed. They were withdrawn of any food intake 3 hrs before immunization. The study protocol including handling, care and immunization were approved and followed under guidelines of Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. Animals were immunized by two intradermal injections with two weeks interval, i.e. at day 0 (priming) and at day 14 (boosting) with one of the following: (i) free OVA (10 µg per mouse) in 50 µl of PBS, (ii) blank aquasomes 100 µg in 50 µl of PBS; (iii) OVA aquasomes (equivalent of 10 µg of OVA per mouse), and (iv) OVA in alum (OVA alum) and (v) sterile PBS 50 µl only. OVA alum was prepared by dissolving the protein (50 µg/ml) in PBS (pH 7.4, 0.01 M) and subsequently mixing by sonication with aluminum hydroxide gel (1 mg/ml).

Blood sample collection

Blood was collected from the saphenous vein of mice (under mild ether anesthesia) at day 0 (pre-immune sera, 3 hrs before immunization), 14 days and 42 days (terminal samples) of priming. The terminal blood samples were obtained by cardiac puncture post euthanasia after 4 weeks of booster immunization. Serum was separated by centrifugation (1500×g for 5 minutes) from collected blood samples after allowing coagulation for 30 minutes at room temperature. Serum samples were stored at −22°C until analyzed for antibody. Spleen of three mice from each group was surgically removed at the end of the experiment for cytokine assay.

Characterization

Characterization of HA core

FT-IR was used for structure analysis of ceramic core. Infrared spectra were recorded in the wave number range of 4000 to 400 cm^{-1} (resolution 4.0 cm^{-1}) using FT-IR spectrophotometer. Phase analysis of HA core was performed using X-ray diffraction (XRD). HA ceramic cores were exposed to Cu Kα radiation in a wide-angle X-ray diffractometer. The instrument was operated in the step-scan mode in increments of 0.03° 2θ. The angular range was 10-50° 2θ, and counts were accumulated for 1 second at each step. The particle morphology was determined by TEM. One drop of aqueous dispersion was placed over a 400-mesh carbon-coated copper grid followed by negative staining with phosphotungstic acid (3%, w/v, adjusted to pH 4.7 with KOH) and placed at the accelerating voltage of 80 kV. The mean hydrodynamic diameter, polydispersity and zeta potential of core was measured by photon correlation spectroscopy (PCS) using Zeta Nano ZS 90) after appropriate dilution (1:200) with PBS pH 7.4 prior to analysis. HA particles prepared by self-precipitation method under controlled process parameters were smaller in size and crystalline in nature. The prepared HA core was analyzed by FT-IR spectroscopy to evaluate their crystallinity. The bands for PO4−3 of the calcined powder observed at 507, 604, 945, 964, 1024, and 1184 cm^{-1}, whereas the medium sharp peak at 633, 2910 and 3570 cm^{-1} was due to the OH−1 bending deformation. The FT-IR spectrum of prepared HA core was in accordance with [55,56,63]. HA ceramic core was characterized by X-ray diffraction patterns to assess their crystalline nature. Characteristic intense absorption peaks at 31-32, 49-50; 25-27 (2θ angle) indicates the crystalline behavior of HA. The XRD pattern of prepared sintered HA ceramic core complied with the standard HA core [55,57,64]. The morphology of the HA core was revealed in TEM image. The microstructure of HA core revealed that the ultrafine nano-sized spherical particles were dense with rough surfaces. Some aggregation was also observed which may be due to drying. DLS results support the finding of TEM and particle size of HA was found to be 39 nm with polydispersity index 0.28.

Characterization of OVA adsorbed aquasomes

The morphological examination, mean particle size and size distribution of prepared aquasomes were determined as described above for ceramic core. Sugar content on surface of HA was determined by anthrone method [55, 58] using glucose as standard. The adsorption of sugar on the surface of HA was confirmed by change in zeta potential using Zetasizer Nano ZS 90. SDSPAGE experiment was carried out to analyze the integrity of OVA antigen under non-denaturing condition. Protein bands were detected by Coomassie blue staining. SDS-PAGE experiment was performed in the presence of strong anionic reducing agent, i.e., SDS In the presence of strong reducing agent and heat, proteins got dissociated before they were applied on the gel [55,59]. OVA was extracted by dissolving the aquasomes in 2 ml of 5% (w/v) SDS in 0.1 N HCl solution. The extracted allergen was concentrated and adsorbed onto a 3.5% stacking gel and subjected to electrophoresis on a 12% separating gel at 200 V until the coomassie dye stained protein band reached the gel bottom.

Aquasomes

Aquasome formulations were also characterized for the size, shape, and antigen adsorption efficiency. TEM image of aquasome systems indicated that the systems were smaller in size, spherical and elongated in shape. Photon correlation spectroscopy also confirmed the nanometric architecture of prepared aquasomes. Both TEM and PCS results revealed that the aquasomes ranged from 47 nm.

Adsorption efficiency and OVA release from aquasomes

The percentage OVA adsorption efficiency of aquasomes was determined as reported [55,57]. To be brief, accurately weighed OVA adsorbed aquasome formulations (200 mg) were suspended in Triton-X 100 (0.01%, w/v) and incubated in a wrist action shaker for 1 hr. The samples were centrifuged at 14,000 rpm for 15 minute and antigen concentration in the supernatant was determined using micro BCA method while setting a blank of plain aquasome formulation treated in the same manner. For in vitro release studies, 200 mg of OVA adsorbed aquasomes was suspended in phosphate buffer (pH 7.4, 10 ml) with slow stirring in magnetic stirrer. Samples were withdrawn (1 ml) after 15 minutes each followed by centrifugation (1700 rpm for 10 minutes at 4°C) and the released protein in the supernatant was measured by BCA protein assay at various times. Equal quantity of fresh buffer was added after each sampling to maintain the sink conditions.

Measurement of humoral response

An indirect ELISA was used to determine the level of OVA-specific IgG antibodies and isotypes in the serum. Each well of microtiter plate was coated with antigen (100 µg/ml, 50 µg/ml, 25 µg/ml OVA in bicarbonate buffer, pH 9.6). Each well was dispensed with 100 µl volume and incubated at 4°C overnight. The plate was then washed three times with PBS-Tween 20 (PBS-T, 0.05%, v/v) followed by blocking with 150 µl of 3% gelatin in PBS for 90 minutes at 37°C and washed thrice with PBS-T. 100 µl of serum (1:800) in gelatin PBS-T20 was added in each well and incubated for 90 minutes at room temperature. The plate was then washed three times as described above. 100 µl of peroxidase labeled goat anti-mouse IgG, IgG2a or IgG1 (1:1000) was added to each well. The plates were covered and after incubation for 1 h at room temperature washing was repeated. 100 µl of substrate ophenylenediamine dihydrochloride (prepared in citrate buffer+H_2O_2) was added to each well followed by addition of 50 µl of H_2SO_4 after 90 minutes. After development of color, the reaction was stopped by adding 25 µl of 2.5M H_2SO_4 and absorbance was measured at 492 nm. Values are expressed as mean±S.D.

Estimation of cytokine levels

Endogenous levels of IL-4 and IFN-γ in mouse spleen homogenates were measured using separate ELISA kits for these cytokines [55,60]. Individual spleens from euthanized mice at the end of experiment were separated, weighed and homogenized in ice-cold RPMI containing 1% 3-(cholamidopropyl-o-dimethylammonio)-1-propanesulphonate

(CHAPS; Sigma) in a micro tissue homogenizer. 10% (w/v) homogenates were stored for 1 hrs at 2-4°C and insoluble debris were removed by centrifugation at 2000×g for 20 minute. Capture ELISA based mouse cytokine assay kits were used to determine the presence of cytokines in spleen homogenates according to the manufacturer's instructions. Data are expressed as picogram per millilitre in the spleen homogenates as computed by comparison with the standard curve provided by the manufacturer for IL-4 and IFN-γ.

Sensitization, vaccination and challenge studies

For sensitization studies, [55,61] protocol was followed. BALB/c mice were sensitized by intraperitoneal injection of 50 μg of OVA emulsified in 1 mg alum adjuvant in a total volume of 150 μl on days 1 and 8. On days 14, 17 and 20, the animals (5 mice per group) received intradermal injections with 3 μg of OVA each incorporated in either OVA-aqausomes, OVA-alum and OVA in PBS. Finally, on day 42 the animals were challenged by an injection of 1 mg of OVA by intraperitoneal route. To monitor serum IgE antibody responses, blood samples from saphenous vein were obtained along the sensitization and immunotherapy periods and stored at −80±1°C until their analysis.

Evaluation of anaphylaxis

IgE antibody level was determined as per reference [55] following the same protocol except the serum dilution ratio was 1:400 and peroxidase labeled goat anti-mouse IgE antibody was used. Histamine release test was performed on heparinized whole blood from the saphenous vein obtained before and 30 minutes after the challenge. Histamine release was assayed by a fluorometric method as previously described [55,62]. The body temperature changes associated with anaphylactic shock were monitored by measuring the rectal temperature without general anesthesia before and 10 minutes after the challenge. Anaphylactic symptoms (activity/lethargy, piloerection or ruffled or erect fur and cyanosis) were evaluated 30 minutes after the challenge and reaction severity was classified according to the categories depending on their gravity: (i) (−) absent, (ii) (+) weak, (iii) (3) moderate, and (iv) (+++) strong. Finally, the survival rate was recorded 24 hrs after intraperitoneal challenge as described [55,61,62].

Adsorption efficiency and release studies

Coating of trehalose using freeze drying showed that HA core adsorbed 2.887% of the trehalose initially added. The zeta potential of the HA ceramic core was slightly positive in pH 7.4 that was reduced to slightly negative value after coating of trehalose, which confirm the coating of sugar layer on ceramic core. Further adsorption of OVA results in negative value of −11.34±1.4 mV. Percent antigen adsorption on aquasome was found to be 60.22±2.24 μg/mg of core. It was previously demonstrated that hemoglobin and insulin intercalated between the trehalose loose epitaxial adsorption around the ceramic core [55,71], similar trend is also postulated in this study, and thus it was assumed that OVA was tightly packed between the trehalose molecules. During the first period, about 40% of the absorbed OVA was released into the medium. Subsequent release of OVA was very small and decreased over time. The cumulative amounts of OVA released at 50 minutes reached at least 90%.

In process stability studies

The ultimate stability of a protein containing preparations can often be a function of the physical and chemical conditions to which it was exposed during processing. Therefore, the primary aim of the *in vitro* stability studies was to determine the structural integrity of antigen protein during the preparation of aquasomes by SDS-PAGE. This in turns revealed that the formulation procedures used did not irreversibly aggregate or cleave the antigen. The data suggested that there was not any deleterious effect on the OVA integrity during the preparation of aquasome formulations. It had been reported that trehalose increases the transition temperature (ΔTm) of proteins to the maximum and polyhydroxy oligomeric coating provides water like environment in extreme desiccated conditions thus it proved to be one of the best candidates as a universal stabilizer because of its inertness toward protein surfaces [55].

***In vivo* immunological response**

The serum levels of OVA-specific IgG isotypes elicited in BALB/c mice after two doses of 10 μg of OVA (equivalent dose with alum or adsorbed on aquasomes) were noted. Comparable titer of IgG1 antibodies was detected in sera of the animals, immunized with OVA adsorbed aquasomes or inoculated with OVA-alum. On the other hand, immunization with naïve OVA in PBS or blank aquasomes was not able to induce any OVA-specific antibody response. Furthermore, an enhanced and significant immune response was also observed after the booster injection in OVA aquasomes immunized mice as compared to OVA in PBS (pb0.001). After the boost, OVA adsorbed aquasomes induced higher antibody response compared with the primary response, indicating induction of memory cells after priming (pb0.05). Negligible IgG2a primary and secondary response was elicited by the OVA alone, while a weak IgG2a antibody titer was elicited by OVA alum in the secondary response only. In contrast, mice injected with OVA adsorbed aquasomes induced high levels of IgG2a. The consistently higher IgG2a anti-OVA titers obtained, in both primary (pb0.001) and secondary (pb0.02) responses, in mice treated with OVA adsorbed aquasomes than in mice treated with OVA alum were observed in all mice. Because IgG1 is driven by IL-4 (Th2), and IgG2a is driven by IFN-γ (Th1) an increase of IgG1/IgG2a ratio after vaccination indicates a Th2 response, and a decrease in this ratio after immunization indicates a Th1 response. Alum induces almost only a Th2 response, while OVA adsorbed aquasomes elicit both Th1 and Th2 responses as demonstrated by the high IgG2a and IgG1 anti-OVA antibody responses. These data were confirmed by studying the cellular immune response. Significantly elevated levels of antigen-specific IFN-γ and IL-4 release were detected in spleens of mice immunized with OVA aquasomes as compared to mice treated with OVA alum (pb0.01) and OVA in PBS (pb0.001).

Mice sensitization and induction of active systemic anaphylaxis

BALB/c mice were sensitized with 50 mg OVA-Alum by intraperitoneal route at days 0 and 7. Once the allergic status to OVA was confirmed, mice were immunized intradermally at days 13, 16 and 19 with either OVA-aquasomeor OVA-alum (as positive control; 3 mg OVA per immunization). Finally, at day 34, mice were challenged with 1 mg of OVA (intraperitoneal) to provoke a systemic anaphylaxis. At day 42, IgE antibody serum titer was significantly lower in group immunized with OVA-aquasomes as compared to the group immunized with OVA alum (pb0.001). Present observation is consistent with previous report which demonstrated that calcium phosphate nanoparticles (CAP) induced lower IgE titer as compared to alum [55]. In order to estimate the IgE-mediated mast cell degranulation, the serum histamine level from the immunized animals was quantified 30 minutes after the intraperitoneal challenge. The histamine secretion in mice treated with OVA aquasomes was significantly reduced as compared to the OVA-alum (pb0.05). Likewise, the decrease of the body temperature was significantly lower in animals treated with OVA aquasomes than with OVA Alum (pb0.01 and pb0.001) compared with basal body temperature. The severity of the anaphylactic shock induced in the animals by both formulations was evaluated by measuring cyanosis, activity and piloerection (ruffled or erect fur). OVA aquasome immunized mice displayed a normal activity and motion with no cyanosis, while mice treated with OVA-alum exhibited a total lack of mobility and a high degree of cyanosis. Hence, animals treated with OVA aquasome displayed less intense symptoms than the control group. Finally, the death rate was also lower for the aquasome immunized animals than those treated with OVA-alum (50% vs. 80%, respectively).

FUTURE PERSPECTIVES

Tin oxide, diamond and brushite (calcium phosphate dihydrate) were generally used as core for preparation of aquasomes [55,65-68]. Owing to biodegradability, cost, stability, and safety, HA was selected as a core for the preparation of aquasomes. Moreover, it is widely used for the preparation of implants, drug and antigen delivery [55,63,69]. They were particularly suitable for protein delivery because of their high adsorption capability [55,70].

Table 9: Patent of immunonanoparticles

Drug	Patent detail	Delivery system method	Observed result	Reference
Antibody-nanoparticle conjugates and methods for making and using such conjugates	EP 2564203 A2 November 10, 2011	Antibody conjugated nanoparticle having two or more nanoparticle (gold, silver etc.) directly linked with antibody through metal – thiol bond	Methods of making the antibody- nanoparticle conjugates disclosed herein include reacting an arylphosphine-nanoparticle composite with a reduced antibody to produce an antibody-nanoparticle conjugate. Also disclosed herein are methods for detecting a target molecule in a sample that include using an antibody-nanoparticle conjugate (such as the antibody- nanoparticle conjugates described herein) and kits for detecting target molecules utilizing the methods disclosed herein.	Ashworth-Sharpe J. Antibody-nanoparticle conjugates and methods for making and using such conjugates. EP 2564203 A2. (2011)
Immunotheraphy of brain tumors using a nanoparticle CpG delivery system	US20130210896 A1 August 15, 2013	NANO-CpG delivery	Preparation methods using the NANO-CpG to improve CpG delivery into brain tumor associated inflammatory cells as well as for treating and/or preventing a brain tumour.	Badie B, *et al*. Immunotheraphy of brain tumors using a nanoparticle CpG Delivery System. US20130210896 A1. (2013)
pH-sensitive immunoliposomes and method of gene delivery to the mammalian central nervous system	US 5786214 A July 28, 1998	Direct injection in CNS	pH-sensitive immunoliposomes with a conjugated antibody sensitive to cells of the mammalian CNS, a method for introducing genetic material into the cells of the mammalian CNS in vitro through these liposomes, and a method of introducing genetic material into the cells of the mammalian CNS through direct injection of the liposomes into the CNS.	Holmberg EG. pH-sensitive immunoliposomes and method of gene delivery to the mammalian central nervous system. US 5786214 A (1998)
Antibody fragment-targeted immunoliposomes for systemic gene delivery	US 8859274 B2 October 14, 2014	Tumour-targeted, systemic delivery	Immunoliposomes that are capable of tumor-targeted, systemic delivery of nucleic acids for use in human gene therapy. High efficiency transfection of various mammalian cell types that express the transferrin receptor.	Xu L, *et al*. Antibody fragment-targeted immunoliposomes for systemic gene delivery. US 8859274 B2 (2014)
Methods of preparing targeted immunoliposomes	US 20070092558 A1 April 26, 2007	*In vivo* delivery	Useful as the entrapped agent in liposomes targeted for neoplastic disease indications.	Wu S, *et al*. Methods of Preparing Targeted Immunoliposomes. US 20070092558 A1 (2007)

Thus, it can be concluded that OVA adsorbed aquasomes have the ability to induce a strong T cell specific proliferative response with a cytokine profile suggestive of a Th1 response, prevention of anaphylactic reactions and maintenance of low titers of IgE, without abrogation of Th2-mediated responses. This suggests that aquasomes could have possible implications in the future of peptide-based vaccines against allergic disorders.

MARKETED PRODUCTS

Table 8 shows immunonanoparticles for therapeutic use.

PATENTS ON IMMUNONANOPARTICLES

Table 9 shows patents enlisted with details.

CONCLUSION

Comprehensively, after a detail overview of each and every aspects of antibody as well as nanoparticles, the conjugation of antibody with nano-carriers for improved therapeutic management of CNS diseases imparts innovative opportunities as pharmaceutical market is more trending towards biotechnology products. Keeping in account of BBB, the only highlighted issue of CNS delivery and one of the most vital limitation is to circumvent this complex barrier where the combination of antibody and nano-carriers can notably prove to be beneficial. Furthermore, the biotechnology-based products having high molecular weight is unable to traverse the BBB, which can be feasible significantly by the use of nanoparticles based conjugation of biological milieu like antibody.

The antibody is naturally produced but at the same time nanoparticle is human engineered, so the versatile combination of both of them for targeting drug delivery system can be useful in clinical scenario of CNS nanomedicine. Since this system is still in infant phase, a more in-depth study along with evaluation and in detail toxicological effect of brain targeting with species-specificity study is a need which will help to incorporate advanced study with more specific and efficient target availability for the improvement of safe and efficient CNS targeting antibody conjugated nanocarriers useful for commercialization in future.

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