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Review Article

Targeting Aspects of Nanogels: An Overview

Taha Umair Wani, Muzamil Rashid, Manish Kumar, Sushant Chaudhary, Puneet Kumar and Neeraj Mishra*

Department of Pharmaceutics, Department of Pharmacology, I.S.F. College of Pharmacy, Moga-142001, Punjab, India.

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ABSTRACT

Nanogels have shown a great potential for the delivery of large number of drugs to different organs of the body owing to their high biocompatibility, high drug loading capacity, high biodegradability (and hence low cytotoxicity), good permeation capabilities and tissue mimicking properties. Their high water retention makes them ideal capable of incorporation of bulky drugs like proteins, peptides, oligonucleotides and other macromolecules. All these properties of nanogels make them able to carry number of drugs to vast number of organs. Nanogels have shown potential in many fields including chemotherapy, diagnosis, organ tergeting, gene delivery and many others. The main areas of the target for the nanogels have been tumors of brain, liver, skin etc. Other uses of the nanogel are in diabetes, inflammation, wound healing, local anesthesia etc. This review concentrates over the targeting potential of nanogels in different organs for various conditions.

KEYWORDS: Nanogel; Polymer; Swelling; Loading capacity; Organ targeting; Cancer therapy.

Introduction

Nanogels are potential polymeric nanoparticulate systems having tremendous biomedical applications that can offer time-controlled drug delivery as well as active drug targeting (Oh et al., 2008; Asadian-Birjand et al., 2012). Structurally nanogels are spherical shaped nanometer sized (10s-100s of nm i.e., upto about 700 nm (Rigogliuso et al., 2012). Hydrogels also known as hydrogel nanoparticles possessing an internal polymeric network for incorporation of drug molecules or other biomolecules. Nanogels arecomposed of synthetic polymers or biopolymers which are chemically or physically cross-linked (Uthaman et al., 2014). They have been largely studied for the incorporation and release behavior of bioactive molecules e.g., drugs, peptides, proteins (Sekine et al., 2012), antigens (Yuki et al., 2013) oligonucleotides, genes, carbohydrates, DNA etc. and also for the incorporation of inorganic molecules e.g. quantum dots (Hasegawa et al., 2005), silver nanoparticles, magnetic nanoparticles (Jiang et al., 2013) etc. Nanogels can be precisely tuned to increase their circulation time in the blood (Vinogradov 2007), avoid clearance by the reticuloendotheial system (Yadav et al., 2011) and hence prolong the drug release (Rigogliuso et al., 2012) and can be conjugated with other molecules for targeted delivery of drugs that release the active substances at the targeted site (Blackburn et al., 2009). Nanogels can be administered with two basic strategies viz. passive targeting and active targeting. Drug release from nanogels follow mechanisms based on above two strategies. In case of passive targeting the nanogels show drug release with respect to their surface charge, size, swelling and other physico-chemical properties.

In case of active targeting nanogels are conjugated with some specific moieties that specifically recognize and bind to some of the receptors that are over expressed at the target sites e.g., in case of tumors many types of receptors are overexpressed that leads to the accumulation of conjugated nanogels at the target site (Rigogliuso et al., 2012).

Nanogels can imbibe large amounts of water and swell to large volumes that increase their loading capacity to accommodate a large quantity of drug. Fig. 1 shows the general structural network of nanogel and their drug loading capacity. With nanogels it is possible to attain greater than 98% of loading efficiency (Chen et al., 2013). Since nanogels are basically hydrogels so another unique property that they shear is their tissue mimicking ability (Mallefet and Dweck) due to the large amount of water they contain and the biocompatible materials used in their preparation. Topical application of such type of a gel gives a soothing effect that is very effective in treatment for conditions like wounds (Mallefet and Dweck). Among some other outstanding properties of the nanogels is their self-healing ability wherein new bonds in them spontaneously form upon the breakage of the older bonds (Kolmakov et al.,).

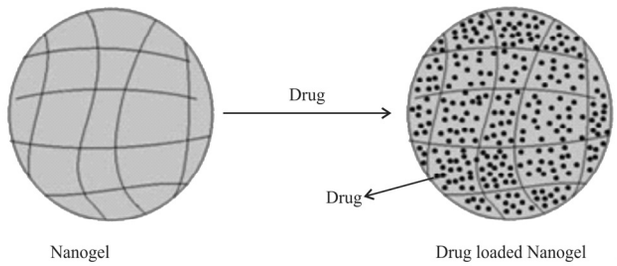

Fig. 1. Polymer network of nanogel demonstrating high drug loading capacity.

Advantages of Nanogels

Nanogels are quite smart to carry drugs to the biological sites of actions besides offering several other advantages ranging from drug loading to the pharmacokinetic characteristics. Nanogels are known for their high drug loading capacities and controlled release regulated by varying crosslinking densities of the polymers used in the preparation of the nanogels that affect the swelling characteristics of nanogels (He et al., 2010; Ryu et al., 2010). Following are some of the advantages that make nanogels unique as delivery systems:

(a) Highly biocompatible (due to high water content and hence behave like natural tissue) and therefore immunological responses
(b) Biodegradable, that makes these nanocarriers nontoxic
(c) High drug loading capacity
(d) Easily escape entrapment byreticuloendothelial system (Yadav et al. 2011)
(e) By tuning crosslinking densities drug release can be regulated (Ryu et al., 2010)
(f) Better permeation via biological membranes due to extremely small size
(g) Can incorporate both hydrophilic and hydrophobic (Murphy et al., 2011; Look et al., 2013) drugs and charged solutes
(h) Excellent transport characteristics.

Limitations of Nanogels

With a vast number of advantages, nanogels offer some limitations that may limit their use in some cases. Following are the limitations of nanogels.

(a) Complete removal of the solvent and surfactants requires expensive techniques.
(b) Traces of the surfactants or monomers may be left and can cause toxicity.
(c) Manufacturing variance, wherein the typical properties of nanogels are possible only within a certain range of sizes (Vinogradov 2007).

Types of Nanogels

Nanogels can be divided into various classes based on type of cross linking, response to an external stimuli (e.g., pH, temperature, light, ionic strength etc.) and methods of preparation. Fig. 2. Gives a brief account of different types of nanogels.

Polymers used in the preparation of nanogels: Nanogels form mainly from hydrophilic polymers that imbibe a large quantity of water between their cross-linked structures. The 3-D nature of the polymers used in the preparation of nanogels imparts some distinctive properties to these nanogels e.g., their biologically being inert and water sorption properties that makes them swell up to 1000 times in water. Following are some of the commonly used polymers in the synthesis of nanogels and can be classified as represented in Fig. 3.

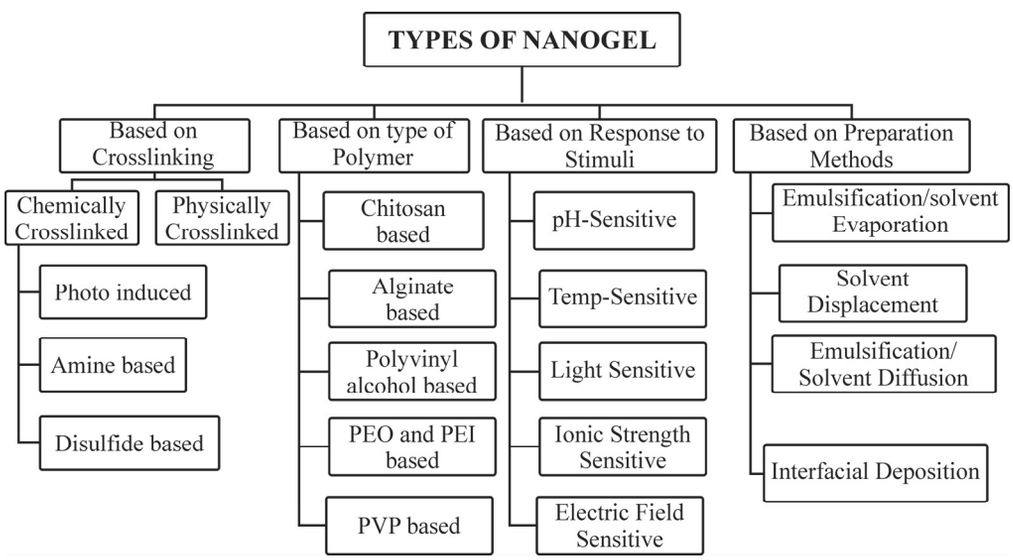

Fig. 2. Types of nanogels.

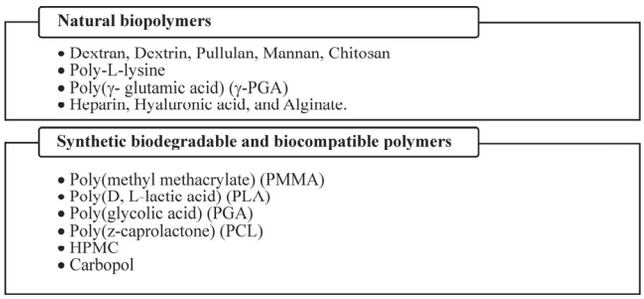

Fig. 3. Types of polymers used in the formulation of nanogels.

Preparation Techniques of Nanogels

Nanogels can be prepared by various techniques as mentioned below.

Preparation of nanogels can be categorized into four classes viz.

1. Preparation by polymerization of monomers
2. Crosslinking of preformed polymers
3. Physical association of polymers through interactive forces
4. Nanofabrication involving template assistance.

Following are the table and the schematic representing various methods of nanogel preparation.

TABLE 1

Methods of preparation of Nanogels

Method	Composition	References
Ionic gelation	Chitosan, TPP	Azadi et al., 2012
Emulsion polymerization	NIPAM, MBA Aac	Samah and Heard 2013
	NIPAM, MAA, PEGMA	Peng et al., 2013
	AMPS, NIPAM, PEG	Atta et al., 2013
Surfactant free emulsion polymerization	NIPAM, BA	Singka et al., 2010
Solvent emulsification/ evaporation method		Watts et al., 1989
Solvent displacement method (or nano-precipitation)	Tri block PLA-PEG-PLA Copolymers	Asadi et al., 2011
Inverse nanoprecipitation	Polyglycerol, *p*-PBDMA	Steinhilber et al., 2013
Modified solvent displacement method		C. G. Oster et al., 2006.
Emulsion-solvent evaporation technique	PEI, PEG	Vinogradov et al., 2004 Moya-Ortega et al., 2012
Semi-continuous emulsion polymerization	MAA-EA linked with DAP, PVC, PE-co-PVA-CO	Tan et al., 2007
Precipitation polymerization	PNIP/Aam	Wang et al., 2008
	NIPAM,	An et al., 2007
Desolvation method	Carbopol 940, HPMC	Hamidi et al., 2008
Emulsion-droplet coalescence method	Cabopol 940	Hamidi et al., 2008
Microemulsion template method	Carbopol 940, Cetylpalmitate, Propylene glycol	Khurana et al., 2013
Free radical precipitation polymerization	NIPAM, Acrylic acid (AA)	Su et al., 2013
Dispersion polymerization	PEG, Oligo (ethylene glycol) (meth)acrylates	Shen et al., 2011
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MBA: N,N'-methylenebis-acrylamide; NIPAM: N-isopropylacrylamide; MAA: Methylacrylic Acid; PEGMA: poly(ethylene glycol) methylether methacrylate, BA: Butylacrylate; PLA: polylactic acid; PEG: polyethylene glycol; PE-co-PVA-co-CO: poly(ethylene-co-vinyl acetate-co-carbon momoxide; MAA-EA: Methacrylic acid-ethyl acetate; DAP: Di-allyl-palmitate; AMPS: 2-acrylamido-2-methylpropane sulfonic acid; PBDMA: Poly butane diol monoacrybeta

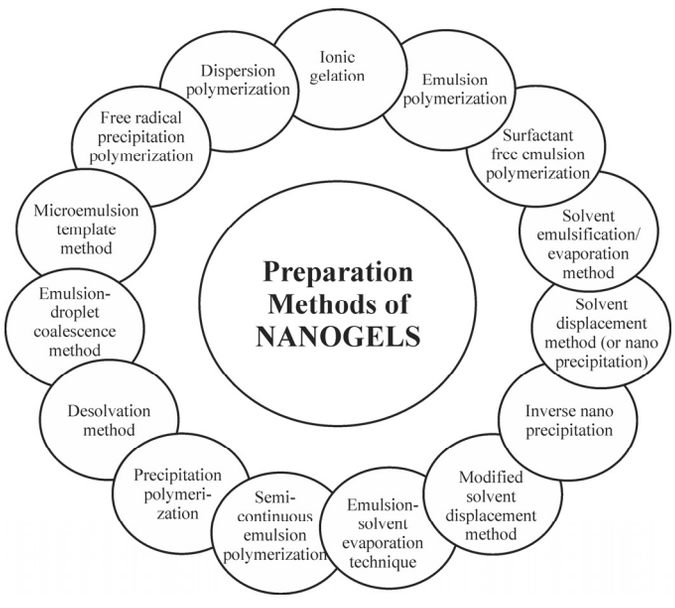

Fig. 4. Schematic representation of methods of prepararion of Nanogels.

Mechanisms of drug release from the nanogels: Present drug carrier systems are aimed to deliver the drugs by equipping the delivery systems with smart techniques that would sense the type and the amount of the change caused by a pathological condition and release the drug accordingly. Table 2 gives an outlook of some of the important mechanisms to describe drug release from nanogels.

TABLE 2

Mechanisms involved in drug release from Nanogels.

Mechanism	Description
pH responsive	Alteration of the pH causes swelling or deswelling of the polymers that contain weakly acidic or basic groups in their structures. Amount of swelling and deswelling is governed by the extent of ionization of the polymer that in turn is determined by the pH of the medium. Hence by regulating the extent of swelling of the polymer pH governs the rate and the extent of release of the drug. e.g., Poly(*N*-vinylformamide) Nanogels releasing proteins (Shi et al., 2008)
Temperature responsive	Specific polymers are tuned to impart them sensitivity for a temperature which results in the expansion of the polymeric chains and hence allowing the diffusion of the drug through (Ward and Georgiou 2011).
Volume transition	Volume transitions of nanogels feature an important application wherein nanogels increase their volume when subjected to a change in pH, temperature, light etc. This volume change triggers the drug release from the nanogel. Such a feature may also utilized for the necrotic damage of the cells as reported by (Lee et al. 2009) wherein the nanogels enter the necrotic cells as small spheres and upon imparting a cold shock the nanogels expand to high volumes that exerts a high pressure on the walls of the cell and leads necrotic cell burst. Alternatively pH change may trigger the volume expansion.
Photo responsive	In these types of nanogels, swelling and deswelling is controlled by employing photocontrollable crosslinking between the polymers. Upon incidence of the light over the nanogels their crosslinking densities alter that change their volume e.g., in case of the nanogels prepared by (He et al., 2009).

Drug Targeting Through Nanogels: Nanogels have gained a wide acceptance in the field of nanotechnology due to the immense potential of targeting vast no. of organs. This owes to the dual nature of these gels i.e. (i) being a hydrogel system that increases their drug loading capacity and (ii) nanoparticulate system that helps them access deeper organs and tissues.

Table 2 lists some of the drugs that have been developed in nanogel form.

TABLE 3

Drugs incorporated in various nanogels

S. No.	Drugs	Nanogel	Condition	References
1.	Methotrexate	Chitosan	Brain disorder	Azadi et al., 2012
2.	Methotrexate	NIPAM-co-BA	Inflammation	Singka et al., 2010
3.	Methotrexate	i) Poly(NIPAM-co-AAc) ii) Poly(NIPAM)	Inflammation	Samah et al., 2010
4.	Doxorubicin	Chitin-PLA	Liver carcinoma	Arunraj et al., 2014
5.	Doxorubicin	PNA	Hyperthermia	Xiong et al., 2011
6.	Doxorubicin	poly(L-aspartic acid)	Antitumor	Oh et al., 2013
7.	Doxorubicin	P(NIPAM-co-AAc)	Tumor targeting	Su et al., 2013
8.	Doxorubicin	Chitin	Prostate, breast, lung and liver cancer	Jayakumar et al., 2012
9.	5-Fluorouracil	Chitin	Skin Cancer	Sabitha et al., 2013
10.	5-Fluorouracil	poly(N-vinylcaprolactam)	Anticancer	Madhusudana Rao et al., 2013
11.	5-Fluorouracil	PNIP/Aam	-	Wang et al., 2008
12.	5-Fluorouracil	PEG-Chitosan	Anticancer	Zhou et al., 2013
13.	BSA	PNIP/Aam	-	Wang et al., 2008
14.	Curcumin	Chitin	Skin Cancer	Mangalathillam et al., 2012
15.	Curcumin	Dextrin	Anti-cancer	Gonçalves et al., 2012
16.	Photosensitizers: (TPPS4), (TPCC4) and (Ce6)	Chitosan	Joint disorders	Schmitt et al., 2010
17.	Bupivacaine	poly (N-isopropylacryl-amide)	Local Anesthesia	Hoare et al., 2012
18.	Dexamethasone	Gamma cyclodextrin	Eye	Moya-Ortega et al., 2012
19.	Insulin	p(VPBA–DMAEA)	Diabetes	Wu et al., 2012
20.	Cisplatin	PEO-b-PMA	Ovarian cancer	Nukolova et al., 2011
21.	Cisplatin	NIPAM	Anticancer	Peng et al., 2013
22.	siRNA anti EGFR	PNIMA	Ovarian cancer	Blackburn et al., 2009
23.	Paclitaxel/Lonidamine	PCL	Breast and ovarian Tumors	Milane et al., 2011; Milane et al. 2011
24.	Spantide II and Ketoprofen	Chitosan and PLGA	Inflammation	Shah et al., 2012

Table 3 *Contd…*

S.No.	Drugs	Nanogel	Condition	References
25.	Gentamicin	Chitosan	-	Zabihian et al., 2012
26.	Aceclofenac	Eudragit RL 100 and RS 100	Inflammation	Phatak Atul and Chaudhari Praveen 2012
27.	Caffeine	Poly(NIPAM-co-AAc)	-	Samah and Heard 2013
28.	Fluconazole	Chitin	Corneal fungal Infections	Mohammed et al., 2013
29.	pDNA and PA_2	Cycloamylose	-	Toita et al., 2011
30.	Fludarabine	PEG/PEI	Human breast and colon carcinoma	Vinogradov et al., 2005
31.	Taxane		Breast and Pancreatic tumor	Murphy et al., 2011
32.	Paclitaxel, docletaxel, Bortezomib, sorafenib, sonitinib, bosutinib.	RGD	Anticancer	Murphy et al., 2011
33.	Paclitaxel	i) Pluronic-F127/PEI ii) PBCA/ Pluronic-F127	Anticancer	Li et al., 2011
34.	Interleukin-12	CHP	Tumor immunotherapy	Shimizu et al., 2008
35.	Hydroxycampt-Othecin	i) Pluronic-F127/PEI ii) PBCA/ Pluronic-F127	Anticancer	Li et al., 2011
36.	Fe_3O_4	PNA	Glioma imaging	Jiang et al., 2013
37.	Quantum Dots	CHP	Imaging	Hasegawa et al., 2005
38.	Vaccines	Chitosan, PLGA, PLA, PGA	Immunomodulation	Ferreira et al., 2013
39.	Vaccines	Alginate coated Chitosan	Immunomodulation	Démoulins et al., 2013
40.	Ribavirin	Polyethelenimine	Influenza A infection	Kohli et al., 2007
41.	Interferons	PEG/PEI	Norovirus infection	Kim et al., 2011
42.	Meloxicam	Carbopol 940	Inflammation	Khurana et al., 2013
43.	Decitabine	NIPAM	Cancer	Vijayaraghavalu and Labhasetwar 2013
44.	Antisense oligonucleotide	PEG-PEI	Managing CNS conditions as brain tumors, HIV encephalaopathy, Alzheimer's disease & acute ischemic stroke	Wong et al., 2012
45.	Gemcitabine	PVA	Drug resistant tumors	Senanayake et al., 2013
46.	Naproxen	-	Musculoskeletal pain	Braroo et al., 2013
47.	Rapamycin	PNIPAM, PEG-MA, VP	Restenosis	Yallapu et al., 2008
48.	Monoclonal antibodies	PEG-*b*-PMA	Tumors	Nukolova et al., 2011
49.	Zidovudine 5'-phosphate	PEI, hydroxypropyl poly(amidoamine), PEG	HIV type-I infection	Vinogradov et al., 2010
50.	Procaine HCl	(MAA–EA), DAP	-	Tan et al., 2007

pDNA and PA2: plasmid DNA and Phospholipase A2; NIPAM co-BA: N-isopropylacrylamide copolymerized with butylacrylate; PNIP/AAm: poly(N-isopropylacrylamide-co-acrylamide); PNA:poly(N-isopropylacrylamide-co-acrylic acid); TPPS4: tetra phenyl-porphyrin-tetra-sulfonate; TPCCA: tetra-phenyl-chlorin-tetra-carboxylate; Ce6: Chlorin e6; p(VPBA–DMAEA): poly(4-vinylphenylboronic acid-co-2-(dimethylamino) ethyl acrylate); PEO-b-PMA: diblock copolymers of Polymethylene oxide and polymethacrylate; PNIMA: poly(N-isopropylmethacrylamide); EGFR: Epidermal Growth Factor Receptor; PCL: poly(epsilon-caprolactone); Poly(NIPAM-co-AAc): Poly(N-isopropylacrylamide) copolymerized with acrylic acid; PEG/PEI: polyethylene glycol and polyethylenimine;PBCA: Polybutylcyanoacrylate; CHP: Cholesterol based pullulan; PVA: Polyvinyl alcohol; (MAA–EA): methacrylic acid–ethyl acrylate; DAP: di-allylphthalate; PEG-MA: poly(ethylene glycol)-maleic anhydride; VP: vinyl pyrrolidone

Following section describes in detail how nanogels are beneficial in targeting conditions related to various organs. The various conditions that the nanogels have been so far developed for are listed below and described thereafter.

A. Chemotheraphy
   (a) Brain delivery
   (b) Liver delivery
   (c) Lung delivery
   (d) Skin Cancer
   (e) Ovarian Cancer
B. Organ Targeting
   (a) Wound healing
   (b) Transdermal delivery
   (c) Joint delivery
   (d) Eye delivery
C. Diagnosis
   (a) Imaging
D. Immunity
   (a) Vaccine delivery
   (b) Monoclonal antibody delivery
   (c) Lupus Erythematosus
E. Anesthesia
F. Diabetes
G. Oral delivery

A. Chemotherapy

(a) **Brain delivery:** A large no. of drugs are being used to treat brain disorders and other CNS related diseases but poor bioavailability of these drugs in such an organ due to poor permeability through blood brain barrier (BBB) has ever limited the entry of these drugs into the brain (Misra et al., 2003). Many approaches now-a-days towards brain

targeting in the field of nanotechnology are leading to improved drug access to the brain but yet a more efficient nanosystem can lead us to an even better treatment of brain disorders. Polymeric nanoparticles have ever been very promising to improve drug bioavailability and some of these have great potential to cross BBB also (Tao et al., 2013). One of the effective nanoparticulate systems to achieve an efficient brain targeting is the use of nanogels. Some drugs have been tried with nanogels for improvement of drug delivery to the brain. An anticancer drug, Methotrexate (MTX), is a widely used chemotherapeutic agent with a prominent position in the treatment of different cancers and autoimmune diseases and this has been formulated as nanogel. MTX was incorporated in the nanogel system and surface functionalization with polysorbate was also done to improve BBB permeability (Azadi et al., 2012).

The drug loading capacity and loading efficiency have increased largely with the use of nanogel. The *in vitro* characterization tests carried out on the prepared nanogels showed results that confirm the suitability of the nanoparticles for brain delivery target.

The study clearly showed that even with the decrease in plasma concentrations (as the drug was given as intravenous injection), the brain concentrations went increasing up to the last time point hence indicating that the drug influxed in a slow and controlled manner from plasma to brain. Both types of the nanogels (surface modified and unmodified nanogels) showed significantly higher methotrexate concentrations in the brain when compared to the free drug. The study reported an increase in the methotrexate conc. in the brain up to 10-15 folds with the use of drug-loaded nanogels, and hence offers highly prospective future to nanogels for brain delivery in future.

Other class of drugs, oligonucleotides (ODN), has also been formulated in nanogel form for targeting to CNS against some neurodegenerative disorders. The study showed that nanogel incorporated ODN formulations effectively crossed the BBB. The transports efficacy is further increased when the surface of the nanogel is modified with transferrin or insulin. After 1 hr of intravenous injection of nanogel it was found that the accumulation of a phosphorothioate ODN increased by greater than 15 fold in the brain while decreased in spleen and liver by 2-fold upon comparison with free ODN (Vinogradov et al. 2004).

Vinogradov et al., (Vinogradov et al., 2010), developed nanogel of NRTIs (nucleoside reverse transcriptase inhibitors) for delivery to the brain for inhibition of HIV type-I in macrophages. Macrophages are regarded as reservoirs of infection that latently harbor infections like viruses and hence give them a chance to escape identification and hence treatment. This may later on lead to still major infection that would be resistant to the existing treatment. Hence in an attempt to fight latent HIV type-I viruses in macrophages, nanogel of NRTIs was formulated. The NRTIs taken were zidovudine 5'-phosphate or didanosine 5'-phosphate. The nanogel was evaluated in HIV type-I infected monocyte-derived macrophages (MDMs) for cytotoxicity, antiviral activity and intracellular accumulation. The nano-NRTIs showed high antiviral efficacy against HIV type-I in MDMs and hence established their potential for delivery to macrophages in the brain. The main advantage demonstrated with nanogels of NRTIs as against standard NRTIs was 3-fold reduction in mitochondrial toxicity caused by mitochondrial DNA depletion by NRTIs that plays main role in NRTI neurotoxicity hence reducing the chances of neurotoxicity.

For the world's most aggressive and frequent brain disorder, Human glioblastoma, Baklaushev et al., formulated cisplatin loaded nanogels conjugated with monoclonal antibodies to target the highly expressing tumor specific membrane protein known as connexin 43 (Cx43) and BSAT1 (a brain-specific anion transporter). The study reported an increased rate of survival of rats of around 27 days than the control and an increased efficiency of the formulation for the treament of gliomas (Baklaushev et al., 2014).

From the above description it can be concluded that nanogel is an encouraging system for potential delivery of drugs to the CNS and has a definitive prospectus in future for the treatment of CNS disorders.

Other studies related to brain delivery of nanogels are outlined in Table 4.

(b) Liver delivery: Hepatic carcinoma (HCC) is regarded as the third leading cause of cancer deaths in the world (Andrade et al., 2009; Liu et al., 2010) (Tam 2013). Doxorubicin (Dox) is among the most frequently used chemotherapeutic agent for the treatment of liver cancer (Tam 2013), (Huynh et al. 2007). Although Dox is amongst the leading drugs in the treatment of cancers but still the drug lacks an efficient treatment potential as a result of the reduced efficacy of Dox due to its serious

toxicity. In addition, Dox has been known to suffer fast metabolization to inactive derivatives that further reduce its efficacy. To overcome these toxic effects of Dox like cardiotoxicity (Shi et al., 2011) and the currently prevailing problems of hepatic carcinoma (HCC) a pH sensitive composite biodegradable nanogel for local delivery by injection was developed. It was evident from the study that the nanogel systems were able to accumulate in tumor tissues through enhanced permeability and retention (EPR) effect and they released the drugs in endosomes or lysosomes by pH-controlled hydrolysis after being taken up by the cell via the endocytic pathway as demonstrated in Fig. 5.

TABLE 4

Brain Delivery of Nanogel incorporated drugs.

Drug	Purpose of study	Approaches	Comments	Reference
N-hexylcarbamoyl-5-fluorouracil	To increase the permeability of the drug to the brain	Coating with polysorbate 80	Increased retention in blood Increased accumulation in brain (0.52% for coated as compared to 0.1% for uncoated nanogels)	Soni et al., 2006
Nucleoside reverse transcriptase inhibitors (NRTIs)	Decrease neurotoxicity and increae antiviral activity against HIV infection in the brain	Conjugated with Peptide (AP) binding brain-specific apolipoprotein E receptor	Suppression of retroviral activity by 10-fold and reduction of associated inflammation in humanized mouse model caused by HIV-1 infection in the brain	Gerson et al., 2014
Blank Nanogels of CHP, cholesterol bearing pullulan, as artificial chaperone	Inhibit the formation of amyloid β-protein fibrils (Aβ) in Alzhiemer's Disease (AD)	Simple CHP and amino-group-modified CHP ($CHPNH_2$) were used.	Both CHP and $CHPNH_2$ nanogels inhibited the association of β-protein fibrils by ingesting 6-8 Aβ and hence cytotoxicity	Ikeda et al., 2006

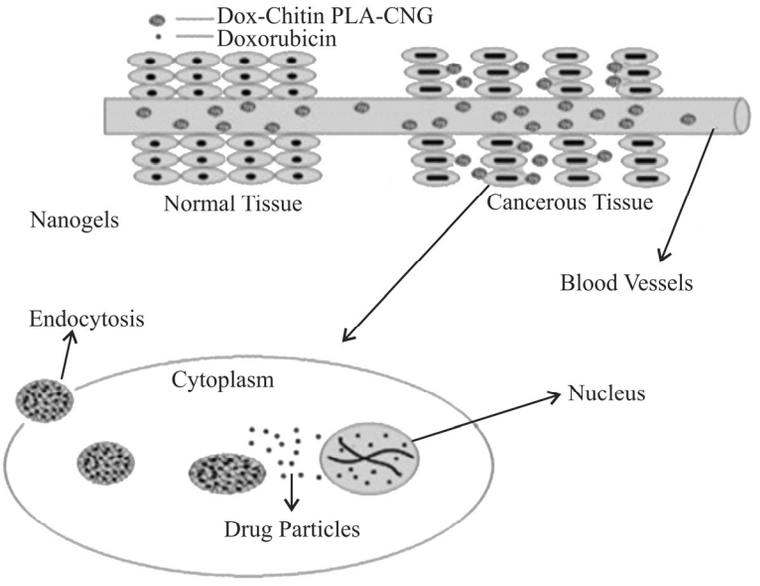

Fig. 5. Drug released from nanogel and taken by tumor tissue through endocytic pathway.

This resulted in the specific delivery of the drug while reducing the side effects, hence potentiating the efficacy of cancer chemotherapy. Chitin-PLA CNGs efficiently incorporated Dox showing an entrapment efficiency of 86%. Increased swelling and improved drug release was attained at acidic pH with both chitin-PLA CNGs and Dox-chitin-PLA CNGs. Also the nanogel system showed no hemolysis of RBC's hence confirming the safety via systemic route. *In-vitro* studies have shown an increased cytotoxicity of the tumors by use of pH-sensitive nanogels that deliver the drug at low pH exactly at the site of tumor while avoiding the toxic side effects by systemic administration of the bare drug (Arunraj et al., 2014).

For an another type of tumor associated with liver, Duan et al., prepared a pH responsive galactosylated nanogel of oridonin that was shown to demonstrate a high antitumor activity that increased with increase in the glycosylation of the nanogel. Also a higher uptake of oridonin by hepatoma cells (HepG2) via asialoglycoprotein receptor-mediated endocytosis was achieved with the use of the nanogel (Duan et al., 2011).

Lee et al., formulated a low molecular weight pluronic nanogel loaded with heparin that inhibited TGF-β/Smad signaling pathway for impeding liver fibrosis. The nanogel was shown to manage the decrease in the amount of liver fibrosis by decreasing the expression of transforming growth factors β(1) (TGF-β(1)), p-Smad 3, and p-Smad 2, which are all involved in the TGF-β/Smad signaling pathway (Lee et al., 2011).

PEGylated siRNA loaded biodegradable nanogels developed by Naeye et al., have been used to demonstrate the efficiency of their uptake by certain human cancer cell lines that included human hepatoma cells and cytometric and confocal microscopic studies confirmed the same. The PEGylation was attributed to increase the circulation time of the nanogels in the blood (Naeye et al., 2010).

(c) **Lung delivery:** Deshmukh *et al.,* prepared stabilized aggregated nanogel particles (SANP) for injectable passive lung cancer targeting by crosslinking 8 Arm PEG thiol with 1,6-Hexane-bis-vinylsulfone (HBVS) using 0.1% v/v Tween™ 80 contained in phosphate buffer (PB, pH 7.4) to incorporate prodrug of camptothecin, norvaline α-amino acid (Deshmukh et al., 2012). The formulation was developed as an alternative to the inhalation for pulmonary delivery of the drug. The anticancer efficacy of the formulated nanogels was evaluated in the lung cancer models of rats against the free camptothecin given as a bolus. The study revealed that 40% of the drug containing nanogel (0.22 mg/kg) treated animals showed 100% cancer treatment and the others also showed a significant response to the drug loaded nanogels and also to the free drug (2 mg/kg). This meant that the efficacy of the drug was increased by 10 times by incorporation in the nanogel.

(d) **Skin cancer:** Melanoma is a malignant tumor of melanocytes predominantly found in skin, but can develop in any part of the body containing melanocytes. It is a potentially fatal cancer. Cutaneous melanoma comprises only 3% of all skin tumors (Sharma et al., 2009) but still about 75% of all the deaths caused by skin cancer comprise of cutaneous melanoma (Halperin et al., 2013). Methods adopted for countering the skin cancer include surgery, radiation therapy and chemotherapy. Usually a combination of surgery with either of later is preferred. Topical formulations, for chemotherapy, in case of skin cancer have proved beneficial in imparting local action over the affected area and in increasing bioavailability of the drug (Proniuk et al., 2002). Today a lot of research is going on for including cytotoxic drugs in novel carrier systems for topical delivery as they offer a great deal of benefits over the conventional carrier systems. In case of topical delivery of drugs the upper layer of the skin, stratum corneum, poses a barrier for the penetration of the drug (Moghadam et al., 2013). Nanoformulations with appropriate size and surface charge provide immense platform for cytotoxic drugs to target specific sites as they possess great penetration abilities via biological membranes. Nanogels among the modern nanoformulations are becoming interesting regarding the topical delivery of drugs via these tiny gels. So far developed nanogels of skin cancer drug include chitin nanogel of 5-fluorouracil (5-FU). The study resulted in an efficient loading of about 90% of the drug in the nanogel that possessed an increased ability of swelling and drug release, at acidic pH. The nanogel was also compatible with the blood. Although the penetration of 5-FU could not improve by incorporating it in nanogel for various reasons but retention time of the gel in the deeper layers of the skin was increased up to 4-5 times. This is an advantage because melanocytes which are the targets of the therapy are present in deeper layers (Sabitha et al. 2013).

Another drug, curcumin, intended for topical delivery to skin cancer has been developed in nanogel form using chitin as polymer. The nanogel showed improved drug release kinetics and cytotoxic action. The study also revealed specific toxicity, at conc. range of 0.1-1 mg/ml, on melanoma cells. Also a steady state flux through the layers of the skin was had by using curcumin nanogels as against control curcumin solution (Mangalathillam et al., 2012).

Hence the above discussion shows that nanogel delivery is a best choice for incorporating cytotoxic drugs, used in skin cancer, for topical delivery.

(e) **Ovarian cancer:** Presently, like many other cancers, ovarian cancers are being managed by surgery followed by chemotherapy (Trimbos et al., 2003). Among various approaches that are being tried for the management of tumors, targeted delivery of the drugs is considered as revolutionary. The era of nanotechnology has diverted considerable attention towards the formulation development for targeted delivery of drugs to the tumors. Nanogels provide for the chief delivery systems that can carry large amounts of therapeutic agents to the tumors specifically. (Nukolova

et al. 2011) Nukolova *et al.,* have developed nanogels of different drugs (doxorubicin, cisplatin) for the treatment of ovarian cancer by using di-block polymer poly(ethylene oxide)-b-poly(methacrylic acid) (PEO-b-PMA) and conjugated with folic acid. The nanogels are designed for the tumor specific delivery of the drugs. Nanogels provide for the multiple drug carrier systems due to their extraordinary high loading capacities. Further, due to the over expression of folate receptors in ovarian tumors (and certain other human tumors also), folate conjugated nanoparticles could be directed specifically towards these tumors. The study resulted in a successful development of folate conjugated nanogels that confirmed tumor specificity through both *in vitro* as well as *in vivo* studies. The study has shown superior anti-tumor efficacy of cisplatin loaded folate nanogels with decreased renal toxicity also.

(Blackburn et al. 2009). Blackburn *et al.,* have worked to develop a thermosensitive nanogel of poly(N-isopropylmethacrylamide) that contained surface localized peptides that target specifically the ovarian tumor cells. The nanogels are strongly hydrated at physiological temperature and undergo a collapsed transition at 43 ^{o}C. These nanogels are utilized to carry siRNA to the desired cell types or tissue that take active part in silencing of mRNA. The nanogels protect the siRNAs and help them escape endosomal uptake cell viability and toxicity studies confirmed the non-toxicity of these nanogels after their delivery. When mRNAs stop working, no proteins are synthesized and cell death follows.

B. Organ Targeting

(a) Wound healing: When considering topical application of drugs, nanogels are among the best carriers to incorporate them in, considering their highly consistent nature and proper retention over skin (Keshavarz and Kaffashi 2013). Presently it is believed that wounds provided with a moist environment show better healing than the dry dressing and gels like nanogels offer the best option for wet dressing as the quality of recovered tissue is best in wet dressing wounds. Further the cooling effect provided by the hydrogel nanoparticles (i.e. nanogel) helps in reducing the erythema and swelling by decreasing capillary circulation at the site of application (Mallefet and Dweck). Many drugs till date have been developed in nanogel form for application over wounds.

Kobayashi *et al.,* developed cholesterol bearing pullulan (CHP) nanogel, a highly biocompatible polymer, in combination with prostaglandin (PGE1) for the treatment of wounds (Kobayashi et al., 2009). PGE1 has been used clinically for the treatment of wounds and chronic skin ulcers. The wound healing efficiency of the formulation was evaluated in full thickness skin defect model animals (Wistar rats). Full thickness wound of 1 × 1 cm^2 was made over the dorsal side of the rats and then treated with CHP/PGE1-nanogel and CHP without PGE1. The parameters measured were the rate of wound size reduction and by histological analysis. It was found that the rate of reduction in the wound size was higher in CHP/PGE1 combination formulation treated animals than the mere CHP treated ones. Neoepithelialization, neovascularization, and wound closure was higher for CHP/PGE1-nanogel. Hence the study established the efficacy of CHP based formulations in promoting wound healing.

Similar polymer (i.e., cholesterol bearing pullulan, CHP) was used by (Miyahara et al. 2012) Miyahara *et al.,* to formulate a nanogel crosslinking membrane for guided bone regeneration (GBR) (in GBR a membrane is used to direct the growth of a new bone). For the study a 5 mm diameter full thickness parietal bone defect was created in wistar rats with a bone trephine burr. The defects were covered with collagen, CHP nanogel membrane, and untreated. Past 2, 4 and 8 weeks the animals were analyzed histologically and radiologically after sacrifice. The results were promising as the CHP nanogel (as well as collagen) treated animals demonstrated higher bone volumes than control with the regenerated bone showing a regular and uniform surface and indistinguishing histology from the original bone for the CHP treated animals.

Another study conducted by Chen *et al.,* for the development of nanogel of micronized sacchachitin (mSC) for wound healing effect has come up with potential benefits for cutaneous wound healing by helping induce cell proliferation and probably inhibit proteolysis (Chen et al., 2012). The authors have been easily able to increase cell proliferation of the Statens Seruminstitut rabbit cornea (SIRC) and treat the corneal wound with the developed nanogel at significant concentrations of 200, 300, 400 µg/ml. The animal study has also shown accelerated corneal wound healing.

(b) Transdermal delivery: Permeation of the drugs via skin by transdermal delivery has improved by the use of nanogels (Shah et al., 2012; Sabitha et al., 2013). A similar type of

nanogel was developed by (Shah et al., 2012), for a combination of two anti-inflammatory drugs, spantide II and ketoprofen by dispersing chitosan coated PLGA nanoparticles of the drugs into HPMC or carbopol. For increasing the permeation into the skin the nanoparticles were modified by impregnating the coated nanoparticle surface with permeation enhancer, oleic acid, a monostructured fatty acid which acts to fluidize biological membranes. The oleic acid modified PLGA-chitosan bilayered nanoparticles were dispersed in a gel system (nanogel) to increase their retention time over skin and obtain controlled release drug - delivery. The nanogels were obtained with optimum particle sizes (226 nm). The gel possessed an excellent rheology being non-Newtonian and showing thixotropic behavior which is desirable for topical formulations.

In vivo studies for determining the permeation into the skin were carried out on dermatomed human skin. DNFB (2,4-dinitrofluorobenzene) induced ACD (allergic contact dermatitis), and imiquimod (IMQ) induced psoriatic plaque models were used to evaluate anti-inflammatory efficacy of the formulation in mice. The results showed that the application of the combined drug formulation improved its effectiveness in bringing down the inflammation.

Samah *et al.,* have prepared topical nanogels of Poly-(N-isopropylacrylamide-copolymerized-acrylic acid) known as [poly(NIPAM-co-AAc)] and poly(N-isopropylacrylamide) known as (polyNIPAM) incorporating the drug methotrexate for determining the mechanism by which nanogels cross the skin (Samah et al., 2010). The authors have used specifically prepared porcine ear membrane to evaluate the fraction of the drug crossed through the skin after the application of specific doses of polyNIPAM (control) and polyNIPAM-co-AAc nanogels. Overall control membranes were those treated by de-ionized water. The cells were disassembled after 24 hr and receptor phases recovered and analyzed by TEM for the presence of nanogels. The TEM results confirmed the enhanced migration of polyNIPAM-co-AAc nanogels than polyNIPAM nanogels which is attributed to the dual properties i.e., response to both temperature and pH of the polyNIPAM-co-AAc nanogels compared to the mere temperature responsive property of polyNIPAM nanogels.

Singka et al., have used activated nanogels of NIPAM (copolymerized N-isopropylacryl-amide) and butylacrylate to incorporate methotrexate for improving its topical delivery and enhance anti-inflammatory action (Singka et al., 2010). Uniform sized and spherical shape nanogel particles were confirmed by TEM images. Nanogels carrying methotrexate greatly aided in increasing the drug bioavailability in the epidermis that increased the uptake by the keratinocytes and hence reducing the PGE_2 production. Permeation of the methotrexate was assessed by using heat-separated epidermal and silastic membranes.

Samah *et al.,* (Samah and Heard 2013) prepared another nanogel, pH and temperature sensitive, for enhancing transdermal delivery of caffeine using poly (NIPAM-co-AAc), poly-N-isopropylacrylamide-co-acrylic acid.

Atul *et al.,* (Phatak Atul and Chaudhari Praveen 2012) developed nanogel for transdermal delivery of aceclofenac to reduce inflammation.

(c) **Joint delivery:** Macrophages are considered as keys to play role in initiation and maintenance of inflammatory disorders (Fujiwara and Kobayashi 2005),(Kinne et al., 2000), (Ma and Pope 2005). Macrophages are responsible for the increased vascular and tissue permeability observed at sites of inflammation e.g., in inflamed articular joints of rheumatoid arthritis patients which suggests that their exclusive elimination may prove productive in treating inflammatory disorders. Through production of various cytokines and other chemicals during inflammation, macrophages conduct three major processes including antigen presentation, phagocytosis, and immunomodulation (Schmitt et al., 2010), (Fujiwara and Kobayashi 2005). And hence possess a great role to initiate, maintain, and reduce inflammation (Fujiwara and Kobayashi 2005). Therefore, they may be beneficial targets of treatment aiming at their local destruction at inflammatory sites. Many studies on rheumatoid arthritis in animal models through photodynamic therapy (PDT) using the photosensitizers, photofrin, mTHPCB PDMA, or hexyl ester of 5-aminolevulinic acid have proven beneficial for PDT.

A photosensitizer is defined as a chemical compound that upon absorption of light can be raised to an excited state and produces singlet oxygen upon interactionwith molecular oxygen (DeRosa and Crutchley 2002). This species rapidly attacks any organic compound it encounters, thus being highly cytotoxic (Weishaupt et al., 1976; Schiff et al., 1987) and hence beneficial in cancer therapy. However, one major problem

of photodynamic therapy is the leakage of therapeutics due to enhanced permeability resulting from inflammation. Photo-sensitizers or other low molecular mass therapeutics when injected in inflamed joints result in their rapid efflux out of the joints (Schmitt et al., 2010). In order to avoid such drainage, photosensitizers could be loaded in a carrier system that would immobilize them and lead them to specific targets. To impart site specific uptake and enhance drug retention in inflamed sites, chitosan based hydrophilic nanogels, and surface impregnated with hyaluron-ateandloaded with anionic photosensitizers[tetra-phenyl-porphyrin-tetra-sulfonate (TPPS4), tetra-phenyl-chlorin-tetra-carboxylate (TPCC4) and chlorine 6 (Ce6)] were developed. In a study over mouse model for rheumatoid arthritis, injection of free photosensitizers resulted in their rapid clearance from the joints, while nanogel encapsulated photosensitizers were retained in the inflamed joints over a longer period of time. The *in-vitro* studies showed an optimal uptake of these functionalized nanogels by murine RAW 264.7 or human THP-1 macrophages after less than 4 hr incubation. *In-vivo* studies were carried wherein the nanogel containing the photosensitizers was injected in the arthritic knee of mouse that retained in the knee for much longer time than the free forms of the photosensitizers.

(d) **Eye delivery:** About 90% of the commercially available drug formulations for eye delivery are topical (Le Bourlais et al. 1998) (Jansook et al., 2010). But the biggest drawback of those topically applied drugs to the eye is the poor retention and hence instant drainage of the formulation from the corneal surface. With the result, only < 1% of the drug gains access to the interior of the eye to reach intraocular tissues (Gupta et al., 2010). Besides, in case of retinal drug delivery, many invasive methods e.g., subconjunctival, intravitreal and retrobulbar administration, are adopted to locally deliver the drug to the retina as an alternative to the systemic delivery (Cardillo et al., 2010). Because systemic delivery leads to low bioavailability due to the poor drug permeability via the retinal endothelial lining, nanotechnology has been used to overcome these limitations of ocular drug delivery and many nanoformulations for sustained drug release have already been developed that include liposomes, microparticles, ocular mini tabs. Nanogels are among the recent drug delivery systems that are gaining an increasing interest in the inclusion of drugs for ocular delivery due to a large no. of advantages that nanogels owe over other carrier systems. A nanogel of dexamethasone with gama-cyclodextrins integrated with the nanogel was developed by (Moya-Ortega et al., 2012) to prolong the retention time on the corneal surface. The nanogel possessed many advantages over the present ocular drug delivery systems. The dexamethasone eye drop formulation demonstrated a greater, longer and steady drug release and hence concentration on the eye surface than the marketed formulation Maxidex. With the nanogels the drug loading attained was upto 25 times greater than the commercial formulation. Thus a 3-fold increase in drug concentration and drug retention for about 6 hr in the aqueous humor after the delivery of the eye drops was attained. The nanogel was also found to be non-irritating when demonstrated in vivo on rabbits. Following table provides an overview of the nanogels developed for eye delivery: Table 5.

TABLE 5
Eye Delivery of Nanogel incorporated drugs.

Drug	Purpose	Comments	References
Fluconazole	Improve corneal bioavailability	The prepared Flu-CNGs showed controlled release of fluconazole	Mohammed et al., 2013
Pilocarpine	Improve stability and bioavailability	Long and sustained release of pilocarpine	Abd El-Rehim et al., 2013
Timolol maleate	As contact lens with lysozyme triggered release of drug	Controlled and sustained drug release attained.	Kim et al., 2014

C. Diagnosis

(a) **Imaging:** Outlining of a tumor is very significant in its effective removal and in that the normal tissue remains intact without any harm since visual contrast between the normal brain tissue and glioma is very poor due to their large infiltration in the normal tissues of brain. (Jiang et al., 2013) Jiang et al., have synthesized new pH/ temperature sensitive nanogels conjugated with Cy5.5-labelled lactoferrin (Cy5.5-Lf-MPNA nanogels) for imaging of glioma (a primary tumor of brain). In case of glioma, neurosurgery is presently the first line treatment. But the tumor mass is so merged with the normal tissue that it becomes very difficult to completely remove the tumor while letting no harm to the normal tissue. If the gliomas are precisely outlined it could help in their complete removal. Many techniques currently exist for imaging of tumors. The newly developed technique for

the imaging of the gliomas is superior in that it offers the combined features of the existing ones. For example the presently developed formulation is a combination of MRI (magnetic resonance imaging) and optical imaging. Both MRI and optical imaging are the current standard non-invasive techniques for imaging. Hence combination of both can give a precise and accurate outline of the tumor mass and help in both pre-surgical planning stage as well as surgical resection stage. The *in vitro* study was carried out on rat C6 glioma cells (possessing high Lf receptor) and human umbilical vein endothelial cells (ECV 304). The results showed a higher uptake of the Lf-nanogels at pH 6.8 than at 7.4 by C6 and ECV 304 cells for both Cy5.5-Lf-MPNA and MPNA nanogels. The C6 cells were further used for the evaluation of MRI and fluorescence imaging capability of the prepared nanogels. The cells were treated with both types of nanogels (Cy5.5-Lf-MPNA and MPNA). Iron was added in increased concentrations to the (Cy5.5-Lf-MPNA and MPNA) nanogel treated C6 cells and decay in the MRI signal was observed. The decay in case of Cy5.5-Lf-MPNA nanogel treated C6 cells was greater than that of MPNA nanogel at the same iron concentration. This indicated specific internalization of Cy5.5-Lf-MPNA nanogels into the C6 cells hence confirming the tumor specificity of the formulated Cy5.5-Lf-MPNA nanogels (Jiang et al., 2013).

Hasegawa et al., have developed a technique for live cell imaging by formulating a nanogel-quantum dot (QDs) hybrid nanoparticle (Hasegawa et al., 2005). This was done by simple mixing of QDs with the amino acid modified nanogel of cholesterol bearing pullulan ($CHPNH_2$). Due to some of the outstanding properties of QDs like bright fluorescence, high photostability, broad excitation and narrow emission they have been long used for long term imaging. QDs are required in large amounts and incorporation of these QDs into nanogels has improved their otherwise low uptake by the living cells. In *in vitro* study $CHPNH_2$-QD complexes demonstrated a uniform internalization into the cells without any sort of aggregation. When compared with the liposome-QD complex for the mean fluorescence intensity, the formulated $CHPNH_2$-QD nanoparticle showed 3.4 times higher value which is attributed to its small size and highly concentrated positive particulate charge in the nanogel system. The $CHPNH_2$-QD nanogel system was investigated for uniform dispersion of the QDs after internalization in various types of cell lines including urinary bladder carcinoma, human glioma cells, lung carcinoma, human osteosarcoma and glioblastoma. The $CHPNH_2$-QD nano-particles were internalized regardless of the cell type and without aggregation. Hence amino acid conjugated CHP (i.e., $CHPNH_2$) nanogels possess great potential for long term cell imaging.

D. Immunity

(a) Vaccine Delivery: Vaccination is all about generation of antigen specific immune response through the use of biological agents that may be weakened or killed forms of the microbe or agents that resemble disease causing microorganisms. Development of immunogenicity includes: I) generation of antibodies and II) induction of cell-mediated immunity (i.e., stimulation of T or B cells to kill the antigen; Disis et al., 2009). For the development of immunogenicity antigens are must to enter APCs (antigen-presenting cells) to be processed internally and taken for surface presentation to T cells, to either a CD8 (cytotoxic) T-cell which directly kills the foreign cells, or to a CD4 (helper) T-cell which help cytotoxic T-cells to kill foreign cells by releasing chemical signals and also induce B lymphocytes to produce antibodies. Besides antigen presentation, T cells also require co-stimulation via the surface co-stimulatory molecules or secreted factors such as cytokines, without which T cells are not able to produce adequate response. In natural immunization, especially after infection, co-stimulation is induced by specific ligands associated with the pathogen. In case of vaccines this co-stimulation is generated by the use of adjuvants that mimic the signals usually produced by natural pathogens.

Currently only a few adjuvants are approved for human use e.g., MF59, alum (aluminum salts), Montanide ISA 51, Adjuvant System 04 (AS04), Adjuvant System 03 (AS03) and virosomes (Leroux-Roels 2010), (Reed et al., 2009), (Mbow et al., 2010). A limitation of such an approach to induce immunogenicity is that these adjuvants provide immunity by merely the stimulation of antibody production and do not potentiate the cell-mediated immunity (Guy 2007). This can result in the hindrance in the prevention of diseases caused by intracellular pathogens or cancer where cellular immunity is must for the encounter (Disis et al., 2009). In order to overcome this limitation novel formulations

for vaccines are being developed. Nanogels are among one of those novel delivery systems that have efficiently been able to induce long-lasting and strong immunity besides providing cell mediated immunity based on $CD4^+$ and $CD8^+$ T-cell immunity (Look et al., 2010).

Temmerman et al., have reported that OVA (ovalbumin) loaded nanogel particles efficiently activated in vitro $CD4^+$ and $CD8^+$ T-cells compared to soluble proteins (De Temmerman et al., 2011). Polymeric nanogels also have the significant advantage to reduce the toxicity caused by inflammatory cytokines that often follows after injection and which is a common side effect of immunostimulants, when directly APCs. Many studies regarding this approach have been done that show that nanogels can stimulate or suppress the immune system to regulate immune response for countering many infections or autoimmune diseases or allergies and cancer (Dobrovolskaia and McNeil 2007). Particulate delivery system, besides carrying vaccine, itself possesses the ability to stimulate APCs and trigger stimulus which resembles that of pathogens. Studies have evidenced that stimulation of dendritic cells (the most versatile APCs) by nanogels resulted in upgradation in the expression of many co-stimulatory molecules, secretion of cytokines, chemokines and expression of chemokine receptors. These activated dendritic cells enter the lymphatic nodes where they trigger cellular immunity by presenting antigens to the T cells which in turn initiates humoral immunity which is an advantage imparted in addition by the nanogel particles (Bivas-Benita et al. 2004). (Bal et al., 2010).

Today a large no. of vaccines based on polymeric nanogel system (Yuki et al., 2013), (Nochi et al., 2010), (Li et al., 2013) have been developed that include; peptide-based vaccines with the use of chitosan, PLGA, gamma PGA; protein based vaccines using mannan and pullulan, chitosan and derivatives, PLA and PLGA, PCL, PMMA etc.; DNA based vaccines using chitosan, gamma PGA, PLGA and PLA; and RNA based vaccines (Ferreira et al., 2013).

Following Table 6 gives a brief description of some types of vaccines developed as nanogels.

TABLE 6

Vaccine delivery of drug via Nangels

Antigen used	Purpose	Route	Description	Reference
A non-toxic subunit fragment of *Clostridium botulinum* type-A neurotoxin BoHc/A	mucosal infectious diseases	Intranasal	The vaccine induced IgG and IgA secretion without any adjuvant being used.	Nochi et al., 2010
CHP-HER2, a cut protein (146HER2) complexed cholesterol pullulan (CHP)	Humoral Immunity	Injected subcutaneously	The vaccine induced IgG Antibodies against the tumor specific HER-2. The action was enhanced with the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) concurrently.	Kageyama et al., 2008
Recombinant *N. caninum* protein disulphide isomerase (rec NcPDI)	*Neosporum caninum tachyzoites* infection (neosporosis)	Intranasal or intraperitoneal injection	recNcPDI with nanogel increased the response and decreased the cerebral parasite load especially in case of i.n injection.	Debache et al., 2011
Pneumococcal surface protein A (PspA)	Pneumococcal respiratory infections caused by *Streptococcus pneumoniae*	Intranasal	Vaccine induced protective immunity against lethal *S. pneumoniae* Xen10, decreased infection by the bacteria in the upper and lower respiratory tracts, and induced systemic and nasal Th17, PspA-specific IgG, and IgA antibody responses.	Kong et al., 2013
Ovalbumin (OVA)	Influence of surface decoraton and amount of vaccine on targeting and activating dendritic cells	Topical Route	Surface modification of nanogels with mannose induced production of interferon-γ by T-lymphocytes with higher efficiency than by unmodified nanogels and free ova. Both surface modification and vaccine cargo interact in different ways with dendritic cells that determines the properties of the immune response.	Thomann-Harwood et al., 2013

(b) **Monoclonal antibody delivery:** (Nukolova et al., have developed nanogels of monoclonal antibody CC49 (mAb CC49) for targeting tumor-associated glycoprotein 72 (TAG-72) to improve cancer diagnosis and therapy by using diblock copolymers of poly(ethylene glycol)-b-poly(methacrylic acid) (PEG-b-PMA) Nukolova et al., 2011).A large number of primary and metastatic human carcinomas are known to express TAG-72 including lung, ovarian, breast carcinomas and other cancers like gastric pancreatic, esophageal cancers etc. (Thor et al., 1986) and mAb CC49 is regarded to possess strong immunoreactivity and high specificity to TAG-72 and has so far clinical trials of mAb CC49 for imaging and treatment of various types of carcinomas have also been conducted. Surface plasmon resonance (SPR) technique was used to analyze the antigen binding selectivity of the mAbCC49 nanogel where antigens bovine submaxillary mucin (BSM) was used as positive control and BSA as negative for the binding of mAb CC49 nanogel. The results were compared with the unmodified nanogel and non-specific IgG-nanogel binding to the same control antigens i.e., BSM and BSA. Also, free CC49 and IgG were analyzed for the antigen specificity by the same SPR technique. The data of the results interpret that decorating the nanogels with the mAb increases the specificity and the strength of interaction of the nanogel with the immobilized antigen.

(c) **Lupus Erythematosus:** In the course of addressing autoimmune diseases in general and Lupus Erythematosus in particular, Look et al., conducted development of mycophenolic acid nanogel that imparted immunosuppression in the experimental animal, mice. The nanogels were productive in that both prophylactic treatment as well as treatment after the development of serious side effects like renal damage could be attained wherein prophylactic treated mice survived to extra 3 months and 2 months in later case (Look et al., 2013).

E. Anesthesia

(a) Local Anesthesia and

(b) Infiltration Anesthesia

Both types of anesthesia are referred in following paragraphs.

Duration of local anesthesia aftera surgery is a very important clinical demand. Most of the anesthetics used today possess very less duration of action. A way out of prolonging the local anesthetic release is to associate the drug with a carrier system. With the use of nanogels it has been possible to achieve prolonged duration local anesthesia for as long as 9 hr. Using poly(N-isopropylacrylamide), in a study, thermosensitive-nanogel of bupivacaine was developed that attained nerve block for about 9hr. 3T3 mouse fibroblasts, J.1774 macrophage-like cells, and C2C12 mouse myoblasts in cell culture were used to evaluate the cytotoxic effect of nanogel. An important observation made in the study was that higher acid functionalization of the nanogels led to slightly less cell viability than with lesser acid functionalization. Also, particle size had a little influence over the cell viability. In the end the study concluded that large nanogels with high acid functionalization led to greater cytotoxicity and increased inflammatory response. As against this, small nanogels with less acid functionalization produced lesser cytotoxic effects and very mild inflammatory response (Hoare et al., 2012). Table 7 lists some nanogel anesthetics.

F. Diabetes

Researchers at the Massachusetts Institute of Technology (MIT) and Boston Children's Hospital are in progress to make a self-operating insulin delivery system using novel nanotech approach which involves single injection of a nanogel that can stabilize blood glucose level for as long as 10 days. The nanogel is glucose sensitive, detects blood-glucose levels and secretes insulin accordingly.

The MIT approach has used a nanogel containing a mixture of oppositely-charged dextran nano-particles that experience electrostatic attraction and make the gel mechanically consistent. The nanoparticles are composed of an inner core of insulin, modified dextran and glucose oxidase enzymes. As the enzyme exposes to the high glucose level in the blood it converts glucose into gluconic acid. The gluconic acid so formed disintegrates the dextran spheres and hence releases insulin which then lowers the glucose level of the blood to the normal. Being biocompatible the gluconic acid and dextran finally dissolve in the body, (Gu et al., 2013; Gu et al., 2013). Recently an optical sensitive, insulin loaded silver nanoparticle nanogel of poly(4-vinylphenylboronic acid-co-2-(dimethylamino) ethyl acrylate) [p(VPBA–DMAEA)] for the management of diabetes have been designed (Wu et al., 2010) (Fig. 6). The study conducted the introduction of the glucose sensitive p(VPBA–DMAEA) shell onto Ag NPs that made the polymer-bound Ag NPs to response against glucose. Any change, over a clinically relevant range (0–30 mM), in the conc. of glucose in the blood is detected by the glucose sensitive polymer, p(VPBA–DMAEA) and converted in an optical signal that is detected by the optical sensitive silver core (10 ± 3 nm) (Wu et al., 2010).

TABLE 7
Anesthetics

S. No.	Drug	Objective	Type	Description	References
1.	Procaine HCl	Determination of Release kinetics	Local anaesthesia	High concentration gradient and high pH increased the release rate due to high driving force and reduced diffusion barrier and decreased with the increased drug loading.	Tan et al., 2008
2.	Lidocaine (subcutaneous)	Prolong duration of anaesthesia	Infiltration anaesthesia	Prolonged duration of anaesthesia was obtained witout any severe toxic effect as demonstrated in rats.	YIN et al., 2009
3.	Bupivacaine	Determine scavenging ability of nanogels	Local anaesthesia	Nanogels proved as highly efficaceous tool for local anaesthetic overdose treatments as acid functionalized nanogels possess the ability to bind catonic drugs at high concentrations that increases with increase in acid functionalization.	Hoare et al., 2012

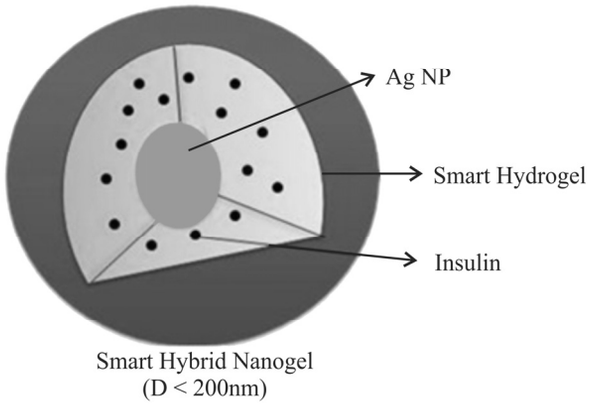

Fig. 6. Nanogel loaded with Insulin.

Wu et al., in another study introduced a new poly [N-iso-propyl-acrylamide (NIPAM)-co-acrylamide (AAm)-co-2-acrylamidomethyl-5-fluorophenylboronic acid (FPBA)] nanogel for diabetes that would sense glucose with high sensitivity and release insulin in its response and hence provide for a full automated system for the control of glucose level in the blood (Wu et al., 2010). The sensitivity for glucose was imparted to the nanogels through optical reading by fluorescent material, zinc oxide (ZnO) quantum dots incorporated in the polymer chains of nanogel network. Hence nanogels possess a great future scope for the efficient management of diabetes.

Pulmonary delivery of drugs at times is beneficial in increasing the bioavailability of the drug as the alveolar surface provides for large area from which the drug absorption greatly increases as demonstrated by the peptides delivered to lungs by inhalation (Patton et al., 2004). Lee et al., 2012 formulated self-assembled nanogel inhalation system from chitosan of an antidiabetic drug, palmityl-acylated exendin-4 peptide (Ex4-C16). The workers were successful in providing sustained release from the Ex4-C16 loaded nanogels compared to non acylated exendin-4 peptide (Ex4). The nanogel provided an effective management of type 2 diabetes as demonstrated from the *in vivo* studies in mice (Lee et al., 2012).

G. Oral delivery

(Kim et al., 2011) Kim et al., have reported interferons (IFNs) to be responsible for reduction in the replication and pathogenicity of murine norovirus (MNV) (RAW267.4 cells) where they demonstrated large reduction in the replicationof RNA and proteins due to various interferons (IFN-α, IFN-γ) and suggested them as therapeutic agents for norovirus infections, a common cause for gastroenteritis. But short half-life of IFNs due to their low stability poses a problem for their delivery to specific infection sites. Hence for a profound action of IFNs their stability was improved by incorporating them in nanogels prepared from cross linked polymers, polyethyleneimine (PEI)-polyethylenglycol (PEG) in their acetylated form (acetylated for prevention of cellular penetration & toxicity). The AcNG-acetylated nanogel and IFN-AcNg both were found to be stable in PBS solution for two weeks at room temperature as observed by AFM (Atomic Force Microscope). The study, through various assays, demonstrated AcNg to highly stabilize IFNs and also significantly increased the activity of IFNs. *In vivo* studies using rats showed no side effects by administration through systemic or oral routes. Hence it could be concluded that AcNg could serve as potential carriers for IFNs to fight norovirus infections through oral route.

Conclusions

From the discussion above it is clear about nanogels that they possess a vast no. of applications regarding the targeted delivery of drugs to various organs. Nanogels exhibit the features of both the hydrogel and nanoparticles that make them a unique carrier system in that the hydrogel properties allow nanogels to accommodate a large quantity of water and hence increase their drug loading capacities, impart tissue like properties, make them flexible while nanometric size of these particles allow them to enter deeper tissues, escape invasion by reticuloendothelial system, provide site specific delivery etc. The so far research over nanogels have collected enough evidence to prove nanogels as potential targeting carriers that can deliver bioactive

substances to large number of organs ranging from topical delivery of skin for conditions like skin cancer, wounds, inflammation, local anesthesia upto CNS delivery. Besides drugs, nanogels have also been further exploited to incorporate macromolecules like proteins, peptides, carbohydrates, oligonucleotides, antigens, monoclonal antibodies, genes and some inorganic molecules like quantum dots, silver nanoparticles, magnetic nanoparticles etc. Considering the above aspects of nanogels it is clear that these versatile carriers possess enough potential to offer any potential breakthrough in the treatment of diseases infuture.

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Address correspondence to: Dr. Neeraj Mishra, *Associate Professor, Department of Pharmaceutics, I.S.F. College of Pharmacy, Moga-142001, India.*
Mob.: + 91 8054746714, 08437190413
E-mail: neerajdops@gmail.com, tahaumair43@gmail.com