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Review Article

NANOGELS AS NOVEL AND VERSATILE PHARMACEUTICALS

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ABSTRACT

The brief review aims at providing comprehensive illustrations on the novel applications, release mechanisms and recent synthesis methodologies of nanogels. Further insight on clinical trials and patents on nanogels have been summarized. Major therapeutic focus have been on anti-neoplastic applications and exhaustive discussion on variedgatedness of therapies anticipated in future by the advent of nanogels.

Keywords: Nanogels, Synthesis, Clinical trials, Patent.

INTRODUCTION

Nanogels may be defined as nano-sized hydrogel systems which are highlycross linked systems in nature involving polymer systems which are either co-polymerised or monomers[1]. Sudden outbreak in the field of nanotechnology have introduced the need for developing nanogel systems which proven their potential to deliver drugs in controlled, sustained and targetable manner. With the emerging field of polymer sciences it has now become inevitable to prepare smart nano-systems which can prove effective for treatment as well as clinical trials progress[2]. Nevertheless, these systems have been investigated from a longer period of time for making advancements in synthetic procedures not only for drug delivery but for miscellaneous agents like quantum dots, dyes and other diagnostic agents [3,4,5,6]. Traditionally in the name of gels we have heard of semisolid formulations with three dimensional network of organic systems encompassing fluids and drugs. Majorly these systems have been the part of traditional system of topical drug delivery for local effects. Prospects of targeted drug delivery perhaps could not been established with these preparations [7]. The significance of nano-sized microgel and hydrogel have arisen due to specific delivery system anticipation. Wide variety of polymer systems and the easy alteration of their physico-chemical characteristics has given advantage for versatile form of nanogel formulations [8]. Recent studies at clinical level have shown promising value of nanogels [9]. Nanogels have revolutionized the field of gene therapy, since delivery of gene has now become possible within cellular organales for gene silencing therapy systems [10]. Nanogels are typical formulations mainly of the size range of 100 nm, by varying solvent quality and branching the volume fraction can be altered variably to maintain a three dimensional structure [11]. The overall review suggests that innovation in this field shall bringforth sound support to cancer therapy in future.

DRUG RELEASE MECHANISMS OF NANOGELS

Mechanism of drug release have been investigated broadly based on the sensitive characteristics of polymer systems such as temperature, pH, volume transition and light responsive behavior either effecting the loading or release capacity of nanogels as described below.

1. pH responsive mechanism

Platinum nanoparticle containing nanogel showed on and off catalytic activity for scavenging reactive oxygen species due to acidic skin pH and for the reason of protonation of Cross linked poly[2-(N,N-diethylamino)methacrylate] core and PEG [8]. The polymers methacrylic acid-ethyl acrylate generally exist at low pH in insoluble 3D structures, by applying variable increasing pH ranges acidic group ionizes due to which the polymeric chains repulsions begins and lead to a particular release profile of procaine hydrochloride [12]. (Figure 1)

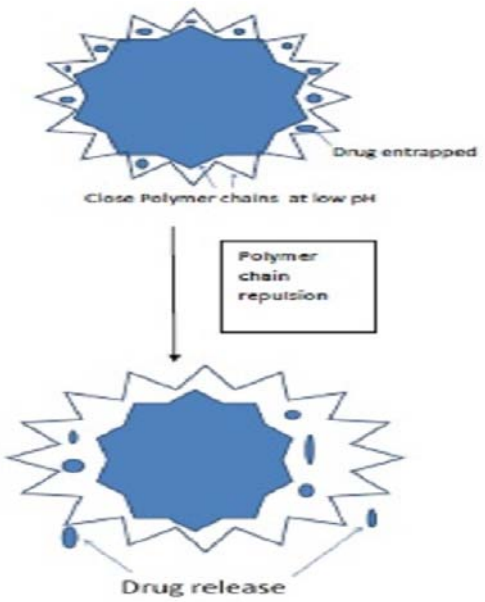

Fig. 1: Drug release from nanogel due to pH responsive polymer chainrepulsion.

Anticancer drug temozolodine release kinetics showed controllable mechanism due to swelling action of pH sensitive polyacrylic acid chains [4]. The release of doxorubicin was significantly increased due to glycol chitosan nanoparticles sensitivity to pH stimuli due to grafting of diethylaminopropyl groups [13]. (Figure 2)

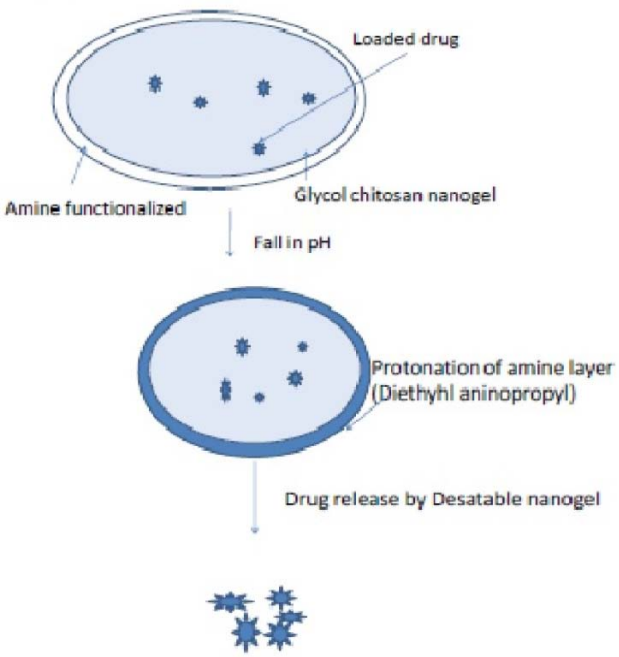

Fig. 2: Drug release due to protonation of amine shell and destabilization of nanogel due to pH changes

Significant mesh size alteration have been seen in dietylaminoethyl methyacrylate cationic nanogel for release of medium size molecules by virtue of pH sensitivity [14].

2. Thermosensitive and volume transition mechanism

Poly(N-isopropylacrylamide) developed nanogels have thermosensitive characteristic leading to sudden shrinkage in gel volume and efflux of indomethacin drug due to maintainance of temperature above lower critical solution temperature (LCST) [15]. The in-situ gelation of 5-fluorouracil poly(N-isopropylacrylamide-co-acrylamide) nanogel in rats was advantageous for drug loading at low temperatures and release at body temperature [16]. Polyethyleneimine nanogels on superficial modification of surface by pluronic had thermoresponsive characteristic with regard to size and were successfully used as gene delivery systems [17]. Thermally trigerrred expanded volume nanogels of poly alkyylene oxides showed physical destruction of cellular network on expansion of 1µm in nanogel size[18] (Figure 3).

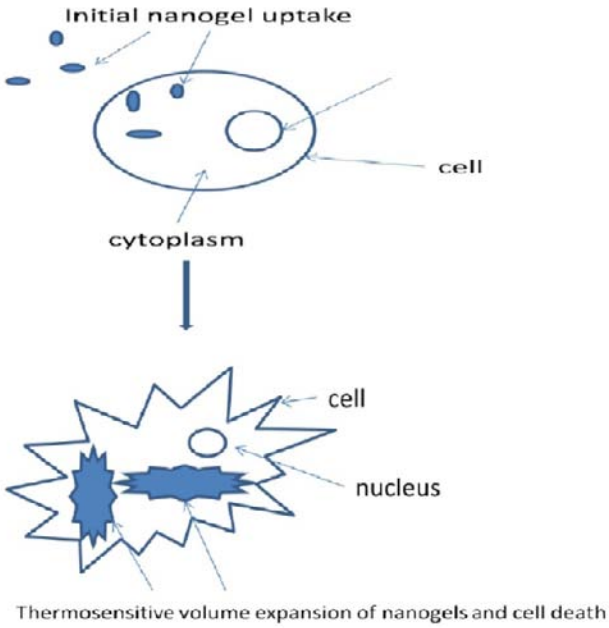

Fig. 3: Drug release due to thermo-volume reaponsiveness of nanogels

Further modifications recently made in nanogels for temperature sensitive drug delivery was biocompatible magnetic field targetibility of poly(N-isopropylacrylamide) and chitosan nanogel in which the lower critical solution temperature could be modified by changing ratio of polymers and hence found application in hyperthermic cancer treatments[19]. By utilizing the thermoresponsiv feature of the shell of hydrophilic core synthesized by amidoximation of hydrophilic acrylonitrile core provided suitable source for two times propanolol loading and release characteristics of the nanogel[20]

3. Photochemical internalization and photoisomerisation

Excitation of photosensitizers loaded nanogels leads to production of singlet oxygen and reactive oxygen species which cause oxidation of cellular compartment walls such as endosomal barrier walls which effects release of therapeutics into cytoplasm easily which is otherwise hindered by intracellular compartments [21] (Figure 4). Cis-trans isomerisation of azobenbenzene by photoregulation in azo-dextran nanogel loaded with aspirin as model drug exhibited that E-configuration of azo group lead to better release profile of drug than z-configuration at 365 nm radiation [22].

4. Miscellaneous examples

It include degradation of nanogel structure as in the case of action of reducing agents which degrade disuiphide linkage in crosslinked hylauronic acid nanogels [10], simple diffusive process involve polymeric release of doxorubicin for a week long period by simple diffusion [23] and control on initial release by coating with cationic and anionic polyelectrolytes increases the size of nanogel and layer by layer release of drug is possible without sudden initial outburst [24](Figure 5).

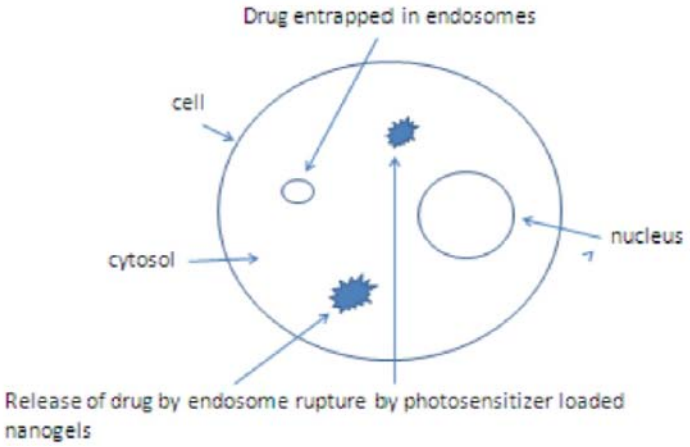

Fig. 4: Drug release due to endosomal rupture caused by photosensitizers loaded

RECENT METHODOLIGIES FOR NANOGEL SYNTHESIS

1. Novel pullulan chemistry modification

Synthesis of cholesterol based pullulan nanogel(CHP) was done by reacting mixture of cholesterol isocynate in dimethyl sulfoxide and pyridine. Pullulan was substituted with 1.4 cholesterol moieties per 100 anhydrous glucoside units. The preparation was freeze dried and in aqueous phase it formed nanogel which was complexed with W-9 peptide for delivery in osteological disorders. The capacity of pullulan has been known to act as good protein carrier hence was used in nanogel formulation for drug delivery [25]. Further CHP has been modified with acrylate group and their thiol group was modified with polyethylene glycol by adopting Michael addition reaction, this allowed reduction in mesh size to 40 nm encapsulating 96% interleukin-12 [26]. These nanosystems have also been investigated by modifying cholesterol units by 1.1 units of cholesteryl group per 100 glucose units of parent pullulan shown significant interaction with Aβ oligomer and monomer for alzhiemer's disease treatment by

enhancing microglia and cortical cell viability [18].More recently pullulan have been used in folate receptor targeted system in which folate was substituted to pullulan by 1.6 glucose units. Further Coupling of pullulan and photosensitizer(pheo-A) was done with carbodimide to produce the conjugate which was converted to nanogel by dialysis in DMSO against deionised water., investigated for photodynamic therapy and were successfully localized at tumour cells to cause cell death by photodestruction [27].

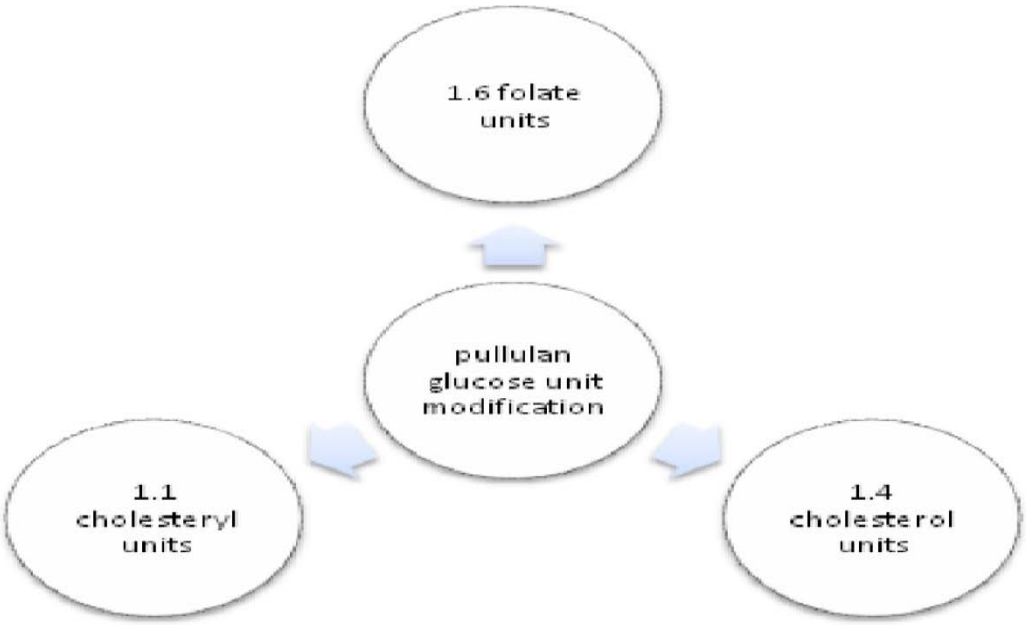

Fig. 6: Schematic presentation of pullulan glucoose unit modifications.

2. Novel photochemical approach

Photochemical approach have been developed to produce ferric oxide nanoparticles nanogel for MRI application by coating oxide with N-(2-aminoethyl)methyiacrylamide and N,N'-methylene bis acrylamide treared with UV radiation at 388 nm for 10 minutes recovering the product after washing with water [5], likewise, diacrylated pluronic and glycidyl methyacrylated chitooligosacchride were loaded with plasmid DNA at different ratio's and were photo irradiated with long wave length UV light at 365 nm ,the photo initiator was igracure added to the mixture for cross linking [28] offering advantage to gene delivery.

3. Novel free radical polymerization process with inverse miniemulsion technique

Free radical polymerization methodology was adopted more recently for poly(N-isopropylacrylamide) chitosan nanogel prepared by redox polymerization at elevated temperatures of 60°C using sulphate initiators producing highly hydrophobic chains with optimized LCST above 42°C relevant for hyperthermic cancer treatments [19] (Figure 7). Atom transfer radical polymerization process reported involves use of lower oxidation state metal complex added as activator to the substrate producing a non-reactive species and a free radical which propagates chain reaction of monomer addition giving a cross linked structure of poly oligo ethylene glycol monoethyl ether methyacrylate nanogel by further adopting a inverse mini emulsion technique in which surfactant are oil soluble. Upon addition of initiator, polymerization occurs in aqueous droplets dispersed in oil droplets, finally nanogel can be obtained in the form of dispersion in organic solvents. Additional thiol-disulfide exchange process made the product target specific as reported for doxorubicin [29]. For the same nanogel, rhodamine B isothionate labeled dextran loaded were prepared in cyclohexane inverse mini emulsion, It was entrapped in 3D matrix by photopolymerisation for 10 minutes under UV producing control release product[30].Inverse emulsion polymerization was also adopted to generate diethylaminoethyl methacrylate nanogel, the method produced pH responsive mesh size controlled release product accepted for controlled drug delivery system [14](Figure 8).

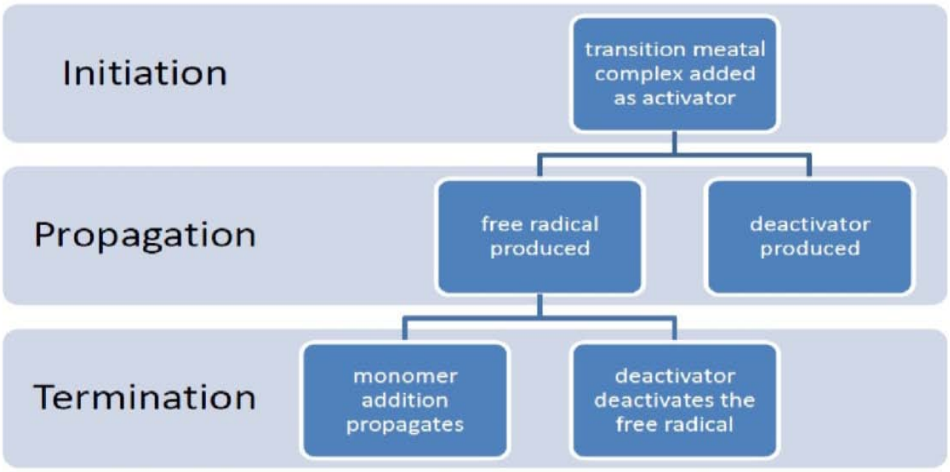

Fig. 7: Schematic presentation of general Atom transfer radical polymerization process

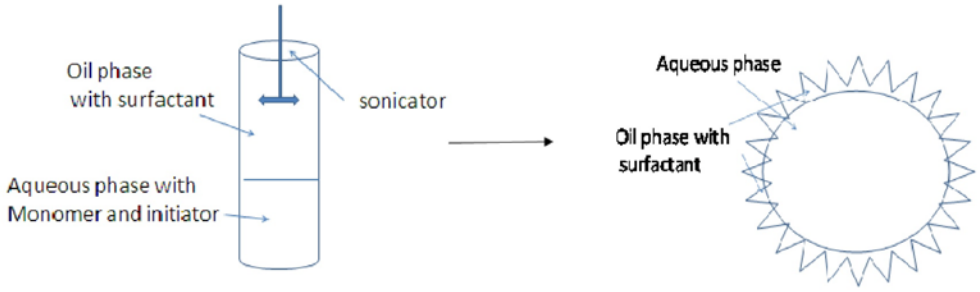

Fig. 8: Reverse miniemulsion process

4. Addition fragmentation transfer(RAFT) process

Reversible addition fragmentation transfer (RAFT) process adopted a single step of synthesis for PEGylated poly (N,N'-dimethylaminomethyl methacrylate) nanogel utilizing an ampiphilic macroRAFT agent trithiocarbonate with hydrophobic dodecyl chain supporting polymerization rather than two step process which produced 500-800 nm size. However single step process presented advantage of reduced radii of nanogel (10 nm) apt for gene delivery [31] (Figure 9).

5. Emulsion photopolymerisation process

Emulsion photopolymerisation using UV was utilized for preparing cationic dextran nanogel in which the dextran hydroxyethyl methacrylate was emulsified with ABIL EM 90 as emulgent in mineral oil , the product was obtained in acetone:hexane(1:1), the precipitate was centrifuged, lyophilized and dessicated. The photosensitizer meso-tetraphenylporphine disulfonate was introduced in the preparation to cause breakage of endosomal membranes in cell and release of genes in cytoplasm and nuclease. Hence internalization accomplished for genetic material [21] (Figure 10).

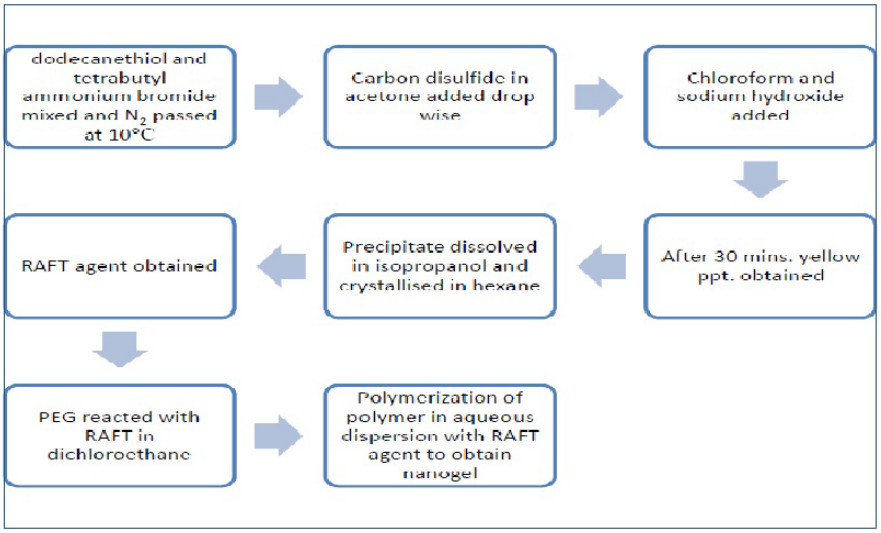

Fig. 9: Schematic presentation of RAFT process

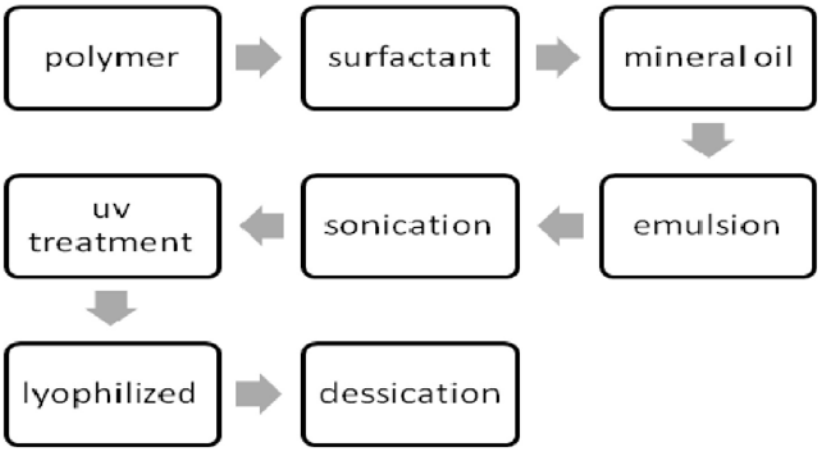

Fig. 10: Schematic presentation of emulsion photopolymerisation process

6. Chemical modifications

Chemical modification of polymers also support release of drugs from the nanogels. Acetylation of chondriotin sulfate can appreciably release doxorubicin in HeLa cells over a 3 week period suggesting antineoplastic prospects [32]. Introduction of quarternary group core to nanogels of polyion complexes consisting of poly2-(N,N-diethyl aminoethyl) methacrylate increases siRNA binding capacity provides prospects for cancer treatment and gene delivery [33]. Methotrexate release profile can be altered from nanogel of co-polymerised N-isopropylacrylamide and butylacrylate saturated with sodium carbonate leads to further drug absorption and release from formulation [34].Modification of hydroxyl group of hydroxypropylcellulose-polyacrylic acid nanogel could sequester cadmium(II) ions quantum dots and polyacrylic acid pH sensitive behavior was utilized for bioimaging of cells by sensing physicochemical environment in a pH dependent manner at emission of 741 nm emission and excitonic vibration at 592 nm [4].use of heparin in pluronic nanogels containing RNase A showed better heparin RNase A conjugation and hence the enzyme was internalized with ease [35]. Grafting of 3-diethylaminopropyl to glycol-chitosan nanogel leads to deaggregation of product at lower pH, doxorubicin release pattern have been investigated [13].

NANOGELS IN CANCER TREATMENT

Undoubtedly cancer treatment involves targeted delivery of drugs with expected low toxicities to surrounding tissues and high therapeutic efficacy. Nanogels technology assures all these advantages as listed below in the table 1.

NANOGELS APPLICATION

Nanogels provide suitable examples and advantages to biotechnological skills dealing with genetics, enzyme immobilization and protein synthesis providing efficient tool to cater with novel therapeutic systems in medicine.(table 2)

CLINICAL TRAIL STATUS OF NANOGELS

Cholesteryl pullulan(CHP) nanogels have shown tremendous potential in delivering peptides. The CHP-HER-2 vaccine was administered to nine patients biweekly dosing of 300µg with booster doses. The vaccine was well tolerated with some skin sensitivity at site of subcutaneous injection. All the patients showed $CD4^+$ and $CD8^+$ T- cell response suggesting better therapeutic activity [49, 9]. CHP nanogels have further proved their prospects for clinical trails by reducing cytotoxicity of nervous system cells by showing increase in binding capacity to Aβ oligomer in treating Alzheimer's disorder [18] it has also been clinically investigated for bone loss disorder and it proved its worth by reducing the dosage of W9 peptide by only two times a day than tedious eight time dosage of drug which was clinically impossible [25]. Recent prospects in diabetes management by optical sensitive insulin loaded silver nanoparticle nanogel of poly(4-vinylphenylboronic acid-co-2-(dimethylamino)ethyl acrylate) have been designed opening new era in the field of clinical trials [50]. Development of antibiotic conjugated nanogels and their in-vivo application have given promising approach towards phase 1 clinical trails [51].

Table 1: Applications of nanogels in cancer treatment

Nanogel constitution	Type of nanogel	Application
Acetylated chondroitin sulfate	Self organizing nanogel	Doxorubicin loaded [32]
Cross linked polyethyleneimine and PEG/pluronic	Biodegradable nanogel	5'-triphosphorylated ribavirin reduced toxicity [36]
Glycol chitosan grafted with 3-diethylaminopropyl groups	pH-responsive	Doxorubicin uptake accelerated [34]
Pullulan/folate-pheophorbide	Self quenching polysaccharide based	Minimal phototoxicity of pheophorbide [27]
Crosslinked branched network of polyethyleneimine and PEG	Polyplex nanogel	Elevated activity and reduced cytotoxicity of fludarabine [37]
Heparin pluronic nanogel	Self assembled nanogel	RNaseA enzyme delivery internalized in cells [35]
Cross linked poly[2-(N,N-diethylamino)methacrylate] core and PEG	PEGylated and partially quarternized amine nanogel	Efficient siRNA delivery [33]
Polyethyleneimine nanogels	Size dependent property nanogel	Suicide gene hTERT –CD-TK delivered for lung cancer [38]
Cholesterol bearing pullulan nanogels	Sustained release nanogel	Recombinant murine interleukine-12 sustained tumour immunotherapy [39]
Reducible heparin with disulfide linkage	Reducible nanogel	Internalization of heparin for apoptotic death of melanoma cells [40]
Pluronic polyethyleneimine/DNA complex	Temperature responsive and volume transition nanogels	Thermo responsive endosomal rupture by nanogel and drug release [17]
Cross linking of oligo(l-lactic acid)-poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)-poly(l-lactic acid) grafted poly(l-lysine)	Thermally triggered and volume transition nanogel	Traumatic cell death due to physical stress and good source for loading anticancer drugs [18]
Cross linked folic acid with pullulan	Self organized with minimal fabrication nanogel	Doxorubicin targeting [41]
Acetylated hylauronic acid	Specific targeting nanogel	Doxorubicin loaded nanogel [42]
Poly(N-isopropylacrylamide) and chitosan	Thermosensitive magnetically modalized	Hyperthermia cancer treatment and targeted drug delivery [19]
Polyacrylamide	Novel core shell magnetic nanogel	Radiopharmaceutical carrier for cancer radiotherapy [6]
Acylate group modified cholesterol bearing pullulan	Cross linked raspberry like assembly nanogel	Efficient interleukin-12 encapsulation and plasma levels [26]
Cross linked poly(ethylene oxide)and polythyleneimine	Nanosized cationic hydrogel	Enhancing oral and brain bioavailability of oligonucleotides [43]
Poly(N-isopropylacrylamide-co-acrylamide)	In-situ gelatinized thermosensitive nanogel	Drug loading capacity of low mol.weight 5-flourouracil was higher than that of biomacromolecules, bovine serum albumin [16]
Cholesterol bearing pullulan with modified amino group	Nanogel quantum dot hybrid	Probe for bioimaging [3]
Hydroxypropylcellulose-poly(acrylic acid)	pH and temperature responsive cadmium(II) ions quantum dots	Optical pH sensing, cell imaging and drug loading of temozolomide [4]

Table 2: Applications of nanogels in gene delivery, enzymology and protein folding:

Nanogel constitution	Type of nanogel	Application
poly[2-(N,N-diethylaminoethyl)methacrylate] PEGlyated macroRAFT agent	One step PEGylated cationic nanogel.	Potential in gene therapy [31]
Cholesterol bearing pullulan	Self assembled artificial molecular chaperone	Assisted protein refolding of carbonic anhydrase and citrate synthase during GdmCL denaturation [44]
Cholesterol bearing pululan and amino group modified Cholesterol bearing pululan	Biocompatible nanogel as artificial chaperone	Treatment of alzheimer's disease by inhibiting aggregation of amyloid β-protien [45]
Cholesterol bearing pululan	Polysaccharide chaperone nanogel	Comparision of chaperone with natural molecular chaperone of E. coli for refolding activity of green fluorescent protein [46]
Methylacrylic acid and N,N'-Methylene-bis-(acrylamide)	Supermagnetic nanogel functionalized with carboxyl group	α-chymotrypsin immobilized on aminated nanogel [47]
Methylacrylic acid and N,N'-Methylene-bis-(acrylamide)	magnetic nanogel hydrophilic polymers	α-chymotrypsin immobilized on carboxyl group [48]
Thiol functionalized hyaluronic acid	Target specific degradable nanogel	siRNA delivery to HCT-116 cells [10]
Dextran hydroxyethyl methacrylate-co-[2-(methacryloyloxy)-ethyl]trimethylammonium chloride	Nanogels with photochemical internalisation	Endosomal escape of siRNA [21]
Di – acrylated pluronic 127 and glycidyl methacrylated chitooligosacchride	DNA nanogel with photo cross linking.	Controlled delivery of plasmid DNA [28]

PATENT STATUS OF NANOGELS

Patents on nanogels have been mainly filed for their synthetic procedures.''Ringing gels''(system vibrates with a little tap for returning to original configuration) describes a surfactant free preparation of oil in water metastable nanaoemulsion which has mainly silicone as component prepared by high shear rate devices converting it into nanogel [5)]. Polyacrylic acid nanogel prepared by free radical polymerization method using gamma radiations for cross linking of polymers for dispensing biologically active molecules [53]. Nanogel prepared from metal salts containing solid particulate matter or solid dispersions utilized for either barrier system or loading system for chemicals, gases, drugs etc [54]. Invention of water soluble degradable bioimaging and drug particle conjugating to free carboxyl groups of polymers hydrogel have been made [55]. Targeted delivery of anti-neoplatic agents non-covalently bound to polymer core in nanogel has been filed[56].

CONCLUSION

Nanogels and biopharmaceuticals have gone hand in hand promising future developments widening the prospects for drug delivery at large scale. Every new research entails to discovery of new polymeric systems and novel mechanistic approaches with promising role in therapies and new innovation on fabrication of nanogel design. Hence, we may certainly hope that these novel carrier systems hold promising virtues to pharmaceutical sciences.

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