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Nanogels: Smart Novel Nanocarriers for Targeted Drug Delivery in Breast Cancer Therapy

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ABSTRACT

Breast cancer is a widespread global health concern that mainly affects women. The significance of prevention, early detection, and effective treatment cannot be overstated. Recently, there has been growing interest in nanotechnology for its potential to develop drug-delivery systems aimed at fighting this disease. One particularly promising innovation is nanogel (NG), which has gained attention for its significant capabilities in cancer therapy and responsiveness to environmental stimuli. Nanogels are hydrogels with a three-dimensional (3D) tuneable porous structure, typically measuring between 20 and 200 nm. NG is viewed as a next-generation delivery technology due to advantages such as size tunability, high drug loading capacity, responsiveness to stimuli, extended drug release through in situ gelling, stability, and the ability to offer personalized therapy based on genetic insights related to cancer. Nanogels can be modified for active targeting, improving drug accumulation at disease sites. Stimuli-responsive NGs can react to physical or environmental triggers (internally or externally) like pH, temperature, light, or redox conditions in the tumor environment, making them smart nanomedicines for breast cancer detection and treatment, and they can also be tailored for personalized medicine. This review article's major objectives include the application of stimuli-responsive NGs in breast cancer treatment, highlighting early preclinical successes and future possibilities.

Keywords: breast cancer, stimuli responsive, targeted drug delivery, nanogels, future prospects, cancer therapy, clinical trials, chemotherapy

1. INTRODUCTION

Breast Cancer

Breast cancer (BC) is the most prevalent cancer diagnosed in women, with approximately 4.1 million cases recorded in the United States as of January 1, 2022 [1]. Global BC survival rates vary widely, with 5-year survival rates estimated at 80% in developed countries and less than 40% in developing countries [2]. BC can be divided into four categories according to molecular subtyping: luminal A BC, luminal B BC, human epidermal growth factor receptor 2 (HER2)-positive BC and triple-negative breast cancer (TNBC) [3].

- *Luminal A BCs* that are estrogen receptor (ER) positive and progesterone receptor (PR) positive but HER2 negative usually have a better prognosis and can be treated with endocrine therapy alone, with chemotherapy as an option.

- *Luminal B BCs* that are ER positive, PR negative and HER2 positive tend to grow faster than luminal A BCs, have a slightly worse prognosis and require chemotherapy combined with endocrine therapy.

- *HER2-positive BCs* that are ER and PR negative and HER2 positive tend to grow faster and have a worse prognosis than luminal BC but can often be successfully treated with targeted therapeutic agents that target the HER2 protein.

- *TNBC* which is negative for ER, PR and HER2, has the worst prognosis of the four molecular types and is usually aggressive, unresponsive to endocrine therapy and targeted therapy, and effectively treated only by chemotherapy [4][5].

Molecular subtypes are mainly based on genes expressed by cancer cells that can control the way cells behave. Understanding common BC cell lines that express different genes and molecular subtypes can help determine the best treatment [6].

Available Breast Cancer Therapies and their Limitations

Existing therapies for breast cancer include surgery, radiation, and chemotherapy. Surgery and radiation are no longer effective when tumors metastasize to other tissues, and unfortunately, metastasis is highly possible for breast cancer. Chemotherapy delivers cytotoxic drugs to suppress tumor cell growth, proliferation, and metastasis; however, it uses poorly water-soluble drugs with limited delivery to target tissues, develops resistant tumors, has high drug toxicity in normal cells, and causes severe side effects (infection and heart damage), rapid degradation, low specificity, and limited targeting, ultimately leading to unsatisfactory clinical outcomes.

Moreover, some studies have found that with prolonged treatment, cancer cells are able to develop resistance (multidrug resistance–MDR) to chemotherapy and radiotherapy [7]. According to the American Cancer Society, over 90 % of patients who succumb to the effects of chemotherapy do so due to the appearance of MDR during the treatment process. MDR refers to the phenomenon of cross-resistance of cancer cells to a variety of drugs with different functions, structures, targets, and mechanisms [8]. For example, the antibiotic doxorubicin (DOX) is most commonly used to treat cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and Hodgkin´s lymphoma but is known for its poor tumour specificity and significant side effects. The use of DOX may lead to the development of multidrug resistance (MDR) in tumour cells, resulting in treatment failure, tumour recurrence, and ultimately, a poor prognosis for patients [9-11].

Some chemotherapy drugs for breast cancer treatment are listed below:

- Taxanes: Paclitaxel (Taxol), docetaxel (Taxotere), and albumin-bound paclitaxel (Abraxane)
- Ixabepilone (Ixempra)
- Eribulin (Halaven)
- Anthracyclines: Doxorubicin (Adriamycin), liposomal doxorubicin (Doxil), and epirubicin (Ellence)
- Platinum agents (Cisplatin, carboplatin)
- Vinorelbine (Navelbine)
- Capecitabine (Xeloda)
- Gemcitabine (Gemzar) [12]

Current therapies are still ineffective in eradicating the disease as a whole, and therefore require improvement using far more specific treatments [13]. Compared with healthy tissues, breast tumors display many distinct characteristics, such as hormone overexpression, acidic pH, excessive adenosine triphosphate (ATP), redox potential, and dysregulated enzymatic activity. These disparities can be exploited to guide the development of targeted delivery systems. Alternatively, external stimuli such as light, ultrasound, and magnetic fields can also be harnessed to trigger physiochemical changes in nanocarriers, enabling therapeutics to be released and take effect only at the intended site. To date, a wide variety of targeting strategies have been successfully established. Pre-vailing perspective has been that these strategies can be divided into passive targeting and active targeting [14-15]. In general, passive targeting is referred to processes passively driven by the physiological property of tumors (e.g., vascular permeability), with the enhanced permeability and retention (EPR) effect being the principal means; and active targeting is referred to processes involving the active participation of an operator (e.g., through engineering surface ligands). For example, nanocarriers (Fig. 1) artificially designed to release drugs at low pH (a property of tumor) are considered passive, whereas those employing specific ligands to target cell receptors (a property of tumor as well) are considered active. Targeting process is predominantly based on internal (e.g., EPR, pH, redox, receptor) or external factors (e.g., light, ultrasound, magnetic waves).

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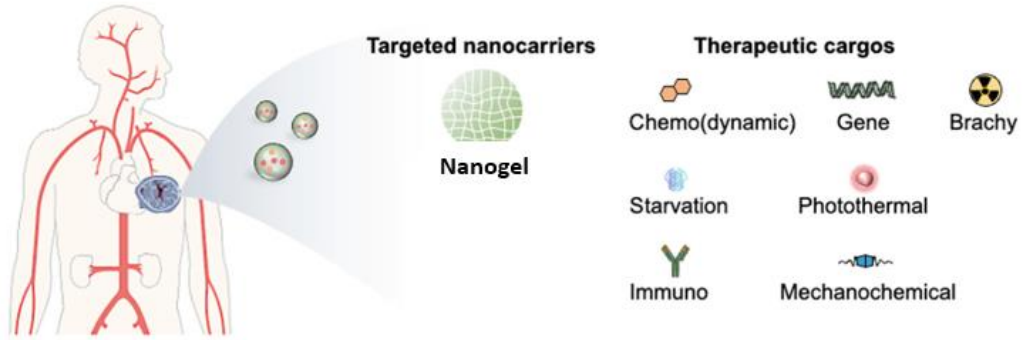

Fig. 1 – Nanocarrier Discussed in this Chapter to Target Breast Cancer

2. NANOGELS AS NOVEL TARGETED DRUG DELIVERY SYSTEM

Drug delivery systems designed using nanotechnology deal with a large variety of materials that have certain valuable characteristics that are dependent on their nano-size. Nanocarriers offer targeted drug delivery to specific sites reducing off-target/side effect. The nanoscale size can provide certain benefits in cancer treatment; particularly in enhancing the dissolution rate of poorly soluble drugs, increasing the accumulation of drugs in tumors, improving the therapeutic agents' stability toward chemical/enzymatic degradation and reducing cytotoxic side effects in cancer therapy [16-17].

Nanogels, a typical nanocarrier, are 3D nanosized particles prepared by cross-linked polymer networks that rapidly swell upon solvent penetration. According to Soni et al. (2016), "nanogels are three-dimensional hydrogel materials in the nanoscale size range formed by cross-linked swellable polymer networks with a high capacity to hold water, without actually dissolving into the aqueous medium."[18] The term 'Nanogel' (NanoGel™) was introduced for the first time by Vinogradov et al. in 1999 to define a bifunctional system consisting of a polyionic and nonionic polymer; in other words, cross-linked polyethyleneimine (PEI) and poly(ethylene glycol) (PEG) (PEG-cl-PEI) [19].

Properties Of Nanogel

Nanogels possess the combined characteristics of nanoparticles and hydrogels, and their size ranges from 20 to 200 nm [20]. Being nanosized, they possess the ability of crossing biological barriers and membranes with ease. They have been proven to be instrumental in the administration of drugs across the blood-brain barrier [21]. Nanogels are effective in avoiding the rapid renal exclusion. They also have the ability to evade the reticuloendothelial system [20].

High swelling capacity (rapid swelling and de-swelling) of nanogels when in contact with physiological fluids, makes them flexible and soft enough to enhance drug-loading capacity, be in close proximity with the targeted site, permeate tumor membrane, and enhance diffusion of drugs and other therapeutics (good biodistribution). This is due to their high affinity functional group of polymers initiating the hydrogen bond formation or van der Waals forces of interaction within the gel network [22].

Nanogels are characterized by biocompatibility, high colloidal stability, biodegradability. They are synthesized by using natural or synthetic polymers for preventing the deposition in systemic circulation. These polymers are biodegradable, stable, hydrophilic, nontoxic in nature [23].

Nanogels in general may be developed from heterogeneous polymerization of monomers or synthesized from polymer precursors. Like hydrogels, nanogels possess the capacity of retaining a great quantity of water or biological fluids within its structure while preserving its arrangement, which is devoted to the existence of hydrophilic groups such as –OH, –CONH–, –CONH2– and –SO3H in the polymer [24]. Yet, nanogels display swelling feature instead of being liquefied due to the presence of cross-links between polymers. Water-soluble non-ionic polymers like hydroxyl propyl methylcellulose as well as ethyl cellulose are especially used to stabilize nanogel dispersions [25].

Due to their hydrophilic characteristics and through hydrophobic and electrostatic interactions, nanogels are compatible with accommodating small drug molecules to big biomacromolecules (e.g., proteins, peptides and nucleic acids) through suitable structure alteration; but not affecting the gel-like performance. Nanogels thus have the unique capability to encapsulate more than

one bioactive ingredient in the same carrier by diverse physical assets. This capability is not feasible to most other categories of nanoparticles, like dendrimers, micelles, liposomes or solid lipid nanoparticles [26].

In addition, the nanogels can be formulated for drug release under specific physiological conditions. They allow possible chemical modifications of the surface for active targeting by attaching ligands that recognize cognate receptors on the target cells or tissues which can be used for targeted drug delivery. Nanogels can be designed to be stimulus responsive, and react to internal or external stimuli such as pH, temperature, light and redox, thus resulting in the controlled release of loaded drugs. This "smart" targeting ability protects the encapsulated biological particles from in vivo degradation and elimination, and endows the delivery process to achieve a retained, controlled, triggered release at specific sites, prevents drug accumulation in non-target tissues and minimizes the side effects of the drug [27].

The drug loading mechanism must be selected carefully to ensure that the large surface area provided by the nanogel network is utilized and the maximum amount of the drug is loaded. Physical entrapment, covalent conjugation, and self-assembly are some of the methods by which the drug is loaded into the nanogel [28]. Drug release characteristics of the nanogel system are influenced by the molecular weight of the polymer, the thickness of the cross-linked network of the gel, the degradation rate of the polymer, and the interaction of the drug and polymeric chains in the gel [29].

Nanogel drug delivery system usually doesn't produce any immunological responses. Thus, smart nanogels with several unique characteristics are versatile for the potential treatment of several diseases – cardiovascular diseases, diabetes, and pulmonary diseases, cancers and other malignancies, to mention a few. Nanogels can also be applied as optical imaging agents and as thermo-chemotherapeutic agents.

This review covers an updated overview of applications of nanogels in breast cancer drug delivery.

Routes of Administration of Nanogel [30]

- ✓ Oral
- ✓ Pulmonary
- ✓ Nasal
- ✓ Parenteral
- ✓ Intra-ocular
- ✓ Topical

Advantages Of Nanogel

a) Free-flowing pearlescent solution of the nanogels is easily dispersed in aqueous media [31].

b) Can be easily administered by parenteral and mucosal route [32].

c) Biggest advantage is reduced premature leakage of the drug from the solution [33].

d) Crosslinking densities of drug delivery can be controlled by tuning.

e) Both hydrophilic and hydrophobic drugs can be formulated in nanogels formulation [34].

f) High biocompatibility and high biodegradability, which is crucial to avoid accumulation of nanogel material in the bodily organs which can lead to toxicity and adverse effects.

g) Nanogels are inert in the blood stream and the internal aqueous environment, meaning that they do not induce any immunological responses in the body.

h) Extremely small size, which induces a number of effects such as:

- Enhanced permeation capability.
- Avoidance of rapid renal exclusion. Escaping renal clearance leads to prolonged serum half-life.
- Avoidance of clearance by phagocytic cells and the uptake by reticuloendothelial system, which permits both passive and active drug targeting.
- Capability to cross the Blood Brain Barrier.
- Enhanced penetration of endothelium in pathological sites like solid tumors, inflammation tissue and infracted areas which increases the amount of drug delivered and the selectivity of the drug delivery.
- Improved ability to access areas that is not accessible by hydrogels, upon intravenous administration.

i) Rapid responsiveness to environmental changes such as pH and temperature for targeted drug delivery.

j) Controlled release of drug from the formulation can be regulated by the addition of a polymeric network [35].

Limitations Of Nanogel

a) Expensive technique to completely remove the solvents and surfactants at the end of preparation process.

b) Surfactant or monomer traces may remain and can impart toxicity [36].

c) Some portion of the formed particles tend to be in the micrometre range [37].

d) Sometimes a strong interaction between drug and polymer decreases the hydrophilicity of the nanogels and causes the structure to collapse, hence irreversibly entrapping the drug molecules and enhancing the hydrophilicity of the nanogel matrix [38].

e) Scale up isn't simple because of mean size and weight [39].

f) Limited drug-loading capacity and suboptimal regulation of drug release [40].

Polymers used in Nanogel

To control the release of the drug from nanogel, a combination of natural and synthetic polymers or modified natural or synthetic polymers are used in their formulation.

Natural polymers are obtained from nature. Some examples of natural polymers used in nanogels include cellulose, chitosan, gelatin, pullulan, alginate, heparin and hyaluronic acid. Natural polymers have desirable characteristics of being biodegradable, non-toxic, biocompatible and abundant in nature but often have the disadvantages of gel difficulty and poor mechanical ability [41].

Synthetic polymers are usually by-products from petrochemical processes. They have attracted much attention due to their easy access to materials and strong controllability of performance [42]. However, unlike natural polymers, they are not easily renewable. There have been concerns about their limited biocompatibility with physiological membranes and their non-degradability and possible cytotoxicity (Sultan et al., 2021). Examples of synthetic polymers include poly (N-isopropylacrylamide), poly (N-isopropylacrylamide-co-acrylic acid), poly (ethylene glycol)-b-poly (methacrylic acid), poly (ethylene glycol)-coMethacrylamide-co-Acrylic acid, and poly (2-(N, N-dimethylamino) ethyl methacrylate), pluronic [43].

Polymers are present in the nanogel network either by physical or chemical crosslinking. There are various intermolecular forces involved in the physical cross-linking of the nanogels, which include hydrophobic interactions, hydrogen bond formation, electrostatic interactions, and guest-host interactions. Creation of cross-linking through chemical reactions is based on covalent bond formation, The links are based on disulfide bonds, amine reactive groups, click chemistry, or light-triggered crosslinking. The cross-linking process is accomplished in three locations of the nanogels themselves – including the center of the micelles, the surface, and the area between the surface and the center.

The composition of polymers in the gel network can be tuned to ensure the biocompatibility and the biodegradability of nanogels. These mentioned characteristics have rendered nanogel formulations advantageous.

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Loading of Drugs in Nanogel

Loading of drugs in nanogels can be accomplished by three methods:

i. ***Covalent conjugation***

During nanogel synthesis or by employing preformed nanogels, conjugation of bioactive entities may be accomplished covalently resulting in the formation of a dense, drug-loaded core [44]. For example, the alteration of acrylic groups occurs when it interacts with enzymes, and after this copolymerization with acrylamide either in dilute aqueous solution so as to achieve nanogel or inverse micro-emulsion [28].

ii. ***Physical entrapment***

This involves the linkage between the hydrophilic chains of the polymer and the hydrophobic portion or the dissolution of hydrophobic particles in a hydrophilic carrier. In cholesterol-modified pullulan nanogels, the loading of proteins has been accomplished through physical entrapment in nonpolar domains via incorporation of hydrophobic molecules that is existent in particular nanogels [28]. Molecular size of enzymes and pore size of nanocarriers determine the physical entrapment. [45].

iii. ***Self-assembly***

The phenomenon of self-assembly has been stated as the spontaneous, autonomous and reversible organization of molecular units into structurally stable and definite aggregates where deficiencies are omitted vigorously. This method applies to polyelectrolyte-based nanogels, where interaction between oppositely charged electrolytes leads to high loading efficiency. This can be seen is cholesterol bearing pullulan nanogels (Fig. 2).

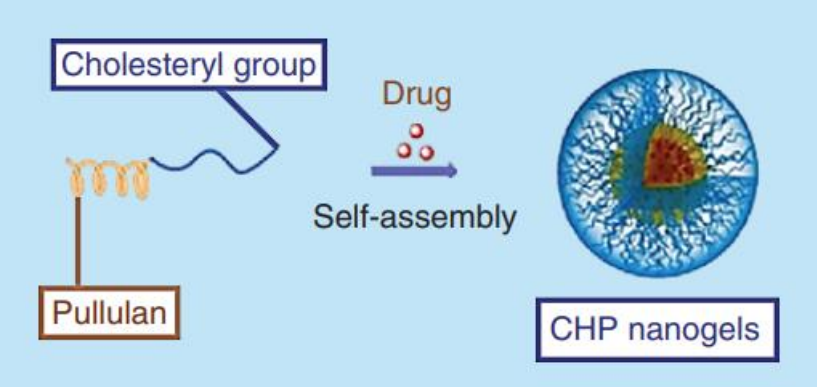

Fig. 2 – Graphical Representation of Cholesterol-Bearing Nanogel

Classification of Nanogels

Classification of nanogels is based on their structure, synthesis method, mechanism of response to stimuli.

i. Based on structure:

Table 1 [46] represents various nanogels based on structure.

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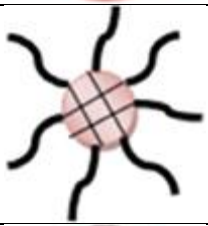
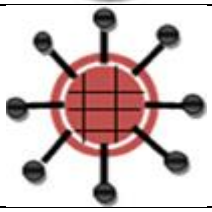
Table. 1 – Nanogels based on structure

S.No	Type	Schematic structure	Network structure	Example
1.	**Simple Nanogel**		a) Cross-linked b) Semi-interpenetrating polymer(semi-IPN) c) Self-assembled	a) Artificial chaperone, cholesterol-bearing pullulan (CHP) nanogel [47] b) Quantum dot nanogel [48] c) Artificial chaperone cholesterol enzymatically synthesized glycogen (CHSEG) nanogel [49]
2.	**Hollow nanogel**		Interpenetrating polymer	Stimuli sensitive/responsive nanogel [50]
3.	**Core—shell nanogels**		Cross-linked	Stimuli sensitive/responsive nanogel [51]
4.	**Hairy nanogels**		Cross-linked	Stimuli-responsive nanogel [52]
5.	**Multilayer nanogels**		Cross-linked	Stimuli sensitive/responsive nanogel [53]
6.	**Functionalized nanogels**		Cross-linked	Polyethyleneglycol-b-poly (methacrylic acid) [PEG-b-PMA] with PEG terminal aldehyde functionality [54]

ii. Based on method of synthesis:

Nanogels, being structures of several polymeric networks consisting of linked matrices which enable them to retain their structures and ensure optimal drug release, are classified as either physically or chemically cross-linked nanogels based on their method of synthesis.

Physically cross-linked nanogels

Physically cross-linked nanogels are supramolecular particles consisting of polymer molecules formed through non-covalent interactions – ionic, hydrophilic-hydrophobic balance, Van der Waals and hydrogen bonds, without crosslinking agents [55]. Size of nanogels can be customized for efficient drug delivery by modification of physical conditions during formulation [56].

Chemically cross-linked nanogels

Chemically cross-linked nanogels consist of polymers formed through covalent chemical interactions [57]. The starting materials are low-molecular weight monomers, polymer precursors or polymers with specific terminal or pendular reactive groups [56]. The linkages formed by chemical cross-linking methods are more stable than those formed by physical methods as a result of the stronger, irreversible bonds formed through covalent interactions [55].

Chemical crosslinking methods include "polymerization by emulsion, reversible addition-fragmentation chain transfer (RAFT), click chemistry crosslinking, and photo-induced crosslinking, precipitation polymerization, polymerization by inverse microemulsion (w/o)," [58].

Hybrid nanogels

Hybrid nanogels are defined as "composites of nanogel particles dispersed in organic or inorganic matrices" [59]. Composites are materials made up of two or more components intimately merged together to combine the advantages of each individual component. For instance, a hydrophilic component can be combined with a hydrophobic component to enable the loading of both hydrophilic and hydrophobic drugs into the hybrid nanogel. Some stimuli-responsive polymers can also be incorporated into hybrid nanogels to facilitate targeted drug delivery.

iii. Based on response to stimuli:

Nanogels can be classified as stimuli-responsive nanogels based on the stimuli they react to for drug release.

Nanogels could be designed to release drugs only under certain physical or physiological conditions in the body. These conditions may include specific temperatures, pH, magnetic fields, ultraviolet light, reduction reaction, oxidation-reaction, hypoxia conditions, among others. This feature of nanogels makes is possible for controlled and targeted drug delivery.

Stimulus response may initiate the interactions within the nanogel framework or cleavage of physical or chemical bonds formed during the design of the nanogel, and swelling of the nanogel matrix which subsequently leads to drug release. It may also lead to degradation of the nanogel materials in which the drug is encapsulated, leading to drug release.

Some nanogels can be developed to respond to one stimulus (single-responsive stimulus), two stimuli (dual-responsive stimuli), or three stimuli (triple-responsive stimulus) or multiple stimuli [60][61][21].

3. "SMART" STIMULI RESPONSIVE NANOGELS: APPLICATIONS IN BREAST CANCER THERAPY

In recent years, nanogels have gained much attraction as potential drug delivery systems to overcome limitations of available conventional cancer therapies, since they can easily pass through the cell membrane of solid tumors by enhanced permeation and retention (EPR) effect owing to the small size, hence improving targeting. The encapsulation of hydrophobic drugs in nanogels makes them readily soluble in aqueous solution, prolongs retention and improves bioavailability towards cancer cells.

Stimuli-responsive nanogels have the potential to conduct controlled and site-specific drug delivery with minimal adverse effects and reduced toxicity, unlink non-selective mechanism of conventional chemotherapy which targets both cancerous and non-cancerous cells. They are also known as "smart" or intelligent nanogels. Chemical triggers like pH, enzymes, and ionic change, and physical triggers like temperature, pressure, and magnetic field, alter the nanogel's swelling behavior or lead to the breakdown of its polymeric network. This affects drug release from the nanogel (Fig. 3) [62].

Hormone therapies are efficacious for cancers associated with hormones however, some hormone therapies have been associated with increased risk factors for diabetes mellitus [63] and blood clots [64]. Such risk factors are not associated with nanogels formulated for targeted drug delivery in such cancer types.

Cancer immuno-therapy is an improvement over many conventional cancer therapies, however, there are several physical barriers and metabolic factors limiting optimal cancer immune-therapy which are not applicable in the use of nanogels. In addition, cancer immuno-therapy could increase toxicity to non-cancerous cells. Tang et al. (2018) succeeded in developing protein nanogels to deliver optimum quantities of chimeric antigen receptor (CAR) T-cells for immunotherapy. The nanogels were designed to respond to T-cell receptor (TCR) activation by releasing optimum quantities of CAR T-cells into the tumor microenvironment. The release of proteins was modulated to ensure the significant release of drug cargo which increased efficacy without increasing toxicity. Their research results reveal the potential use of nanogels in T-cell immunotherapy. [65]

Angiogenesis inhibitors are efficient to some extent in arresting the growth of certain tumor which require production of new blood vessels. However, they may not be efficient if the cancer utilizes existing blood supply [66]. The efficient tumor-targeting ability of nanogels overcomes this limitation of angiogenesis inhibitors. Su et al. (2013) synthesized thermo- and pH-responsive poly (Nisopropyl acrylamide co-acrylic acid) nanogel for tumor targeting. [67]

Radiation therapy also shares the same limitation with most conventional cancer therapies which is non-selectivity and subsequent cell toxicity [68].

In addition to reducing cell toxicity by ensuring targeted drug delivery, nanogels can be formulated to combine the benefits of two or more conventional cancer therapies. Novel research by Liu et al. (2018) reported a biodegradable and pH-sensitive nanogel system as a drug nano-carrier for combinational chemotherapy and radiotherapy. This stable and uniform nanogel was fabricated through self-assembly of carboxymethyl cellulose and bovine serum albumin. The successful loading of the radionuclide, ^{131}I and camptothecin into a hybrid nanogel showed a high drug loading pH-controlled drug release profile capacity of 16.72 wt% with excellent biocompatibility and low hemolysis. This formulation enhanced drug accumulation at the tumor site, improved cell uptake and prolonged circulation in the blood [69]. Tyler et al. (2010) proved that OncoGel containing 6.3 mg/ml paclitaxel in 18 Fischer-344 rats bearing glioma was safe for intracranial injection but most effective when administered with radiation therapy [70].

Nanogels have been developed for the purpose of cancer therapy through integrating the following drugs: cisplatin, 5-flurouracil, heparin, doxorubicin, temozolamide and so on. For management of prostate, breast, lung and liver cancer, chitin-polymerized doxorubicin nanogels have been successfully utilized. Cisplatin loaded nanogels (thermo- and pH-responsive nanogels) were developed for the treatment of breast cancer.

In the following, a few examples of nanogel applications in breast cancer are presented with respect to their mechanism of response to stimuli:

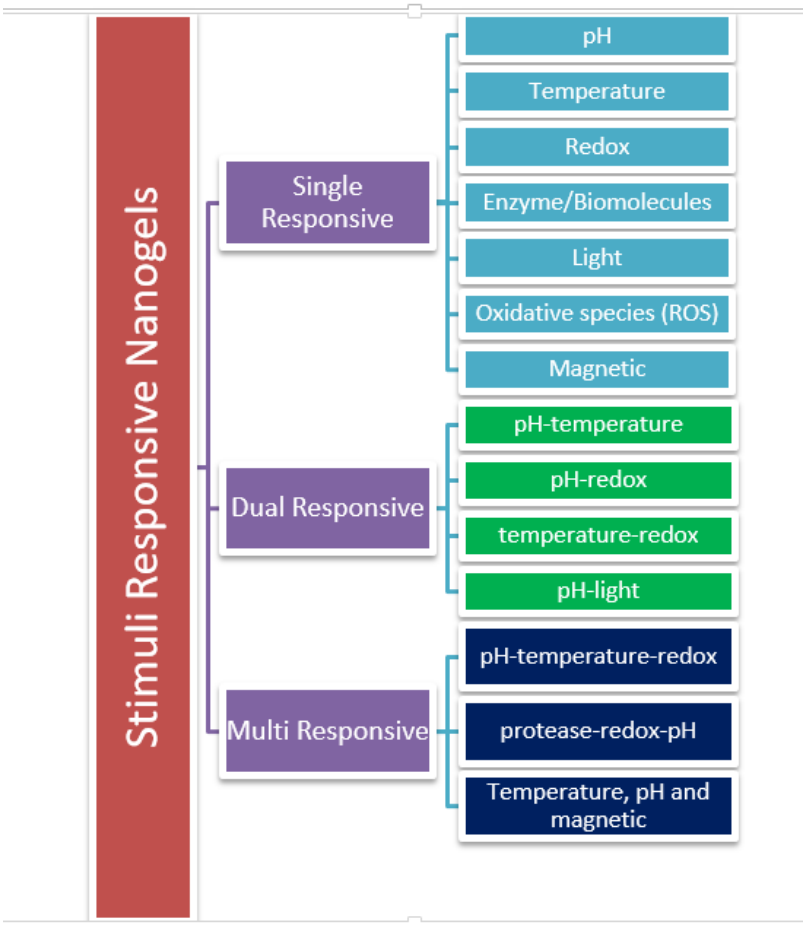


Fig. 3 – Types of Stimuli-Responsive Nanogels

Single stimuli-responsive nanogels

i. pH sensitive nanogels

The pH of the human body has a wide distribution. The normal physiological pH is 7.4 (blood, and directly in contact areas), in the stomach it is around 1–2.5, and in the small intestine and colon it is around 7.2–7.5 and 7.9–8.5, respectively. Furthermore, all eukaryotic cells during the endocytosis process build special vesicle-like endosomes and lysosomes. It has been proven that these vesicles have lower pH than the physiological pH (i.e., 5–6.5 and 4 in endosomes and lysosomes, respectively) [71]. The extracellular environment of tumor tissues is usually more acidic (pH 5–6.8) compared to normal tissues due to the accumulation of acidic compounds such as lactic acid and some acidic metabolites [72]. It has been reported that cancer cells consume 40 times higher amounts of glucose compared to normal cells. Therefore, lactic acid can be produced and accumulated at higher rates as a result of anaerobic glycolysis of glucose due to the hypoxic conditions of the tumor tissues.

In recent years, pH-sensitive polymers have been widely used for the synthesis of stimuli responsive nanogels for cancer therapy. pH-sensitive polymers usually contain pH-sensitive cleavable bonds or basic and acidic functional groups.

When these polymers are utilized for the synthesis of NGs, the resulting product can respond to the pH change of the environment through different mechanisms, including pH-cleavable bonds, ionic interactions, and change in the swelling degree of the network.

For the synthesis of a pH-responsive nanogel network, pH-cleavable bonds such as acetal and hydrazone can be improvised in the network. This action can be carried out by using a linker or crosslinker containing such bonds. Therefore, the final structure can respond to a drop in pH environment by losing/degrading its primary structural network. Furthermore, the desired anticancer drug molecule can be attached to the nanogel network using pH-sensitive linker so the drug can be released as a response to the pH change of the environment.

Another mechanism that is utilized for the synthesis of pH-responsive NGs is change in the swelling degree of the nanogel network by use of ionic interaction as the crosslinking method. When a polymeric precursor such as alginate and chitosan contain acidic (pendant carboxyl, imine) or basic (amine) functional groups respectively, it can be crosslinked using ionic interactions. The product would be sensitive to the environment pH since it contains functional groups that undergo protonation/deprotonation process as a result of pH change. Therefore, when a nanogel synthesized from acidic polymeric precursors is subjected to an appropriate acidic pH, the acidic functional groups would be protonated and the ionic interaction between the polymeric chains and the ionic crosslinker is lost, osmotic pressure is produced in the gel due to the electrostatic repulsion forces between ionized functional groups resulting in an expansion, which affects the swelling degree of the nanogel. The swelling and the collapsing of the polymer chains will trigger the release of the therapeutic encapsulants [73].

The pH-responsive behavior of nanogels may be utilized not only for drug release but also for drug loading [74].

Applications:

- Kang et al. demonstrated a pH-responsive NGS from 4-carboxyphenyl boronic acid-conjugated lactose modified CS, dopamine-nitric oxide (NO) linked, partially carbonized hyaluronic acid (HA) for dual delivery of Doxorubicin (Dox) and NO. The release of Dox from the NG was evaluated under different pH conditions and found that Dox was released rapidly to tumor cells by the synergy of pH and HAase. The Dox and NO were released from the NG as a consequence of pH labile cyclic boronate ester, were also vulnerable towards enzymatic activities. Finally, the Dox release kinetics was confirmed by adsorption properties as well as guided-imaging, the NO release was confirmed via DAF-2 diacetate assay, and biocompatibility of the NG was assessed on KB, breast cancer (MDA-MB-68 cells), and kidney (MDCK cells) [75].

- Zhang et al. reported an adenosine-5′-triphosphate as well as pH-responsive dendritic polyglycerol (DPG) based NGs based on the reaction of 1,2-diols in DPG, boronic acids, which are conjugated with DPG, and the NG was employed for the delivery of Methotrexate (MTX). The drug-loaded NG internalized to the tumor cells leads to the incomplete release of MTX in the mildly acidic environment and an enhanced release occurred in cytoplasm. Conclusively, the cytotoxicity and the anti-cancer activity of MTX loaded NG was assessed in HeLa and MCF-7 cells showed the blank NG have no cytotoxic effects up to a wide range of concentrations. Notably, the MTX loaded NG showed equal or greater cytotoxicity than free MTX for both cell lines [76].

- Ding et al. developed a pH-responsive E3/K3 peptide cross-linked HA-NGs was reported for intracellular protein (saporin) delivery to MCF-7 cells. Herein, saporin-loaded HA-NGs were efficiently internalized by MCF-7 cells, and the HA-NGs facilitated the proteins to leak out from endosomes, through fusion of uncoiled E3/K3 peptides with endosomal membranes. These HA-NGs exhibited a high antitumor efficacy against MCF-7 cells in nanomolar concentrations [77].

- pH-sensitive hybrid nanogel particles were formulated via an oil-in-water emulsion process using fluorescently doped nanoparticles of silica encapsulated inside a positively charged poly (2-diethylamino ethyl methacrylate) hydrogel. siRNA loaded into the nanoformulation displayed acidic pH-triggered release. The formulation and the genetic materials were able to avoid sequestration by endosomes and degradation by enzymes. The nanogel was found to decrease the expression of the membrane receptor protein CXCR4 in MDA-MB-231 or human breast cancer cell lines. The formulation was preferentially accumulated in the tumor tissue and thus a potent chemotherapeutic effect was obtained [78].

- Li et al. developed pH-responsive nanogels containing benzimine bonds for the delivery of hydrophobic antitumor drugs. The pH-responsive nanogel system was prepared by crosslinking of a polypeptide-based copolymer of poly(ethyleneglycol)-b-poly[N-[N-(2-aminoethyl)-2-aminoethyl]- L-glutamate] (MPEG-b-PNLG) using terepthalaldehyde (TPA) as a crosslinker. It displayed an excellent DOX loading capacity, high stability, and specific pH-controlled drug release properties. The pH-sensitive benzoic imine bond can be degraded under acidic tumor conditions, which leads to the destruction of the nanogel and rapid drug release. Experimental results proved that in MDA-MB-231 human carcinoma cancer cells, the cytotoxicity of DOX loaded in nanogels is higher than that of free DOX. This indicates that the polypeptide-based nanogel has great potential for antitumor drug delivery [79].

Although a large number of studies have shown that pH-sensitive HA nanogels can be used as excellent drug carriers and have certain targeted drug delivery effects, most of the current research is still in the experimental stage in vitro and lacks in vivo research. In addition, the development and utilization of HA as a drug carrier is still limited in industrial applications.

ii. Temperature-responsive nanogels

Temperature can be utilized as an external stimulus for manipulation of stimuli responsive nanogels, for intelligent drug delivery to inflamed areas and tumor tissue when they have elevated temperatures between 40°C and 45°C.

Temperature-responsive nanogels show shrinking-swelling behavior triggered by environmental temperature. This facilitates controlled drug release from the smart nanogel [55]. For this, temperature-responsive polymers can be used in their synthesis. Solubility of some polymers decreases upon heating as a result of alteration of their hydrophobic/hydrophilic ratio. Such polymers are characterized by a lower critical solution temperature (LCST), above which the polymer becomes insoluble and the formulated nanogel collapses/shrinks, whereas below LCST the nanogel swells. On the other hand, the solubility of some other polymers increases upon heatin. The corresponding parameter for these polymers is upper critical solution temperature (UCST), below which the polymer becomes insoluble and the nanogel formed collapses, whereas above UCST, swelling of the nanogel occurs. At a specific temperature point, called the volume phase transition temperature (VPTT), the physical characteristics of temperature-responsive nanogels in a given solvent show a sudden change in their solvation state. This temperature point is correlated to the LCST and UCST of the polymeric precursors used for the preparation of the nanogel network.

To obtain temperature-responsive polymers, it is necessary for the polymeric structure to contain both hydrophobic and hydrophilic domains. At temperatures below VPTT, the polar-polar interactions between the hydrophilic domain of the polymeric structure and water molecules dominate the nonpolar-nonpolar interaction of the polymeric hydrophobic domains with themselves in the nanogel network. The degree of swelling of the nanogel is enhanced by improving the solvation ratio of the polymeric chains. However, at above VPTT, the nonpolar-nonpolar interactions dominate and the water molecules are ejected to the solution phase, leaving behind the deswelled polymeric network [80][81].

Poly(N-isopropyl acrylamide) PNIPAM is a good example of an LCST polymer that has been widely utilized for the preparation of temperature-responsive nanogels for biomedical applications, with an LCST around 32°C [22]. Below LCST, the polymer is hydrophilic, while above LCST the polymer aggregates and starts showing hydrophobic characteristics. The system is thus found to shrink above LCST. It forms strong hydrogen bonds through its amide functional groups with water molecules below the LCST, it swells leading to the dissolution of the polymeric network. Above LCST, the hydrogen bonds between the polymeric chain and water molecules are broken, resulting in the deswelling of the polymeric network. Therefore, the polymeric structure collapses due to the water molecules expelled from the network leaving behind the insoluble polymeric chains.

The distinct hyperpyrexia (increased body temperature) locally in malignant tumors is always used as a bio-signal to induce the changes of thermosensitive nanogels. The external temperature stimulus should be generated using well-localized heating sources for selective targeting the desired tissue to prevent severe damage of neighbouring healthy tissues. Although some traditional heating sources, which utilize radio frequency, microwave, or ultrasound, can be used for this aim, the application of these sources is limited to superficial applications due to poor penetration of these waves inside thick tissues [82]. Fortunately, there has been some progress to generate well-localized temperature at the desired tissues. Most of these progresses are based on utilization of near-infrared (NIR) and alternating magnetic fields in combination with transducer moieties. The transducer enables the conversion of the energy input to heat at the targeted site [83].

In recent years, NIR radiation has found a wide range of application in cancer therapy. NIR waves can be effectively localized on the desired tissue deep under the skin; therefore, causing minimum damage to the other tissues in its way [84]. It can be directly focused on the tumor site to cause heat-destruction of the tumor cells or it can be used as a stimulus to trigger temperature-responsive NGs in the tumor site. In the latter case, it is necessary to implant a moiety as a transducer in the nanogel network that is sensitive to NIR light and can absorb and convert the light energy to heat (photothermal therapy, PTT) or can produce ROS (photodynamic therapy, PDT) [85-86]. Such functionalities can be produced by trapping the transducer in the nanogel network, core-shell implanting the transducer, transducer formation inside the pre-obtained nanogel, and using NIR light-sensitive polymeric precursors [87-88]. Various transducers can be utilized for this purpose including chromophores such as organic dyes, metallic nanomaterials such as gold, silver, and other plasmonic nanoparticles, carbonous nanomaterials, and conductive polymers [87][89].

Applications:

- Shirakura et al. developed thermo-sensitive nanogels using the reverse micelle polymerization technique based on the combination of acrylic acid and acrylamide for controlled delivery of cisplatin (CP). The temperature-dependent release of CP was evaluated at different temperatures and it was found that percentage of CP release was highest at 42 °C after 48 h of release experiments. It was interesting that with the combination of acrylamide and acrylic acid a UCST like system could be constructed based on the cleavage of hydrogen bonds between them at elevated temperature, causing swelling of the nanogel matrix rather than de-swelling. These CP-nanogels were uptaken by breast cancer MDA-MB-435 cells, via endocytosis, and then mostly localized in the lysosomes. In vitro cytotoxicity experiments confirmed that CP-nanogels had a remarkably greater efficacy at slightly higher temperatures [90].

- 5-fluorouracil and megestrol acetate were loaded into a fibrinogen-graft-poly (N-vinyl caprolactam) nanogel to obtain a biocompatible, temperature-sensitive formulation. The formulation was directed towards specific receptors present on breast cancer cells. The drug release was found to be significant above LCST rather than below it. The formulation displayed improved toxicity, apoptosis, and cellular uptake when studied using the MCF-7 cell line. The drug combination was released in a sustained manner in vivo [91].

iii. Redox-responsive nanogels

The "redox" stimulus originates from the electrochemical response of a specific redox-responsive functional group, which undergoes a difference in its oxidation state. The common examples which respond towards an electric impulse are disulfides (SS), dithienylethenes, ferrocene, diselenide, ditelluride etc [92][93].

Reduced glutathione (GSH) is maintained to regulate cells, which include cell differentiation, proliferation and apoptosis. The concentration level of glutathione tripeptide (GSH) is about 2-3 orders higher (approximately 2-10 mM) in the cytosol and nuclei than in the extracellular fluids (approximately 2-20 μM) [94][95]. Furthermore, the GSH concentration in some tumor tissues is about 4-fold higher than that in normal tissues.

GSH were able to reduce SS bonds or other reducible moieties by serving as an electron donor. It was also reported that a substantial amount of γ-interferon-inducible lysosomal thiol reductase (GILT) is present in endosomes and lysosomes, which could cleave disulfide bonds at low pH [96]. This significant difference in GSH levels has led to the design of redox-responsive nanogels, which respond to the higher GSH concentration in cancer tissues, and containing disulfide bonds which can be disassembled in the cytosol and nuclei of cells.

The disulfide bonds can be introduced either into crosslinkers (polymer chains), using disulfide containing molecules (e.g. lipoic acid and cystamine) or thiol-disulfide exchange reactions [97-98]. When the anticancer drug molecules are attached to the polymeric backbone of nanogel network through disulfide bonds, under the reductive environment of the targeted cancer tissue, the disulphide bonds and thus the stable cross-links were easily reduced/cleaved inside the cells. These bioreduction responses destabilizes the drug encapsulated NGs, which causes the sustained release of encapsulated therapeutic drug, in order to minimize damage to healthy surrounding cells [99].

Three main advantages of these nanogels are low biological toxicity due to their relative stability in healthy tissues, immediate drug release in tumor cells following destabilizationin the presence of high GSH concentration, and a relatively higher release into the cytoplasm resulting in maximum therapeutic efficacy [100].

Applications:

- Zhong et al. developed reducible nanogels with a hydrodynamic size of 152-219 nm via self-assembly of HA-lysine-lipoic acid conjugates followed by DTT-catalyzed self-crosslinking. These reducible HA nanogels mediated active targeting delivery and fast release of DOX to CD44-positive breast cancers in vivo, effectively overcoming drug resistance (ADR) and prolonging mice survival rate [101]. To further improve drug loading capability and reduce nanogel sizes, lipoic acid was linked to HA via poly(γ-benzyl-L-glutamate) (PBLG) [102]. These nanogels exhibited a high DOX loading ability of 25.8 wt.% and small size of 72–80 nm due to presence of π-π interactions between PBLG and DOX. These compacted HA nanogels showed little drug leakage under physiological conditions while quickly releasing 92% DOX in 30 h in a cytosol-mimicking reductive environment. The in vivo studies showed a superb tolerated dose of over 100 mg DOX equiv./kg by the injection of DOX-loaded nanogels with an extraordinary breast tumor accumulation of 8.6%ID/g in mice, exerting effective tumor growth inhibition in MCF-7 human breast tumor-bearing nude mice. These tumor-targeted multifunctional HA nanogels are derived from natural compounds and easy to prepare, rendering them highly appealing for clinical translation.

- Zhang et al. designed CXCR4 chemokine targeted reducible dextrin nanogels via self-crosslinking of thiolated dextrin followed by coating with AMD3100, a CXCR4 antagonist. These multifunctional dextrin nanogels exhibited a high anti-metastatic effect by inhibiting CXCR4-mediated invasion of 4T1 and U2OS cells [80].

- Zhu et al. developed reducible and fluorescent HA-iodixanol nanogels (HAI-NGs) from HA-cystamine-tetrazole (HA-Cys-Tet) conjugate and polyiodixanol-methacrylate (SS-PI-MA) by combining nanoprecipitation and photo-click crosslinking reaction. HAI-NGs exhibited significantly enhanced X-ray computed tomography (CT) imaging of MCF-7 breast tumors in nude mice following either intratumoral or intravenous injection as compared to free iodixanol control and targeted chemotherapy of MCF-7 human breast tumors [81].

- HA nanogels from HA-cystamine-methacrylate (HA-Cys-MA) and HA-lysine-tetrazole (HA-LysTet) by combining inverse nanoprecipitation and photo-click chemistry. These photo-click HA nanogels exhibited a high loading of CC and granzyme B (GrB), and could effectively target and release proteins to CD44 positive MCF-7 and A549 cancer cells. GrB-loaded HA nanogels at a low dose of 3.8–5.7 nmol GrB equiv./kg completely suppressed growth of subcutaneous MCF-7 human breast tumor and orthotopic A549 human lung tumor xenografts with minimal adverse effects [103].

- Reducible and fluorescent photo-click HA nanogels were also prepared from hyaluronic acid-graft-oligo(ethylene glycol)-tetrazole (HA-OEG-Tet) using L-cystine dimethacrylamide (MA-Cys-MA) as a crosslinker, which were used to achieve targeted protein therapy of MCF-7 breast tumor in mice with significant tumor growth inhibition at dosages of 80 and 160 nmol CC equiv./kg [104].

- CD44 and EGFR dual-targeted functional nanogels were prepared by incorporating GE11 peptide (YHWYGYTPQNVI)-functionalized HA. These dual-targeted reducible nanogels showed significantly increased uptake by CD44 and EGFR-positive SKOV-3 ovarian cancer cells compared with CD44 single-targeted nanogels. GrB-loaded nanogels induced nearly complete growth suppression of both SKOV-3 human ovarian carcinoma and MDA-MB-231 human breast tumor in mice at a low dose of 3.85 nmol GrB equiv/kg, elucidating that dual targeting approach is potentially interesting in targeted tumor therapy [105].

- Hong et al. fabricated reduction-sensitive siRNA/PEI complex nanogels from thiol-terminated siRNA and thiolated linear PEI (LPEI) followed by oxidation for targeted gene silencing. These siRNA/LPEI complex nanogels were highly stable, and exhibited higher cellular uptake by MDA-MB-435-GFP cells and more efficient gene silencing than siRNA/LPEI physical complexes [106].

- Li prepared gene concentrated bio-reducible nanogels with locally enriched positive charge but low cytotoxicity for intracellular Bcl2 siRNA delivery towards breast cancer treatment. A self-crosslinked reducible siRNA-nanogel complex was formed by reacting thiolated PEI of 1.8 kDa and thiolated dextrin using the suspension method, followed by siRNA loading. The intracellular GSH-triggered siRNA release strategy exhibited the same level of deregulation of Bcl2 protein expression compared with the use of cationic PEI of 25 kDa in vitro, while the cytotoxicity was decreased and almost no hemotoxicity was found. Bcl2 siRNA-loaded nanogels significantly inhibited tumor growth in 4T1-luc tumor bearing BALB/C mice, in which the tumor volume was about 24% of the average tumor volume found in the saline group [107].

- For the management of metastatic breast carcinoma, a bioreducible and Dox loaded dextrin NG was established. Herein, the NG was decorated with AMD3100 to perform CXCR4 directed chemokine and suppression of cancer metastasis through bioreduction-triggered delivery of Dox in the cellular level. The NG displayed a high DLE of 13.45%, and toxicity of Dox encapsulated NG was assessed on 4T1 cancer cells. Dox loaded NG showed substantial growth inhibition to 4T1 cells, and also in animal model compared to free Dox [80].

- A glycopolymer based redox responsive fluorescence active NGs was developed by Bhattacharya and co-workers, through RAFT polymerization followed by modified by gelatin quantum dot (QD) to impart fluorescence property. Dox was introduced into the NG system followed by cross-linking with redox responsive cross-linker through "click" reaction made the NG redox responsive results in the cleavage under high GSH to release the Dox in tumor cells. The viability and anticancer action of the Dox-NG was confirmed with MBA-MD-231 cells through fluorescence spectroscopy and flow cytometry [108].

- A poly-α,β-polyasparthydrazide based NGs (average size around 200 nm) was prepared by the crossing-linking reaction with 3,3′-dithiodipropionic acid with redox-responsive SS bond for the intracellular delivery of 5-Fluorouridine. Herein, the NGs showed 90% of DLE and 20% of DLC, and in GSH milieu, gradual cleaving SS crossing-linking induced for sustained drug release. The blank NG confirmed negligible cytotoxicity, however cell viability drastically decreased once the 5-Fluorouridine NG treated with B16F10 and MCF-7 cells [109].

iv. Enzyme-responsive nanogels

Enzymes are known to be not only efficient but also highly specific catalysts for chemical transformations under aqueous conditions. It has been reported that the concentration levels of certain enzymes are significantly higher in cancer cells compared to the corresponding normal cells. Several enzymes that are sensitive to proteins, sugars, and phosphorylation, such as cathepsins, plasmin, urokinase-type plasminogen activator, b-glucuronidase, and kinases, are overexpressed in different cancer types.

The overexpression of a certain enzyme can be utilized as a stimulus for triggering enzyme-responsive NGs [110-111]. It is interesting to note that enzymes in the tumor environment can not only trigger drug release but also improve tumor penetration and cellular uptake.

The utilization of enzymes as external stimuli provides some certain advantages over other stimuli such as pH or temperature since, in contrast to other stimuli, the chemical or physical changes caused by enzymes are usually irreversible and are done with high selectively.

The substrate (enzyme-sensitive moieties) for the enzyme of interest can be the crosslinker itself, a linker in the polymeric structure, or a linker for the attachment of the anticancer drug to the nanogel network. When such substrates are exposed to an overexpressed enzyme, certain bonds in the enzyme-sensitive moieties can either be cleaved (nanogel degradation) or formed (nanogel formation) leading to a chemical or physical change in the nanogel network, causing nanogel degradation for triggering the release of the encapsulated drugs. Other physical or chemical changes in the nanogel network under the enzyme action can occur called as nanogel morphology change that include change in the hydrophilicity/hydrophobicity, steric effects, ionization or neutralization of different functional groups, and a change in the polymeric chain length [71].

Hyaluronic acid (HA) is a natural material with intrinsic targetability towards CD44-overexpressed tumor cells. The degradation of HA can be triggered by extracellular overexpressed hyaluronidases (HAase) in tumors, resulting in enhanced DOX accumulation at the tumor site and significant tumor inhibition.

Applications:

- Jiang et al. developed a programmed drug-delivery nanogel-liposome system composed of a liposomal core and a crosslinked HA shell for the sequential and site-specific delivery of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and DOX. The rapid degradation of the HA shell by HAase in the tumor environment induced fast extracellular release of TRAIL and subsequent internalization of the liposomes. The IC50 of TRAIL and DOX co-loaded system was 83 ng mL–1 toward MDA-MB-231human breast cancer cells, which was 5.9-fold higher than that of the DOX only (single drug) system [112].

v. Light Responsive Nanogels

Light irradiation can be easily achieved by the exposure of a sample to light of a specific wavelength. Use of light as a stimuli-trigger offers an "on-off " feature to control over the exposure time towards a particular system. The most common examples for materials which shows light-responsive properties are azobenzene, spiropyran (spiro- to merocyanine isomerization form), spirooxazine and fulgide (photochromic behaviour) and its derivatives.

Several light responsive NGs for therapeutic applications are reported, and the light-dependent release was generally due to the hydrophobic to hydrophilic transformation of the NGs as a result of light induced isomerization/photodegradation of the corresponding light-responsive species.

Applications:

- HA based NIR and UV-responsive degradable NGs (HA-CM NG) (DH of 147-165 nm) for CD44-targeted Dox delivery was developed. The Dox delivery was explored upon UV or NIR irradiation remarkably increased the delivery kinetics due to photo activated destruction of the NG. The intracellular release of Dox in receptor over-expressing cells were performed with NIR and without irradiation. Notably, antitumor effect of Dox-NG with light activation resulted enhanced growth inhibition towards MCF-7 cells than without irradiation [113].

- Thayumanavan group has developed a NGs based on an amphiphilic random copolymer through RAFT polymerization and subsequently conjugated an NIR probe cyanine 7 dye (Cy7). The NIR-labelled NGs were demonstrated for in vivo fluorescence molecular tomography (FMT) imaging (700−900 nm) in mammary carcinoma models. Herein, the distribution was found through FMT imaging at various time intervals postinjection, and the retention of fractions of total administrated dose in various major organs were 43 obtained, by using the model of triple negative MDA-MB-231-luc-D3H2LN cells. The ex vivo studies of distinct tissues from the necropsy after 3-day post injection presented a correlation with the FMT study [114].

vi. ROS-responsive nanogels

Reactive oxygen species (ROS) have been closely related to important pathophysiological events including atherosclerosis, aging, and cancer. A moderate level of ROS is involved with normal cell functions, but excessive amounts of ROS cause oxidative stress and damage of critical components of cells at all levels including DNA, proteins, and lipids. Cancer cells are reported to overproduce ROS and are thus under increased oxidative stress. This phenomenon can be used as one of the promising intracellular stimuli for selective delivery of drugs to diseased sites by targeting oxidative microenvironments at different levels [115].

Applications:

- Dong et al. prepared a ROS-responsive poly (*N*-isopropylacrylamide-co-Cinnamaldehyde-co-D-α-tocopheryl polyethylene glycol 1000 succinate, P_{ss}NCT) nanogels, which employed two exogenous ROS inducers, cinnamaldehyde (CA) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), to selectively induce apoptosis by regulating ROS levels in tumor cells. Mean fluorescence intensity values (MFI) were less than 20 in L929 cells, but more than 30 in MCF-7 cells. After incubated by different preparations, continuous elevation of ROS signal appeared in MCF-7 cells, and leading to significant cytotoxicity. Moreover, due to the combined application of CA and TPGS, P_{ss}NCT nanogels exhibited the strongest ROS inducing ability, which was correlated well with the synergistic function of CA and TPGS in elevating ROS level [116].

Dual and multi-responsive nanogels

In addition to single stimuli-responsive nanogels, advanced nanogels of dual and multi-responsivity have been synthesized [117-119]. They can be designed and synthesized in special ways so they can respond to two or more stimuli, leading to an enhanced responsivity, site-selectivity and efficacy in the releasement of the encapsulated/loaded anticancer drug. Multi-responsive nanogels may respond not only to dual stimuli but also to other stimuli like glucose concentration, redox conditions, light, pressure, etc. Greater control over drug delivery can be obtained with these nanogels leading to an enhancement in the therapeutic efficacy of the formulation. Among various dual and multi-responsive investigated systems, pH-temperature responsive, pH-redox responsive, temperature-redox responsive, pH-light responsive, pH-temperature-redox responsive, and protease-redox-pH responsive systems are more abundant [71].

Applications:

- Chen et al. developed “epidermal growth factor receptor (EGFR) and CD44 dual-targeted hyaluronic acid nanogels (EGFR/CD44-NGs) that enhanced targeted delivery of protein therapy for metastatic 4T1 breast cancer in vivo.” Analysis of the evaluation revealed there was over 6-fold higher cellular uptake of the protein from the dual-targeted nanogels when compared with the monotargeted nanogels [120].

- Wang et al. developed redox-sensitive nanogels based on dextran grafted PAA with disulfide-containing junctions (Dex-SS-PAA) through a one-step self-assembly assisted methodology (SAA). DOX was conjugated onto nanogels via an acid-labile hydrazone bond. The release of DOX exhibited pH and redox dual-responsivity, significantly inhibiting the growth of MDA-MB-231 tumors [121].

- Wu et al. designed and prepared FA-decorated pH and reduction dual-responsive nanogels from reflux-precipitation copolymerization of AA and CBA and folate PEG conjugation via carbodiimide chemistry as a co-delivery system for DOX and

cisplatin (CP) to overcome drug resistance. DOX and CP-loaded dual-responsive nanogels could introduce more drugs into MCF-7/ADR cells than free drugs, exhibiting a superior cell-killing activity. The in vivo test revealed that the combination therapy was effective for the multidrug-resistant MCF-7/ADR tumor with reduced side effects [122].

- Hu et al. designed core-shell nanogels (CS-NGs) based on a core of emulsion polymerized acrylamide (AAm) and N-(3-aminopropyl) methacrylamide (APMAAm) with a pH-degradable glycerol dimethacrylate (GDA) crosslinker, and a shell of UV-crosslinked acrylated HA and N,N'-methylene-bisacrylamide (MBA) assembled by the carbodiimide crosslinking reaction. These CS-NGs were responsive to overexpressed hyaluronidase (HAase) and acidic pH in the tumor microenvironment for co-delivery of tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) and antiangiogenic cilengitide. After intravenous injection into MDA-MB-231 tumor-bearing nude mice, CS-NGs were accumulating in the tumor tissues, where the overexpressed HAase degraded the HA matrix, and previously loaded transglutaminase (TG) was released from the nanogel shells, catalyzing the formation of micro-sized "drug delivery depots". The cellular uptake of the oversized depots was restrained, which facilitated the interaction of plasma membranes of the cells with TRAIL/cilengitide released from the depots due to the degradation of nanogel core in the acidic tumor microenvironment [123].

- A near infra-red (NIR) PS, cyanine dye Br2-IR808 incorporated polypeptide derived reduction-responsive NGs were reported by Jing and co-workers. The NG showed size in between 100-250 nm with a DLC and DLE of 3.94% and 15.6% was observed for the cyanine dye. Dox was also entrapped into the NG, and release confirmed that, under reduction-insensitive conditions, Dox release was only 27%, while in the presence higher GSH concentration, while the release was 50% for the same time period. In summary, the cytotoxicity of the dye-loaded NG was negligible in the non-irradiated condition, however, Br2-IR808-loaded NG we exhibited a substantial decrease of cell viability in irradiated environments equated to free cyanine in the same concentrations for HepG2 and MCF-7 cells [124].

- A biocompatible, pH and temperature responsive core–shell NG based on PDEAEMA and PVCL were developed from various dextran methacrylates as cross-linking macro molecules. The reversible swelling characteristics of the NGs was studied and was capable of encapsulating Dox effectively due to the hydrogen bond interactions among Dox and the polymer chains. Herein, drug was loaded with a DLC 40 %, and Dox diffusion was faster at pH 5.2 and 37 ^{o}C. In conclusion, the cytotoxicity assays were confirmed that, blank NGs were cytocompatible, however, Dox-loaded NG was significantly suppressed the proliferation of HeLa and MDA-MB231 cells [125].

- A pH, temperature reactive NGs composed of NIPAM, itaconic acid and 2-Acrylamido2- methylpropane sulphonic acid was synthesized by random copolymerization and subsequent cross-linking with EGDMA for releasing Dox in MCF-7 cells. Herein, the swelling and the NG phase change at physiological temperature and pH 5.4 leads to controlled Dox release kinetics. The cytotoxic characteristics was assessed in multiple cells lines and found that NG displayed improved cytotoxicity than free Dox in MCF-7cells [126].

- Matusiak et al. synthesized a new pH-redox-photo multi-responsive nanogel for the delivery of the antitumor drug DOX. The multi-responsive nanogels were obtained by crosslinking poly(acrylic acid-spiropyran methyl methacrylate) with N,N-bis(acryloyl)cystamine containing disulfide bonds as a crosslinking agent. There is electrostatic interaction between the antitumor drugs and the acrylic acid in the nanogel, so they could be effectively encapsulated in the nanogel. The cleavage of the acid-cleavable bonds at low pH caused the destruction of the nanogel, resulting in an increase in the release rate. In addition, the protonation of the hydrophobic spiropyran in the acidic environment of the tumor enhanced the hydrophilicity of the nanogel, causing it to swell and release the drug. Compared to free DOX, the DOX-loaded nanogels induced decreased viability of MCF-7 cells. The DOX-loaded nanogels after UV irradiation exhibited higher cytotoxicity against MCF-7 cells than those without UV irradiation [127].

- Temperature, pH and magnetic-triple responsive NG composed of PNIPAM, poly(2-dimethylaminoethyl) methacrylate SA, and MGO were developed through radical precipitation polymerization for Dox delivery. Herein, the Dox release rates were found to be accelerated in acidic pH and higher temperature as well as in the present magnetic field. The cytotoxicity of the NG was assessed on MCF-7 cells, confirmed noncytotoxic nature of blank NG. However, Dox-loaded NG had a negligible impact towards viability of MCF-7 cells in comparison of free Dox [128].

4. CLINICAL STATUS OF CHOLESTERYL PULLULAN (CHP) NANOGELS IN BREAST CANCER

Cancer vaccines based on cationic CHP (Cholesteryl pullulan) nanogels are the most promising applications of nanogels in cancer therapy and have been in clinical trials since the last two decades with promising results (Fig. 4).

CHP serves as a vehicle to protect and transport of antigen to antigen-presenting cells of the immune system. The commonly encapsulated antigens for cancer vaccines using CHP nanogels are Human epidermal growth factor receptor type 2 (HER2), New

York esophageal squamous cell carcinoma 1 (NY-ESO-1) in esophageal cancer and malignant melanoma treatment, and melanoma related antigen A (MAGE-A4) [129-132]. Data from several clinical studies performed using the cholesterol-pullulan NGs based anticancer vaccines displayed safety of the systems even after frequent administrations as well as to prove the accomplishment to inducing both antigen-specific response with humoral immunity.

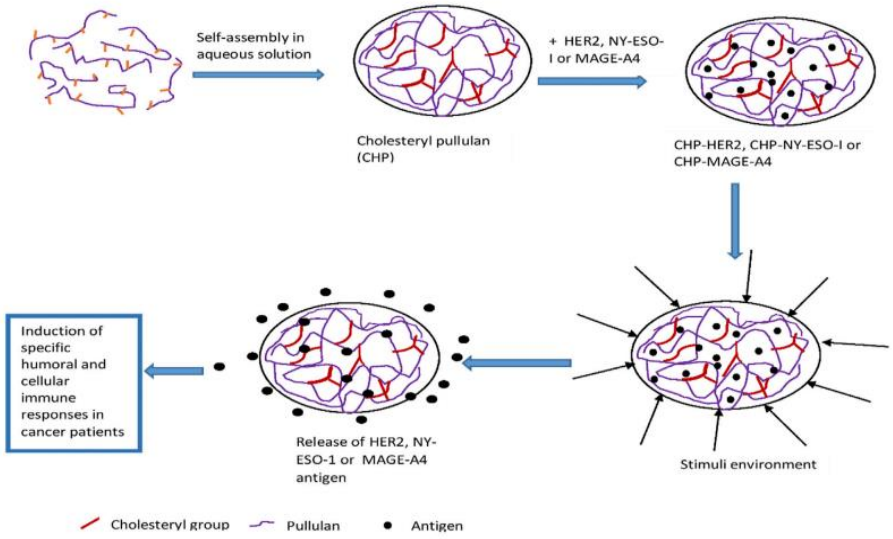


Fig. 4 – Mechanism of antigen release from CHP nanogel based vaccine.

Clinical trials on CHP-HER2 vaccine

HER2 is a growth-promoting protein on breast cells. HER2 protein overexpression has been found in several tumors including breast, esophageal, lung, cervical, bladder, pancreatic, ovarian, and stomach cancers.

Table. 2 – Current Clinical Trial based on Nanogel Vaccine CHP-HER2

Nanogel Based Vaccine	Adjuvant	Type of Cancer	Clinical Phase	Reference
CHP-HER2	–	Lung, breast, pelvis, pancreatic, nasal cavity	I	Kitano et al. [133]
	GM-CSF/ OK-432	Breast, ovarian, non-small cell lung cancer	I	Kageyama et al. [129]

- In a clinical trial by Kitano et al. (2006) (table 2) to investigate the safety as well as specific immune responses to HER2, nine HER2-expressing cancer patients were administered 300 μg of the CHP-HER2 vaccine subcutaneously thrice at interval of 2 weeks. The vaccine was well tolerated with only grade 1 reaction at injection sites. There was induction of specific CD8+ and CD4+ immune responses in five patients [133].

- In another similar clinical trial by Kageyama et al. [129], fifteen patients with HER2-expressing tumor were enrolled to investigate the safety and specific cellular and humoral immunological responses. Adjuvants were added to the antigen and their ability to enhance the immune response was assessed. The study was split into two phases. For the first phase of the study, nine patients were injected with the vaccine subcutaneously biweekly. After receiving the fourth dose of the vaccine, the patients were given one of these two adjuvants–human granulocyte-macrophage colony-stimulating factor (GM-CSF) or OK-432. OK-432 is a low-virulence Streptococcus pyogenes strain that has been killed and lyophilized. In the second phase of the study, a combination of CHP-HER2 vaccine and GM-CSF was administered to six patients.

The vaccination with CHP-HER2 vaccine induced the production of IgG antibodies in 14 formerly seronegative patients. The findings show that CHP-HER2 vaccine elicited HER2-specific humoral responses in patients with HER2- expressing malignancies, and that GM-CSF appears to speed up these responses. Despite the fact that the CHP-HER2 vaccination was successful in enhancing the induction of HER2 specific antibodies, there was no evidence of tumor regression in any of the participants.

Clinical translation of the majority of the stimuli-responsive NGs reported for other diverse applications should pass through clinical phases to realize their potential in particular therapeutic and diagnostic application.

5. CONCLUSION:

In the last decade, nanogels for anticancer drug delivery has been extensively researched. Different polymer tuning and methods used in the synthesis affect the nanogel size, drug loading, stimuli-responsive features and drug release capacity. This review also summarizes their classification. In particular, we have reviewed "smart" stimuli-responsive nanogels under the action of pH, temperature, redox species, ROS, and enzymes, as a single or any two or multiple stimuli. Smart nanogels are unique such that they have good aqueous stability, can encapsulate different drugs ranging from hydrophilic chemotherapeutics to proteins and siRNA, exhibit fast response to biological stimuli, and are amenable to functionalization with cell targeting ligands. They present a revolutionary approach to targeted breast cancer therapy with enhanced specificity in the tumor environment and minimized impact on healthy cells, overcoming the limitations of conventional therapies such as toxicity to healthy cells, premature drug release etc.

The results of a clinical trial using CHP-based anticancer vaccine showed that the vaccines were safe after repeated subcutaneous injection and that they were effective in generating antigen-specific T-cell responses as well as humoral immunity, however more research is needed to properly comprehend CHP's true effectiveness in cancer vaccination. As research advances, the potential for these versatile platforms to revolutionize cancer treatment continues to grow, paving the way for promising, more effective and personalized therapeutic strategies.

6. FUTURE PERSPECTIVE:

Future perspectives seem to be mainly focused on the synthesis of dual and multi-responsive NGs with cancer cells targeting properties.

There is a need to standardize processes with a good manufacturing practice guideline for nanogel formulations. Methods to upscale the production and to and confirm the safety profile of these nanogels must also be investigated.

Even though large volume of literature is available, the in vivo studies and clinical trials with nanogels in different domains is limited. Despite available clinical trials, the clinical efficacy of nanogel in cancer vaccines has been found to be insufficient, and the intended clinical outcome of tumor regression has not been detected in a significant number of clinical trials. Understanding the precise immunological pathways of immune response generation is critical for improving the efficacy of nanogel based cancer vaccines. Identification of more immunological biomarkers that could allow for a more precise assessment of the clinical outcome to cancer vaccination will surely be advantageous in this context.

Thus, the perspective of the efficient stimuli-responsive nanogel system desires to be focused on optimization of formulation to possess the greatest therapeutic potential and pre-clinical and clinical studies to accelerate the transition from bench to bedside. This will revolutionize the treatment of many different diseases like cancer, inflammatory disorders, diabetes, etc.

7. CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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