Synthesis and applications of nanogels via covalent cross-linking strategies

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Abstract

In the last decades, the need of new therapies and treatments for various pathologies have increased the attention to drug delivery systems due to their multiple advantages. In this context, nanogels, a specific type of nanoparticles, have emerged as potential drug carriers due to their interesting properties in terms of biocompatibility, biodegradability and tunability. In this chapter we focused our attention on different strategies to prepare nanogels using covalent cross-linking strategies and then on manufacturing and different biomedical applications. The results obtained by many different research groups in different medical applications underlined the high promises behind these nanomaterials. Indeed their high biocompatibility, together with their ability to be functionalized and loaded with different drugs, guarantee strong potentialities and development in the field.

1. Introduction

The term nanogel was originally introduced in 1999 by Vinogradov et al. (1999) to describe drug carriers composed of cross-linked networks of PEG and PEI. Today, this term is used to define aqueous dispersions of hydrogel particles formed, physically or chemically, by cross-linked polymeric networks of nanomeric size, generally between 20 and 200 nm (Kabanov et al., 2009). They are included in the nanoparticle family. Nanogels are biodegradable and biocompatible materials characterized by the ability to incorporate large amounts of water and biological fluids due to their structure and the presence of different functional groups such as hydroxyl, carboxyl, amide and sulfonic. Due to their small size, nanogels can easily cross biological barriers and can therefore be subjected to cellular uptake. Their main characteristics are:

Nanomeric dimensions: Involve the increase of the surface to volume ratio and allows them to penetrate through different tissues and cellular pathways (Zarekar et al., 2017).

Swelling behavior: Nanogels have swelling/deswelling characteristics that can be regulated based on structural features, such as the degree of

crosslinking, and can be modified due to external stimuli such as pH, temperature and specific biomarkers (Shah et al., 2020). As already mentioned for hydrogels, nanogels can also swell and de-swell when placed in aqueous environment: this behavior is determined by the structure of the nanoparticle, by the chemical nature of the polymer chain, by the inner cross-linking and by external parameters, such as temperature and pH. When the structure, assumed initially dry, is immersed in aqueous media, the fluid approaches the surface and penetrates in the polymeric matrix: as a result, the mesh of the network expands and solvent's molecules can enter inside the polymer scaffold. There are cases in which the drug loading can also reduce the volume: in fact, the drug can interact with the polymer chains and induce a condensation or collapse of the network. Obviously, the nanogel dimensions are determined by the balance between osmotic pressure, influenced by the ratio between the ion concentration inside and outside the nanogel, and the polymer elasticity. Commonly, nanogels show a faster swelling capacity compared to bulk hydrogels, due to their larger surface area that ensures a higher possibility of fluids exchange.

1.1 Drug loading

Nanogels can be loaded by covalent conjugation, physical entrapment and passive/diffusion-based drug loading.

1.2 Controlled and sustained drug release

Drugs can be released by diffusional release, by nanogel degradation and/or in response to environmental stimuli.

1.3 Viscoelasticity

The high level of solvation of nanogels allows them to perform as both liquids and solids, enabling their flow through needles and the extracellular matrix.

1.4 Shape control

The ability to control the shape of nanogels can increase their performance: for example, non-spherical nanogels circulate for longer periods in the circulatory system and avoid phagocytosis.

1.5 Colloidal stability

Compared to micelles, nanogels have higher stability in biological fluids.

1.6 Possibility of intravenous injection

It is possible to administer this typology of nanocarrier using the intravenous injection sending it directly in the veins using a needle or a tube.

2. Nanogels manufacturing

There are currently four methods for the preparation of nanogels (Beiranvand et al., 2018): physical self-assembly or interactive polymers, polymerization of monomers in homogeneous phase or micro- or nano-heterogeneous environment, chemical cross-linking of preformed polymers and template-assisted nanofabrication.

2.1 Physical self-assembly

In this formulation method, the formation of nanogels is obtained thanks both to the hydrophobic or electrostatic interactions and due to hydrogen bonding between the hydrophilic polymers constituting the nanoparticle. Polymers concentrations and environmental factors (pH, temperature, operative conditions) influence the size of the resulting nanoparticles. This strategy is useful because occurs in mild conditions and aqueous medium and it gives the chance to encapsulate bio-macromolecules and obtain protein loaded systems.

2.2 Polymerization reactions

This technique can use either oil-in-water or water-in-oil emulsion. The NGs formation is started in a homogenous aqueous solution of water-soluble monomers and it commonly ends with the formation of a colloidal suspension of a growing polymer. This strategy gives the possibility to introduce cross-linkers inside the nanogel during the process, in order to facilitate its degradation and the drug release.

2.3 Covalent cross-linking of preformed polymer chains

Nanogels synthesized with this method are obtained through the cross-linking of polymers previously formed. It is a very useful method to produce NGs for drug delivery: in fact, as with polymerization reactions, it

is possible to introduce degradable bond to facilitate the release of the payload. Nanogels obtained by linking of polymer precursors are generally made of self-assembling amphiphilic or triblock copolymers or composed of polymers having different types of reactive sites that can be directly used in the formation of covalent bonds. This chemical cross-linking strategy includes a wide range of experimental pathways based on the principles of click chemistry, thiol−disulfide exchange, Schiff base reaction, photo- or thermally induced cross-linking, amide bond formation, enzyme-mediated cross-linking, catalyzed coupling and the chemistry of ketones, aldehydes, epoxides, or other groups. Moreover, they can be used for the preparation of core−shell structures or micelles by tuning of the spatial organization of the molecules and the consequent interaction with external biomolecules.

2.4 PRINT technology

The *Particle Replication In Non-wetting Templates* (PRINT) is a new technique to produce polymeric particles with dimensions from 10 nanometers to several microns. This method favors the formation of a mono-disperse and shape-specific nanoparticle thanks to the utilization of non-wetting elastomeric molds of a perfluoropolyether network that avoids the formation of a film interconnetting the molden objects. The great advantage is the formation of a particle with a great control of shape, size, composition, surface functionality, and with the possibility to load high quantities of different typologies of drugs.

3. Drug release applications of covalent cross-linked nanogels

3.1 Drug loading

Corss-linked NGs can achieve high drug loading capacities of up to 50% by weight due to their structural properties, which are quite high compared to the 25% that other nanocarriers can achieve.

As reported in Kobanov et al. (2009), there are three main drug loading strategies for nanogels: covalent conjugation, physical entrapment, and controlled self-assembly.

Covalent conjugation of biological agents: this method is based on the covalent interaction formed between the biological agent and the

nanogel structure. This bonding can be obtained during the synthesis of the nanogel or after its formation.

Physical entrapment: this technique is based on the linking between the hydrophilic vehicle and the hydrophilic or hydrophobic portions of the molecules or the dissolution of hydrophobic molecules.

Controlled self-assembly: this process is based on the autonomous organization of components into the nanogel structure. It is characterized by an initial diffusion followed by a specific association of the drug molecules by weak interactions such as electrostatic and/or hydrophobic association. This weak association can be fortified by using chemical entities that are able to compact the structure ensuring stronger bonds.

3.2 Drug release mechanisms

The release of the drug from the charged nanogels can occur through several mechanisms. Some examples are diffusion, degradation, pH change, ion displacement or by application of external energy to the nanogel structure that induces degradation or structural transition of the polymer chains.

Diffusion: is based on the diffusion process that occurs in the nanostructure due to the gradient concentration.

Degradation: the nanogel degradation leads to the release of the encapsulated molecules.

pH responsive mechanism: due to a change in the pH, deionization of the polymer structure can occur releasing the drug that has been electrostatically bounded.

Displacement by ions present in the environment: the presence of multivalent low molecular mass cations or poly-ions may displace the drugs with the same charge that have been previously loaded by electrostatic forces.

Application of external energy: this can induce nanogel degradation or structural transition of its polymeric chains.

3.3 Targeted drug delivery release

One of the main foreseen advantages of using nanogels as nanocarriers for drug delivery is the ability to selectively target (Eckmann et al., 2014; Pinelli et al., 2020) specific sites such as organs, tissues, and cells. There are two different strategies for targeted drug delivery: passive targeting and active targeting.

Passive targeting: this technique is based on the accumulations of nanoparticles in specific tissues, being the circulation time a key role in this process.

Active targeting: is based on the use of ligands coupled to the nanocarrier. This strategy can be much more effective than passive targeting when coupled with an appropriate design of the nanogel structure. Active targeting can be achieved by functionalizing the surface, through the so-called "surface coating" process. As reported in Pinelli et al. (2020), several strategies can be considered: coating with polymers, using cell membranes, coatings with proteins and antibodies and hybridized DNA structures. In the last decades, the interest in the study of drug delivery systems has increased considerably due to their interesting properties in terms of safety, efficacy and patient convenience. These systems can be administrated through different routes and strategies: oral delivery, transdermal delivery, implants, pulmonary delivery, injectable systems, nasal delivery and ocular delivery.

Due to their properties, nanoparticle-based drug delivery systems have emerged as interesting drug carriers. They allow to avoid pharmacokinetic limitations that traditional drug formulations have.

Moreover, some studies have been proved that a correct design of this kind of drugs can not only overcome biological barriers[7] but also targeting specific sites and cells. In this context, nanogels can be specially considered due to their interesting drug delivery properties associated with their biocompatibility, high water content, high surface area, high loading capacity, soft nature and being able to deliver hydrophilic and hydrophobic drugs. Their surface can be also modified to overcome biological barriers and targeting specific sites. In this present thesis work we will focus on the analysis of different functionalized nanogel formulations for drug delivery applications, including biological tests.

3.4 In vivo drug delivery applications

Research groups around the world have recognized a potential in research and development of nanogels for drug delivery. Several cross-linked nanogel structures have been developed and tested in vivo for the treatment of a wide range of pathologies such as cancer, spinal cord injury, ischemic stroke, cardiovascular diseases, wound healing, bone regeneration, psoriasis, other inflammatory diseases and other pathologies. They are also used for anesthetic drugs delivery. In the following sec-

tions is reported a review of nanogels tested in vivo for treating different diseases to stress the importance that these delivery systems have acquired.

3.5 Cancer

Tumors are chronic diseases that cover more than 277 types of cancer diseases. Several approaches have been developed to treat them, examples being surgery, radiation therapy and targeted therapy. Nanogel have attracted the attention of several research groups that believed on their potential for the specific delivery of active compounds for the treatment of cancer diseases. An example of this kind of pathology that can be treated with nanogels is the breast cancer. In this context Zhang et al. (2017) developed a dextrin nanogel coated with AMD3100 (Plerixafor) and loaded with doxorubicin (DOX).

Through in vivo tests, they proved that this system was able to target CXCR4 chemokine receptor and to have an anticancer and anti-metastatic effect. In another study, Chen et al. (2019) produced an epidermal growth factor receptor and CD44 dual-targeted hyaluronic acid nanogel loaded with saporin that afford enhanced targetability and protein therapy for metastatic 4T1 breast cancer in vivo, without remarkable side effects. Another important approach was proposed by Si et al. (2020). In this case the nanogels were fabricated using host-guest interactions between azobenzene (Azo) and β-cyclodextrin (βCD) conjugated to poly (L-glutamic acid)-*graft*-poly (ethylene glycol) methyl ether (PLG-g-mPEG) loaded with Ribonuclease A (RNase) that was proved through in vivo tests to be capable to achieve a tumor suppression rate of 68.7% that was improved until a 91.7% when was combined with PLG-g-mPEG/combretastatinA4 nano-formulations (Fig. 1).

Another important strategy that is widely studied is cholesterol based nanogels for treating cancer. Shimuzu et al. (2008) developed cholesterol-bearing pullulan (CHP)-based nanogels loaded with interleukin-12. This formulation demonstrated to induce grow retardation of fibrosarcoma. In a different study, Fuji et al. (2014) used a cholesterol-bearing cycloamylose with spermine group nanogel for delivering vascular endothelial growth factor-specific short interfering RNA that were proved to be able to suppress neovascularization and growth of renal carcinoma cells in mice models. Another important polymer used for producing nanogels that have been used for treating cancer is hyaluronic acid. In

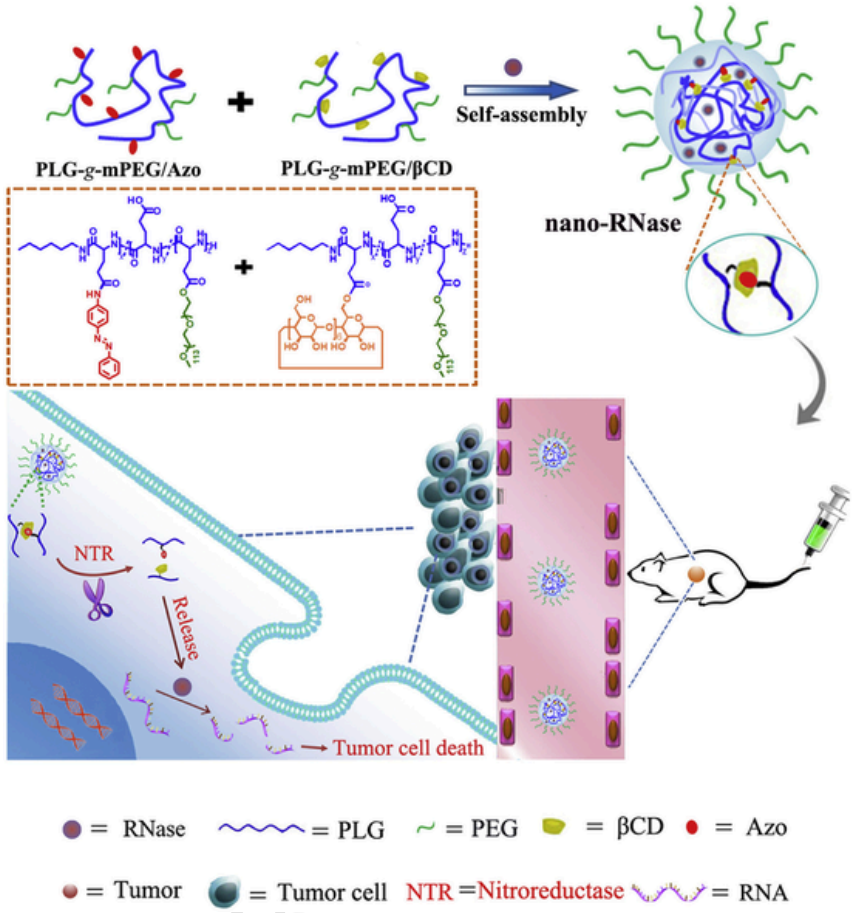

Fig. 1 Schematic illustration of the hypoxia-sensitive nanogels used for RNase delivery. RNase was loaded into the supramolecular nanogels through supramolecular interactions between Azo and βCD grafted onto PLG-*g*-mPEG. Following intravenous injection, the nano-RNase accumulated in tumor tissue by virtue of enhanced permeability and retention (EPR) effects. Following internalization into cancer cells, RNase was released in response to the NTR in tumor cells. This catalyzed the decomposition of RNA promoting tumor cell death. *Reprinted with permission from Si, X., et al. Hypoxia-sensitive supramolecular nanogels for the cytosolic delivery of ribonuclease a as a breast cancer therapeutic. J Control Release* ***2020****, 320, 83–95.*

this context, a cisplatin (CDDP)-crosslinked hyaluronic acid (HA) nanogel is prepared by Zhang et al. (2018) for effective delivery of doxorubicin (DOX) to treat osteosarcoma. They obtained positive in vivo

results due to the synergetic effect of DOX and cisplatin that enhanced the antitumor potency of the formulation with lower toxicity. The same polymer was used by Seok et al. (2018). In their study they developed novel hyaluronic acid cross-linked zein nanogels (HA-Zein NGs) to deliver the potential anticancer agent curcumin (CRC), a naturally occurring phytochemical drug in cancer cells that was proved to have antitumor efficacy in CT26 tumor model. Anyway, those just presented are not the only available formulations; the use of other polymers has been also explored. For example, Huang et al. (2015) used a reduction-responsive polypeptide nanogel loaded with DOX that showed positive in vivo results due to its excellent properties in terms of security and tumor inhibition. Pullulan nanogels have been also produced by Zheng et al. (2019).

In this case, two multi-functional pullulan nanogels for delivering DOX were produced using two different crosslinking agents obtaining a tumor growth inhibition up to 83.37%, this result was obtained through in vivo tests working with an acid-labile ortho ester-modified pluronic used as a crosslinking agent.

Another nanogel formulation was studied by Cheng et al. (2018) in which acid-degradable nanogels were prepared by cross-linking methacrylated soy protein with an acid-labile ortho ester cross-linker, and then modified with lactobionic acid (LA) to give tumor-targeted nanogels for delivering DOX. In vivo tests showed that this formulation was effective to target tumors and obtain a better therapeutic efficacy.

In addition to what we have just presented a very interesting feature of nanogels is the possibility to modify their structure for selective target drug release applications. In this context Peng et al. (2020) developed Zwitterionic polysulfamide nanogels modified with transferrin (Tf) for the loading with DOX. In vivo tests showed that this formulation had on-demand tumor targeting properties being able to enhance cancer chemotherapy. Another strategy was used by Xu et al. (2019). In this case they used an irinotecan loaded cross-linked gelatin nanogel coated with platelets membranes. This formulation showed in vivo antitumor activity being able to suppress tumor cells growth presenting minimal side effects.

3.6 Spinal cord injury

Spinal cord injury (SCI) is a neurological disorder that can be caused by both traumatic and non-traumatic events. The “primary injury” is the ini-

tial neurological damage in the spinal cord followed by the "secondary injury", which is a cascade of biological and inflammatory events.

The latter, in fact, is one of the most important aspects of this second phase and has been extensively studied to find effective treatments to reduce it. Different polymer-based drug delivery carriers have been developed for treating SCI, including nanogels and nanoparticles and in any case the pivotal point is to be able to selectively reach the cells of the central nervous system. In a recent study Vismara et al. (2020) developed a cross-linked polyethylene glycol (PEG) and polyethylene-imine (PEI) nanogel coated with primary amines loaded with rolipram, an anti-inflammatory drug. In vivo tests showed that these formulations were able to selective target astrocytes, improving the motor performance in the early stage after the spinal cord injury in mice models, reducing the pro-inflammatory response mediated by astrocyte activation. Similarly, Nazemi et al. (2020) developed PTX-encapsulated poly (lactic-*co*-glycolic acid) PLGA microspheres along with MH incorporated into an alginate hydrogel alongside with minocycline hydrochloride. In vivo rat model tests showed that this dual-drug treatment was able to reduce inflammation after 7 days, decreasing the scar tissue formation and increasing neuronal regeneration after 28 days (Fig. 2).

Another important strategy using polymeric nanoparticles was developed by Papa et al. (2016). In their study minocycline loaded poly-ε-caprolactone-based nanoparticles were used for targeting microglia. The acute administration of this formulation in SCI mouse model was able to reduce the pro-inflammatory response by modulating the resident microglia cells and being able to maintain a pro-regenerative milieu up to 63 days after injury occurs. Minocycline was also used by Wang et al. (2019) for treating SCI. In their study an E-selectin-targeting sialic acid-polyethylene gly*co*l-poly(lactic-co-glycolic acid) (SAPP) copolymer was designed for delivering hydrophobic minocycline to achieve combinational therapy of SCI. In vivo tests showed that these micelles were able to efficiently be accumulated in SCI lesion sites in rats, reducing the area of the lesion cavity and increasing the survival rate of axons and myelin. Polymeric nanoparticles were also used by Li et al. (2016) in their study. In this case, lipidoid nanoparticles containing IRF5 (interferon regulatory factor 5) siRNA were infiltrated into the SCI mice wound. The administration of this nanoparticles was able to modulate the anti-inflammatory response due to the decrease in the number of M1 macrophages and the increase in the number of M2 macrophages in the wound.

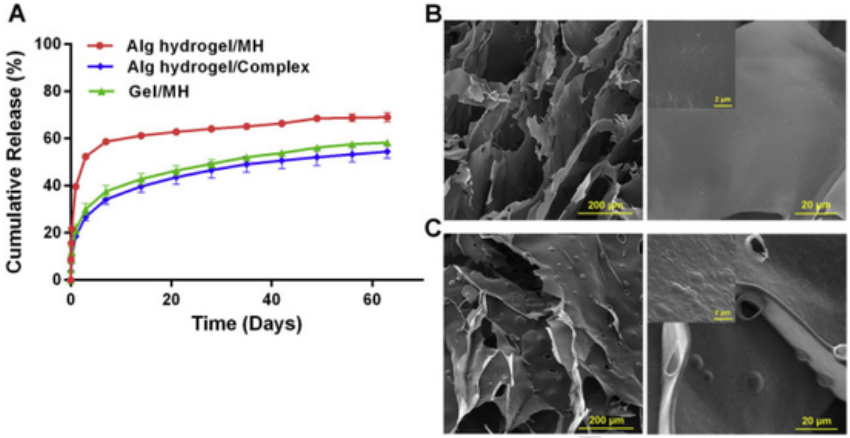

Fig. 2 Physicochemical characterization of hydrogels. (A) The in vitro release of MH from the Alg hydrogel (Alg hydrogel/MH), Alg-AlgS hydrogel (Gel/MH) and Complex-incorporated Alg hydrogel (Alg hydrogel/Complex). SEM images of the cross-sectioned lyophilized hydrogels: (B) Alg hydrogel/MH and (C) Gel/MH. Data shown are average ± SD. *Reprinted with permission from Nazemi, Z., et al. Co-delivery of minocycline and paclitaxel from injectable hydrogel for treatment of spinal cord injury. J Control Release* ***2020****, 321, 145–158).*

3.7 Ischemic stroke

Stroke is a clinical syndrome based on the rapid development of clinical signs of brain function disorder, with a vascular origin, which can last even more than 24 h or can lead to death. It is the second most common cause of death worldwide and the third most common cause of disability, as 68% of stroke cases are ischemic. Thrombolytic therapies can be used in this type of pathologies using many different thrombolytic agents such as urokinase, streptokinase, tissue plasminogen activator or anistreplasis. In this context, Cui et al. (2016) developed a pH sensitive PEG-conjugated urokinase nanogels for delivering urokinase, a thrombolytic agent. The nanogel is designed for releasing urokinase when pH decreases, that is a consequence that occurs in this kind of diseases due to the lack of oxygen caused by microcirculatory clots. Through in vivo tests it was observed that urokinase was delivered 1 h after middle cerebral artery occlusion, decreasing the severity of ischemic injury by protecting the blood-brain barrier, improving the ischemic brain tissue, decreasing neurotoxicity and inhibiting apoptosis. In a subsequent study, Cui et al.

(2020) observed that by using this same formulation outside the usual thrombolysis time window, positive results were observed through in vivo tests. In their study, the loaded nanogels were able to not only preserve the integrity of blood-brain barrier but also were able to reduce the excito-neurotoxicity in the permanent middle cerebral occlusion in rats. In another study, Jin et al. (2012) developed hollow nanogels, which are generated by the reaction of glycol chitosan and aldehyde capped poly(ethylene glycol) (OHC-PEG-CHO) through a one-step approach of ultrasonic spray, encapsulating urokinase for delivering it under diagnostic ultrasonic conditions. It was observed through in vivo tests that this kind of formulations were able to enhance the circulation time of urokinase and proving that the loaded nanogels were sensitive to diagnostic ultrasound since delivered urokinase, in a faster rate under these conditions, enhancing the thrombolysis of clots. By using the same urokinase loaded hollow nanogels, Teng et al. (2017) proved through in vivo tests that this kind of nanogels were capable to treat acute ischemic stroke by enhancing the thrombolysis effect of urokinase compared with the effect of free urokinase, protecting the blood-brain barrier integrity avoiding adverse brain hemorrhage without causing animal death after 1 week of its administration.

3.8 Cardiovascular diseases

Cardiovascular diseases are the leading cause of death worldwide. They cover a wide range of diseases: rheumatic and congenital heart disease, coronary heart disease, peripheral arterial disease, venous thromboembolism and cerebrovascular disease. Several factors can lead to cardiovascular problems, from genetic problems to hypertension, obesity, diabetes. Different approaches have been studied to treat this type of disease, among which recently the use of nanogels for drug delivery applications has been explored by several research groups. Hypertension can be treated with many different approaches. In the context of drugs nanocarriers, Azegami et al. (2018) developed an innovative intranasal vaccine that simultaneously targets both hypertension and pneumonia and offers prolonged therapeutic effect and reduced frequency of administration. To do this a cationic cholesteryl-group-bearing pullulan (cCHP) nanogel loaded with angiotensin II type 1 receptor (AT1R) and pneumococcal surface protein A (PspA) was developed. AT1R has been demonstrated to be able to decrease blood pressure without adverse events in rat

models, meanwhile PspA is a surface protein expressed by *Streptococcus pneumoniae* that can be used for inducing immunization against it. In vivo results showed that this vaccine was able to protect from lethal pneumococcal infection attenuating hypertension. Another therapy for treating hypertension using nanogel formulations was developed by Laha et al. (2019).

In this study, self-assembled nanogels of amphiphilic Karaya gum with a degree of propyl group substitution of 3.24 for delivering bosentan monohydrate, a poorly soluble antihypertensive drug, were developed for preferentially deliver the drug in the intestine. This loaded self-assembled nanogels were demonstrated, through in vivo tests, that were able to decrease blood pressure over 10 h after its administration, obtaining the maximum of blood pressure level decrease after 8 h, when it was decreased about a 31%. Anyway, hypertension is not the only cardiovascular disease where it can be found prospective therapies based on drug delivery applications using nanogels. In fact, Cheraghi et al. (2017) developed N-isopropylacrylamide-methyl methacrylate nanogels for delivering N, α-L-rhamnopyranosyl vincosamide for studying their cardioprotective properties. In vivo tests showed very interesting results, demonstrating how these structures have interesting cardioprotective properties being able to reduce cardiac toxicity in doxorubicin-induced toxicity models. In another study Tang et al. (2017) developed thermosensitive poly(N-isopropylacrylamine-*co*-acrylic acid) or P(NIPAM-AA) nanogel loaded with human cardiac stem cells for treating myocardial infraction. The injection of the loaded nanogels proved, through in vivo tests in mice and pig models, that were able to preserve the cardiac function reducing the scar size without creating systemic inflammation.

3.9 Wound healing

Wound healing is a complex biological process that can be divided into three main stages that can be superimposed: inflammation, cell proliferation and synthesis of different elements that make up the extracellular matrix and final remodeling. Several elements such as immune surveillance cells, microvascular cells and fibroblasts, platelets and keratinocytes, play a key role in this type of biological processes. Traditionally, several compounds such as those obtained from medicinal plants have been used to improve wound healing processes. In recent times,

studies have been carried out on different strategies based on the use of nanovectors, such as nanogels, to improve the efficiency of therapies.

Infection is a problem that can occur during wound healing, to overcome this problem, El-Feky et al. (2017) developed alginate coated chitosan nanogels loaded with silver sulfadiazine, an antibacterial drug, and compared their performance with commercial creams containing this molecule. It has been observed, through in vivo tests, that the loaded nanogels were effective formulations in healing burn wounds, hypothesizing that this fact was related with the infection control, and they also observed that the silver sulfadiazine required to achieve this result was lower compared with the one of the commercial creams. Another study that reached a similar goal was proposed by Zhu et al. (2018). In their work they formulated a lysine-based nanogel loaded with chlorhexidine diacetate, an antiseptic and disinfectant molecule. The loaded nanogels were incorporated into hybrid hydrogels with rapid hemostasis and sustainable antibacterial property obtained combining aminoethyl methacrylate hyaluronic acid (HA-AEMA) and methacrylated methoxy polyethylene glycol (mPEG-MA) hybrid hydrogels. Through in vivo tests it was observed that when the hydrogels containing the loaded nanogels were used, no bacterial biofilm was formed, confirming its antibacterial effects. It was also seen a rapid hemostasis effect, accelerating the healing process. Other strategies have been developed to regulate inflammation in wound healing processes. In this context, Manconi et al. (2018) developed gellan-cholesterol nanohydrogels loaded with baicalin, that is a flavone widely used in different inflammatory diseases. Through in vivo tests it was observed that baicalin loaded gellan-cholesterol nanoparticles were able to inhibit certain inflammation markers such as tumor necrosis factor-α and myeloperoxidase in a more efficient way compared with commercial creams, dexamethasone solutions and baicalin in PBS formulations. Other natural compounds such as curcumin have shown interesting properties in wound healing processes. Pathan et al. (2019) developed fish scale collagen-hydroxypropyl methyl cellulose nanogels loaded for treating this kind of diseases. It has been observed through in vivo tests that these formulations lead to higher wound contraction values and lower irritation symptoms compared with other curcumin formulations proving the benefits of combining collagen and curcumin.

Interleukin-2 is another interesting molecule due to its beneficial effect on the proliferation of T lymphocytes. Aslan et al. (2017) developed chitosan-based nanogels loaded with Interleykin-2 obtaining inter-

esting in vivo tests results due to the decrease of the malondialdehyde levels (a biochemical marker of lipid peroxidation) and the increase of glutathione levels (a well-known antioxidant) leading to interesting results for wound healing applications. In another study, Yang et al. (2014) produced heparin-modified supramolecular pluronic nanogels containing basic fibroblast growth factor and VEGF195 genes (vascular endothelial growth factor) that were pre-coated with PEI for inducing neovascularization of wound sites. In vivo tests proved that this kind of formulations were effective promoting neovascularization and promoting endothelial cell differentiation.

3.10 Bone regeneration

Diseases that affect bones are the main cause of disability worldwide. Fractures, osteoporosis and tumors are the main examples of diseases that affect bone tissue. In normal health conditions, bones show a unique bone healing ability without forming scar tissue. However, many times it is required special treatments such as the use of bone substitutes with osteoinductive, osteoconductive and biocompatible properties or the use of cells, for example the use of human mesenchymal stem cells, and different growth factors such as bone morphogenic proteins, vascular endothelial growth factor, fibroblast growth factor, platelet-derived growth factor, transforming growth factor-β1 (TGF-β1) and insulin-like growth factor-1.

In this context, Fujioka-Kobayashi et al. (2012) developed a cholesteryl-group and acryloyl group-bearing pullulan nanogels that were aggregated to form fast degradable hydrogels to develop recombinant human bone morphogenetic protein 2 (BMP2), an osteogenesis modulator, and recombinant human fibroblast growth factor 18 (FGF18), used for enhancing the activity of low doses of BMP2 (Fig. 3). Through in vivo tests, it has been observed that these formulations were able to induce bone repair compared with the use of free BMP2 or the combination of free BMP2 and FGF18.

Another formulation strategy to induce bone regeneration by using BMP2 was chosen by Gong et al. (2018). In their study they developed a mixture of poly(ε-caprolactone) (PCL) and redox responsive c-6A PEG-PCL nanogel for delivering poly(ethylene oxide) and bone morphogenetic protein 2 (BMP2) forming nanofibers with a core-shell structure. Through in vivo tests it was observed that the controlled delivery of

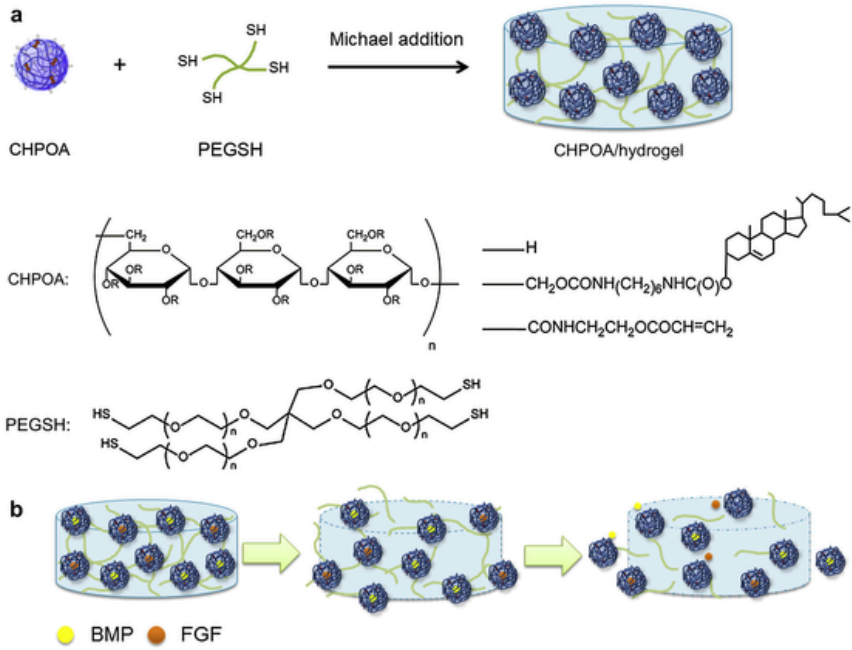

Fig. 3 Structure of the CHPOA nanogel and synthesis of the CHPOA/hydrogel. (A) Synthesis of the CHPOA/hydrogel block and chemical structure of the CHPOA nanogel and PEGSH. CHPOA nanogels were cross-linked with PEGSH by Michael addition to form the CHPOA/hydrogel. (B) Schematic representation of the FGF18- and BMP2-releasing from nanogels after the disintegration. *Reprinted with permission from Fujioka-Kobayashi, M., et al. Cholesteryl group- and acryloyl group-bearing pullulan nanogel to deliver BMP2 and FGF18 for bone tissue engineering. Biomaterials* ***2012****, 33, 7613–7620.*

BMP2 of this nanogels was able to promote bone defect repair. In another study, Zhang et al. (2017b) developed a temperature-sensitive p(N-isopropylacrylamide-co-butyl methyl acrylate) nanogel (PIB nanogel), to form scaffolds, used as biomaterial for the delivery of mesoporous bioactive glass. The in vivo results showed that this nanoparticle formulation has been able to enhance regeneration of femur defects in osteoporotic animals. Another important molecule that can be used to induce bone regeneration is the W9-peptide, a TNF-α and RANKL antagonist. Alles et al. (2009) developed a cholesterol-bearing pullulan (CHP)-nanogel as the drug delivery system for W9-peptide. In their study they observed, through in vivo tests, that this kind of structures

were able to prevent bone loss in bone resorption models. Prostaglandin E2 (PGE2), a non-peptide anabolic agent can also be used to promote bone regeneration. However, the main drawbacks of this compound are its side effects at high doses and its short half-time circulation. To overcome these problems, Kato et al. (2007) developed a CHP nanogel for delivering PGE2. In vivo tests showed that PGE2 was able to induce new bone formation when it was in combination with nanogel cross-linking hydrogel sphere.

3.11 Psoriasis

Psoriasis is an inflammatory autoimmune skin disease that affects 1–3% of the worldwide population. It is an immunological disease caused by the activation of T lymphocytes in the epidermis and dermis. It has been proved to be closely related to dysregulation of immune cell function as well as keratinocyte proliferation/differentiation. Traditionally, the main psoriasis treatments occur with methotrexate (MTX), cyclosporine and retinoids in different forms and dosages. Recently, several nanogel formulations have been developed to deliver this kind of drugs with positive results.

In this context an important strategy has been proposed by Panonnummal et al. (2017). They developed chitin based nanogels loaded with clobetasol (an anti-psoriatic drug) (CLCNG), for its topical application. In their study, it has been reported, through in vivo tests, that by using CLCNG nanogels, anti-psoriatic activity was achieved inducing lower skin irritation, making them good candidates for their topical use. Following a similar approach, Panonnummal et al. (2018) developed a methotrexate loaded chitin based nanogel (MCNG) for its topical use. Using this formulation, in vivo tests showed a reduction of Comulative Psoriatic Area and Severity Index (PASI) between 73.11% and 89.22% depending on drug's dose level, higher than the ideal reduction rate to consider them clinically effective between 73% and 75%. It has also been shown to be advantageous by comparison with a commercial methotrexate gel, whose PASI reduction is lower, by observing a lower toxicity induction.

Another effective molecule used to treat psoriasis is babchi oil. It is a natural essential oil that has lower side effects than other synthetic drugs. In this context, Kumar et al. (2019) developed a cyclodextrin-based nanocarriers loaded with babchi oil for topical use in order to study their

efficacy for the treatment of psoriasis comparing their performance with the native babchi oil gel. In their study it was demonstrated through in vivo tests, the anti-psoriatic activity of babchi oil loaded nanogels without apparent skin irritation or inflammation and erythema.

In another study, Feng et al. (2020) used another strategy to treat psoriasis using nanogel carriers. In previous works, it has been demonstrated the important role that miRNA-210 plays in this kind of pathologies. In this study, they developed a biomimetic reconstituted high-density lipoprotein (rHDL) nanocarrier gel containing miR-210 antisense (NG-anti-miR-210) to investigate its effect on imiquimod (IMQ)-induced psoriasis-like dermatitis in mice. Its efficacy was demonstrated thanks to the psoriasis-like inflammation reduction, proving its potential use in topical applications.

3.12 Other inflammatory diseases

In the previous points several strategies have been described for the treatment of inflammatory diseases such as psoriasis. Currently, different strategies are being developed that exploit nanogels as drug carriers for the treatment of other acute and chronic inflammatory diseases such as peritonitis, edema, periodontitis, allergic rhinitis and ulcerative colitis. In this section are presented some of the most important strategies studied.

Yurdasiper et al. (2018) developed a poly (N-isopropylacrylamide) nanogel loaded with naproxen to treat oedema. Topical administration of this formulation proved through in vivo tests to have anti-inflammatory activity being able to decrease the relative levels of COX-2 expression. This decrease of the COX-2 expression was enhanced when it was activated with sodium carbonate.

In another study, Yeo et al. (2020) developed a phenylboronic acid-tannic acid nanogel to treat peritonitis through the release of tannic acid. They developed a nanogel formulation that, through degradation, was able to release tannic acid, that is a natural antioxidant. In vivo tests showed that this formulation was able to alleviate the induced inflammation by changing the pro-inflammatory cytokine levels in serum, peritoneal lavage and peritoneal cell population.

In this context, it is very important to stress that nanogel applications can also have dual action. This aimed to develop Aminu et al. (2019) to treat periodontitis. They synthesized an anti-inflammatory and antimicrobial nanogel result of the combination of poly-ε-caprolactone loaded with

triclosan, an antimicrobial drug, and chitosan-based hydrogel loaded with flurbiprofen, an anti-inflammatory drug. This nanogel formulation proved through in vivo tests its dual effect.

Another inflammatory disease that can be treated with similar strategy is ulcerative colitis. To treat it, Onishi et al. (2019) produced a conjugate between chondroitin sulfate (CS) and glycyl-prednisolone (GP), named CS-GP, that gave a nanogel in aqueous solution, loaded with prednisolone. In vivo test it has been showed that when this nanogel was loaded with a 21.1% (w/w) of prednisolone the therapeutic efficacy observed was quite better than the one obtained when free prednisolone was used, proving the efficiency of the nanogel carrier.

Allergic rhinitis is another inflammatory disease that is generally treated with intranasal glucocorticoid spray, but there are still some side effects in this kind of treatment. Wu et al. (2019) developed a poly(methacrylic acid) nanogel loaded with anti-IL-1β IgY for treating this disease. Through in vivo tests it was observed that this nanogel formulation relieved allergic rhinitis symptoms inducing lower toxicity compared with the use of free anti-IL-1β IgY, proving to be an efficient drug release system.

3.13 Local anesthetic

Analgesic treatments are commonly used to reduce the sensation of pain. Generally, the molecular structure of local anesthetics contains: lipophilic aromatic ring, intermediate ester or amide bond and tertiary amine. The main examples of local anesthetics are bupivacaine, levobupivacaine, lidocaine, tetracaine, ropivacaine and prilocaine. Various nanogel strategies have been studied and developed for the delivery of these active substances.

One of these strategies has been developed by Hoaere et al. (2012). In their study it was developed a poly(N-isopropylacrylamide) nanogels loaded with bupivacaine. Through in vivo tests, it was observed that the loaded nanogels having a diameter lower than 300 nm were able to achieve up to 8–9 h of sciatic nerve blockade without inducing severe inflammatory response.

Another study in which bupivacaine has also been used is carried out by Rodrigues et al. (2020). In this case an injectable in situ forming nanogel composed of nanostructured lipid carriers to encapsulate bupivacaine was developed. It has been observed, through in vivo tests, that the

formation through injection of the nanogel was able to prolong the anesthetic effects for more than five times (more than 24 h) compared with free bupivacaine at clinical doses. However, also other types of anesthetics, in addition to bupivacaine, has been used in drug delivery applications using nanocarriers strategies. Beirenvand et al. (2018) developed magnetic nanogels based on poly(N-isopropylacrylamide) loaded with Artemsia aucheri. L extract, an anti-inflammatory and anesthetic compound. It was observed by in vivo testing that the injection of this loaded magnetic nanogels induced shoulder block in rat.

3.14 Other diseases

As it has been shown in the previous sections, the use of nanogels in drug delivery applications has been studied for various purposes in order to treat multiple diseases. However, there are still many diseases not covered by the previous sections in which it is possible to apply nanogels as nanovectors, witnessing the versatility of this type of devices.

For example, in Liu et al. (2018) thioketal-based ROS-responsive polymeric nanogels containing DFO moieties (rNG-DFO) were designed to treat iron overload. In their study they observed that using this nanogel formulations the ferritin levels and iron concentrations decrease in major organs in iron overload mice models without inducing relevant toxicity.

Nanogels have been also used by Yoon et al. (2016) to treat growth retardation caused by human growth hormone deficiency (hGH). In their study cinnamoyl alginate, cinnamoyl Pluronic F127 and cinnamoyl PEG were self-assembled into nanogels and used for delivering hGH. In vivo pharmacokinetics tests showed that the one-time injection of this kind of formulations allowed to maintain a substantial blood level of hGH for 2 weeks.

Wu et al. (2018) used lung-targeted genipin-crosslinked deacetylated chitosan (GEN-CS) nanogel particles loaded with isoniazid and rifampin to treat tuberculosis. In vivo tests proved the antibacterial activity with low toxicity levels of the formulated nanogels when they were inhaled. Moreover, the pulmonary doses of the loaded nanogels were able to achieve up to 24 h of therapeutic drug concentrations in lung and other organs.

Otomo et al. (2015) used nanogel carriers for treating encephalomyelitis and lupus. In their study, a KN93 loaded nanolipogel was used. KN93 is an inhibitor of CaMK4. These loaded nanolipogels

proved through in vivo tests to be able to inhibit molecules involved in the pathogenesis of autoimmunity in this kind of diseases.

A rapidly developing area in which nanogel applications are studied is the field of vaccines. A clear example is represented by the study of Fukuyama et al. (2015). They tested the nasal vaccination efficacy and safety of cationic cholesteryl group-bearing pullulan nanogels (cCHP nanogels) containing pneumococcal surface protein A (PspA) to treat pneumococcal infection obtaining positive results in nonhuman primates. Similarly, Azegami et al. (2017) used cCHP nanogels containing ghrelin-PspA to develop a vaccine against obesity. The intranasal administration of this vaccine proved to be able to decrease fat accumulation and increase the energy expenditure on in vivo mice models.

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