Review

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Posted Date: 10 July 2024

doi: 10.20944/preprints202407.0821.v1

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 doi:10.20944/preprints202407.0821.v1

Review

Nanogels: Recent Advances in Synthesis and Biomedical Applications

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Abstract: in the context of advanced nanomaterials research, nanogels have recently gained considerable attention for their versatility and promising biomedical applications. To date, a significant number of nanogels have been developed to meet the growing demands in various fields of biomedical research. Summarising preparation methods, physicochemical and biological properties and recent applications of nanogels may be useful to help exploring new directions for their development. This article presents a comprehensive overview of the latest nanogel synthesis methodologies, highlighting advances in formulation with different types of hydrophilic or amphiphilic polymers. It also underlines recent biomedical applications of nanogels in drug delivery and imaging, with a short section dedicated to biosafety considerations of these innovative nanomaterials. In conclusion, this article summarises recent innovations in nanogel synthesis and their numerous applications, highlighting their considerable potential in the biomedical field.

Keywords: nanogels; nanoparticle synthesis; hydrogels and microgels; batch chemistry; flow chemistry; microfluidics; nanomedicine; theranostics; cross-linking; drug delivery

1. Introduction

Recent advancements in nanomedicine have led to the development of nanogels (NGs) for drug delivery, gene therapy, and nanotheranostics [1–3]. NGs are polymeric nanomaterials that possess the advantages of both hydrogels and nanoparticles (NPs). Indeed, both hydrogels[4] and nanogels are polymeric materials with a three-dimensional cross-linked structure (at the nanometer level) capable of retaining large amounts of water or other fluids without dissolving, and are often biocompatible, making them suitable for biomedical applications. Hydrogels can vary widely in size, from a few micrometres to several centimetres, and are used in a wide range of applications, such as medical devices, cosmetics, tissue engineering, and bandages. Their structure can be moulded into various shapes to suit different needs. On the other side, nanogels are defined by their sizes between few and few hundred nanometers, which allow for specific interactions at the cellular and tissue level. Moreover, like for NPs, the functionalization of their surfaces allows various moieties to be linked at high densities (because of the high surface-to-volume ratio of nanoparticles), and this can be used e.g., for targeted delivery and/or for modulating the protein corona in biofluids [5]. Due to their inherent porosity, NGs can encapsulate very efficiently both hydrophilic and lipophilic payloads, protecting them from rapid renal clearance and from degradation (e.g., by hydrolysis or enzymatic degradation) during storage or blood circulation, increasing therefore their circulation half-life. Finally, they can be made responsive to specific external stimuli such as pH, temperature, or specific molecules by exhibiting changes in the gel volume and water content ("swelling"), colloidal stability, mechanical strength, and/or other physical/chemical properties;[6] this enables controlled and precise

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release of drugs or therapeutics. For all these reasons, nanogels are indeed often used in advanced applications such as targeted drug delivery, biomedical imaging, and diagnostics. However, NGs share with nanoparticle a relatively low translation: e.g., despite extensive research in the last decades, only 15 nanoparticle-based pharmaceuticals for cancer treatment are currently on the market [7]. The bottlenecks in NP translation include limited scalability, poor control over reaction parameters, extensive NP polydispersity, unsatisfactory batch-to-batch reproducibility, and large volumes of chemicals and therapeutics used. These issues affect encapsulation efficiency and release profiles, hindering optimal treatment performance [8]. Moreover, NGs usually suffer of a low mechanical and biological stability under physiological conditions, due also to physical interactions, since they are often "softer" than other kinds of NPs.

Nanogels can be composed of a variety of natural or synthetic polymers, depending on the specific applications and desired properties. Natural polymers include, e.g.,: (i) chitosan, derived from chitin, known for its biocompatibility, biodegradability and antimicrobial properties; (ii) alginate, extracted from algae, prized for its ability to form gels in the presence of divalent ions such as calcium; (iii) hyaluronic acid, a natural component of the extracellular matrix, chosen for its ability to retain water very efficiently. Among synthetic polymers, polyacrylamide is used for its ability to form strong gels and for the ease of derivatization due to the presence of the amine group, while poly 2-hydroxyethyl methacrylate (Poli(2-HEMA)), known for its biocompatibility and transparency, is often used in contact lenses and medical devices. Poly(N-isopropylacrylamide) (PNIPAM) is a thermoresponsive polymer useful for controlled drug release, featuring a lower critical solution temperature (LCST) of around 32°C in water: it forms temperature-sensitive gels that swell or shrink in response to temperature changes. Polyvinyl alcohol (PVA), chosen for its biocompatibility and solubility in water, forms tough films and gels,[9] while poly(ethylene glycol) (PEG) is used to improve biocompatibility and reduce immunogenicity of nanogels. Polyglutamic acid (PGA), a biodegradable and biocompatible polymer, is utilized for its ability to enhance the stability and drug-loading capacity of nanogels [10]. Nanogels are commonly classified according to the types of bonds involved in forming the polymer network, their stimulus-responsive capabilities[11], routes of administration[12] and applications[13].

In this review, we will mainly focus on the synthesis methods of nanogels, highlighting their strengths and weaknesses; we will also describe synthesis protocols based on flow chemistry, in particular on microfluidics, since this can help in solving some of the bottlenecks for NPs translation in clinics mentioned above. In addition, we will illustrate the various applications of nanogels in different fields while also presenting a brief analysis of possible challenges for clinical translation (Figure 1). Focusing mostly on relevant research from the past five years, this review aims to provide a comprehensive overview of the topic, highlighting current challenges and offering insights for researchers to overcome them.

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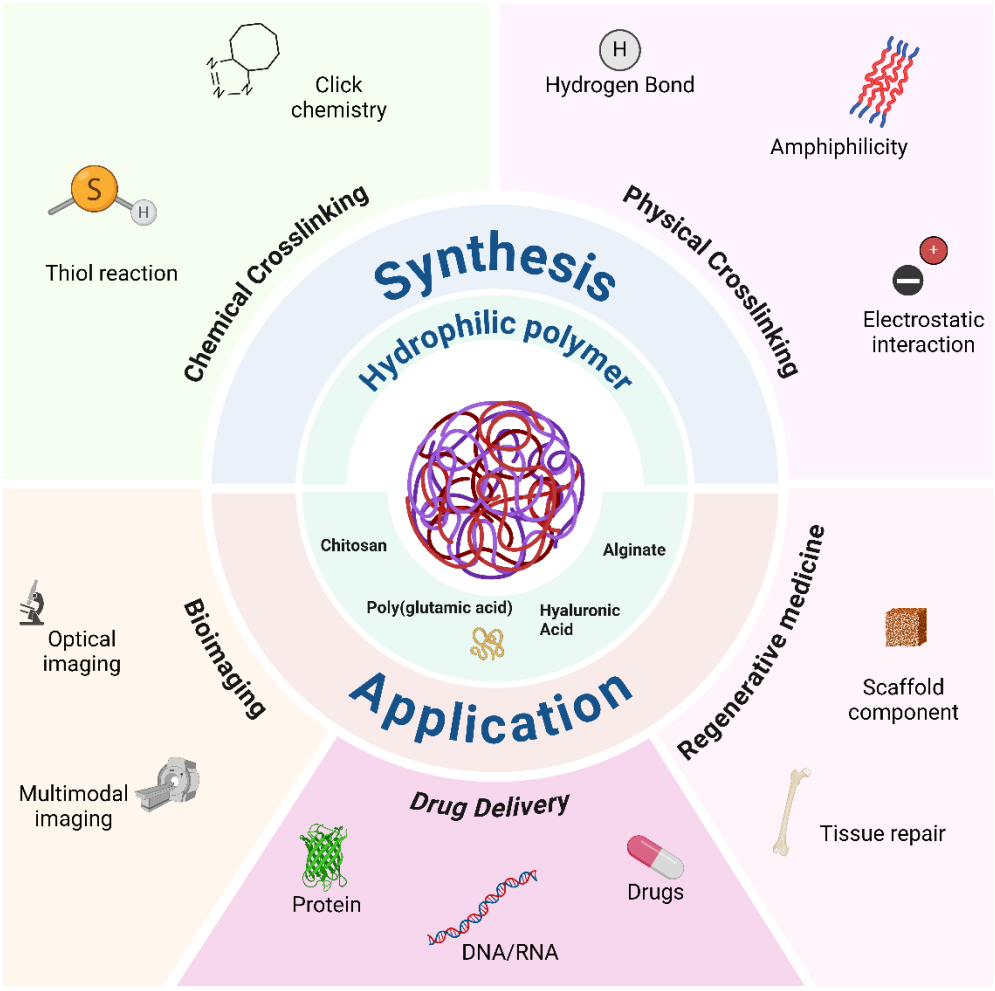

Figure 1. Schematic synopsis of possible synthesis routes for, and applications of, nanogels.

2. Batch Synthesis

In the last several years, various techniques for creating nanogels have been discovered. NGs can be made via simultaneous polymerization and crosslinking or by polymerization first and subsequent crosslinking, depending on the used starting ingredients. There are several methods to synthesize them, such as ionic gelation, emulsion polymerization, precipitation polymerization, inverse nanoprecipitation, self-assembly and (micro)template-assisted polymerization [14,15]. For NGs, many polymerization processes can happen in an aqueous environment, due to the water solubility of most of the monomers and crosslinking agents used for the formation of nanogels.

Precipitation polymerization and reverse emulsion polymerization are the most widely used techniques based on simultaneous cross-linking and polymerization for creating nanogels. Precipitation polymerisation is a method in which soluble monomers are polymerised in a solvent in which the resulting polymer is insoluble. This process leads to the formation of polymer nanoparticles or nanogels through several key steps. Initially, the (multifunctional) monomers and a cross-linking agent are dissolved in a suitable solvent. A polymerisation is then initiated, usually by a thermal or chemical initiator, which leads to the formation of free radicals. These radicals initiate polymerisation of the monomers, and the cross-linking agent creates bonds between the polymer chains, forming a three-dimensional network. As polymerisation proceeds, the growing polymer chains become insoluble in the solvent and precipitate, forming nanogels. The size and structure of the nanogels can be controlled by adjusting the monomer concentration, the amount of crosslinking agent and the polymerisation conditions, such as temperature and time; in particular, controlled radical polymerization techniques, such as atom transfer radical polymerization (ATRP) and reversible addition–fragmentation chain transfer polymerization (RAFT), can slow the reaction rate

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to promote the formation of uniform particles [13,15,16]. For instance, Ribovski *et al* [17]. used precipitation polymerization to produce fluorescently tagged PNIPAM nanogels, with the degree of polymer crosslinking determining the nanogels hardness. In another example, Kusmus *et al* [15]. described the development of versatile epoxide-functional precursor nanogels via controlled crosslinking polymerization; in a subsequent post-formation step, the epoxide moieties were functionalized with various amines, azides and thiols, as well as hydrolyzed to the corresponding diol.

Nanogels can also be synthesized using appropriate emulsification techniques with an oil-soluble emulsifier. Reverse emulsion polymerisation, also known as water-oil emulsion polymerisation, involves the dispersion of an aqueous phase containing monomers and a cross-linking agent in a continuous oil (or organic) phase (immiscible with water), with the aid of a surfactant. Polymerisation takes place within the dispersed water droplets, with the monomers reacting to form cross-linked polymer chains, leading to the formation of nanogels[18]. The concentration of monomers and crosslinkers, the pH of the reaction medium, the choice of surfactant, and other parameters significantly influence the size of the nanogels formed during reverse emulsion polymerization. The drawbacks of this method include the use of an organic solvent as the reaction medium and the difficulty in purifying the resulting nanogels due to the presence of emulsifiers and co-emulsifiers.

While the simultaneous approach is widely adopted, nanogels can alternatively be synthesized through two sequential steps: polymerization and crosslinking. This sequential approach offers enhanced control over nanogel properties and functionalities, a topic that will be explored further in subsequent sections. In terms of post-polymerization crosslinking, nanogels are categorized into chemically crosslinked or physically self-assembled types: the first type rely on covalent bonds amongst different polymers, while the second one on non-covalent interactions amongst them. However, many nanogels incorporate both chemical and physical connections in their formation. Thus, this section will elaborate on these interactions and showcase examples of nanogels formed through these mechanisms. These diverse synthesis techniques provide versatile tools for tailoring nanogels with specific properties, making them highly adaptable for applications in drug delivery, diagnostics, and tissue engineering within the biomedical field.

2.1. Physical Methods

Physical methods for synthesizing nanogels rely on spontaneous interactions and arrangements of their components, resulting in rationalized, self-organized, and self-assembled supramolecular structures without covalent bonds. Despite having lower mechanical strength than covalently bonded systems, these physically constructed nanogels are favored because they do not require additional crosslinking agents or polymerization initiators, enhancing their safety and biocompatibility. The primary forces driving the formation of these entangled polymeric materials, including NGs, are often called host-guest interactions, and can include hydrogen bonds, electrostatic interactions, van der Waals forces, π-π stacking (interactions among aromatic rings), and hydrophobic interactions.

2.1.1. Electrostatic Interactions

Nanogels with electrostatic interaction are created by the attraction between molecules with opposite charges, which facilitates their self-assembly and stability. These structures are typically constructed using polymers containing ionizable or ionic functional groups such as carboxylates, amines or quaternary ammonium ions. Polymers commonly used to create nanogels through electrostatic interactions include chitosan[19], alginate[20], hyaluronic acid (HA)[21] and poly(glutamic acid) (PGA)[22]. The synthesis of nanogels by physical cross-linking involves a simple and efficient procedure, usually conducted in an aqueous environment where the polymer chains are completely solvated and initially non-strongly interacting. When conditions are changed, e.g., by changing temperature, pH or ionic strength, the polymer chains begin to interact and form cross-links. For example, pH adjustment can protonate or deprotonate certain polymer functional groups,

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causing them to attract each other and form ionic cross-links. Another type of synthesis based on electrostatic interaction is ionic gelation, based on the use of polyelectrolytes forming cross-links in the presence of ions; it is a simple and rapid synthesis, but it is difficult to control given the very rapid assembly of polymers and crosslinkers, causing batch to batch variations in physiochemical properties such as particle size, polydispersity, surface charge and drug release profiles.

In general, electrostatic interactions cause the polymer chains to organize themselves into a nanoscale gel structure, effectively trapping water within the network. This straightforwardness not only simplifies production, but also makes the resulting nanogels ideal structures for biomedical applications, with reduced risk of toxic effects and an improved biological safety profile due to their synthesis without chemical cross-linking, which often requires non-biocompatible crosslinkers. One of the remarkable advantages of physically cross-linked nanogels is their adjustable size: by adjusting parameters such as polymer concentration, ionic strength, temperature and pH during synthesis, researchers can precisely control the size of nanogels[23]. This tuning is crucial for adapting the properties of nanogels to the requirements of specific applications, such as drug delivery or tissue engineering. Furthermore, the ability to modulate the size of nanogels allows optimizing their pharmacokinetic profiles, cellular uptake and in vivo biodistribution. The electrostatically driven formation of nanogels allows for precise encapsulation of charged or polar drugs or biomolecules, which interacts with the charged groups in the nanogels components. This versatile system finds applications in encapsulating chemotherapeutic agents for improved treatment efficacy[24], and more generally in theranostics[25], where nanoparticles are formulated for simultaneous therapy and diagnostics. An example of synthesis of a drug-containing nanogel where also electrostatic self-assembly is involved is reported in Figure 2.

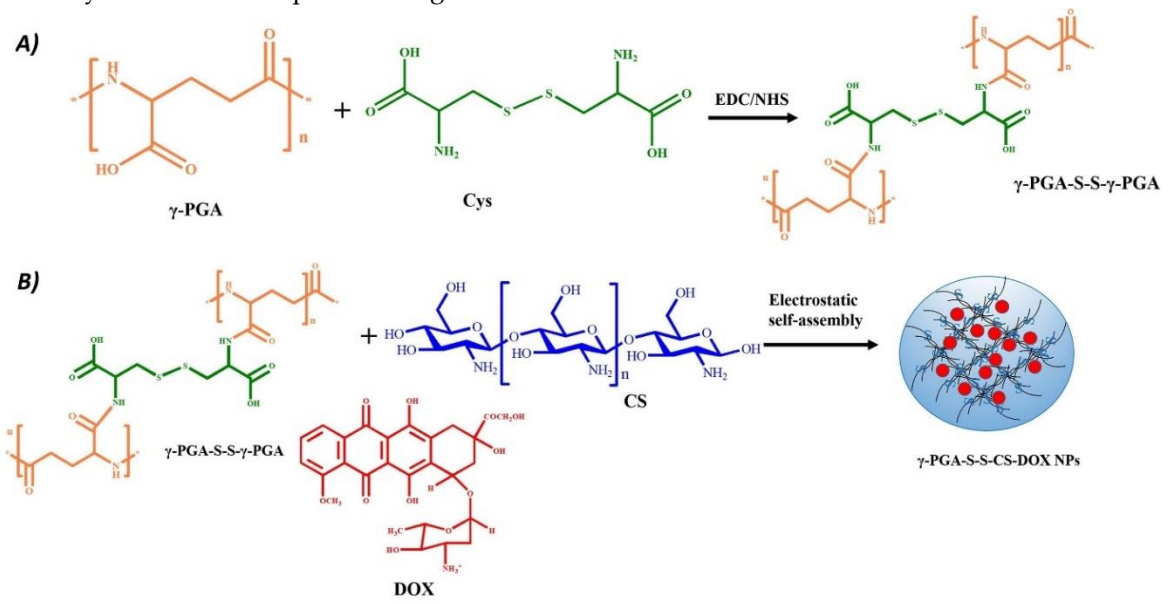

Figure 2. Schematic illustration of the synthesis of a nanogel using also electrostatic self-assembly. A) Polyglutamic acid (PGA) is covalently modified with cystine (Cys) to obtain a pH/redox responsive nanogel. B) The modified PGA is mixed with doxorubicin (DOX) and Chitosan (CS) in an aqueous environment to formulate nanogels by electrostatic self-assembly. Reprinted with permission from Ref. [22].

2.1.2. Amphiphilic Properties

Polymers composed by hydrophobic and hydrophilic parts are called amphiphilic and can be used in the generation of amphiphilic nanogels. The synthesis of amphiphilic nanogels involves the dissolution of amphiphilic polymers in an aqueous solution, in which the hydrophilic segments interact with water and the hydrophobic segments tend to avoid it. By changing environmental conditions, such as temperature, pH or ionic strength, the polymers self-assemble, with the hydrophobic parts aggregating; often the structure of these kinds of nanoparticles is constituted by a

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hydrophobic core surrounded by a shell of hydrophilic segments. Amphiphilic nanogels offer many benefits. They possess the ability to swell in aqueous and organic media due to the mixture of hydrophilic and hydrophobic compounds. However, they exhibit a lower propensity to swell in water than nanogels consisting exclusively of hydrophilic polymers, consequently ensuring superior mechanical properties. In addition, they offer greater thermophysical and structural stability. In this formulation, the fundamental characteristics of the colloidal structure are driven by the primary structure of the polymer, including its composition, molecular weight, and branching. The type and abundance of hydrophobic groups along the polymer chains, which act as physical bonds, are particularly important, thus defining the network formation in the nanogel structure[26]. Moreover, this formulation facilitates the encapsulation of poorly water-soluble payloads: the cargo loading capacity and release kinetics are set by the interaction between the hydrophobic cargo and the hydrophobic nanodomains[27]. In addition, the flexible hydrophilic matrix allows to control the mechanical properties of the nanogel and to reduce the potential toxic effects of the hydrophobic groups. The regulation of these interactions can be achieved by varying the type and content of hydrophobic side groups on the polymer. This allows precise control over loading and release profiles[28]. Consequently, the ability to finely tune the hydrophobicity of the nanogel, or the hydrophilic/hydrophobic balance, will open new therapeutic options for various administration routes[29]. The amphiphilic structure offers the opportunity to regulate and tailor their interactions with biological systems; for example, Bewersdorff et al. produced amphiphilic nanogels with more or less idrophobic groups on their surface, and they were therefore able to control the protein corona formation and modulate interactions with biological barriers[30]. Even if the self-assembly strategy offers considerable versatility, counting exclusively on physical bonds can be restrictive. To overcome this limitation, reactive groups can be incorporated into amphiphilic copolymers, facilitating covalent cross-linking after the self-assembly process (Figure 3). This method stabilizes the properties of the nanoparticles, resulting in functional amphiphilic nanogels whose network characteristics can be precisely controlled by a combination of hydrophobic physical interactions and covalent cross-links. Covalent cross-linking of these self-assembled systems can be achieved through two main synthetic approaches. One incorporates all the necessary reactive groups directly into the amphiphilic copolymer. The other involves the reaction of the reactive copolymers with (bi)functional crosslinkers. These strategies, which employ crosslinking agents and chemical synthesis, will be discussed in detail in the following sections.

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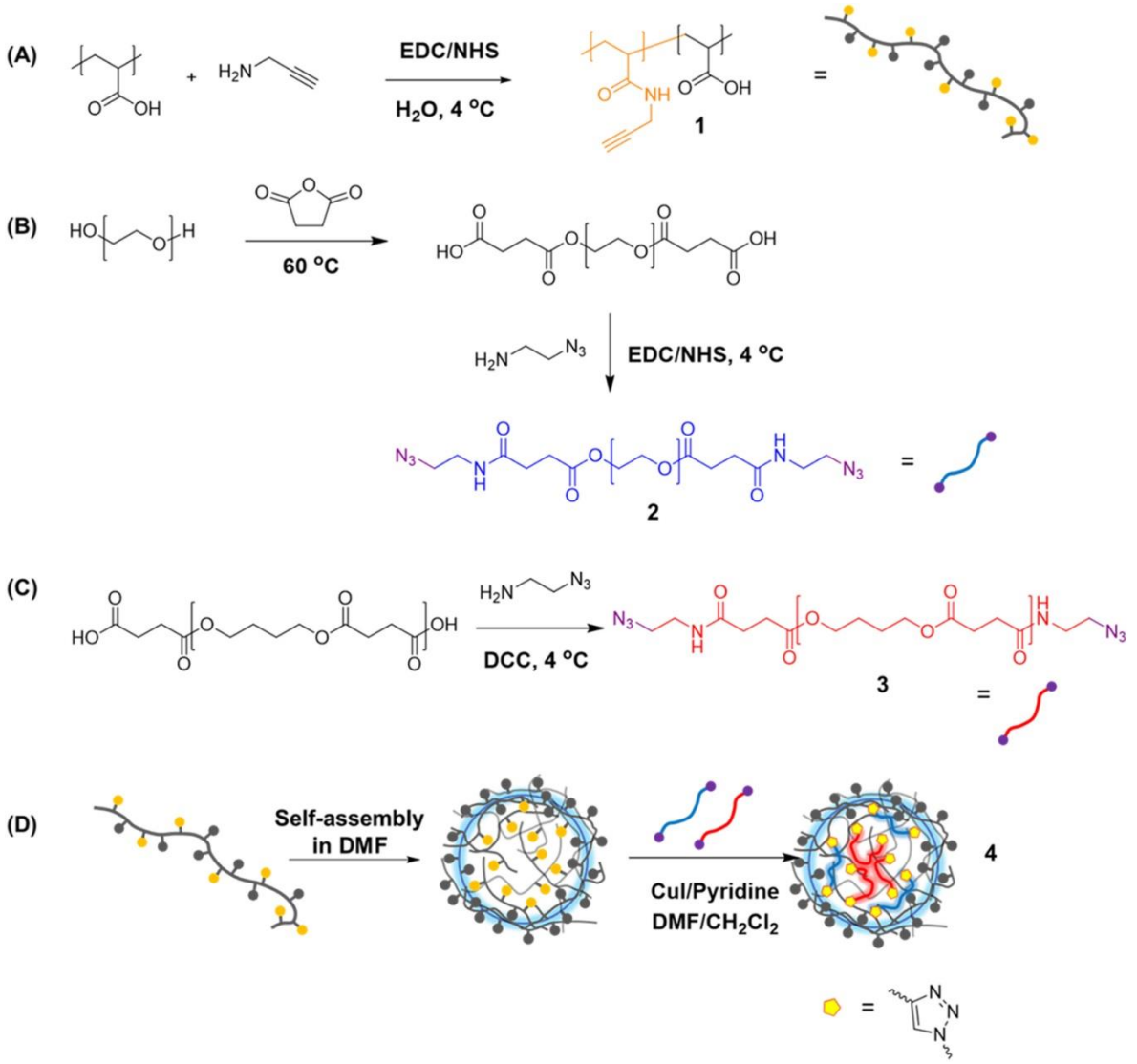

Figure 3. Illustration of the synthetic route for the preparation of random amphiphilic copolymers using poly(acrylic acid) (PAA) modified with alkyne groups (A) and their macromolecular crosslinkers poly(ethylene glycol) (PEG)(B) and poly(butylene succinate) (PBS) (C) modified with azides. Amphiphilic nanogels (D) were created by hydrophobicity-driven self-assembly of the copolymers (A) and subsequent crosslinking with a mixture of hydrophilic (B) and hydrophobic (C) crosslinkers, resulting in amphiphilic nanogels with biodegradable and/or water-soluble crosslinkers. Reprinted with permission from Ref. [31].

2.2. Chemical Methods

Covalent interactions in the synthesis of nanogels can provide essential stability and functional properties. Various chemical reactions are employed to form these bonds, including free radical polymerization, click chemistry, disulfide bond formation, and carboxyl-amine reactions. Each of these reactions offers distinct advantages in terms of specificity, efficiency, and stability, helping to ensure the integrity and functionality of the network under physiological conditions.

2.2.1. Covalent Crosslinking Reaction

Nanogels formulated using covalent crosslinking techniques have stable chemical bonds between polymer chains forming a three-dimensional network that can retain water without dissolving. By selecting appropriate polymers and crosslinking agents, the properties of the nanogels can be tailored for specific functionalities, such as pH sensitivity, temperature responsiveness, or

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redox responsiveness. The incorporation of various functional groups during the crosslinking process also enhances the versatility of chemically crosslinked nanogels, making them suitable platforms for a wide range of biomedical applications. Both the choice of the starting materials and the particle formulation methods (e.g., microemulsion[32], precipitation) can be tailored to effectively shape and stabilize the nanogel three-dimensional structure. In a study by Tian et al [33]., the initial fabrication of a nanogel exploited a modified emulsion crosslinking technique: poly(ethylene glycol) diglycidyl ether acted as a long binding agent, linking hyaluronic acid (HA) chains within the emulsions to create a loose nanogel structure. Next, cystamine was introduced as an additional binding agent between HA chains via an EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide) reaction to create a more compact nanogel. In this way, the nanogel became reactive to the glutathione (GSH) present in a tumor environment and in particular in the cytoplasm of cells: GSH breaks the disulfide bridge in the cystamine, reverting the nanogel to a loose structure with consequent release of the drug loaded within.

The formation of amide bonds, used e.g., for the reaction with cystamine mentioned above, is a common approach to chemical crosslinking: these bonds are typically formed from carboxyl and amine groups using carbodiimide chemistry or by generating activated esters. In the synthesis of nanogels, amide bonds can serve a dual role. They can act as crosslinking points within the nanogel network, contributing to its structural integrity. Moreover, they can function as linkers that facilitate the conjugation of drug molecules or other bioactive compounds to the nanogel structure. Apart from amide bonds, numerous other chemical bonds can be used for crosslinking in nanogel synthesis. These include disulfide bonds, which will be discussed later, and ester bonds formed by hydroxyl and carboxyl groups

2.2.2. Click Chemistry

Click-chemistry (Figure 4) involve reactions commonly used for joining two molecular entities of choice with very high chemical yield towards a single product; click-reactions are widely insensitive towards solvent parameters, oxygen, and water, and happen in mild (e.g., physiological) conditions[34]. Typically, these reactions take place in an aqueous environment, ensuring compatibility with sensitive biological components. Furthermore, these reactions evolve according to a mechanism, called regiospecificity, whereby one of the possible functional isomers is generated preferentially, if not exclusively. Click chemistry is widely used in nanogel synthesis due to its efficiency, specificity and ability to form stable covalent bonds. This approach has significant advantages in nanogel production, including short reaction times, higher productivity and improved purity. These characteristics make click chemistry an ideal method for tailoring nanogels with precise control over their properties. Reactions such as azide-alkyne cycloaddition and thiol-ene reactions are particularly useful for cross-linking polymer networks within nanogels. Azide-alkynic cycloaddition, which includes variants such as Cu(I)-catalysed (CuAAC) and strain-promoted (SPAAC) reactions, involves the covalent binding of azide and alkynic groups to create 1,2,3-triazole bonds[34]. CuAAC offers high specificity and fast kinetics under mild conditions; however, the application of CuAAC is somewhat limited by the potential cytotoxicity of copper ions and their ability to generate reactive oxygen species (ROS), which can damage biomolecules; sometimes, it is possible to mitigate these effects by removing the copper ions. Duro-Castano et al. optimised CuAAC coupling conditions in aqueous solution prior to the preparation of polyglutamic acid-based NGs to achieve higher cross-linking efficiency using the minimum amount of catalyst. Furthermore, they were able to establish washing protocols that used acidic conditions to protonate the carboxylic acid groups and thus hinder their complexation with the remaining copper ions[35] (Figure 5). Copper-free click reactions, including SPAAC, which utilize strained alkenes like dibenzocyclooctyne (DBCO), generally involve bioorthogonal cycloadditions, which allows them to take place within living systems without interfering with native biochemical processes and are therefore widely applied in nanogel synthesis due to their biocompatibility[36]. For example, Nagel et al. reported a peptide-crosslinked nanogel in which dendritic polyglycerol (dPG) modified with bicyclononyne groups (BCN), to form dPG-BCN, was crosslinked by a matrix metalloproteinase (MMP)-sensitive

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peptide ligand modified with two azides[37]. These reactions, together with thiol-ene coupling, disulfide exchange reactions and Michael reactions, which will be discussed in detail below, highlight their exceptional suitability for nanogel preparation.

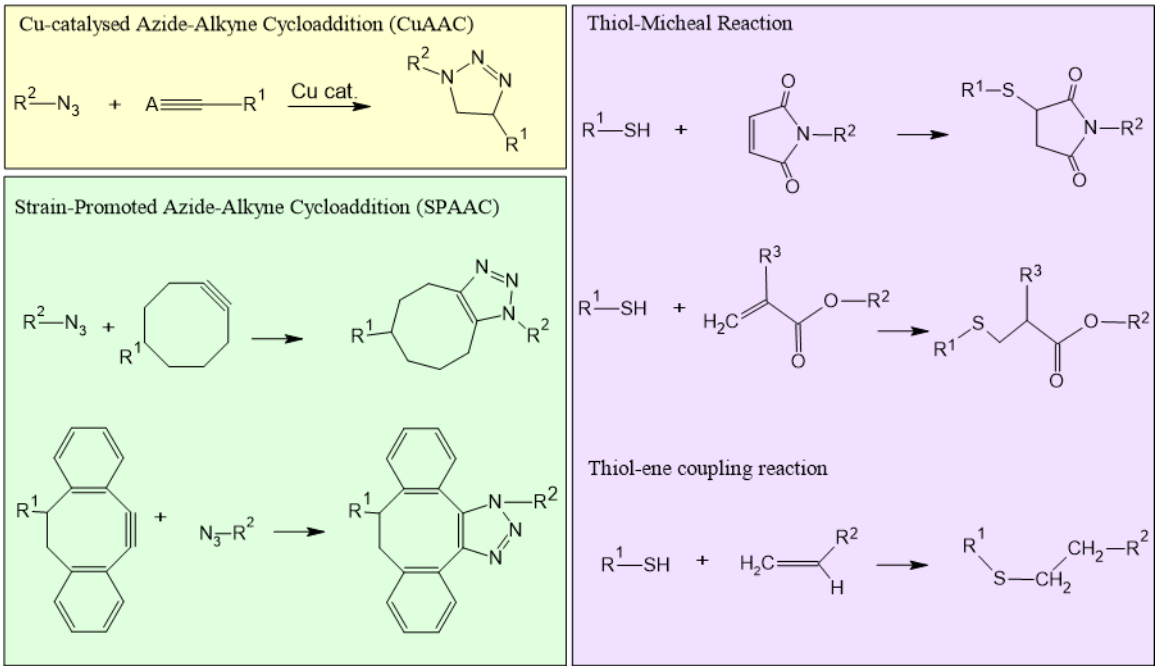

Figure 4. Schematic illustration of different types of click reactions. CuAAC in yellow, copper-free click reactions (SPAAC) in green and click-like reactions in violet.

2.2.3. Click-Like Reactions

Other reactions that are included in the category of "click chemistry" are thiol-click chemistry (Figure 4), which covers various thiol-based reactions, such as thiol–alkene and thiol–alkyne reactions, Michael addition, and disulfide exchange. Thiol-based reactions are advantageous due to their moderate orthogonality, allowing them to proceed in the presence of various functional groups without interference. They are highly reactive and easy to perform, typically under mild conditions, and they generally produce high yields. Their efficiency, undemanding reaction conditions and high specificity make them particularly suitable for nanogel synthesis and other biomedical applications. In organosulfur chemistry, the thiol-ene reaction, also called alkene hydrothiolation, involves the reaction between a thiol (R-SH) and an alkene ($R_2C=CR_2$) to produce a thioether (R-S-R – note that "R" is a generic organic group, possibly different wherever it appears). Thiol-ene additions can occur through two mechanisms: free-radical additions and Michael-catalyzed additions. Free-radical additions can be triggered by light, heat or radical initiators, which generate thiyl radicals, while thiol-ene-Micheal addition is catalysed by a base or a nucleophile.

Thiol-ene-Michael addition is a significant member of the "click" chemistry family, which has many parallels with CuAAC and SPAAC reactions. However, a crucial difference is the natural presence of reactive groups. Azides and alkynes are absent in native biomolecules, allowing CuAAC to effectively functionalize only the particles in complex, living environments. In contrast, thiol-ene chemistry is very advantageous for conjugating or functionalizing colloids with biomacromolecules, such as proteins, due to the presence of thiols in cysteine-containing macromolecules. It can be used for the complete cross-linking of nanogels during their synthesis, but, to a reduced extent, also for the surface functionalization of nanogels. These functionalized nanogels can then be employed in various fields, including biosensing, bioimaging, drug delivery, and theranostics[38–40]. Among the most efficient Michael-type additions, there are the reactions between thiols and maleimides. The primary driving forces for this reaction are the electron-withdrawing effect of the two adjacent activating carbonyl groups and the release of ring strain upon product formation. The reaction

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between maleimides and thiol-containing biological molecules has been employed since 1949 [41]. However, it was not until 1980 that thiol-maleimide reactions were recognized as potential tools for nanocarrier functionalization. The maleimide-thiol reaction is widely used in functionalization protocols due to the high reactivity of maleimides under mild conditions, their selectivity for thiol groups at physiological pH and the stability of the resulting thioether bond under physiological conditions. In addition to surface functionalization applications, the thiol-maleimide reaction is also used in the synthesis and modification of nanogels for various biomedical purposes[42,43] (Figure 5). E.g., Altinbasak et al. prepared a nanogel system cross-linked through the Michael thiol-maleimide addition reaction, which can be degraded in a reducing environment through a thiol-disulfide exchange reaction [44]. A significant disadvantage in this reaction is the potential hydrolysis of maleimide groups in aqueous solutions, resulting in the formation of maleamic acid, which inhibits reaction with thiols. This secondary reaction can significantly reduce the degree of functionalization and negatively affect the properties of the resulting system overall. Disulfide cross-linking, a reaction similar to the other reactions discussed so far, is also a commonly used method in nanogel synthesis, which offers unique advantages for various applications. In this approach, disulfide bonds (-S-S-) are formed between polymer chains having thiol moieties, resulting in a three-dimensional network structure. In addition, the cleavable nature of disulfide bonds in response to certain stimuli, such as glutathione or reactive oxygen species (ROS),[45,46] enables the controlled release of encapsulated therapeutic agents within target cells or tissues. This responsiveness to environmental stimuli enhances the therapeutic efficacy and biocompatibility of disulfide-crosslinked nanogels, making them promising candidates for various biomedical applications[47–49].

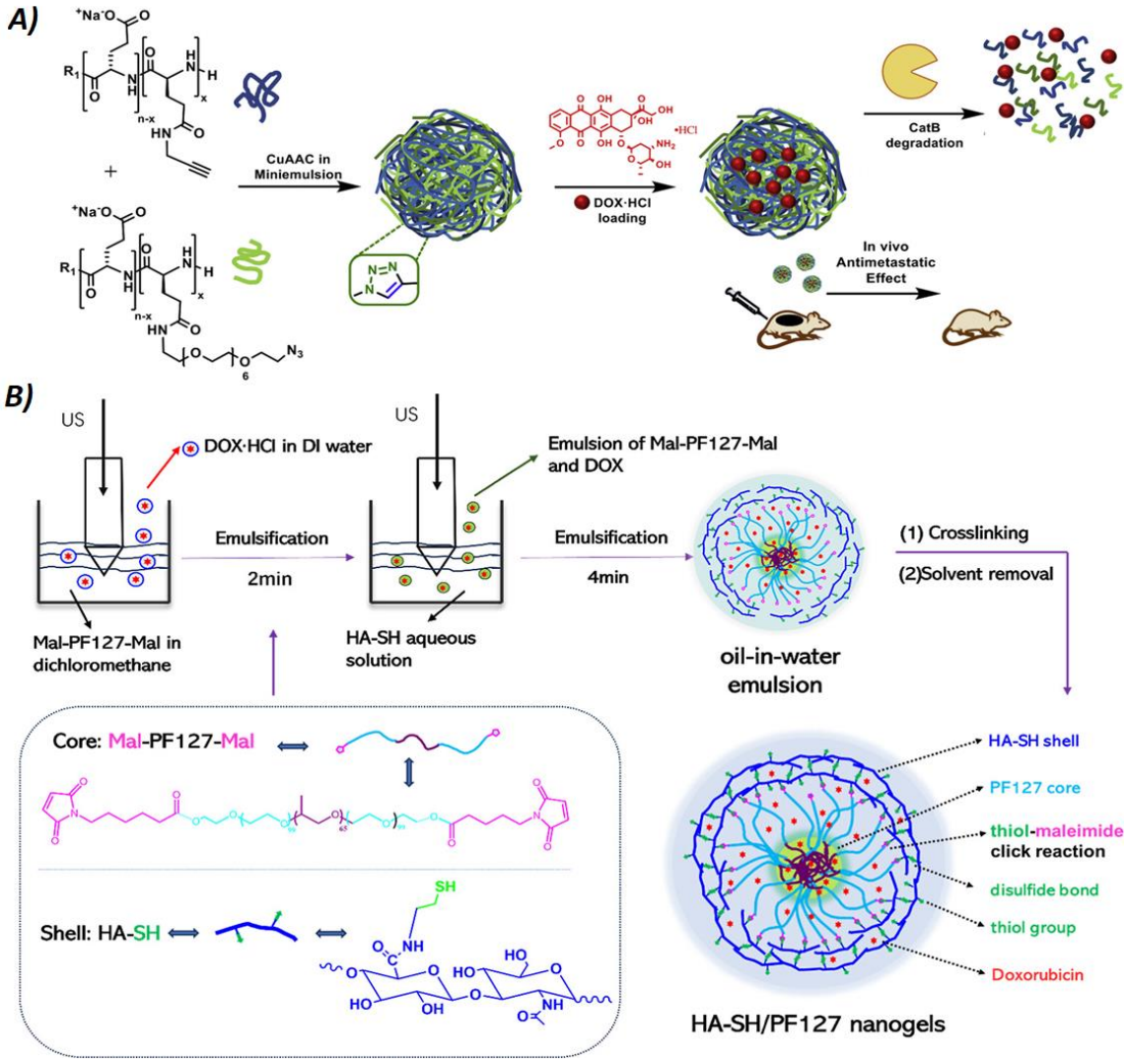

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Figure 5. A) Synthesis and mode of action of bioresponsive polyglutamic acid nanogels: nanogels are obtained by miniemulsion of azide- and alkyne-modified polyglutamic acid (blue and green filaments, respectively) cross-linked by click reactions (CuAAC) and then loaded with Doxorubicin (DOX). The image also shows the degradation mechanism mediated by Cathepsin B (CatB), a lysosomal enzyme that is overexpressed in the stroma of some types of tumors. Reprinted with permission from Ref. [35]. B) Illustration of the preparation of mucoadhesive hyaluronic acid thiol (HA-SH)/pluronic acid (PF127) nanogels by thiol-maleimide click reactions. Using an ultrasonicator probe (US), nanogels were produced by a double emulsion technique: first by sonicating maleimide-modified PF127 with doxorubicin, and then by adding in the second emulsion hyaluronic acid with thiol. Reproduced from ref. [43]. with permission from copyright © 2024, American Chemical Society.

3. Flow Chemistry Synthesis

All the synthesis routes described above can be used with flow chemistry. In flow chemistry, also known as continuous processing or continuous flow chemistry, two or more streams of different reagents are pumped at specific flow rates into a chamber, tube, or microreactor. A reaction occurs, and the flow containing the resulting compound is collected at the outlet. To generate the final product, the solution can also be directed to subsequent loops of continuous reactors [50–52]. This route offers significant benefits over traditional batch chemistry, including enhanced mass and heat transfer, improved safety and reproducibility, increased reaction efficiency, reduced waste, and better scalability [53]. Due to the intrinsic characteristics of continuous-flow reactors, it is possible to exploit reaction conditions not achievable in batch processes, allowing for precise control over reaction conditions and real-time monitoring, resulting in high-quality products and streamlined processes. Advancements in 3D-printing flow setups and affordable electronic toolkits have made flow chemistry more accessible [54]. Continuous manufacturing, often coupled with photochemistry and photocatalysis, improves performance and safety while reducing costs [55–57]. Key challenges include solvent compatibilities and byproduct formation, necessitating inline analysis and purification strategies. Optimizing mass and heat transfer is crucial in flow chemistry: efficient mass transfer correlates with mixing efficiency, while heat transfer can be optimized by a high surface-to-volume ratio and large heat exchange surfaces in microchannels. Efficient heat transfer allows for isothermal or superheated conditions, improving chemical selectivity and safety. Small reactor volumes and precise reaction conditions provide, beside of better mass and heat transfer, also an increased safety; hazardous or impractical reactions under conventional conditions can be safely conducted in flow conditions [58]. Flow chemistry enables the use of lower reagent amounts, reducing costs and environmental impact. Scale-up can be achieved through numbering up or sizing up, maintaining the benefits of microreactor environments [59,60].

3.1. Microfluidics Systems

Several flow chemistry-based devices have been developed and optimized, and among them microfluidics stand out to be one of the most promising one. Microfluidics technology is based on fluidic circuits where the channels have lateral dimensions of ten to hundreds micrometers, and aims at miniaturizing the manufacturing nanoparticle production (Figure 6). Compared to bulk synthesis, this approach leads to narrower size distribution, higher encapsulation efficiency, sustained drug release profiles, and highly controlled synthesis conditions. Moreover, in the photochemistry case, the small lateral dimensions of the microchannels allow a better and more homogeneous illumination even when molecules with high extinction coefficients are present. Additional benefits of microfluidics include low variation between batches, high throughput, reduced reagent volumes, low instrumental footprint, and scalable production yields. For these reasons, microfluidics is deemed a promising approach for the design of advanced micro- and nanoparticles and micro- and nanogels [61]. Microfluidic approach can be employed for the production of several types of nanoparticles, from polymeric to lipidic to inorganic ones, by changing the microfluidic set-up and mixing parameters [62]. E.g., for polymeric nanoparticles in general, several production strategies can be used exploiting microfluidics, like: (i) nanoprecipitation, with the mixing time between solvent and

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non-solvent phases more easily tweakable for a better control of nanoparticle nucleation and growth; (ii) self-assembly, which leverages the pH- or temperature-responsive behavior of amphiphilic derivatives; (iii) droplet-based methods, which adopts immiscible phases to create flowing micro-emulsion droplets that act as micro-reactors. While nanoprecipitation and self-assembly methods are prone to channel clogging due to undesired reagent interactions, droplet-based approaches provide rapid heat and mass transfer, enabling faster reaction kinetics and precise control over droplet size and composition [63].

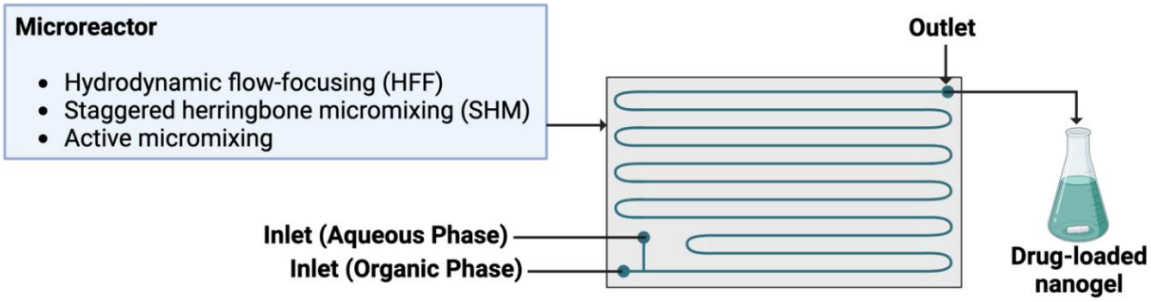

Figure 6. Schematic diagram of the most simple microfluidic experimental setup.

Common microfluidic designs are hydrodynamic flow focusing (HFF) and staggered herringbone micromixer (SHM) [62]. HFF exploits the laminar flow regime typical of microfluidic platforms: a narrow stream of NP precursor (e.g., lipid or polymer dissolved in a solvent) flows parallel to an antisolvent (e.g., water or buffer) from two side channels. In this case, mixing occurs through diffusion due to the laminar flow [64]. In contrast, SHM induces micromixing through chaotic advection caused by structures on the microchannel floor or walls, achieving shorter mixing times at lower flow rates, reducing therefore dilution and synthesis times [65,66]. Such passive mixing technologies can be coupled with active methodology to reduce the mixing time, control the nanoparticle size, and increase the throughput; active micromixing utilizes external energy sources to enhance mixing by disrupting the laminar flow regime, leading to faster homogenization and producing smaller NPs with narrower size distributions [67,68]. In this contest, ultrasound are noteworthy as they are generally employed to boost nucleation rates and significantly reducing NP mean size and polydispersity compared to other passive synthesis [69–71].

However, the employment of microfluidics faces several challenges. A possible difficulty arises from the fact that many materials used for microfluidic devices, like polydimethylsiloxane (PDMS), swell upon contact with organic solvents, potentially adsorbing small molecules and affecting device structure and nanoparticle production efficiency [72]. Moreover, the strong dependence of nanoparticle properties on multiple experimental factors calls for the application of a proper design of Experiments (DoE). DoE, and in particular response surface methodology (RSM), is meant to identify critical variables and their interactions in order to predict the most suitable experimental conditions while minimizing costs and reagent waste [73–75].

3.2. Nanogels Produced through Microfluidics

Various methods for producing NGs via microfluidic platforms have been explored:[13,16,76] (i) chemical gelation, involving emulsifying low-viscosity monomer solutions, followed by photoinduced cross-linking or polymerization, resulting in mechanically strong microgels; however, without proper optimization, these particles may not be easily enzymatically cleaved or metabolized, and photo-irradiation can be harmful to encapsulated biological species. (ii) Gelation induced by temperature changes after emulsification: e.g., heated agarose solutions can be cooled to form micro- or nanogels; this approach has challenges in maintaining temperature differences across the microfluidic device and potential harm to bioactive species. (iii) Coalescence-induced gelation: this strategy involves the coalescence of biopolymer droplets with crosslinking agent droplets, as demonstrated with alginate hydrogel microbeads; the productivity depends on droplet collision

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probabilities. (iv) Reversible shear thinning: droplets are formed from shear-thinning polymers that restore their network structure after emulsification to form microgels. (v) Internal gelation: droplets contain a gelling polymer and a bound crosslinking agent; a compound in the continuous phase diffuses into the droplets, releasing the crosslinking agent and causing gelation; (vi) external gelation: droplets of gelling polymer are emulsified in a continuous phase containing a crosslinking agent. Diffusion of the agent into the droplets causes gelation.

These kinds of techniques were initially used to produce microgels, and some of the optimizations carried out in those cases, and the applications of those microgels, can be considered also for NGs. E.g., Zhao et al [77]. developed an injectable scaffold using droplet-based microfluidic technology and photo-crosslinking to synthesize bone marrow stromal cell (BMSC)-laden microgels; with a similar technique, Feng et al [78]. engineered multifunctional microgels with a precursor suspension containing kartogenin-loaded cyclodextrin nanoparticles (KGN@CD NPs), BMSCs, gelatin methacryloyl (GelMA), and phenylboronic acid-grafted methacrylate hyaluronic acid (HAMA-PBA). These microgels were then assembled using dynamic crosslinking between dopamine-modified hyaluronic acid (HA-DA) and phenylboronic acid groups on the surface of the microspheres. Upon injection into a cartilage defect, HA-DA facilitated adhesion to native tissue, and the microporous microgel assembly along with sustained KGN release promoted BMSC chondrogenesis and cartilage repair [78]. Seiffert and Weitz[79] used microfluidic devices to produce monodisperse poly(N-isopropylacrylamide) (pNIPAAm) microgels via a polymer-analogous crosslinking reaction, achieving higher crosslinking efficiency and greater homogeneity compared to classical free-radical crosslinking copolymerization techniques. Zhang et al [61]. prepared droplets-formed sodium alginate biomicrogels (hydrogel microbeads derived from biopolymers), whose synthesis is usually done in two-stage: emulsification followed by gelation of the resulting droplets by chemical or physical crosslinking of the biopolymer. They generated stable alginate nanogels through Ca^{2+}-mediated crosslinking, after having compared external and internal gelation in their microfluidic preparation. They showed that internal gelation had a limited application in this field, as it did not allow control over morphology and the resulting microgels were soft and not colloidally stable. In contrast, external gelation produced stable microgels with a good control of the structure [61].

Microfluidic synthesis of nanogels has demonstrated significant improvements in control and efficiency.[16,76,80–82] E.g., Bazban Shotorbani et al [83]. synthesized alginate nanogels via ionic gelation using hydrodynamic flow-focusing microchips, achieving precise size control and monodispersity. Mahmoudi et al [84]. fine-tuned the flow rate ratio on microchips to form alginate nanogels, while Huang et al [85]. created hyaluronic acid nanogels via photo-click cross-linking on a microchip platform. Majedi et al [86]. utilized modified chitosan on a T-shaped microfluidic chip, and Chiesa et al [87]. formed chitosan/sodium triphosphate (TPP) nanogels using a staggered herringbone micromixer.

Pessoa et al [88]. used microfluidic microchips in order to attempt to mitigate fouling issues in the production of chitosan/ATP nanoparticles, while Whiteley and Ho[89,90] addressed these fouling challenges by creating a 3D flow-focusing profile on a coaxial flow reactor (CFR), producing nanoparticles with smaller size and higher monodispersity than 2D methods. They also tried to predict the interaction effects of process factors (component concentrations and flow ratio) on the size, PDI and encapsulation efficiency of the nanogels [89,90]. Giannitelli et al [1]. synthesized hyaluronic acid (HA) and linear polyethyleneimine (LPEI)-based nanogels for controlled doxorubicin delivery using a pressure-actuated microfluidic chip. The formation of NGs was confirmed by nuclear magnetic resonance (NMR) and Fourier-transform infrared (FTIR) analyses. NG specimens were also evaluated in terms of size and morphology through dynamic light scattering (DLS), atomic force microscopy (AFM), scanning electron microscopy (SEM) and tunneling electron microscopy (TEM) analyses, in order to define a correlation between the active tuning of the flow focusing geometry and the physical features of the resulting nanoscaffolds. The optimized DOX-containing nanogels demonstrated significant antitumor and anti-metastatic effects in vivo [1].

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4. Stimuli Responsive Nanogel

Nanogels for biomedical applications require stability under many conditions but can also be engineered to adapt and respond to external signals by altering their chemical and physical properties. Nanogels can respond to a variety of stimuli, including physical (temperature, light, ultrasound, magnetic/electric fields, pressure), chemical (pH, redox potential), and biological (enzymes, specific biomolecules) ones. Stimulus-responsive nanogels are promising nanoformulations that by their selective response to environmental signals improve therapeutic precision, offering versatile platforms for targeted therapies and diagnostics and minimizing systemic toxicity. Continued research into optimizing these nanomaterials has the potential to realize revolutionizing biomedical technologies, while also advancing precision medicine and patient care. Other reviews have described in more detail the stimuli-response properties of nanogels,[11,91,92] so only a brief introduction will be provided here.

Among the various stimuli, pH is one of the most commonly used in biomedical science: healthy tissues have a pH around 7.4, tumor tissues range from 6.5 to 7.0; within cells, the cytosol has a pH similar to blood (7.4), the Golgi apparatus is at about 6.4, endosomes range from 5.5 to 6.0, and lysosomes are the most acidic at 4.5 to 5.0. pH-reactive nanogels exploit these variations to deliver drugs in a targeted manner, minimizing off-target drug loss. These nanogels are typically synthesized by incorporating acidic[93] or basic functional groups into the polymer backbone or using cross-linking molecules that degrade under specific pH conditions[94]. This design enhances the swelling and degradation of the nanogel, promoting the release of the encapsulated cargo[95].

Redox-responsive nanocarriers show great promise for delivering payloads within cells by utilizing the natural redox gradient between intracellular and extracellular environments to trigger the release of encapsulated substances. The antioxidant glutathione (GSH) primarily regulates these redox potentials, and its cytosolic concentration in cancer cells is significantly higher than in normal tissues. This difference highlights the importance of redox-responsive drug delivery systems for targeted therapies[96]. Recent studies on nanogels have focused on incorporating disulfide-based crosslinking monomers to achieve precise control over degradation kinetics. This strategy optimizes the balance between crosslinking and the encapsulation/release of therapeutic agents, leading to the development of nanogels specifically aimed at intracellular release in antitumoral applications[44,97–99].

Recent years have seen significant advances in nanogel technology, particularly in the development of systems that can recognize and respond to multiple stimuli. This progress involves the integration of various response functions into a single platform, thereby improving the versatility, effectiveness, and/or specificity of nanogel-based therapies [100,101]. By incorporating two or more stimulus-responsive components within a single nanogel delivery system, researchers aim to achieve a higher level of precision in therapeutic applications[102]. These sophisticated nanogels can dynamically adjust their behavior in response to a combination of environmental stimuli such as pH, temperature, redox potential, and light exposure, enabling therapeutic payloads to be released in a controlled manner [103] (Figure 7).

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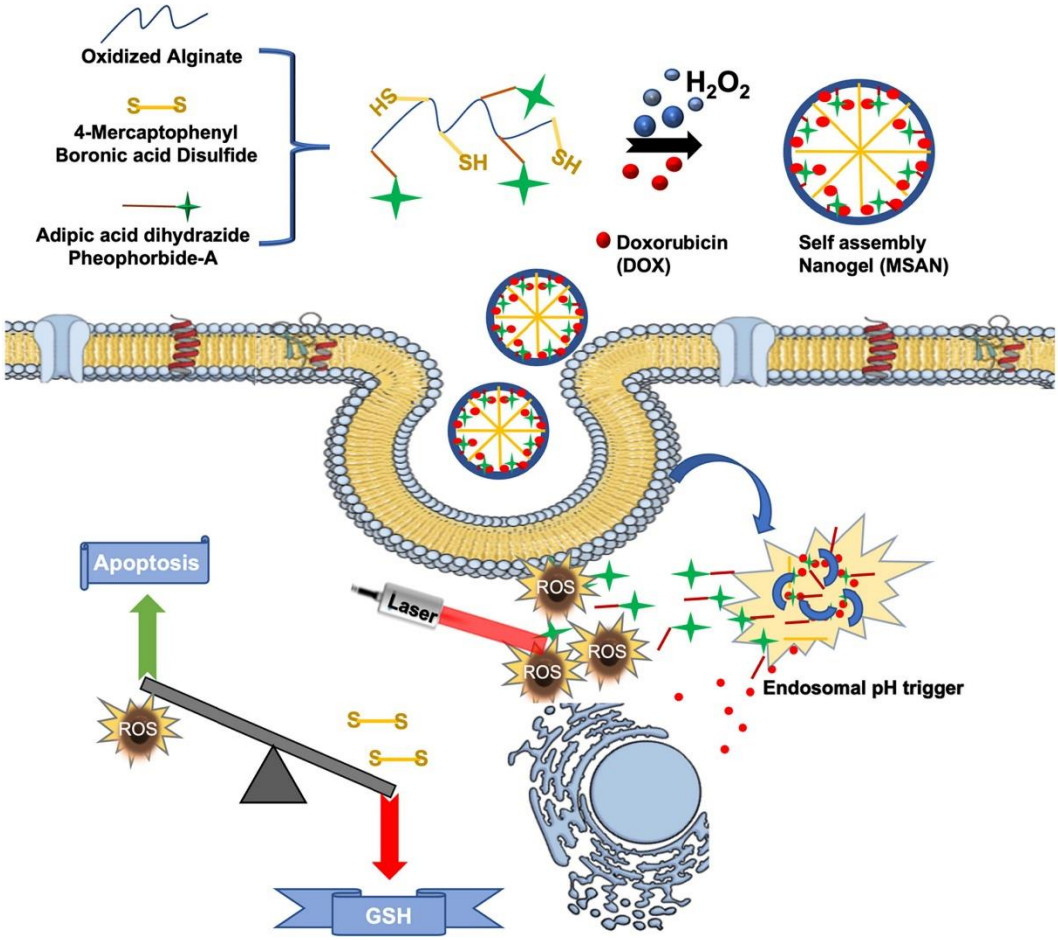

Figure 7. Schematic illustration of the redox-responsive nanogel (MSAN) for chemo-photodynamic therapy. Using a skeleton of Oxidated Alginate conjugated with 4-mercaptophenyl boronic acid and Pheophorbide-a (photosensitizing agent), the nanogels were formed by self-assembly in the presence of hydrogen peroxide (H_2O_2) and doxorubicin (DOX). The image also shows the mechanism of action within a cell with particle degradation triggered by acidic pH conditions or assisted by phototherapy. ROS: Reactive oxygen species; GSH: Glutathione. Reprinted with permission from Ref. [103].

5. Application

As already mentioned, due to their unique structural and functional properties, nanogels have acquired significant attention in biomedical applications. Their biocompatibility and ability to encapsulate drugs, proteins and other biomolecules allow for targeted and controlled release, minimising side effects and improving treatment efficacy. As already mentioned, the possibility of stimuli-responsive nanogels allows drugs to be delivered in a specific manner. NGs Cellular uptake and biodistribution behavior can also be controlled by different types of surface functionality: factors such as polarity and surface charge impact on the hydrophilicity of the nanogel and on its blood-circulation time. In recent years, nanogels have seen enormous developments in terms of design, optimisation, functionalisation and application. Therefore, in this section we will mainly focus on recently published biomedical applications.

5.1. Nanogels for Drug Delivery

Nanogel-based drug delivery systems have been very efficient in precisely delivering drugs to their target sites, significantly reducing toxicity to surrounding healthy cells. This remarkable potential has led to extensive research into their application for the treatment of diseases with high morbidity and mortality rates, with the aim of improving traditional therapies and patients' quality of life. Many nanogels have a high encapsulation efficiency and drug loading capacity, making them

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suitable for transporting both hydrophilic and hydrophobic drugs, including small molecules such as chemotherapeutic agents and inhibitors, as well as macromolecules such as proteins[104], DNA or RNA[105,106]. Depending on the route of administration, nanogels encounter various physiological barriers during drug delivery. This necessitates specific properties in the nanogels used, such as mucoadhesivity and mucopenetrativity for mucosal routes, clearance-avoidance systems for intravenous routes, or sophisticated mechanisms capable of crossing the blood-brain barrier. Several reviews[107–109] have examined these physiological barriers and the strategies of nanogels to overcome them, illustrating typical cases, and therefore we will not elaborate further on this topic. A big part of the research on nanogels for drug delivery focuses primarily on cancer therapy; nanogels designed for chemotherapeutic drug delivery play a crucial role in improving efficacy and reducing the side effects of cancer treatments. These particles are designed to encapsulate chemotherapeutic agents, protecting them from degradation and improving their delivery to tumor sites[110,111]. She et al [112]. developed a hypoxia-degradable zwitterionic phosphorylcholine nanogel (^{H}PMPC nanogel) using an azobenzene-based crosslinker (Figure 8). This nanogel could degrade under hypoxic conditions, triggering the collapse of its structure and the rapid release of the drug doxorubicin (DOX) into tumor tissues. As a result, the nanogel showed prolonged accumulation in glioblastoma tissues and effectively inhibited the growth of this highly malignant tumor. However, the heterogeneity of tumors and underlying limitations of some anticancer drugs can lead to incomplete eradication of cancer cells when using a single compound for treatment, with possible tumor recurrence. In contrast, combining multiple chemotherapeutic agents with different mechanisms of action, i.e., using a “drug cocktails,” can synergistically increase therapeutic efficacy. As a result, there has recently been an intensification of loading nanogels with multiple drugs[113,114]. For example, Zhang and his colleagues[115] designed a hyaluronic acid nanogel that can deliver doxorubicin due to its cationic nature. The nanogel uses cisplatin to cross-link its structure by binding it to the carboxyl (COOH) side-groups of hyaluronic acid. This cross-linking stabilizes the drug-loaded nanogel, preventing premature release during blood circulation. Besides focusing on specific diseases, many studies have created nanogel-based vectors for various applications, such as ocular administration; this innovative, noninvasive, and safer nanogel-based delivery method shows great potential for treating numerous conditions.

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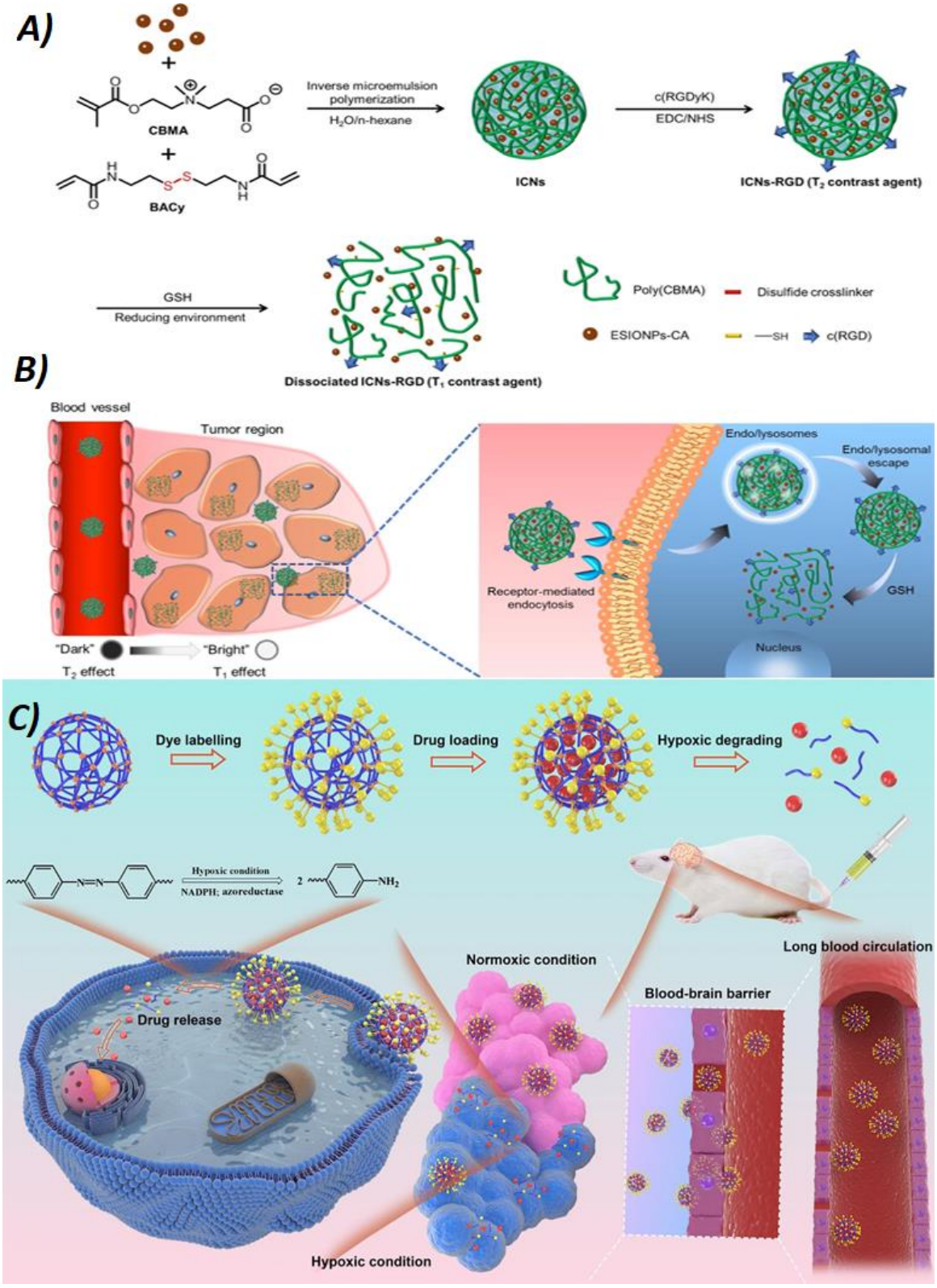

Figure 8. A) Scheme for the preparation of poly(carboxybetaine methacrylate) (CBMA) nanogel crosslinked with N,N-bis(acryloyl)cystamine(BACy), loaded with extremely small iron oxide nanoparticles (ESIONP) and modified on the surface with c(RGD) ligands, which bound $\alpha_v\beta3$ integrins that are overexpressed on the membrane of some tumour cells. These nanogels (ICNs-RGD) act as activatable MRI contrast agents with switchable function from a T2 contrast agent to a T1 one through the stimuli-responsiveness toward GSH. (B) Depiction of the utilization of ICNs-RGD to realize a precise tumor diagnosis based on the transformation from the T2 to T1 contrasting effect at the tumor region. C) Schematic illustration of the poly(phosphorylcholine)-based (HPMPC) nanogel

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with long blood circulation, blood–brain barrier (BBB) penetration and hypoxic controlled drug release for glioblastoma drug delivery. In blue: phosphorylcholine polymers; in orange: azobenzene-containing crosslinker (molecule shown on the left); in yellow: Cy5 dye with a linker; in red: Doxorubicin. Panels A and B reprinted with permission from Ref. [116] with permission from copyright © 2020, American Chemical Society. Panel C reprinted with permission from Ref. [112].

5.2. Nanogels for Bioimaging

Conventional imaging methods include various techniques used to visualize internal body structures for diagnosis, monitoring, and treatment of medical conditions. For example, computed tomography (CT) combines X-rays with computer technology to generate detailed three-dimensional images of organs, bones, and tissues, proving invaluable for visualizing anatomical details and evaluating trauma, tumors, and cardiovascular diseases. Magnetic resonance imaging (MRI) employs magnetic fields and radio waves to produce detailed images of soft tissues, brain, spinal cord, and joints, making it ideal for diagnosing neurological problems, musculoskeletal injuries, and central nervous system disorders. Despite their wide use and significant contributions to medical diagnostics, these methods have limitations. In fact, the low resolution and limited signal-to-noise ratio (SNR) of these imaging techniques have limited their wider application. Therefore, researchers have developed different contrast agents, i.e., substances that improve the visualization and definition of internal body structures by increasing the contrast between normal and abnormal tissues or between different types of tissues. This allows for clearer and more detailed images, aiding the diagnosis and monitoring of medical conditions. Gadolinium is a transition metal used as a contrast agent in imaging techniques such as magnetic resonance imaging (MRI). It is often bound to organic molecules to increase its solubility and stability in a biological environment, and sometimes to increase its concentration in certain tissues or cells. Due to its magnetic properties, gadolinium enhances the MRI-contrast between different tissues in the body, allowing more detailed and accurate images. Despite its effectiveness in improving diagnosis, it has been reported that gadolinium can accumulate in body tissues, especially in the brain, even in patients with normal renal function, although the extent and clinical implications of this phenomenon are still being studied and discussed. Among the various nanocarriers, contrast-agent-containing nanogels are particularly popular due to their high-water content and their ability to encapsulate a wide range of cargo[116,117] (Figure 8). Kimura et al [118]. developed ultra-small gelatin nanogels as contrast agents for MRI that cannot pass through the blood-brain barrier. They used a γ-radiation crosslinking technique for the formation of gelatin nanogels, and conjugated chelating agents to the protein nanogels to load gadolinium (Gd) and form gadolinium-coordinated gelatin nanogels (GdGN). In vivo studies confirmed the safety and effectiveness of GdGN as MRI contrast agents. GdGN were quickly eliminated via renal excretion within 90 minutes and did not cross the brain barriers. In their study, Shi[119] and colleagues synthesized (AuNP)-loaded γ-polyglutamic acid (γ-PGA) NGs using a dual emulsion method for CT imaging of tumors. Initially, γ-PGA was activated with 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (EDC) and then emulsified. Subsequently, polyethylenimine (PEI)-coated Au NPs [(Au0)200-PEI-NH2-mPEG]), synthesized by reduction of ($HAuCl_4$) with $NaBH_4$, were cross-linked in situ. In vivo CT imaging of the tumors showed that the γ-PGA-[(Au0)200-PEI-NH2-mPEG] nanoparticles effectively accumulated within the tumors and provided clear visualization of its site.

Multimodal imaging integrates multiple imaging techniques into a single procedure to provide complementary and detailed information about biological tissues. It combines modalities such as CT and MRI to enhance diagnostic sensitivity and specificity. This approach allows for a comprehensive assessment of anatomical structures and functional characteristics, aiding in early diagnosis, therapy monitoring, and understanding of pathophysiological processes. In the study of Sun et al [120]., PEI, partially modified with polyethylene glycol (PEG), was used to encapsulate AuNPs and load gadolinium through chelation. These alginate nanogels (AGs) were obtained by a double emulsion process where the PEI-Au-Gd particles acted as crosslinkers to crosslink the alginate exploiting the activated carboxyl groups. The AG/PEI-Au-Gd nanogels exhibited higher T1 relaxivity in MRI and

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greater X-ray attenuation in CT compared to PEI-AuGd nanoparticles and conventional iodinated contrast agents. Due to their enhanced cellular uptake relative to PEI-Au-Gd nanoparticles, AG/PEI-Au-Gd nanogels represent a dual-mode MR/CT imaging system for improved visualization of tumor cells in vitro and enhanced imaging of tumors in vivo.

5.3. Nanogels for Regenerative Medicine

Nanogels have emerged as a promising tool in regenerative medicine, offering unique advantages due to their nanoscale size, high water content and versatile structure. For this purpose, these hydrophilic polymeric networks are designed to encapsulate and deliver bioactive molecules like growth factors, cytokines and genetic material, in a controlled and targeted manner. The gels ability to mimic the natural extracellular matrix and provide a supportive environment for cell growth and differentiation makes them ideal candidates for tissue engineering and regenerative therapies. This adaptability increases their potential to facilitate the repair and regeneration of damaged tissues and improve the integration of implanted biomaterials.

A field of application for these systems is bone tissue regeneration, where osteoclasts and osteoblasts handle bone resorption and formation, respectively; this is an intricate process involving other possible cell types and numerous intracellular and extracellular signaling pathways, including cytokines and growth factors [121]. However, natural physiological mechanisms are insufficient for repairing large bone defects. The preferred treatment for critical bone defects is still autologous bone grafting, known for its osteoconductive (via bone fragments), osteoinductive (via growth factors), and osteogenic (via cells) properties. Nonetheless, this method often leads to chronic pain, infections, iatrogenic fractures, and suboptimal aesthetic outcomes. Nanocarriers can replicate the natural nanostructure of bone, potentially forming a precisely controlled porous microstructure that boosts osteoconduction[122]. Nanogel formulations can serve as injectable carriers for systemic or localized delivery of drugs or genetic material or be integrated into scaffolds to accurately host and release active substances during tissue growth and to adjust the scaffold's physical properties[84,123].

Another important application to consider is that of nanogels for wound healing. When the skin surface is damaged, timely restoration of its integrity becomes critical. The wound healing process takes place through stages of hemostasis, inflammation, proliferation, and remodeling. While minor wounds usually heal spontaneously in a few days, conditions such as diabetes, vascular insufficiency, or cancer can cause chronic wounds that resist normal healing. Delayed recovery of chronic wounds prolongs tissue regeneration, causing structural and functional damage. Due to their substantial water content, compatibility with natural extracellular matrices, ability to take desired shapes, and effective drug delivery capabilities, hydrogels have long been favored for wound healing. Nanogels inherit many of these advantages and have been designed for applications in managing bleeding and promoting wound healing[124–126]. For example, Zhang et al [127]. developed a novel nanocomposite consisting of chitin nanogels and rectorite (a mineral with hemostasis properties) for effective hemorrhage control. Chitin chains are intercalated into the rectorite, and vigorous mechanical agitation produces chitin nanogels. These nanogels assemble onto rectorite nanoplates through electrostatic interactions, forming a sandwich-like structure. In experimental models, the nanocomposite achieves hemostasis in 121 seconds in rat tail incisions and shows superior hemostatic activity compared with Celox, a commercial chitosan-based hemostatic, in rabbit artery injury models. The enhanced biocompatibility and hemostatic efficacy of the chitin/reptorite nanocomposite make it a promising and cost-effective option for hemorrhage management. In addition to the applications mentioned above, nanogels have also been studied for various other uses in regenerative medicine: cardiac repair[128,129], regeneration of ischemic limbs[130], coating of biointerfaces[131], etc. Despite the limited examples discussed in this review, extensive research in this area suggests that promising and encouraging applications of nanogels in regenerative medicine are likely to emerge in the near future.

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6. Biocompatibility, Safety and Long-Term Stability

After introducing the various applications of nanogels, this chapter will address the potential challenges for their clinical translatability. Currently, there are no nanogel-based drugs or systems approved for clinical use. However, preclinical studies have shown promising results, indicating significant potential for their future clinical application. Current research efforts involving nanogel-based systems are focused on conducting in vitro and in vivo studies to validate their efficacy and safety in disease treatment. One of the biggest challenges for clinical translatability is reproducibility and scalability. As described in the chapter on synthetic methods, nanogel production involves a multitude of parameters and variables: different conditions and reaction settings produce nanogels of varying sizes, shapes, and behaviors, posing a significant challenge in obtaining reproducible results from batch to batch[132]. This variability can affect the consistency of the nanogels' performance, making it difficult to ensure uniformity in their therapeutic efficacy and safety. Overcoming these challenges is crucial for advancing nanogel-based systems towards clinical application, and this could be achieved also by the use of microfluidic systems as explained in section 4. In any case, it is crucial to implement regulatory guidelines that oversee the synthesis and development of nanogels, ensuring stringent adherence to uphold high standards of quality and safety. These guidelines would streamline the process of clinical translation, thereby facilitating the effective transition of nanogels from preclinical studies to clinical applications.

Another significant challenge associated with nanogel systems is their biocompatibility and safety in humans, which have yet to be fully explored. Potential immunogenicity may result from interactions between the nanogel and the drug delivered into the body, or from interactions with biological components, resulting in allergic, anaphylactic, or hypersensitivity reactions. However, accurately determining the immunotoxicity of nanogels through preclinical studies is difficult. Therefore, it is critical to use validated biocompatible materials during nanogel synthesis and conduct thorough investigations of potential toxicity to minimize immune responses. In addition, the potential toxicity of prolonged exposure to nanomaterials needs to be carefully examined because of their increased accumulation at disease sites. Nanogel-based carriers offer promising therapeutic potential but also present risks, which require further research into their safety, feasibility, and long-term stability as a treatment modality. Summarizing clinical studies on nanogels, it is notable that they are primarily administered through topical application, topical injection, or subcutaneous injection[133]. The intravenous injection route, commonly used in preclinical research, has not yet been widely observed, likely due to significant challenges in addressing safety and to efficacy concerns with systemic administration. Furthermore, while clinical studies involving nanogels are increasing, most are currently in phase I or II trials,[134,135] indicating that their clinical translation still requires substantial advancement.

7. Conclusions and Future Perspectives

Nanogels, first developed in the late 1990s as part of polymer science and nanotechnology,[2] have undergone significant evolution, becoming an integral part in modern nanomedicine studies. This review provides a concise exploration of nanogels, highlights their synthesis techniques and various biomedical applications. Nanogels represent a key advance in drug delivery, potentially capable of precise targeting and controlled release.

Even though there is still a significant journey to bring nanogels from bench to bedside for drug delivery applications, the results presented in literature offer a positive outlook for NGs as a novel drug delivery system: they hold promise for personalized therapies in regenerative medicine and contribute to advances in biomedical imaging. Despite these advances, several challenges hinder their widespread clinical application. Many current processes necessitate extreme pH and temperature conditions, as well as crosslinking agents that are not entirely biocompatible; to address these limitations, the use of peptide-based nanogels (PBNs) could serve as an innovative solution [136–138]. Nanogels are still in the early stages of development and require extensive research to address issues such as reproducibility, scalability, efficient targeting, bioavailability, potential toxicity, and long-term stability. To facilitate the clinical transition of nanogels, future research should

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focus on developing scalable manufacturing methods and thoroughly investigating the benefits of nanogel-based therapies over traditional delivery methods. Standardizing manufacturing processes and ensuring batch consistency are critical for regulatory approval and clinical adoption; using flow chemistry approaches, especially based on microfluidics, could help in this aspect, but the research on quantifying the advantages of this approach for NGs production over conventional ones based on batch chemistry is still in its infancy. Addressing biocompatibility and long-term safety through rigorous preclinical and clinical studies is also imperative. Future studies should aim to establish reliable large-scale production techniques for nanogels without compromising their properties, preparing them for extensive clinical evaluation.

Author Contributions: "Conceptualization, P.M., B.T., S.L.; methodology, P.M., B.T., S.L.; investigation, P.M.; data curation, P.M.; writing—original draft preparation, P.M.; writing—review and editing, P.M., B.T., S.L.; visualization, P.M.; supervision, S.L.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received funding from the European Union Next-GenerationEU through the PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR - MISSIONE 4 COMPONENTE 2) within the National Quantum Science and Technology Institute (NQSTI – INVESTIMENTO 1.3; PE_00000023) and the Tuscany Health Ecosystem (THE - INVESTIMENTO 1.5; ECS_00000017).

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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