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Nanogels for intracellular delivery of biotherapeutics

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ABSTRACT

Many biomolecules, such as proteins and genes, are presently used as therapeutics. However, their delivery to target sites inside cells is challenging because of their large molecular size, difficulties to pass cellular membranes and their susceptibility for enzymatic and chemical degradation. Nanogels, three-dimensional networks of hydrophilic polymers, are attractive carrier systems for these biotherapeutics because they protect the biologicals against degradation and, importantly, facilitate cell internalization. Furthermore, the development of responsive nanogel delivery systems has resulted in particles that release their payloads due to a certain physiological trigger inside cells, such as in the cytosol or endocytic compartments. This paper reviews and discusses the use of nanogels, with special emphasis on biologically responsive systems, for intracellular delivery of biotherapeutics.

1. Introduction

Many biotherapeutics (e.g. proteins and nucleic acids) have their targets inside the cells [1–4]. However, delivery of biotherapeutics to these intracellular targets is challenging due to their unfavorable biopharmaceutical properties (hydrophilic molecules with a high molecular weight), which make them prone to both enzymatic and chemical degradation and prevent them to cross cellular membranes by Fickian diffusion [5–7]. Nanoparticle delivery systems have been shown to be effective in protecting drugs from degradation, overcoming biological barriers, and controlling the rate and duration of drug release [7–13]. Moreover, nano-sized particles can after e.g. intravenous administration accumulate in sites of high vascular permeability (sites of inflammation in e.g. tumors) via the enhanced permeability and retention (EPR) effect [13–15], and nanoparticles can also be rendered cell-specific by coupling of targeting ligands to their surface [16,17]. So far, various types of nanoparticle systems have been developed and applied for (targeted) drug delivery, among which polymer based nanoparticles, micelles, liposomes, as well as inorganic particles [18–24].

Hydrogels are crosslinked networks of hydrophilic polymers that retain a large content of water and can be used for loading and release of biotherapeutics because of this feature [25–27]. Since their discovery and application in the biomedical field, macroscopic systems of hydrogels have been developed and investigated for the design of tissue engineering scaffolds and for local delivery of biotherapeutics [28–31] Nanogels are nano-sized hydrogel particles, which in contrast to macroscopic hydrogel particles, can be injected in the circulation to reach target tissues and deliver their payloads locally and also intracellularly [32–37]. The hydrophilicity of nanogels contributes to some of their desirable features including biocompatibility and high loading capacity for hydrophilic biotherapeutics, and their network protects the encapsulated molecules against degradation because enzymes cannot penetrate into the particles [34–38]. Importantly, the characteristics of nanogels can be tailored by altering their size, crosslink density, and surface properties (PEGylation and surface decoration with targeting ligands) [36,37,39]. However, it is difficult to load and retain molecules with a size that is smaller than the pore meshes in nanogels because the loaded molecules will be released from the particles during their preparation. This can be solved by increasing the crosslink density of nanogels to stably entrap their payloads during gel formation. However, once the biotherapeutics are loaded in hydrogel particles during preparation, this might result in chemical modification of the loaded molecules [40–43]. In other alternative methods, strongly charged biotherapeutics, such as nucleic acids, can be post-loaded into oppositely charged nanogels and stably immobilized by strong electrostatic interaction under physiological conditions [44–48]. For both approaches, the entrapped biotherapeutics can subsequently be released by hydrolytic degradation of the gel network [46–50]. However, this sustained release in turn will result in low concentrations of the released biotherapeutics for prolonged times in the extracellular as well as intracellular environment, which is particularly not wanted for drugs that have their sites of action inside cells. Fast intracellular release of therapeutics can be established by the design of nanogels that are taken up by cells and subsequently degrade rapidly in a triggered manner because of physiological differences between the intracellular environment and the extracellular space. Particularly the low pH of the endo/lysosomes as well the low reduction potential in cells have been exploited to develop nanogels that release their payload in a triggered manner, as discussed in the next sections of this review.

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2. The needs and challenges for intracellular delivery of biotherapeutics

Over the last decades, biotherapeutics have evolved as attractive agents for the treatment of various diseases [1,3,51,52]. Pharmaceutical peptides and proteins as well as nucleic acid based drugs are developed to interfere with key pathways of the target cells to treat both chronic and acute pathologies [1,3,53]. Besides, vaccination with specific antigens provides immunological protection and treatment against different types of cancer and infectious diseases [54,55]. Many peptides and proteins, including antibodies, exert their effect by interactions with cell surface receptors [1,56]. However, a significant number of peptides and proteins have their therapeutic actions inside cells, e.g. in the cytoplasm and specific cellular compartments [2,53,57]. Various forms of RNA based drug (siRNA, mRNA, and miRNA) need to be delivered into the cytoplasm where the cellular translation machinery is located, while pDNA must also cross the nuclear membrane to enable expression of the target genes [3]. In the case of vaccine delivery to induce antigen specific humoral or cellular immune responses, the antigen needs to be translocated in lysosomes or the cytosol of antigen presenting cells (APCs), where it is processed and presented to T cells [58–60].

Biotherapeutics in their free form have some unfavorable pharmaceutical properties. Firstly, these complex molecules are often rapidly eliminated from the circulation by renal filtration (for biotherapeutics ~< 60 kD) or by scavenger cells in the liver (for larger biotherapeutics) and/or inactivated by enzymatic degradation. Secondly, they do not spontaneously pass biological barriers such as lipid membranes of cells. For these reasons, appropriate delivery systems of biotherapeutics are essential to prevent their fast degradation and renal clearance, and to render their intracellular delivery possible. Therefore, in recent years various nano-sized delivery carriers have been developed for encapsulation of biotherapeutics to increase their stability, improve their efficacy by assisting their intracellular delivery to reach to intracellular target sites [5,35,61]. Besides that biotherapeutics need to be retained by the carriers until they reach their target sites, intracellular delivery of these biomolecules with nano-carriers is another key step. These nano-carriers can enter cells from the extracellular space by cell uptake processes including endocytosis and phagocytosis to result in their localization of these particles in endo/lysosomes [62,63]. To reach the aimed intracellular target sites in the cytoplasm or nucleus, the particles and/or the released payload have to undergo endo/lysosomal escape [64–66].

3. Effect of the particle size and surface chemistry on cell internalization

The internalization of nanoparticles and their endocytic processes are impacted by their size and surface chemistry [62,63,67,68]. Larger particles (>1 μm) are taken up by phagocytosis, while the uptake of particles with size between 500 nm to 1 μm occurs essentially via micropinocytosis [67,68]. Particles of about 100 nm are taken up by clathrin mediated endocytosis, while cavelae-mediated endocytosis takes place when the particle size is between 50 and 80 nm [62,67,69]. It has further been shown in many studies that nanosized particles are beneficial to enter the cells rapidly and the preferred size for drug delivery is smaller than 100 nm [62,63,67,70,71]. Further, nanogels with size between 20 and 350 nm all showed more or less internalization by different cells [47,72–78].

On the other hand, many studies suggest that the size of particles may not be that important compared to other factors for cell internalization. In many studies it has been shown that nanoparticles with a positive surface charge bind to the negatively charged cytomembrane via electrostatic interaction, which subsequently results in a rapid entry of the cells through adsorptive endocytosis [30,67,79–85]. However, it should be noted that positively charged nanogels generally speaking are more cytotoxic than neutral or negatively charged particles. These latter particles might interact with cells with hydrophobic domains present on their surfaces [62,67,86–89]. Cellular uptake of nanogels can be promoted by the introduction of targeting ligands on their surface which bind to receptors expressed on certain cells. Hyaluronic acid is often used as a component of nanogels because it can target the CD44 receptor that is overexpressed in many cancer cell lines [90,91]. Furthermore, the surface of nanogels can be modified using antibodies, polypeptides, aptamers and other targeting groups for specific binding with receptor specific cells [92–95].

4. Biologically responsive nanogels as delivery systems

As pointed out in the previous sections, biotherapeutics can be stably encapsulated either in highly crosslinked nanogels or by strong electrostatic interactions with nanogels to minimize their premature leakage. Such nanogels mostly slowly release the encapsulated biomolecules due to hydrolytic degradation of (crosslinks in) the polymer network. However, this sustained release may also lead to too low concentrations of the biotherapeutics at their site of action. Therefore, in recent years, nanogels have been designed with crosslinks that can be broken by external stimuli such as temperature, light, and ultrasound, or by biological triggers, such as differences in pH and/or reduction potential that might result in rapid swelling and/or degradation which in turn is associated with release of the payload [35,36,96,97]. For nanogels that respond to external stimuli, highly functionalized equipment is required to provide a focused trigger after the nanogels reach their targets, which is not always feasible. Therefore, in the following subsections, the emphasis is on the triggered release of biotherapeutics from responsive nanogels by biological stimuli.

4.1. Reduction responsive nanogels

The intracellular environment is characterized by a reducing environment which is due to the fact that the glutathione (GSH) levels in the cytosol and nucleus (approximately 2–10 mM) are hundred-fold higher than that in the extracellular fluids (approximately 2–20 μM) [98]. This substantial difference in GSH concentration can be exploited as a potential stimulus for cytosolic release of biotherapeutics from internalized carrier systems. Particularly disulfide linkages are readily cleavable in reducing environments and converted to thiols [99], which can be exploited for the design of intracellular degradable nanogels. However, as mentioned in Section 2, nanogels and nanoparticles in general enter cells mostly via endocytic pathways [39,100–102]. Therefore, these reduction responsive nanogels are likely to be entrapped in endo/lysosomes in which the GSH concentration is much lower than in the cytosol [103,104]. This means that the nanoparticles need to escape from the endo/lysosomes to access GSH [64–66]. It should be noted that endocytic compartments also provide reducing environments for disulfide reduction by other means [105]. To mention, it has been reported that redox enzymes expressed on cell surfaces or secreted by cells, such as protein disulfide isomerase (PDI), are transported into endosomes during the invagination process [106]. However, PDIs loses their catalytic activity at low pH of the late endo/lysosomes [105]. Thus, the activity of PDI is likely restricted to the early endosomes. The redox potential in endocytic compartments is mainly modulated by a specific reducing enzyme called gamma interferon-inducible lysosomal thiol reductase (GILT), which has its optimal enzymatic activity at a low pH (4.5–5.5) [107]. Furthermore, the reductive activity of GILT has been reported to be maintained by cysteine and GSH [108–110]. Taken together, disulfide crosslinks may also be reduced in the endocytic compartments.

Biotherapeutics can be reversibly immobilized in nanogels via reduction sensitive disulfide bonds exploiting mainly two approaches. Firstly, therapeutics can be covalently conjugated via disulfide linkages to nanogel networks and in this way burst release of the conjugated molecules is avoided [111–113]. The release requires a reductive trigger