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Self-assembled glycol chitosan nanoparticles for disease-specific theranostics

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ABSTRACT

Hydrophobically modified glycol chitosan (hGC) conjugates spontaneously form self-assembled nanoparticles (NPs) in aqueous conditions, and glycol chitosan NPs (CNPs) have been extensively studied for the past few decades. For disease-specific theranostics, CNPs could be simply modified with imaging agents, and the hydrophobic domains of hGC are available for encapsulation of various drugs. Based on the excellent physiochemical and biological properties, CNPs have been investigated for multimodal imaging and target specific drug delivery. In particular, a recent application of CNPs has shown great potential as an efficient theranostic system because the CNPs could be utilized for a disease-specific theranostic delivery system of different imaging agents and therapeutics, simultaneously. Furthermore, various therapeutic agents including chemo-drugs, nucleotides, peptides, and photodynamic chemicals could be simply encapsulated into the CNPs through hydrophobic or charge–charge interactions. Under *in vivo* conditions, the encapsulated imaging agents and therapeutic drugs have been successfully delivered to targeted diseases. In this article, the overall research progress on CNPs is reviewed from early works. The current challenges of CNPs to overcome in theranostics are also discussed, and continuous studies would provide more opportunities for early diagnosis of diseases and personalized medicine.

1. Introduction

In the early 2000s, nanotechnology-based medical techniques emerged as a powerful tool for theranostics. The concept of theranostics, a combination of diagnostics and therapy as a single platform, emerged with progress in molecular imaging and nanomedicine. Advances in nanomedicine contributed to theranostics with targeted drug delivery strategies to reduce the systemic toxicity of drugs. A variety of nanoprobes also have been designed for molecular imaging to visualize cellular function or changes in biomarkers, which reflect the progression and therapeutic response of a disease [1]. These nanoprobes, in most cases, can chemically interact with biomarkers to alter the signals for imaging and provide biological information on pathological lesions [2]. The combination of these two advanced technologies for theranostics is expected to achieve early diagnosis and personalized medicine in the near future, and nanoparticles will play a significant role in the concept of theranostics.

In general, nano-sized particles accumulate more in pathological lesions than in normal tissues. In particular, tumor-accumulation of nanoparticles (NPs) has been extensively studied for cancer theranostics. Based on the size-dependent property, NPs can extravasate from angiogenic blood vessels which consist of coarsely connected vascular endothelial cells. The poor lymphatic systems cause retention of NPs in tumors, and the so-called enhanced permeability and retention (EPR) effect is the most well-known mechanism for tumor targeted delivery of NPs [3]. Besides tumors, NPs also can escape from ruptured or damaged blood vessels and travel to other pathological lesions including trauma, hemorrhagic stroke, and inflammation [4–6]. In inflammation, a variety of immunocytes release chemotactic factors and vasodilators including histamines and bradykinins. Under such conditions, increase in local blood flow and endothelial permeability can be observed, subsequently increasing the accumulation of NPs in the pathological lesions. In addition, NPs can be delivered within macrophages. A recent study showed that macrophage migration is dependent on the size of the treated NPs [7]. The NPs with 100 nm diameters were effectively taken up by the macrophages and showed enhanced vector migration rates compared to smaller NPs 30 and 50 nm in diameter. Indeed, nanoparticle-loaded exogenous macrophages migrated into brain lesions through the disrupted blood–brain barrier [8]. These results suggest that the NPs circulating in the blood also may be phagocytized by macrophages to be delivered to the site

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of blood vessel disruption and the foci of inflammation. These reports suggest that NPs can be applied in targeted delivery to non-tumoral lesions, such as chronic inflammation and autoimmune diseases.

A variety of theranostic NPs have been developed for biomedical applications. In particular, chitosan has been widely used for nanoparticle fabrication during recent decades. Chitosan, a deacetylated chitin, is a natural polymer that has many functional groups in its backbone structure. The abundant functional groups of chitosan allow easy chemical modification, and the inherent cationic charges are useful for developing chitosan as a gene carrier. Furthermore, chitosan and chitosan derivatives are attractive materials for their excellent biocompatibility, biodegradability, and low immunogenicity. In particular, hydrophobically modified glycol chitosan (hGC) spontaneously forms self-assemblies in aqueous conditions, and the glycol chitosan NPs (CNPs) have been extensively studied for the past few decades. The hydrophobic modifications provide glycol chitosan (GC) polymers with interesting properties to form NP structures enabling them to be delivered to pathological lesions in a targeted-manner.

The CNPs have demonstrated great potential as an innovative theranostic system. In particular, a recent application of multifunctional CNPs holds promise for efficient theranostics with low systemic toxicity. In this article, we will review all of the CNPs including the early works on the development of CNPs. Several factors affecting target specificity, physicochemical/biological properties, and practical applications of CNPs in theranostics will be described to understand the research progress of the CNPs-related studies. In addition, the current issues and challenges of CNPs to overcome in theranostics will also be discussed in the last part of this review.

2. Self-assembled glycol chitosan nanoparticles (CNPs)

Polymeric amphiphiles can form micelles or micelle-like aggregates, spontaneously. In aqueous environments, the hydrophobic moieties of the polymeric amphiphiles are facing toward the core, and the hydrophilic moieties are exposed to the surface. Although chitosan has a low solubility above its pKa (6.4) in water, GC is a hydrophilic polymer which exhibits complete solubility in water in broad pH conditions [9,10]. Based on this property, several hydrophobic moieties have been introduced to hydrophilic GC polymers to form amphiphiles for preparing self-assembled NPs since 2003 [10–14]. Among them, 5β-cholanic acid is one of the most commonly used materials for hydrophobic modification of GC. Amphiphilic GC–5β-cholanic acid conjugates have been carefully and consistently studied. As other polymeric amphiphiles, the GC–5β-cholanic acid conjugates naturally build up into nano-sized micelle-like aggregates in aqueous solution (Fig. 1A). To trace the resulting CNPs *in vitro* and *in vivo*, imaging agents would be labeled to GC polymers. For example, GC polymers were labeled with near infrared fluorescence (NIRF) dyes, such as Cy 5.5 for fluorescence optical imaging. By the EPR effects or enhanced vascular permeability, the CNPs can infiltrate into pathological lesions including tumors for enhanced contrast imaging of lesions to provide more accurate anatomical information (Fig. 1B).

2.1. Optimization of CNPs and factors affecting targeting efficiency

The characteristics of CNPs are quite variable, depending on the constituents of the hydrophilic and hydrophobic components. As the hydrophilic part, various types of GCs have been investigated to improve target specificity. The molecular weight and degree of acetylation of the chitosan may affect the properties of hGC polymers and CNPs [16,17]. Consequently, different factors affecting the targeting efficiency have been considered to optimize the CNPs for *in vivo* application, especially in tumor-bearing mice [17]. Different degrees of hydrophobic substitution and the properties of the resulting CNPs were investigated as well [11,18].

In practice, the 5β-cholanic acid conjugated GC based CNPs were prepared with different molecular weights of GC polymers (GC 20 kDa-CNP, GC-100 kDa-CNP, and GC-250 kDa-CNP) [17]. Their physicochemical properties and tumor accumulation of various CNPs were comparatively evaluated in tumor-bearing mice. When the feed mole ratio of 5β-cholanic acid to GC sugar residue was fixed as 1:20, the surface charges of the GC-20 kDa, GC-100 kDa, and GC-250 kDa-CNPs were not significantly different from each other. The average diameter of the particles varied from 231 to 310 nm (Table 1). The GC 20 kDa-CNPs were relatively small with an average diameter of 231 nm, and the average diameter of GC 250 kDa-CNPs was up to 310 nm (Fig. 2A). Despite the relatively large size of the particles, GC 250 kDa-CNPs showed the most efficient accumulation in tumors *in vivo* (Fig. 2B).

The 250 kDa-CNPs with different degrees of hydrophobic substitutions (DSs) were also prepared as in Table 2 [18]. The CNPs with different DSs were almost the same in their sizes and morphology, whereas the stability and flexibility of the CNPs were not the same in substance. In particular, a filtration test confirmed the flexibility and deformability of the CNPs. The changes in NIRF intensity of Cy5.5-labeled CNPs were compared to those of polystyrene NPs, before and after syringe filtration. The polystyrene NPs with rigid structures lost their NIRF signals after the filtration, which suggested that the polystyrene NPs could not pass through a cellulose acetate filter (0.2 μm pore size) at all (Fig. 2C). Meanwhile, despite their larger hydrodynamic sizes of over 300 nm, the CNPs maintained distinct NIRF signals after filtration with a 0.2 μm pore filter, especially for those with under 35% 5β-cholanic acid content (Fig. 2D). Based on the ideal balance of stability and deformability, the CNPs containing 23 wt.% 5β-cholanic acid (CNP-23%) showed higher tumor accumulation than that of the others (Fig. 2E and F). The CNPs with a low DS (12 wt.% 5β-cholanic acid included) showed low *in vivo* tumor targeting and low serum stability, and the CNPs with a high DS (35 wt.% 5β-cholanic acid included) could not penetrate into angiogenic blood vessels in tumors to be recognized by the reticuloendothelial system (RES) in the liver and spleen.

The preparation methods of CNPs have been gradually modified for better targeting efficiency. Recently, GCs (250 kDa) were modified to introduce 150 ± 4.5 molecules of hydrophobic 5β-cholanic acid and 4.8 ± 0.7 molecules of Cy5.5 per each molecule of GC polymer [15,19].

2.2. Physiochemical properties of CNPs

The physiochemical properties of NPs should be carefully considered to achieve target specific delivery of the particles. The primary concern should be the size of the particle, because the enhanced delivery of NPs is mainly due to the size-dependent effect. In general, NPs 100 nm or less in diameter are considered for prolonged blood-circulation and high tumor selectivity minimizing or delaying RES clearance [20,21]. Interestingly, however, the most efficient tumor-specific accumulation was achieved with relatively large CNPs (around 300 nm in diameter). Considering the particle size only, the CNPs are somewhat large to expect the EPR effect. However, as described in Section 2.1, the elastic and deformable structure of CNPs permit the extravasation of NPs to be delivered to pathological lesions, such as tumors.

The particle stability would be also an important factor to maximize the targeting characteristics of NPs. In particular, the stability of NPs in serum is essential for a prolonged blood half-life *in vivo*. The CNPs are generally stable in physiological conditions for a long period, and they maintain the size and size distribution in phosphate-buffered saline (PBS, pH = 7.4, 37 °C) for 2 weeks, regardless of the DS [18]. In blood serum, however, CNPs may differ in stability depending on the DS. The stability of CNPs in serum tends to be in direct proportion to the DS value of the CNPs, and CNPs with a low DS are dissociated by serum proteins for rapid renal excretion [18].