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Review

나노의학용 생분해성 나노입자

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Biodegradable Nanoparticles for Nanomedicines

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초록: 최근 들어 나노의학에 사용되는 생분해성 나노입자는 치료용 제제를 효과적으로 전달하기 위한 전달체로 큰 주목을 받고 있다. 특히, 생분해성 고분자를 기반으로 제조한 나노입자는 친수성 담지 약물의 안정성 유지한 상태로 약물을 전달하고자 하는 부위로 찾아가 약물의 서방형 방출거동을 통해 약물의 효과를 극대화하며 약물에 의한 부작용을 최소화해야 할 것이다. 따라서 본 고에서는 나노의학에 응용이 가능한 생분해성 물질인 단백질과 다당류 등과 같은 천연고분자에 대한 소개와 이를 이용한 나노입자의 제조 방법에 대해 소개하였다.

Abstract: Biodegradable polymeric nanoparticles (NPs) have shown significant therapeutic potential as nanomedicines for delivery of pharmaceutical agents. NPs made of biodegradable polymers can efficiently deliver drugs for sustained, controlled and targeted release, enhancing the stability of hydrophilic bioactive molecules, thereby improving their therapeutic efficacy while reducing their side effects. This paper reviews candidate biodegradable materials for NPs, including proteins and polysaccharides, and their methods of NP fabrication for nanomedicines.

Keywords: biodegradable nanoparticles, polymeric nanoparticles, nanomedicine, drug delivery, tissue engineering.

Introduction

Recent nanotechnology has led to significant progress in various biomedical fields, including controlled therapeutic drug delivery,[1] tissue engineering,[2] imaging of specific sites and probing of nanostructures.[3] Nanoparticles (NPs) have contributed to progress in nanomedicines, especially in the formulation of drug delivery systems (DDS) for treating various diseases, including cancer, diabetes, allergy, infection, and inflammation.[4] NPs developed for DDS are alternatives to liposome technology, formulated to overcome problems associated with the stability of liposome vesicles in biological fluids and during storage.[5] Recent technological developments in NPs have improved the efficacy of the drugs being delivered. NPs have been incorporated into colloidal drug delivery systems, which offer advantages due to surface modifications, including drug targeting to specific tissues and cells and enhanced cellular uptake, thus reducing the toxic side effects of the free drugs.[6] The accessibility of their surfaces can allow for easy modification of functional groups, enabling systemically administered NPs to be transported via the circulation to targeted tissue sites. These NPs can be prepared from a variety of biocompatible and biodegradable materials; including proteins, polysaccharides and synthetic polymers.[7] This article reviews developments in formulating protein and polysaccharide NPs and their methods of fabrication for nanomedicines.

Biodegradable Nanoparticles

The first NPs designed for nanomedicines were composed of biodegradable albumin[8] and non-biodegradable synthetic polymers such as polyacrylamide and poly(methyl acrylate).[9,10] NPs composed of non-degradable polymers, however, were found to be associated with chronic toxicity due to intracellular and tissue overloading, limiting their use for systemic admin-

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istration in humans. Thus, interest focused on NPs made of synthetic biodegradable polymers, including hydrophobic polyalkylcyanoacrylate, poly(lactic-*co*-glycolic acid) and polyanhydride.[11-15] These polymers also had limitations, since most were hydrophobic, whereas their bioactive molecules were hydrophilic. Difficulties therefore arose in efficiently encapsulating drugs and protecting them against enzymatic degradation.[16]

Attention therefore turned to NPs composed of more hydrophilic and naturally occurring materials.[17] The biodegradable NP-based administration of hydrophilic molecules, such as proteins, peptides and nucleic acids (oligonucleotide and genes), has been shown to have great potential in therapeutics. In addition to the general advantages of biodegradable NPs, they are easy to prepare from well-understood biodegradable polymers and have shown high stability in biological fluids and during storage. NPs made of biodegradable polymers, such as proteins and polysaccharides, can efficiently deliver drugs for sustained, controlled and targeted release, enhancing their therapeutic efficiency while reducing their side effects.[18]

Biodegradable Protein Nanoparticles. The first naturally occurring material used for the preparation of NPs consisted of two proteins, albumin and gelatin.[8,19] Colloidal systems based on proteins have several important advantages, including being biodegradable, less immunogenic and non-toxic.[20] Moreover, they are highly stable *in vivo* and during storage and are relative easy to prepare; their size distribution can be monitored, and their manufacture can be scaled up. In addition, because the primary structure of proteins can be determined, protein-based NPs offer various possibilities for surface modification and covalent drug attachment.[19]

Albumin. Albumin, a biodegradable, biocompatible and less-immunogenic protein found in blood plasma, has many functions and applications.[21] In the circulatory system, albumin aids in the transportation, metabolism, and distribution of exogenous and endogenous ligands. It can also act as an extracellular antioxidant and to protect other molecules from free radicals and other harmful chemical agents.[8] Modified serum albumin has been used as a selective agent for tumor detection and/or therapy [21] and as a receptor-mediated delivery system of toxic compounds to eliminate *Mycobacterium tuberculosis*. [22]

Nanomedicines have employed the properties of both human serum albumin (HSA) and bovine serum albumin (BSA) for various purposes. These include as carriers of targeting agents, such as antibodies, interferon gamma, and antiviral compounds,[23,24] as enhancers of anti-cancer drugs,[25] and as modified vehicles for drug delivery across the blood-brain barrier and across the brain to the central nervous system.[26,27]

Collagen. Collagen is the structural building material of vertebrates and the most abundant mammalian protein, accounting for 20-30% of all proteins in the body. Collagen has a unique structure, size and amino acid sequence, resulting in the formation of triple helix fibers.[28] Collagen is regarded as a useful biomaterial because of its high availability and excellent biocompatibility and biodegradability.[29] Further, it can be easily modified, enabling numerous applications in NP fabrication.[30] These modifications include the addition of other proteins, such as elastin, fibronectin and glycosaminoglycans, and using crosslinking agents such as glutaraldehyde, formaldehyde, ultraviolet and gamma radiation.[31] Such modifications have been found to enhance the physicochemical and biological properties of collagen, as well as altering its biodegradability and subsequent release of ligands.[30] Biodegradable collagen-based NPs are thermally stable, easily sterilized, and can be taken up by the reticuloendothelial system, enhancing the uptake of drug molecules into cells.[31]

Gelatin. Gelatin is a natural water-soluble macromolecule formed as a result of the heating and partial hydrolysis of collagen. There are two types of gelatin.[18] Type-A gelatin is obtained by acid treatment of collagen with an isoelectric point (pI) between 7.0 and 9.0, whereas Type-B gelatin is produced by alkaline hydrolysis of collagen with a pI between 4.8 and 5.0.[19] Gelatin offers a number of advantages over other synthetic polymers, including non-irritability, biocompatibility and biodegradability, making it desirable as a carrier molecule.[32] In addition, gelatin is a natural macromolecule that is non-toxic and non-carcinogenic and shows low immunogenicity and antigenicity.[33] Gelatin has a large number of functional groups on its surface, which aid in chemical crosslinking and derivatization. These advantages have led to its use in the synthesis of NPs for drug delivery over the last 30 years.[32]

Silk. Silk fibers are primarily made of two proteins, fibroin and sericin, with the structural protein fibroin enveloped by the gum-like sticky protein sericin. Fibroin is a hydrophobic glycoprotein that makes up over 70% of the proteins in the cocoon.[34,35] This insoluble protein is almost entirely made of the amino acids glycine, alanine, and serine, in the sequence (-Gly-Ala-Gly-Ala-Gly-Ser-), leading to the formation of antiparallel β-pleated sheets in the fibers.[35] Fibroin is semi-crystalline and consists of two phases: a highly crystalline β-pleated sheet phase and a non-crystalline phase.[34] Silk fibroin is also biocompatible, less immunogenic and non-toxic and

can be processed into various forms, including gels, fibers, membranes, scaffolds, hydrogels and NPs.[35,36] Silk fibroin matrices with high specific surface area, high porosity, good biocompatibility and biodegradability have been utilized extensively as biomaterials and for drug delivery.[37] Silk has been used as a suture material for many centuries because it evokes a minimum immune response. Moreover, silk-based biomaterials are highly biocompatible with various cell types and promote cell growth and proliferation.[35]

Sericins are hydrophilic glycoproteins that act as a 'glue', constituting 20-30% of the proteins in the cocoon.[35] Sericins are soluble in hot water, range in molecular weight from 24 to 400 kDa, and have unusually high serine content (40%) along with significant amounts of glycine (16%).[36,37] Structurally, sericins consist of 35% β-sheet and 63% random coil, with no α-helical contents.[38] In addition to having the general advantages of protein NPs, sericin NPs may have other benefits associated with the inherent properties of sericins. Those include antioxidant and antitumor action;[39] enhancement of the bioavailability of such elements as Zn, Mg, Fe, and Ca; and suppression of coagulation when sulfated.[40] Water-soluble silk sericin is non-immunogenic, as well as being biocompatible like silk fibroin.[35] Moreover, sericin NPs have been shown to promote wound healing without inducing any inflammation.[41]

Keratin. Keratins are a group of cysteine-rich structural proteins that exhibit a high mechanical strength owing to a large number of disulfide bonds.[42] Keratin has been used very recently as a nanosuspension that results in ultrathin, transparent keratin coatings, in order to investigate *in vitro* cell proliferation and to determine whether keratin can act as a coating material for standard cultivation.[19] Keratin nanosuspension coatings may provide an inexpensive alternative to materials such as collagen and fibronectin and may be utilized in tissue engineering.[43]

Biodegradable Polysaccharide Nanoparticles. Biodegradable NPs made of intrinsic positively- or negatively-charged polysaccharides are currently in development for pharmaceutical formulations.[7] These polysaccharides are biodegradable, and their surfaces can be modified. NPs made of naturally occurring polysaccharides have been designed for the administration of peptides, proteins, and nucleic acids.[44]

Chitosan. The most commonly used polysaccharide for NPs fabrication is chitosan, a linear cationic polysaccharide of *N*-acetyl-*D*-glucosamine and *D*-glucosamine linked by β-(1–4) glycosidic bonds (Figure 1(A)), obtained by the partial deacetylation of naturally derived chitin.[44,45] Chitosan is hydrophilic

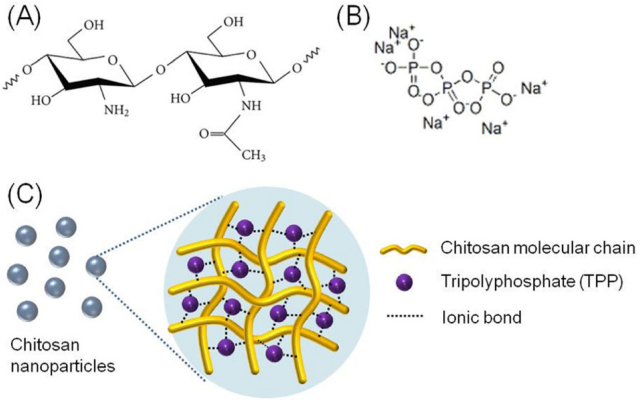

Figure 1. Chemical structure of (A) chitosan; (B) tripolyphosphate (TPP). (C) Schematic illustration of ionically crosslinked chitosan nanoparticles with TPP.

and soluble in acidic solutions due to protonation of its amine groups, and can be degraded by enzymes such as lysozymes, lipases and proteases.[46] Chitosan increases cell membrane permeability, thereby enhancing absorption across the intestinal epithelia and prolonging the residence time of delivery systems at absorption sites.[45] In addition, chitosan can open the tight junctions of cell membranes.[47] Due to their mucoadhesive properties, chitosan based systems have been designed for nasal, ocular, oral, parenteral and transdermal drug delivery.[44] Moreover, chitosan NPs have been used as carriers of growth factors such as epidermal growth factor and fibroblast growth factor, suggesting that they may be introduced into engineered tissue constructs.[48] Growth factors are essential in cellular migration, proliferation, differentiation and maturation.[48,49] The cationic nature of chitosan has led to the preparation of most chitosan NPs by physical crosslinking, with the amino groups of the chitosan backbone interacting with salts such as sodium sulfate, tripolyphosphate (TPP, Figure 1(B)), and other multiply charged anionic bio-molecules.[50] The ionic cross-linking of chitosan is simple and often carried out under mild conditions, without using organic solvents (Figure 1(C)). Moreover, these NPs are generally pH sensitive, a feature suitable for stimuli-sensitive controlled release.[44] TPP, a non-toxic anionic molecule, has been widely used for the preparation of crosslinked chitosan NPs encapsulating several drugs.[50,51] Due to their high physical stability and encapsulation efficiency, the ionically crosslinked chitosan NPs have been used to deliver proteins, oligonucleotides, and plasmid DNA.[51,52]

Alginate. Alginate is another type of polysaccharide often used for NP production.[53] Alginate is a linear anionic polysac-

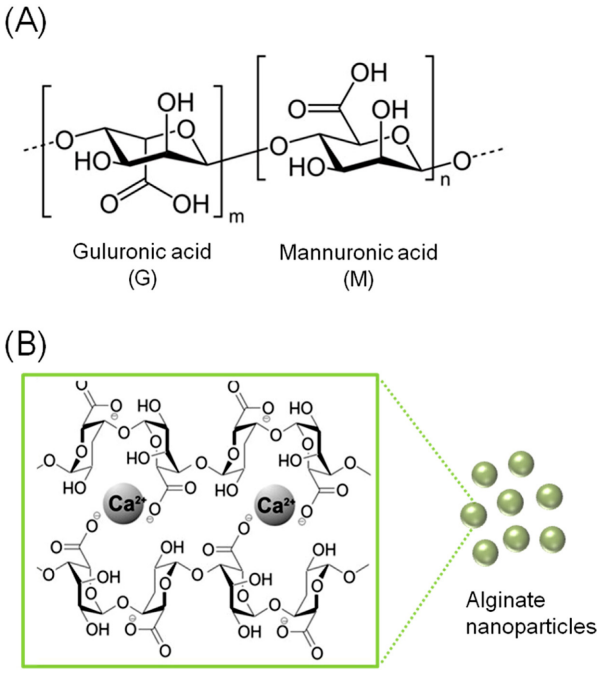

Figure 2. (A) Chemical structure of alginate consist of guluronic (G) and mannuronic (M) acid. (B) Schematic illustration of calcium crosslinked alginate nanoparticles.

charide composed of alternating blocks of 1,4-linked β-d-mannuronic acid (M) and α-l-guluronic acid (G) residues (Figure 2(A)). The advantages of alginate include its high mucoadhesiveness, aqueous solubility, biocompatibility, non-toxicity, and tendency to form gels under certain conditions.[53-55] Alginate can form networks following ionic inter- and intramolecular crosslinking with divalent ions, such as $CaCl_2$, $CaSO_4$ and $CaCO_3$ (Figure 2(B)).[54] Alginate-based NPs are being developed for the delivery of therapeutic agents, such as plasmid DNA and anticancer drugs.[56] For example, $CaCO_3$ crosslinked alginate NPs were shown to successfully encapsulate both plasmid containing p53 DNA and doxorubicin (DOX) using a coprecipitation method, with a higher encapsulation efficiency than p53 and DOX formulations alone.[57]

Methods for Fabricating Biodegradable NPs

Emulsification. Homogeneous sized NPs can be generated by the formation of nano-emulsions, based on the spontaneous emulsification that occurs on mixing an organic phase and an aqueous phase.[12] The organic phase consists of a homogeneous solution of oil, lipophilic surfactant and water-miscible solvent, whereas the aqueous phase consists of hydrophilic surfactant and water.[58] This method can be described as the dissolution of hydrophobic substances in an organic solvent, followed by emulsification with an aqueous solution at very high shear. This results in the formation of very small droplets (50-100 nm).[9] After emulsification, the organic solvent is removed by evaporation, yielding stable dispersions of solid NPs.[59,60] The major disadvantage of this method is the need to add organic solvent and then to remove it. Moreover, residual organic solvents may be toxic to the host.[4]

Desolvation. Another method used to fabricate NPs involves the slow addition of a desolvation factor, such as natural salts or alcohol, to a protein solution.[18] The desolvation factor changes the tertiary structure of the protein.[19] When a critical level of desolvation is attained, a protein clump will form, which, upon crosslinking with a chemical substance (e.g. glutaraldehyde), will result in the formation of NPs.[61]

Alternatively, a two-step desolvation process can be used in the synthesis of gelatin NPs.[32,33] In the first desolvation step, the low molecular gelatin fractions present in the supernatant are removed by decanting, and in the second step, high molecular fractions present in the sediment are re-dissolved and then desolvated again at pH 2.5. The resulting particles can then be easily purified by centrifugation and re-dispersion.[33]

Coacervation. The coacervation method is similar to the desolvation method, in that an aqueous solution of protein is mixed with an organic solvent such as acetone or ethanol to yield tiny coacervates.[7,17] These coacervates are limited by the addition of a crosslinking agent such as glutaraldehyde.[62] The coacervation and desolvation methods differ in the parameters that affect the fabrication process, including initial protein concentration, temperature, pH, crosslinker concentration, agitation speed, molar ratio of protein to organic solvent and the rate of addition of organic solvent.[17,63]

Electrospraying. The electrospraying method produces relatively monodisperse and biologically active protein particles. This method involves dissolving dry protein powder in an electrosprayable solution. Dispersion of the solution followed by solvent evaporation leaves dry residues collected on suitable deposition substrates.[64] Insulin NPs sized between 88 and 110 nm have been prepared by this method. Increasing the applied voltage reduces NP size, whereas increasing the flow rate or concentration of the electrosprayable solution increases NP size.[64] The biological activities of the electrosprayed protein-based NPs were found to be unaffected by the processing conditions.[65]

Conclusions

The use of biodegradable NPs as DDS seems to be a viable and promising strategy for nanomedicines. These formulations have several advantages over conventional drug delivery systems. They can increase the bioavailability, solubility and permeability of many potent drugs, which are otherwise difficult to deliver orally. DDS using biodegradable NPs can also reduce the undesired toxic side effects of the free drugs, as well as reducing dosing frequency, thus enhancing patient compliance. Moreover, such NPs can minimize some of the limitations of drugs by enhancing their stability and preserving their structure. The use of biodegradable polymeric NPs for delivery of pharmaceutical agents has shown significant therapeutic potential in nanomedicines.

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