Chitosan for Tailored Delivery Vehicles in Medicine

Lisa Jacob Literature Seminar March 13, 2014

Delivery of therapeutic agents in medicine is a field that has evolved rapidly over the past few decades. New vehicles such as nanoparticles, liposomes, and hydrogels have demonstrated potential for effective delivery.[1] Polymeric constructs and specifically designing them by incorporating natural polymers has garnered increased research efforts of late.[2] A natural polymer derivative of particular interest is chitosan. Chitosan is the product resulting from the deacetylation of chitin, an abundant polysaccharide occurring in nature, second only to cellulose (**Figure 1A**).[3] Chitin is a major structural component of various animals and is found in shells of various crustaceans and mollusks, insect wings, and fungus walls.[3] Chitin has limited use for biological applications because of extensive hydrogen bonding within the compound, resulting in a semi-crystalline structure and limiting its solubility in a variety of solvents.[4] Upon deacetylation, the resulting amine groups are available for protonation at low pH, rendering chitosan a water-soluble, cationic polyelectrolyte.[4] Chitosan is an attractive and renewable material for medical applications because of its biocompatibility, degradability, low toxicity, and low immunogenicity.[4,5]. Chitosan has three sites for further chemical modification, which are the C2 amine and C3 and C5 hydroxyl moieties, resulting in a highly versatile starting material that can be tailored for a variety of different systems (**Figure 1B**).[6]

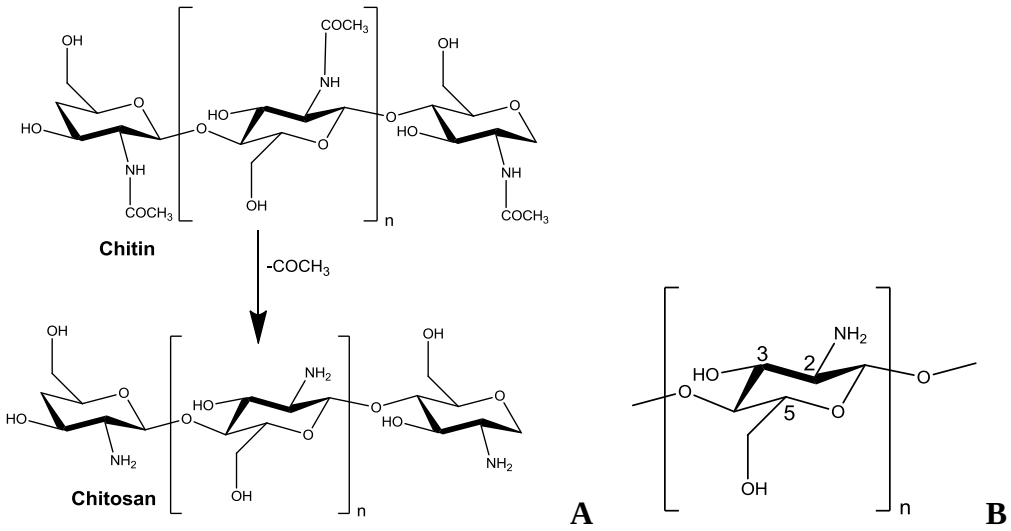

Figure 1. A) Chemical structures of chitin and chitosan. B) Chitosan functional groups for chemical modification.

One avenue of recent exploration is amphiphilically modified chitosan (AMC). Attaching hydrophobic groups such as alkyl chains to the amine groups and hydrophilic groups like sulfate groups to the hydroxyl groups, generates an amphiphilic polymer. The AMC structure will then self-assemble to form polymeric micelles with the hydrophobic moieties point inward and the hydrophilic groups facing polar aqueous media, minimizing the free energy of the system. The core serves as a place to load compounds and molecules, such as drugs[7], genes[8], and proteins[9], in high concentrations far above their intrinsic water solubility.[7] The colloidal properties of such assemblies are important

to their function *in vivo*. This presentation will focus on AMC and highlight recent reports on the resulting nanostructures and their effectiveness in drug delivery.

A recent study of a unique AMC micelle construct investigated how changes to chitosan's chemical backbone changed the resulting micelle structure. Liu et al. examined the role of acyl chain lengths and degree of substitution when modifying chitosan and tested the drug loading efficiency using doxorubicin.[10] A polar carboxylmethyl group was attached to the terminal C5 hydroxyl moiety and the C2 amine was reacted with hydrophobic acyl moieties with varying chain lengths $R=C_{n-2}H_2(n-2)CH_3$ where n=2, 6, 10, 12. With carbon number of 2 ($C_n=2$), the micelles were compact with uniform size distribution of around 25 nm in diameter as presented in transmission electron microscopy (TEM) analysis. By increasing the acyl chain length from 2 to 6, the resulting micelles changed from compact particles to hollow capsules of approximately 100 nm. Scanning electron microscopy (SEM) images of the aggregates clearly show a capsule-like space in the interior of the particle.[11] Doxorubicin, an important anticancer drug, was chosen as the test molecule to determine the drug-loading capacity of the micelles. The encapsulation efficiency (EE) of doxorubicin was measured as a function of acyl chain length and the degree of acyl substitution. With increasing chain length and acyl substitution, the EE reached a maximum of 56% at a C_n of 12 and degree of acyl substitution of 0.5. Such polymeric micelle-based systems are promising because they can self-assemble and have exceptional drug-loading capacity. However, studies on *in vitro* and *in vivo* activity of this system are needed to determine the scientific merit of the research and possible clinical impact.

Marques et al. developed an exciting polymeric delivery system that demonstrates clear clinical potential in targeting breast cancer.[12] They explored two chemical groups, deoxycholic acid (DOCA) and leucine (Leu) to generate two distinct chitosan-derived micelles. DOCA and Leu-modified conjugates self-assemble into micelles at 0.101 mg/mL and 0.065 mg/mL, which are low concentrations indicating a compact hydrophobic core. UV-visible spectrophotometry showed high EE values for loading ibuprofen for both types of micelles; ~66% and ~69% for DOCA and Leu-modified micelles, respectively. These two micelle types demonstrate an improved loading capacity compared to the previous study. MCF-7 breast cancer cells successfully internalized both types of micelles, however the possible mechanism for internalization was not discussed, which is important because these delivery systems rely on passive accumulation. Overall, DOCA-modified micelles loaded with ibuprofen possessed the highest activity against breast cancer cells compared to Leu-modified micelles and was comparable to cells administered free ibuprofen. This work is promising because simply administering free ibuprofen is ineffective as it has low bioavailablility and non-specific toxicity. Long-term and in vivo studies with an animal model are important next steps to understand effectiveness and degradation of the polymeric delivery system.

A new drug-loading system incorporating AMC micelles in a gel network forming a hydrogel illustrates a newer delivery design with wide potential.[13] Chitosan was partially modified with carboxymethyl and hexanoyl groups to increase the hydrophilic and hydrophobic character of the precursor, respectively. It was previously demonstrated that such micelles slowly released the hydrophobic drug, all-transretinoic acid, when embedded in a calcium alginate gel.[14] Building on this work, Hsiao et. al, prepared carboxylmethyl-hexanoyl chitosan solutions and transformed them into hydrogels. TEM analysis of dried gel samples revealed gel consisting of nanocapsules with diameters ranging from 50–200 nm. SEM micrographs of freeze-dried gel samples

show nanocapsules of 100–500 nm. Freeze drying keeps intact the swelling of particles when in solution, whereas dried samples will be dehydrated and shrink in size. Hydrogels loaded with 10 mg/mL of ethosuximide, an antiepileptic drug, showed only 20% of the loaded drug amount released after 20 days compared to hydrogels prepared using different gel formulations, which released half of the loaded drug amount after 2 days. Cytotoxicity of various hydrogel formulations using human retinal pigmented epithelium cells showed no significant effect on cell viability. Rats with seizure-like behavior that can be monitored through spike-wave discharges (SWDs) were used as animal models for *in vivo* analysis of hydrogel efficacy. The experiments spanned seven days. Rats receiving a 1 mL subcutaneous implant of the hydrogel containing 10 mg/mL ethosuximide experienced 50% less SWDs on days 1–3 after the injection. After day 4, the frequency of SWDs increased and by day 6, the number of SWDs returned to pretreatment levels. Magnetic resonance imaging showed that only 20% of the hydrogel remained by day 5 and by day 7, nearly of the implanted hydrogel was gone. Incorporating polymeric micelles into hydrogels is an alternative construct for delivery however, certain questions remain. Hydrogels can allow for easier administration but the demonstrated hydrogel system has only short-term activity. Ability to control release for slow but effective dosage or perhaps burst release is important considering the nature of possible target diseases or conditions.

Using polymeric micelle systems for delivery in medicine is an active area and chitosan shows significant promise as a viable material. Further development is needed to make chitosan-based delivery vehicles effective and demonstrate improved clinical impact compared to other types of delivery systems. Chitosan's physicochemical properties are inherently favorable towards use in biomedicine. Chitosan's versatile chemical platform and ability to self-assemble are the key attributes that make chitosan a worthy starting material to develop and encourages future scientific endeavors.

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