Characterized and synthesis of chitosan nanoparticle as nanocarrier system technology

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Abstract. Chitosan is a natural material produced by deacetylation of chitin from crustacean exoskeleton. Being biocompatible, biodegradable, and affordable, chitosan is widely explored in biomedical field as active agent or drug carrier. Nanoparticles with its unique physicochemical properties, have great potential to maximize therapeutic efficacy. This study used chitosan nanoparticles made by ionotrophic gelation technique. The aim of this study was to characterized chitosan nanoparticles for particle size, surface morphology and degree of deacetylation. The chitosan nanoparticles diameter size was 48nm with polydispersity index was 0.289 and degree of deacetylation less than 75%. Therefore, chitosan nanoparticle is a promising nanocarrier system for drug delivery to enhance drug permeation.

1. Introduction
Nanotechnology is based on the ability to characterize, manipulate, and organize materials on a nanoscale, which gives the products characteristics and behaviors different to those found at the larger scale.[1] Nowadays, nanotechnology has emerged as the third approach, which has opened opportunities for skin drug delivery via nanosystem-like particles, dendrimers, etc. The size of nanosystems for topical and transdermal delivery generally ranges from 1 to 1000nm. They increase skin permeation by enhancing drug solubilization, partitioning of drug into the skin layers, and fluidizing the stratum corneum lipids.[2]

Nanoparticle, as one of nanotechnology products, have unique physical and chemical properties maximized for therapeutic effect, including for wound healing. Therefore, this study based on chitosan copolymer nanoparticle as an active aging for enhancing skin repair (wound healing). Nanocarrier systems as an innovative cosmetic delivery technology have been used widely. Most drug or cosmetic delivery particle technology was based on lipid carriers, such as liposomes and solid lipid nanoparticles of 100-300 nm in diameter.³ Nanoparticles have unique physical properties making them ideal for usage in various skin care products currently on the market.[4, 5]

Polymeric nanocapsules and nanoparticles are relatively rigid nanosystem as compared to others. In nanocapsules, the drug is entrapped in the core whereas in nanoparticle, drug is dispersed in polymeric
matrix, absorbed, complexed, or conjugated on the surface of the particle. Penetration potential of polymeric nanoparticles depends on their size. Roman et al reported that smaller size polystyrene nanoparticles (20nm) accumulated more than bigger one (200nm). Moreover, permeation of these particles through the skin also depends on surface charge. Negatively charged particles can penetrate the SC layer of skin and can reach the viable epidermis.[2]

Chitosan is a marine product derived from the shells of crustaceans, including shrimps. Chitosan was produced by deacetylation of chitin, which is the structural element of the crustacean exoskeleton and the cell walls of fungi.[1,2] Chitosan is a polysaccharide, which consists of acetylated and deacetylated units. The acetylated units are formed by N-acetyl-D-glucosamine while the deacetylated units are composed by β-(1,4)-D-glucosamine. Chitosan is hypoallergenic and has natural antibacterial properties, which qualities make it useful as wound healing agent.[1,2,3] Chitosan is biocompatible and biodegradable and its low costs suggest the biomedical applications in polymer.[4-8] Recently, literature has shown attractive biological chitosan activities, which include immuno-enhancing effects, antimicrobial activities, facilitating wound healing.[6-9]

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The aim of this study is to evaluate the characterized of chitosan nanoparticles made by ionotrophic gelation technique and the in vitro activity of chitosan nanoparticles. The chitosan nanoparticles were characterized for particle sized, surface morphology and degree of deacetylation.

2. Material and Methods

Synthesize of Chitosan Nanoparticle Chitosan nanoparticle were synthesized by ionic gelation method, using chitosan (Sigma Chemical, St. Louis, USA) with degree of deacetylationization less than 75% and Na-Triply phosphate (Na-TPP). The chitosan solution in 0,2% acetic acid was prepared by 24 hours stirring at 300 rpm and room temperature. Further, 6 ml of Na-TPP solution that has been stirred for 2 hours was added dropwise to 30ml of chitosan solution, kept under constant stirring on magnetic stirrer at 1000 rpm For 15 minutes at room temperature.[15] Chitosan nanoparticle was synthesized at Centre for Pharmaceutical and Medical Technology, Agency for the Assessment and Application of Technology, Serpong

3. Result and Discussion

Characteristic of Chitosan Nanoparticles The nanoparticle size distribution was evaluated with a Beckman particle size analyzer CV. At Centre for Pharmaceutical and Medical Technology, Agency for the Assessment and Application of Technology, Serpong.
Figure 1. Characteristics of Chitosan Nanoparticles (CNP)

Figure 2. Transmission electron micrograph (TEM) of chitosan nanoparticles with magnification x100,000. Scale bar 50 nm.

It has been shown that changes in molecular weight had bigger effects than degree of deacetylization in altering the zeta potential of chitosan molecules and chitosan nanoparticles.[16] Therefore, physical characteristics of chitosan play significant roles in determining its effects. Correlation between molecular characteristics of chitosan and its antimicrobial activity has also been reported[10] Proliferative activity of chitosan to fibroblasts and keratinocytes was positively correlated to its degree of deacetylation.[8]
Chitin and chitosan are effective materials for biomedical applications because of their potential agent of their biocompatibility, biodegradability and non toxicity, apart from their antimicrobial activity and low immunogenicity, also as hydrating agent which clearly points to a promising potential agent for future development.[10, 17] Wound dressing is one of the most promising medical applications for chitin and chitosan as active agent for wound healing treatment. Different derivatives of chitin and chitosan have been prepared in the form of hydrogels, fibers, membranes, scaffolds, and sponges.[9, 11, 18] Study by Kang et al. showed that chitosan coated polyvinyl alcohol nanofibers give best result for wound healing.[19] Another study by Howling et al. (2000) demonstrated that chitosan with relatively degree of deacetylation strongly stimulated fibroblast proliferation while chitosan with lower levels of deacetylation showed less activity.[3] In this study, we used chitosan from Sigma with a degree of deacetylation of more than 75%. Howling et al also demonstrated that chitosan with a relatively high degree of deacetylation strongly stimulate fibroblast proliferation compared to chitosan with lower levels of the acetylation.[8], Leonida et al investigated the use of chitosan for wound healing and as antimicrobial and also has anti aging activity. The authors reported that “nano-sizing” enhanced the activities.[12] Draelos et al also reported that nanotechnology could re-invent the “old” one of active agent in cosmetic dermatology to a “new” great one, and greatly enhance the therapeutic efficacy of the substance currently used.[20] Zhang et al reported the correlation between molecular characteristics of chitosan and its microbial activity. Chitosan has found its application in a variety of industries, in cosmetics for delivery, for skin hydration, as film forming and to modify viscosity.[4,5,20] When well-known substances are ground into nanoparticles, they may display completely different properties and can behave in an unpredictable manner. This means that nanoparticles could re-invent the properties of substances currently used in cosmetic dermatology creating new chemical entities from old ones.[20] Nanoparticles chitosan has been fabricated to maximize its biological activity. Chitosan membrane could be used to fasten wound healing and include cell migration and proliferation due its healing effect and antibacterial activity.[21,22] Furthermore, we note that the development of chitosan nanoparticles could lead to stabilization and will reduce its toxicity and irritation effect, therefore clinical study is needed in the future.[4,20] The use of chitosan nanoparticles as biochemical materials is promising especially in bone repair and wound healing and many of the related studies were conducted in vivo. The research, is relevant to trauma related process and the improvement of wound healing.[23] Jayakumar et al study the application of chitosan based nanomaterials in the form of hydrogel, fibers, membrane scaffolds and sponge. The other study explored chitosan silver (Ag) nanoparticles and chitosan – zinc oxide (ZnO) nanoparticles as novel chitosan nanoparticles in biochemical application.[21], Jayakumar et al also demonstrated wound dressing is one of the most promising medical application for chitosan. Biomedical application of chitosan is also based on nanomaterials.[21] Chitosan nanoparticles can be used in regenerative medicine, wound healing, cosmetic anti aging, drug delivery. Chitosan is an effective material for biomedical wound dressing application and hydrating agent.[21,22]. Yulianti et al investigated Centella asiatica extract encapsulated into nanoparticle chitosan increase collagen III synthesis and proliferation in Normal Human Dermal Fibroblast significantly in time and dose dependent manner.[22] Yulianti et al also used Centella asiatica encapsulated into chitosan nanoparticle to enhance expression of aquaporin 3 in Normal Human Dermal Fibroblast and Normal Human Dermal Keratinocytes proliferation and to increase the expression of aquaporin 3.[23] The particle size in nanoemulsions increased the contact surface area, resulting in increased drug efficacy, even in comparison with other existing pharmaceutical formulations.[1]
4. Conclusion
Chitosan nanoparticle can be used in regenerative medicine, wound healing, cosmetic anti-aging as an active agent and drug delivery. Chitosan nanoparticles are an effective material in nanotechnology for biomedical applications. Chitosan nanoparticle as nanocarrier increase drug permeation and reduce drug side effects.

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