In Vitro and *In Vivo* Degradation Behaviors of Acetylated Chitosan Porous Beads

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Introduction: Chitosan, which is derived from chitin, is a linear heteropolysaccharide composed of β-1,4-linked D-glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc) with various compositions of these two monomers [1]. The degree of acetylation (DA) represents the portion of GlcNAc units with respect to total number of units. DA of chitosan influences not only the physicochemical characteristics, but also the biodegradability and biocompatibility [2]. Commercially available chitosans have been obtained by deacetylation of purified chitin with concentrated alkali and high temperature treatment. However, their low solubility (thus processing difficulty) in common solvents caused by block-type distribution of the GlcNAc residues and low DA reproducibility which leads non-uniform physicochemical properties and biodegradability are still remained as some limitations [3]. To overcome these problems, we synthesized DA-controlled chitosans (DA values from 10 to 50%) by the acetylation reaction of deacetylated chitosan and acetic anhydride with different ratio. We also fabricated injectable porous beads (diameter ~500 μm) using the acetylated chitosans with different DA values and their *in vitro* and *in vivo* degradation behaviors were investigated.

Methods: Acetylated chitosans were synthesized by the acetylation reaction of deacetylated chitosan (DA 10%, Regen Biotech, Inc., Korea) and acetic anhydride (Junsei, Japan) with different ratios. The DA values of the synthesized acetylated chitosans were in the range of 10 ~ 50%, as characterized by FTIR and ^{1}H-NMR. The porous beads were fabricated by lyophilization of acetylated chitosan solution (in acetic acid). *In vitro* degradation experiment of the acetylated chitosan beads was conducted in the solutions of lysozyme (10 mg/L in PBS) and *N*-acetyl-β-D-glucosaminidase (5 U/L in PBS) (separately and both together), which are enzymes in the human body inducing chitosan degradation [4]. Their degradation behavior in the enzyme solutions was evaluated by the measurements of end group (UV), molecular weight (GPC) and weight loss. The acetylated chitosan beads were also examined to evaluate their *in vivo* degradation behavior by subcutaneous implantation of the beads into the back of SD rats. The results were evaluated by the measurement of molecular weight (GPC) and histological examination (H&E staining).

Results/Discussion: We fabricated porous beads from the acetylated chitosans with various DA values (10 to 50 %). From the *in vitro* degradation experiment, it was observed that the degradation behavior of acetylated chitosan beads in lysozyme/*N*-acetyl-β-D-glucosaminidase mixture solution is more similar to the *in vivo* degradation behavior than in single lysozyme or *N*-acetyl-β-D-glucosaminidase solution. It may be owing to the sequence degradation reactions of the chitosan beads (1st step, degradation by lysozyme; 2nd step, degradation by *N*-acetyl-β-D-glucosaminidase). The degradation rate of the acetylated chitosan beads was observed as follows, according to their DA values: 50% > 30% > 10% (Fig. 1), similarly to the *in vivo* degradation behavior. It can be explained by synergistic effect of DA value (number of degradation site) and chain interaction (facility of enzyme access to degradation site) [5]. It was also observed that the *in vivo* degradation rate of acetylated chitosan beads is faster than the *in vitro* degradation rate. The acetylated chitosan porous beads with different DA value (and thus different degradation time) can be widely applicable as cell carriers for tissue engineering applications.

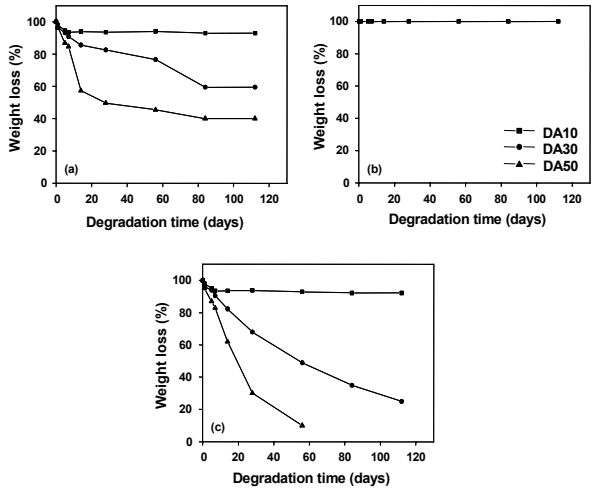

Fig. 1. *In vitro* degradation behavior of acetylated chitosan beads in different enzyme solutions; (a) lysozyme, (b) *N*-acetyl-β-D-glucosaminidase, and (c) lysozyme/*N*-acetyl-β-D-glucosaminidase mixture.

References:

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