The Effect of Different Degrees of Deacetylation and Organic Acids on *In Vitro* Protein Adsorption of Chitosan Films

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Introduction:

The physicochemical and biological properties of chitosan materials are affected by a number of factors such as the source of chitin such as crab, shrimp, fungi[1], molecular weight, degree of deacetylation and molecular structure[2], and type of solvent used to process chitosan materials[3]. Most of the chitosan materials for biomedical applications are made using acetic acid as a solvent[1,2]. But different acids such as hydrochloric, formic, lactic, citric have also been used to make chitosan materials. Studies have shown that the different acids affect the physicochemical properties of chitosan[3], but their effect on the biological behavior of chitosan is not known. The aim of this study was to determine the effect of different degrees of deacetylation DDA and types of solvent on basic *in vitro* protein adsorption of chitosan films.

Methods:

Three chitosan powders (Vanson HaloSource Redmond, WA) of crab origin and with 92.3%, 87.4%, and 78.7 % degrees of deacetylation (DDA) were used. Four organic acids; acetic, formic, citric, and lactic were used to solution cast chitosan films from 1wt% solution. Wettability of chitosan film surfaces was evaluated by measuring the water contact angle (˚) using a VCA OPTIMA video contact angle system (AST Products, INC. Billerica, MA). Image of 100µl of water dropped onto the chitosan film surface was captured in about 5 seconds and contact angle (in degrees) was recorded. Bovine albumin (pH 7.0) (MP Biomedicals, INC) was used for protein adsorption. 500 µg/ ml of albumin was pipetted onto 1cm diameter chitosan discs which were punched from the films. Supernatant that contained unadsorbed protein was removed after 30min, and 2hrs. The films were washed using phosphate buffer saline (PBS) and the wash solution was collected. A BCA analysis was used to determine the concentrations of non-adhered protein and the wash solution using a microplate reader at 562nm. The amount of protein adsorbed was determined by subtracting the concentrations of non-adhered protein plus the wash solution from the original protein concentration.

Two factor ANOVA was used to determine if there were significant differences in the protein adsorption characteristics of chitosan films made using the different DDA and organic acids. A value of $p<0.05$ was considered as significant.

Results/Discussion:

Wettability is an important factor that influences the protein adsorption of chitosan materials. Protein adsorption is an important factor to be determined as it influences cell attachment and tissue interaction. A hydrophobic surface tends to enhance protein adsorption[5]. The cationic nature of chitosan makes it a favorable substrate for protein and cell attachment. Generally chitosans with higher DDA have enhanced protein adsorption, due to the increase in the number of positive amino groups. Chitosan films made using different DDA and acids exhibited varying contact angle values (Table 1).

DDA	92.3%	87.4%	78.7%
Solvent	Contact angle (˚)		
Acetic acid	82.7±2.2	87.3±8.2	92.6±3.8
Citric acid	88.5±2.8	93.3±1.4	85.9±4.4
Formic acid	92.1±0.9	101.3±2.6	99.4±2.8
Lactic acid	98.6±1.3	91.8±8.0	92.4±3.8

Table 1. Contact angle values of chitosan films

Chitosan films made using formic, lactic and citric acids showed increased protein adsorption as compared with those made using acetic acid. This was attributed to a slightly more hydrophobic nature of chitosan film surfaces made in these three acids. There was a significant difference ($p<<0.01$) in the amounts of protein adsorbed on chitosan films within different DDA and organic acids (Figures 1 and 2). These results suggest that using different degrees of deacetylation and/or solvents to make chitosan materials may be used to control cell and tissue responses.

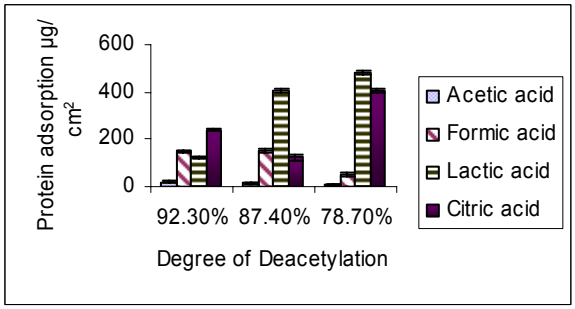

Figure 1. Protein adsorption of chitosan films after 30min

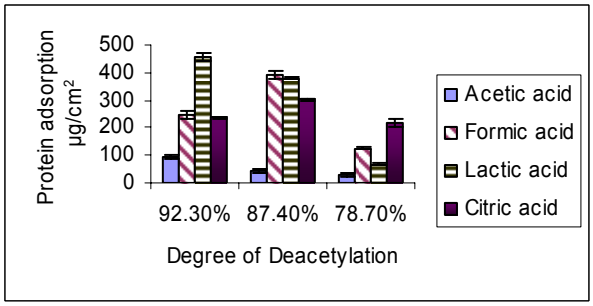

Figure 2. Protein adsorption of chitosan films after 2hours

Conclusions:

Adsorption of protein on chitosan materials is dependent on degree of deacetylation (DDA) and type of solvent used.

References:

1. Jaworska M. et al. Polym. Int. 2003; 52: 198-205
2. Freier T. et al. Biomaterials. 2005; 26:5872-78
3. Leffler CC. et al. Int. J. Pharm. 2000; 194: 229-237
4. Tangpasuthadol V. et al. Carbohydr Res. 2003; 338: 937-942