Chitosan Source Evaluation by Two Degradation Assessment Methods for a Local Delivery Device

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Statement of Purpose: Chitosan is a naturally occurring, linear polysaccharide having both deacetylated and acetylated units, with generally greater than half being deacetylated. Chitosan, *in vivo,* has been reported as enzymatically biodegradable, primarily due to lysozyme's ability to cleave chitosan's glycosidic bonds between repeating units, yielding biocompatible byproducts[1]. When lyophilized into a sponge, chitosan has proven to be an effective, drug delivery device for infection prevention in open wounds[2]. Chitosan's biodegradation has been reported to have an inverse relationship to chitosan's degree of deacetylation (DD)[1]. This study evaluates the *in vitro* degradation profile of several vendors' chitosan products, formed into a sponge, with analysis by both weight loss and molecular weight (MW) changes.

Methods: Unless otherwise noted, all chemicals were purchased from Fisher Chemicals (Thermo Fisher Scientific Inc., Waltham, MA). *Sponge Manufacture:* Multiple chitosan products, with varying DD and MW, from three companies—ChitoClear (Primex, Siglufjörður, Iceland), Chitopharm (Cognis, Monheim, Germany), and Chitoceuticals (Heppe Medical Chitosan, Halle, Germany) as listed in table 2—were dissolved at 1% (w/v) chitosan in a 1% (v/v) weak acid aqueous solution. The chitosan solution was frozen at -20°C and lyophilized into a sponge. The acidic sponge was neutralized in 0.6M sodium hydroxide (NaOH) followed by rinsing with copious amounts of deionized water. The pH neutral, hydrated sponge was frozen at -20°C and lyophilized into the finalized chitosan sponge product. *Degree of Deacetylation (n=3):* Using a standard titration method[3], a chitosan sample was dissolved in 0.1M hydrochloric acid. Using 0.1M NaOH, the acidic chitosan solution was titrated until the solution reached pH ≥ 3.1. The volume of titrant used to reach the pH end point corresponds to the quantity of the free amine groups which allowed for the calculation of chitosan's DD[3]. *Enzymatic Degradation:* Dehydrated chitosan sponge samples were normalized by weight and hydrated in 1mg/ml egg white lysozyme. Samples were continually shaken at 37°C and after 48 hours, the lysozyme solution was completely refreshed. After another 48 hours the chitosan sponge sample was removed, gently rinsed in deionized water to terminate the degradation reaction, and was dehydrated in a vacuum oven at 80°C. *Weight Loss Evaluation (n=3):* Calculations were made comparing the initial sponge weight to the 96 hour, enzymatically degraded, sponge weight to give the average percent remaining. *MW Evaluation (n=3):* Manufactured and degraded sponge samples were taken and dissolved at 1mg/ml in a 0.1M sodium acetate and 0.2M acetic acid aqueous solution. Samples were subjected to gel permeation chromatography with multi-angle light scattering to determine the weight average molecular weight (in daltons) and calculate the degraded sponge's % decrease in molecular weight. *Statistics:* Excel 2010 (Microsoft, Redmond, WA) was used for all statistical analyzes as described in table 1.

Testing Method	*Statistical Test Applied*	*Replicates*	*Confidence Interval*
Titration for DD	One sample t test	3	95%
Weight Loss Degradation Evaluation	One-way ANOVA, with Holm Sidak *post hoc*	3	95%
DD Degradation Evaluation	Paired t test	3	95%
DD and MW Correlation	Regression Analysis	-	95%

Table 1. Statistical analysis performed on each method.

Results: Measured DD results varied with respect to the manufacturers stated DD. The weight loss degradation evaluation indicated that all groups were statistically similar with two exceptions, both having significantly lower % remaining sponge after the 96 hour degradation. All chitosan groups had a statistically significant decrease in MW except for the Chitoceuticals 70/5 product, whose MW significantly increased. Additionally, the Chitoceuticals 70/5 product had the lowest MW of any group tested, whose sponge's MW was $2.829\times10^{+04}$ Da compared to the other products whose MW ranged from $1.673\times10^{+05}$ Da to $8.994\times10^{+06}$ Da. There was no correlation identified between both the reported or measured DD and either the weight loss or MW based degradation evaluation.

Chitosan Product, Vendor (Location)	*Product Variation's Name*	*Measured % DD (n = 3)*	*% of Sponge's Weight Remaining (n = 3)*	*% Decrease in Sponge's MW (n = 3)*
ChitoClear, Primex (Siglufjörður, Iceland)	61%	64.62±0.40*	96.66±0.39	81.96±1.64*
	71%	73.44±1.34*	96.92±0.08	46.05±7.09*
	80%	74.62±1.13*	96.82±0.51	52.94±2.07*
Chitopharm, Cognis (Monheim, Germany)	S	80.60±1.18	97.13±0.31	13.18±2.14*
	M	76.71±1.11*	94.88±0.30	24.56±5.05*
	L	76.66±1.78	96.95±0.37	34.38±4.17*
Chitoceuticals, Heppe Medical Chitosan (Halle, Germany)	70/5	73.01±0.47*	86.60±1.23*	-45.02±32.41*
	70/50	77.42±0.91*	96.96±0.18	50.27±6.44*
	70/1000	74.82±0.76*	95.53±0.08	32.15±17.24*
	75/1000	73.13±1.32	94.11±0.15*	44.37±9.35*
	80/1000	79.58±1.95	97.42±0.26	56.19±10.92*

Table 2. Results are given in average ± standard deviation. "*" indicates significant difference.

Conclusions: Chitosan does show degradation *in vitro* with lysozyme. We were not able to confirm the inversely proportional relationship between DD and MW degradation as reported in the literature. While MW can be an indicator for polymer degradation, weight loss evaluation indicates the overall changes in the local delivery device. A chitosan product showing successful degradation using both degradation evaluation methods will be utilized in future, expanded *in vitro* and *in vivo* degradation tests.

References: [1]Dash M. Prog Poly Sci. 2011;36:981-1014.
[2]Stinner DJ. J Orthop Trauma. 2010; 24:592-597.
[3]Domszy JG. Makromol Chem 1985;186:1671.

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