In Vitro and In Vivo Degradation and Biocompatibility Evaluation of Chitosan Sponges and Paste

Cheyenne S Rhodes, Marsalas Whitaker, Ashley C Parker, Heather Doty, Jessica A Jennings, Warren O Haggard.
The University of Memphis Department of Biomedical Engineering.

Statement of Purpose: When systemic antibiotic distribution is limited, local antibiotic delivery systems are able to provide an increased level of antibiotics without toxic side effects to combat proliferating bacteria that can establish an infection[1-2]. Chitosan sponges (CS) were developed to be a biocompatible and biodegradable local drug delivery system. Chitosan/PEG paste (CPP), which is made from ground up CS using Chitopharm S chitosan (Chitinor AS, Tromsoe, Norway) and 6,000g/mol polyethylene glycol (PEG; Sigma Aldrich, St. Louis, MO), was developed to improve upon CS by adding injectability and adhesivity as material properties. Prior in vitro research established the fabrication procedures for CS and CPP. The objective of these studies was to establish a difference in degradation (DEG) and biocompatibility (BCMP) through cell/tissue response between the CS and the CPP.

Methods: In Vitro CS DEG: 8mm sodium acetate buffered Chitopharm S (BCS) CS of varying pHs including 5.6, 6, 6.3, and 7 pH were weighed then covered with 35mL of a 1mg/mL lysozyme solution. Samples were placed in an incubator on a shaker at 37°C, solution replaced every 2 days, and time points taken at 2, 14, and 28 days. After each time point, the CS was dried, weighed, and percent CS remaining was assessed. In Vivo CS DEG & BCMP: With an established, IACUC approved intramuscular rat model, 8mm CS including neutral ChitoClear (NCC; Primex, Siglufjörður, Iceland), 5.6 pH buffered ChitoClear (BCC), neutral Sentrex (NS; Bionova Medical, Memphis, TN), and 5.6 pH BCS CS were placed into 4 separate back muscle pouches on each rat. After 14 and 28 days, implant sites were excised for comparison using histological analysis. In Vitro CPP DEG: 5mL of dehydrated CPP with varying ratios of acidic to neutralized product including 70:30, 50:50, and 30:70 were weighed, hydrated with a 1xPBS solution, and covered with 50mL of a 1mg/mL lysozyme solution. Samples were placed in an incubator on a shaker at 37°C, solution replaced every 24 hours, and time points taken every 24 hours over 6 days. After each time point, the CPP was dried, weighed, and percent CPP remaining was assessed. In Vitro CPP BCMP: 0.5mL of the three CPP variations and 8mm neutral CS were placed in 12-well tissue culture plates and covered with 2mL of High Glucose Dulbecco's Modified Eagle Medium with 10% FBS and 1% antimicrobial additive. Samples were tested against normal human dermal fibroblasts (NHDF) under normal cell culture conditions, media refreshed every 24 hours, and time points taken at 24 and 72 hours. After each time point, the Promega (Madison, WI) Cell Titer-Glo Luminescent assay was performed to assess viability.

Results: DEG of the CS occurred primarily within the first 2 days of in vitro testing, significantly in the buffered CS, minimally in the neutral, and negligible DEG thereafter for all CS. All CS experienced significant in vivo DEG; however, the BCS CS had the most DEG through 14 days. DEG of the CPP occurred primarily in the first day with less DEG thereafter. The 70:30 and 50:50 CPP both degraded significantly more than the 30:70 over the entire 6 day study. Figure 1 below shows the percent remaining through reduction in dry weight of all CPP and CS made from Chitopharm S chitosan.

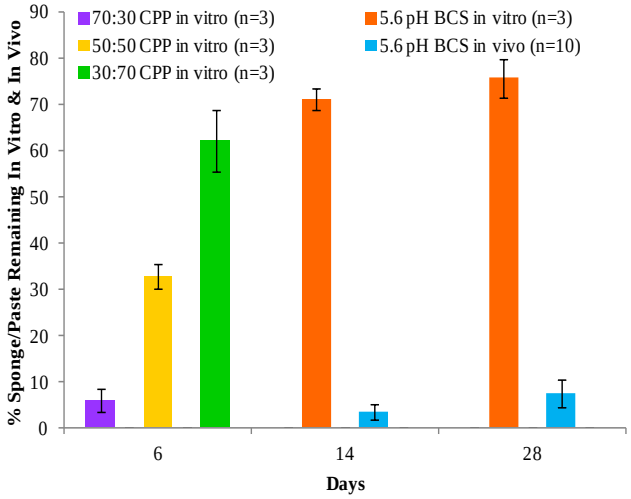

Figure 1. Percent CPP and 5.6 pH BCS CS remaining after 6, 14, and 28 day DEG (results given in avg±dev).

Further histological analysis of the in vivo tested CS revealed that all exhibited similar elevated levels of fibrous tissue with a mild inflammatory response. BCMP analysis of the in vitro tested CPP showed that all CPP variations and the untreated cells (1st control) expressed an increase in cell viability from 24 to 72 hours and better BCMP than the neutral CS (2nd control) at both time points.

Conclusions: The 5.6 BCS CS tested in vivo degraded significantly more than those tested in vitro after 14 and 28 days as did all three variations of the CPP after only 6 days in vitro. While only the 70:30 CPP degraded as much as the 5.6 BCS CS tested in vivo, the CPP variations were only tested for 6 days compared to the 14 and 28 day trials of the CS. Although the 70:30 and 50:50 CPP degraded more efficiently than the 30:70, the 50:50 and 30:70 proved to be more biocompatible than the 70:30 CPP. However, the 30:70 CPP was not injectable through a syringe during BCMP testing. Additional testing of the 50:50 CPP will be pursued as well as testing of 60:40 and 40:60 CPP variations. Extended in vivo DEG and BCMP models would be beneficial for the CPP to determine if the CPP would degrade more and cause less inflammation than the CS. An in vivo infected model would also be beneficial in order to compare if the CPP is equally effective as the CS in treating an infected musculoskeletal wound in a clinical setting.

References: [1]Skinner HB. NY: Lange Med Bks/McGraw-Hill Med Pub Div; 2006.
[2]Hanssen AD. Clin Orthop Relat Res; 2005:91-96.

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