Preparation of Raspberry Ketone Modified Chitosan Microspheres and Effect on Macrophage Activation

Gregory S. McGraw[1], Alex Hoban[1], Daniel Abebe[2], Amber J. Jennings[1],
Tomoko Fujiwara[2], Warren O. Haggard[1], Joel D. Bumgardner[1]
[1]UM-UTHSC Joint Biomedical Engr Program, University of Memphis, [2]Dept Chemistry, University of Memphis.

Statement of Purpose: Inflammation is a key response during the healing process but can be harmful if left unchecked. Inflammation is mediated in part by the release of nitric oxide (NO) by activated macrophages. Excess production of NO can lead to diseases such as cancer and rheumatoid arthritis. Raspberry ketone (RK), a natural compound found in raspberries, has been shown to be an inhibitor of NO production[1]. In this study, chitosan was modified with RK then used to make microspheres. Chitosan has been shown to be biocompatible and biodegradable and to be an effective local delivery mechanism for therapeutic agents for treating inflammation[2]. The goal of this study was to modify chitosan (CS) with RK and (RKCS) for making microspheres to, characterize the composition and size of the microspheres, and to demonstrate that the RKCS microspheres would not activate macrophages.

Methods: RK (Sigma) was added to de-aerated 2 wt% chitosan (87 % DDA) in 2% acetic solution in 1.6:2 ratio. The mixture was stirred overnight at 85°C then lyophilized. The lyophilized RKCS was combined with unmodified chitosan in 0:1, 25:75 and 50:50 ratios to make a 4 w/v% polymer in 5% acetic acid solution. Using a w/o emulsion crosslinking technique[3], the RKCS solution was added to liquid paraffin while stirring and then crosslinked by 5mM Genipin (Wako) in acetone for about 12 hrs. The spheres were recovered by centrifugation and size characterization was performed using ImageJ software on images taken under light microscopy. Fourier transform infrared spectroscopy (FTIR) was performed to determine if RK had attached to the chitosan via imine bonds. This was done on RKCS films for convenience. *In vitro* analysis of the RKCS microspheres was performed by exposing macrophages (RAW 264.7) to the microspheres for 48 hours and measuring the NO response. Five groups (n=4) were analyzed. Macrophages were exposed to the microspheres of the different weight ratios including unmodified chitosan microspheres. Cells exposed to 2.5 µg/ml lipopolysaccharide (LPS) were used as positive controls, and cells with no LPS or microspheres were used as negative controls. Samples were taken at 24 and 48 hour intervals and NO concentration was measured using a Griess Reagent System (Promega).

Results: Light microscopy images of RKCS microspheres showed spherical shape with a range of sizes (Fig. 1, Table 1). Spheres below 10 µm diameters were excluded as it was difficult to observe microspheres under this size in the light microscope The FTIR spectrum displayed a peak at 1680 cm^{-1} which corresponded to the imine bond between the RK and chitosan (Fig. 2). This peak is not present in the unmodified chitosan. Macrophage cells exposed to all microsphere compositions showed low level NO production similar to the negative controls. Only the positive control cells exposed to LPS exhibited high levels of NO production (Fig. 3).

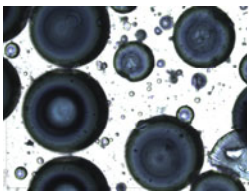

Figure 1. RKCS microspheres at 10x magnification using light microscope.

Table 1. Size distribution of RKCS microspheres by percentage.

Diameter (µm)	% Microspheres
10-30	17.8
31-60	34.2
61-100	26
>100	21.9

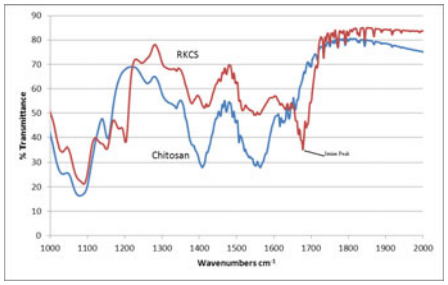

Figure 2. FTIR spectra of unmodified chitosan and RKCS.

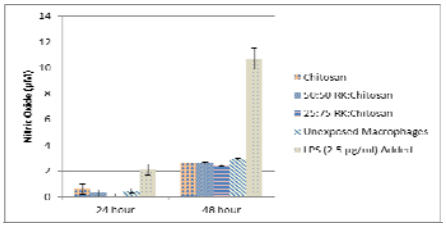

Figure 3. NO production of macrophages at 24 and 48 hours.

Conclusions: We have successfully developed a protocol that allows us to prepare microspheres from RK-modified CS via w/o emulsion technique. While the size range is relatively large, the majority of microspheres fell between 30 and 90 µm diameter. The FTIR spectra demonstrated the imine bond peak appeared after modifying the chitosan with RK indicating that RK was bonded to the chitosan and not just absorbed to the chitosan polymer. The *in vitro* data demonstrates that the modified microspheres did not activate NO production at a higher level than the negative controls or unmodified chitosan groups. Further research is needed to explore whether the RKCS microspheres can inhibit an NO response from macrophages that are exposed to LPS, as well as degradation profiles.

References:

1. Jeong JB. Food Chem. Toxicol. 2010;48:2148-2153.
2. Dash M. Prog. Polym. Sci. 2011;36:981-1014.
3. Jain SK. Indian J Pharm Sci. 2007;69:498-504.

Acknowledgements: Work was supported by the Biomaterials Applications of Memphis (BAM) labs at UM-UTHSC