Effect of Chaotropes on Antigen Removal in Xenogeneic Scaffold Generation

Janelle L. Wong1, Maelene L. Wong1,2, and Leigh G. Griffiths1
1Department of Veterinary Medicine: Medicine and Epidemiology, 2Department of Biomedical Engineering
University of California, Davis, Davis, CA
Contact information: janwong@ucdavis.edu, lgriffiths@ucdavis.edu

OBJECTIVES

The objectives of this study were to determine which component(s) of the Cordwell solution (1) contribute to its potential ability to reduce lipid-soluble protein antigens in BP and (2) result in a potentially detrimental alteration in BP morphology and whether this change in BP morphology can be lessened by reducing the concentration of the component responsible for this effect.

METHODS

Antigen removal. Two-step AR was adapted from the one-step AR method previously described. Briefly, BP pieces were incubated in optimized solubilizing AR buffer (opt SARB) to solubilize and remove water-soluble proteins at 125 rpm and 4°C for 48 h during the first step of AR. Samples were then subjected to a second step of AR using additives to solubilize lipid-soluble proteins for removal in opt SARB at 125 rpm and 25°C for 48 h; adjacent pieces subjected to 1 m incubations served as negative AR controls (n=6 per group).

Assessment of antigen removal. Assessment of AR was performed as described previously. Briefly, residual lipid-soluble proteins extracted from minced BP AR were subjected to electrophoresis and Western blot, probed with rabbit serum generated against native BP and assayed for IgG positivity. Residual antigenicity, defined as the ratio of 48 h to 1 m samples, was determined by densitometry and normalized to the previously.

RESULTS

Our results indicate that chaotropes and ASB-14 are the additives primarily responsible for the efficacy of the Cordwell solution in reducing lipid-soluble protein antigens in BP AR. Unfortunately, the presence of 8 M urea/2 M thiourea also resulted in the thickening of BP AR observed with the Cordwell solution. Decreasing the concentration of chaotropes in opt SARB to 4 M urea/1 M thiourea resulted in BP AR that was not grossly thicker than that generated using opt SARB alone. However, the residual lipid-soluble protein antigenicity achieved using 4 M urea/1 M thiourea was not reduced to the degree observed with 8 M urea/2 M thiourea treatment.

Future studies are needed to determine how the thickening of BP AR, elicited by treatment with 8 M urea/2 M thiourea, has affected the functional properties of BP AR. Furthermore, as treatment with 1% (w/v) ASB-14 significantly reduced residual lipid-soluble protein antigens compared to opt SARB alone without thickening the BP AR, future studies will determine an optimal concentration of ASB-14 for maximal removal of lipid-soluble protein antigens. Additionally, it would be important to determine whether the use of both ASB-14 and 4 M urea/1 M thiourea would further enhance AR of lipid-soluble antigens.

CONCLUSIONS & FUTURE WORK

REFERENCES


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