Polyanhydride Nanovaccines against Viral Infections in Shrimp

Yashdeep Phanse[1], Supraja Puttamreddy[2], Duan Loy[2], Kathleen Ross[3], Julia Vela Ramirez[3], Balaji Narasimhan[3], and Lyric Bartholomay[1]
[1]Pathobiological Sciences, University of Wisconsin, Madison, WI 53706, [2]Entomology, Iowa State University, Ames, IA 50011, [3]Chemical and Biological Engineering, Iowa State University, Ames, IA 50011

Statement of Purpose: The $11 billion shrimp industry continues to suffer major losses each year due to viral disease, such as white spot syndrome virus (WSSV) and infectious myonecrosis virus (IMNV).[1] While there are currently no treatments for these diseases, dsRNA-based vaccines have shown promise in preventing WSSV and IMNV infections.[2,3] Unfortunately, dsRNA-based vaccines have limited stability and short *in vivo* residence times, limiting their implementation in field-relevant scenarios.

Nanovaccines based on polyanhydride nanoparticles have been successfully used for the encapsulation and release of vaccine antigens.[4,5] Polyanhydrides erode via a surface erosion mechanism that limits the exposure of encapsulation payloads to water, and therefore, enhancing their stability.[4,5] In addition, the nanovaccine platform is capable of mass immunization of shrimp via immersion or milling with feed, making their use in field-relevant conditions possible. In this work, dsRNA-nanovaccines against WSSV and IMNV were developed. Herein, we examined the safety, biodistribution, and efficacy of these nanovaccines in shrimp.

Methods: Copolymers composed of sebacic acid (SA), 1,6-bis-(*p*-carboxyphenoxy) hexane (CPH), and 1,8-bis-(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were synthesized as previously described.[6,7] Nanoparticles comprised of 20:80 CPTEG:CPH and 20:80 CPH:SA chemistries were synthesized via nanoprecipitation[8] as blank (*i.e.,* no payload) or encapsulating 11% dsRNA (WSSV), 5% dsRNA (IMNV), or 1% rhodamine dye. The safety of nanovaccines was examined by administering 500 µg of blank nanoparticles to Pacific white shrimp (*Litopenaeus vannamei*) via reverse gavage and monitoring body weight and survival. After 60 days, tissues were harvested for histopathology. The biodistribution of 250 µg of rhodamine-loaded nanoparticles delivered via immersion and reverse gavage to adult and post-larvae shrimp were observed with *ex vivo* imaging for 24 h (immersion) or 28 days (reverse gavage). Finally, the efficacy of dsRNA-loaded nanovaccines was examined by viral challenge three days post-immunization and by monitoring survival for two weeks.

Results: Shrimp were monitored for 60 days post-nanovaccine administration. The normalized biomass (*i.e.,* weight gain) of shrimp administered 20:80 CPTEG:CPH or 20:80 CPH:SA was similar to control shrimp administered saline. In addition, no adverse effects were noted in tissue sections stained with hematoxylin/eosin-phloxine (H&E).

Ex vivo imaging of shrimp demonstrated that rhodamine-loaded nanoparticles localized to the hepatopancreas and stomach for approximately 21 days post-immunization. In addition, the 20:80 CPTEG:CPH nanoparticles localized to the gills and persisted for at least 28 days post-injection.

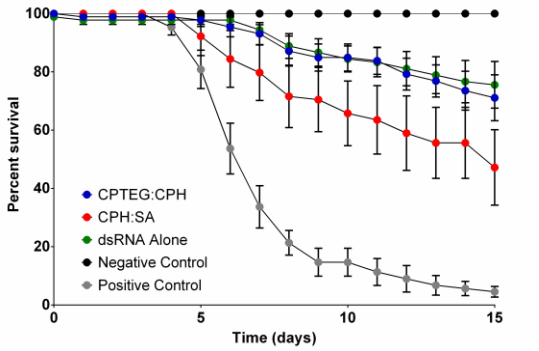

Fig. 1. Survival of nanovaccine-administered shrimp post-challenge. Shrimp were challenge with IMNV three days post-immunization. Shrimp immunized with 20:80 CPTEG:CPH nanovaccines had similar survival in comparison to dsRNA delivered alone.

Finally, shrimp were injected with 100 µg of dsRNA-loaded nanoparticles and challenged with WSSV (two days post-injection) or IMNV (three days post-injection). Animal survival was monitored and both nanovaccine formulations achieved 70-85% protection (Fig. 1).

Conclusions: The polyanhydride nanoparticle platform was shown to successfully encapsulate dsRNA-based vaccines against WSSV and IMNV in shrimp. The nanoparticles did not induce any adverse effects in shrimp post-administration and histopathology showed tissues to be similar to the control. *Ex vivo* imaging demonstrated that the particles localized to organs commonly associated with viral entry in shrimp (hepatopancreas, stomach, and gills) and persisted for at least 28 days. Finally, dsRNA-based polyanhydride nanovaccines demonstrated protection against WSSV and IMNV viral infections.

References:

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