18

Correlation Between *In Vitro* and *In Vivo* Release of Radiolabeled Antigen from PLGA Microspheres Using SPECT/CT Imaging

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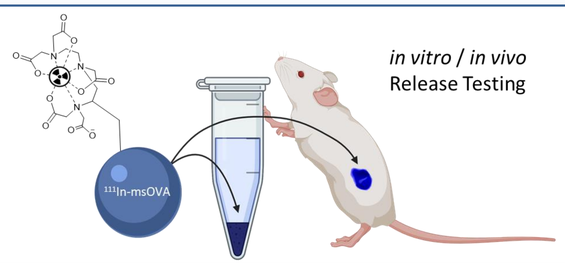
INTRODUCTION

To generate a protective response, subunit vaccines are typically administered as multiple doses (initial priming and following booster injections). Lately, poly(lactide-co-glycolic acid) (**PLGA**) microspheres have been extensively studied as a **drug delivery system** that can enable the **release of antigen** over a controlled series of burst and lag phases from a single injection. This would improve patient compliance as multiple injections would be replaced **by a single dose**.

The release of antigens from PLGA microspheres is well characterized *in vitro* in buffers and physiological media, but their performance *in vivo* remains largely unknown.

OBJECTIVE

To study the **pharmacokinetics of ^{111}In-msOVA** and free ^{111}In-OVA in a murine model via SPECT/CT imaging, and assess the **correlation between *in vitro* and *in vivo* release** of the antigen from PLGA microspheres.

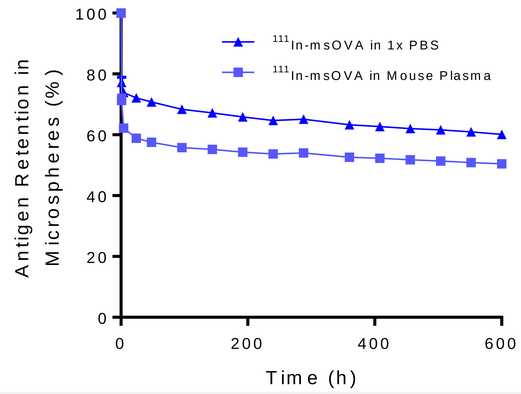


METHODS AND MATERIALS

- Model antigen ovalbumin (OVA) was radiolabeled with ^{111}In and encapsulated into PLGA microspheres using W/O/W emulsion technique, obtaining the final product ^{111}In-msOVA
- Morphology of the particles was assessed through scanning electron microscopy (SEM) and dynamic light scattering
- *In vitro* antigen release assays were performed in 1x PBS and diluted mouse serum over 3.5 weeks
- *In vivo* release was assessed using SPECT/CT imaging following subcutaneous injection of ^{111}In-msOVA and free ^{111}In-OVA (control) in healthy C57Bl/6 mice

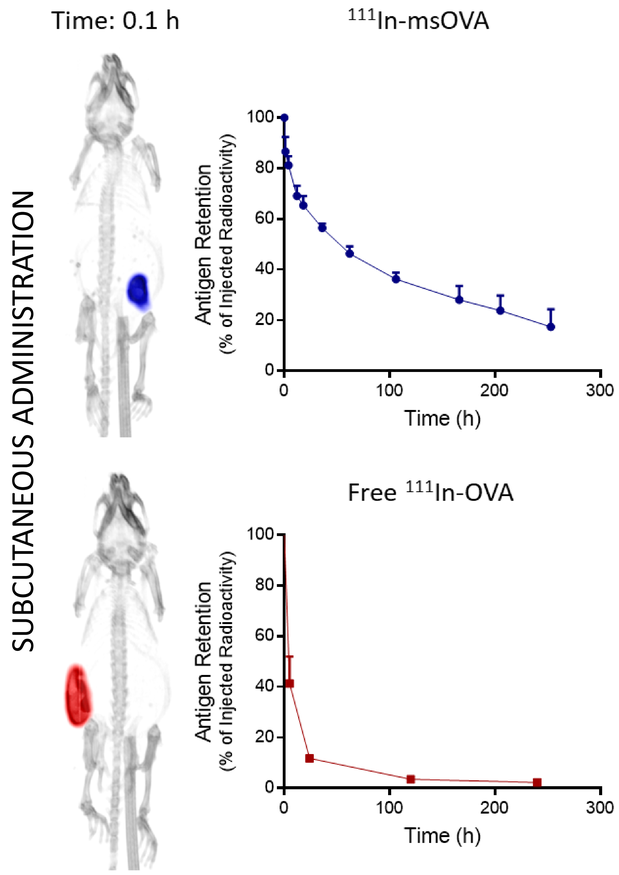
RESULTS

IN VITRO RELEASE ASSAY

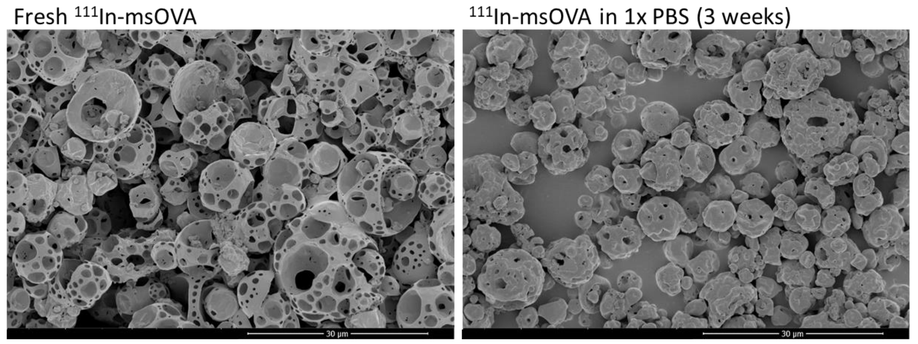


- **Size** of the PLGA microspheres showed a Z-average of **4.6 ± 0.9 µm**
- A p**rime phase** was observed both *in vitro* and *in vivo*, with ~30-40% of the antigen load released **within 24 h**
- PLGA microspheres released a higher antigen load when incubated in mouse plasma compared to 1x PBS
- Following the prime phase, PLGA microspheres tested ***in vitro*** entered a prolonged **lag phase** for the next 2 weeks
- ***In vivo,*** the antigen was **continually released** from the injection site and no apparent boost phase was observed
- PLGA microspheres **prolonged antigen retention** compared to control free ^{111}In-OVA

SPECT/CT IMAGING



SCANNING ELECTRON MICROSCOPY



CONCLUSION

PLGA microspheres showed an initial prime phase both *in vitro* and *in vivo*. However, while microspheres studied *in vitro* entered a prolonged lag phase, ^{111}In-msOVA *in vivo* was continually released from the injection site. Therefore, our data shows that *in vitro* assays do not always reflect what occurs in the body. To optimally design and develop new vaccines, more attention should be address to their pharmacokinetics.

ACKNOWLEDGEMENTS

We'd like to thank Laura Mowbray, from UBC Centre for Comparative Medicine for her assistance during the animal studies. Gethin Owen from UBC Centre for High-Throughput Phenogenomics for his help in the acquisition of SEM images.

Funding was provided by Lundbeck Foundation.

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