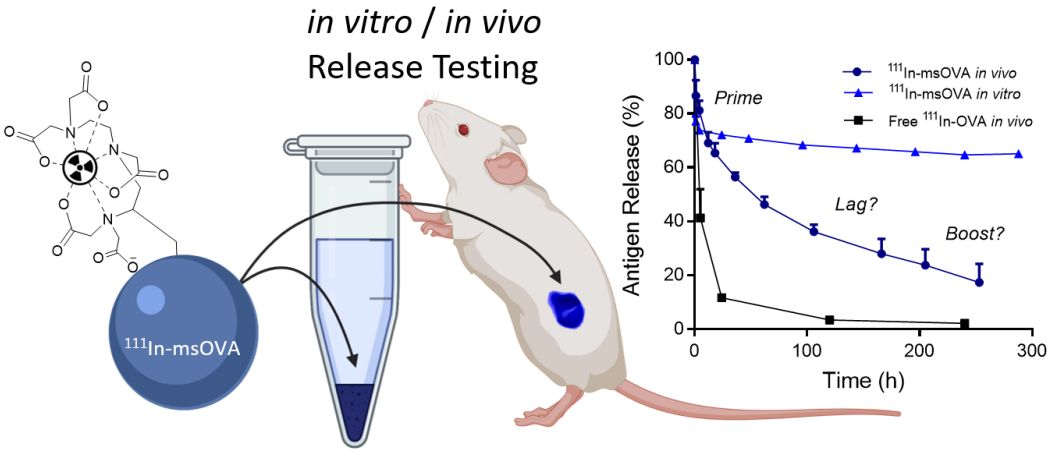

Correlation Between *In Vitro* and *In Vivo* Release of Radiolabeled Antigen from PLGA Microspheres Using SPECT/CT Imaging

Tullio V. F. Esposito[1,2,#,*], Jacqueline C. Y. Lai[3,#], **Marta Bergamo**[1,‡], Colin Blackadar[1], Cristina Rodríguez-Rodríguez[1,4], Katayoun Saatchi[1,*], Jan P. Dutz[3] and Urs O. Häfeli[1,2,*]

[1] Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada. [2] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [3] Department of Dermatology & Skin Science, Faculty of Medicine, University of British Columbia, Vancouver, Canada. [4] Department of Physics and Astronomy, Faculty of Science, University of British Columbia, Vancouver, Canada

Co-First Authors; ‡ Presenting Author; * Corr. Authors: tullio.esposito@alumni.ubc.ca, kathy.saatchi@ubc.ca, urs.hafeli@ubc.ca

Typically, for inactivated subunit vaccines, multiple doses have to be administered to generate a protective response: an initial priming injection followed by one or more booster shots. To improve patient compliance with such vaccination schedules, drug delivery systems have been developed to release antigen over a tightly controlled series of burst and lag phases from a single injection. Biodegradable poly(lactide-co-glycolic acid) (PLGA) have received considerable attention for this application due to their peculiar 'prime-lag-boost' release profile. Although the release of antigens from PLGA microspheres is well characterized on the bench in buffers and physiological media, the *in vivo* performance of these particles remains largely unknown.

To shed a light on the latter, the model antigen ovalbumin (OVA) was radiolabeled with the long-lived gamma-emitter ^{111}In and encapsulated into PLGA microspheres using a W/O/W emulsion technique; these particles are termed ^{111}In-msOVA. The morphology of the particles was assessed using scanning electron microscopy (SEM) and dynamic light scattering (DLS; Z-Ave 4.6 ± 0.9 µm). Benchtop release curves were obtained in 1x PBS and diluted mouse serum using a gamma counter over a period of 3.5 weeks. The same microspheres were also injected into the subcutaneous fat of healthy female C57Bl/6 mice, and using a preclinical SPECT/CT imager, the activity remaining at the site of administration was measured over a 2 week period. A prime phase of release was observed both *in vitro* and *in vivo*, consisting of ~30-40% of the antigen load over the first 24 hours. However, while the *in vitro* microspheres entered a prolonged lag phase for the next 2 weeks, antigen was continually released from the *in vivo* injection site. No apparent boost phase was observed for the *in vivo* microspheres either. However, ^{111}In-msOVA did prolong antigen retention compared to free OVA. The timing of antigen administration is crucial in producing the desired immune response from a vaccine. Our data shows that the *in vitro* release profile of antigen from PLGA microspheres does not always reflect what occurs in the body. In order to design and develop better vaccine delivery systems, a particularly important topic in the time of the COVID-19 pandemic, more attention needs to be addressed to their pharmacokinetics.