



John Garner
Manager

**PolySciTech Division of
AKINA, INC.**
3495 Kent Avenue
West Lafayette, IN 47906
Tel: (765) 464-0501x304; Fax: (765) 464-0820
E-mail: jg@akinainc.com
<http://www.akinainc.com/>

PolyVivo AV006 (PLGA-FPI749) In-Vivo Imaging Study

Purpose

Determine feasibility of in-vivo imaging using PolyVivo AV006 as a near-IR fluorescently conjugated tracer.

Equipment

Imaging done using custom made imaging system consisting of a Nikon D7000 camera with internal near-IR filter removed to increase range of sensitivity. This camera was equipped with a Tamaron SP AF 60mm lens and two sequential filters were mounted on the lens (Hoya R72 filter and Edmond Optics 800nm SC filter) to filter out visible light. Excitation was provided by a 650nm LED in a darkened room and images were collected for 15-30 seconds at an ISO sensitivity of 6400.

Animal

All animal research was performed ethically at Purdue University under the auspices of the Purdue Animal Care and Use Committee (PACUC).

Method

PLGA-FPI749 end-capped polymer (PolyVivo AV006) was dissolved in dichloromethane and swiped across a 50 um micro-patterned hydrogel template to generate microparticles. The particles were subsequently harvested and washed as detailed elsewhere (<http://www.akinainc.com/pdf/140219MicroparticleKitSOPcurrent.pdf>). The collected microparticles were re-dispersed in sterile saline (0.9%) with poloxamer 407 (20% w/v) thermogel included to assist in holding the microparticles in place at injection site.

For the test ~1mg total mass of 50 um sized AV006 microparticles were injected subcutaneously in dorsal location of a balb/c mouse. Images (Fig 1) show normal view and NIR imaging of same location after particles had been in mouse for 22 days. Note the NIR fluorescence appears as a purple color.

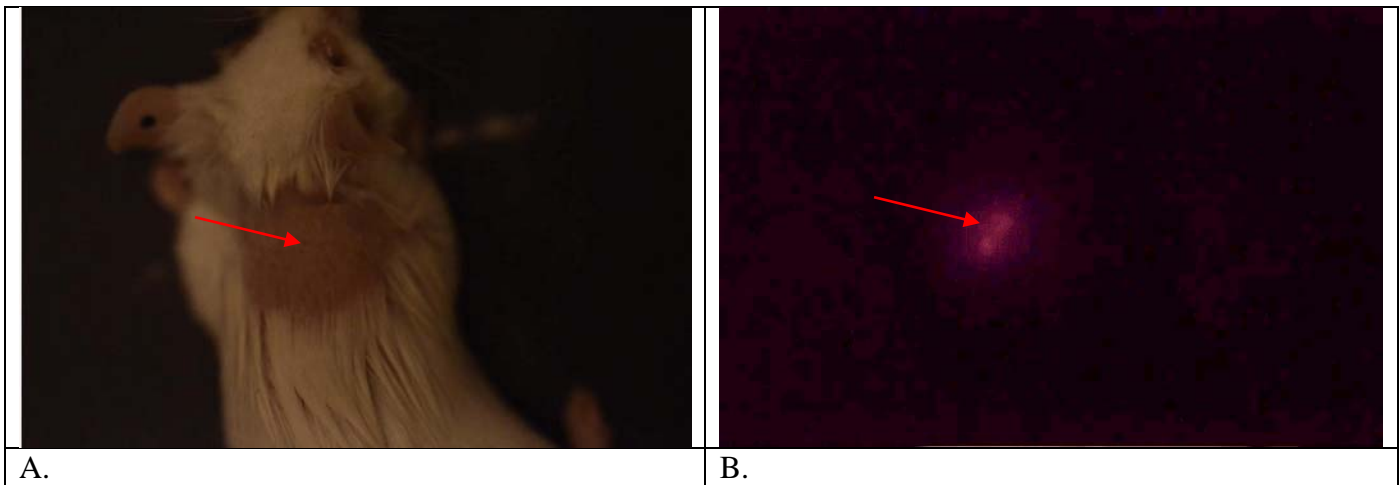


Figure 1. Image series shows mouse 22 days after subcutaneous injection of AV006 microparticles. Photos taken in same location in A) normal view and B) Near-IR imaging modes. Red arrows indicates dorsal injection site.

Additionally the same test was performed using an intramuscular injection into the hind-limb of the balb/c mouse model. The images below (Fig 2) show the same site under both normal lighting and in Near-IR mode.

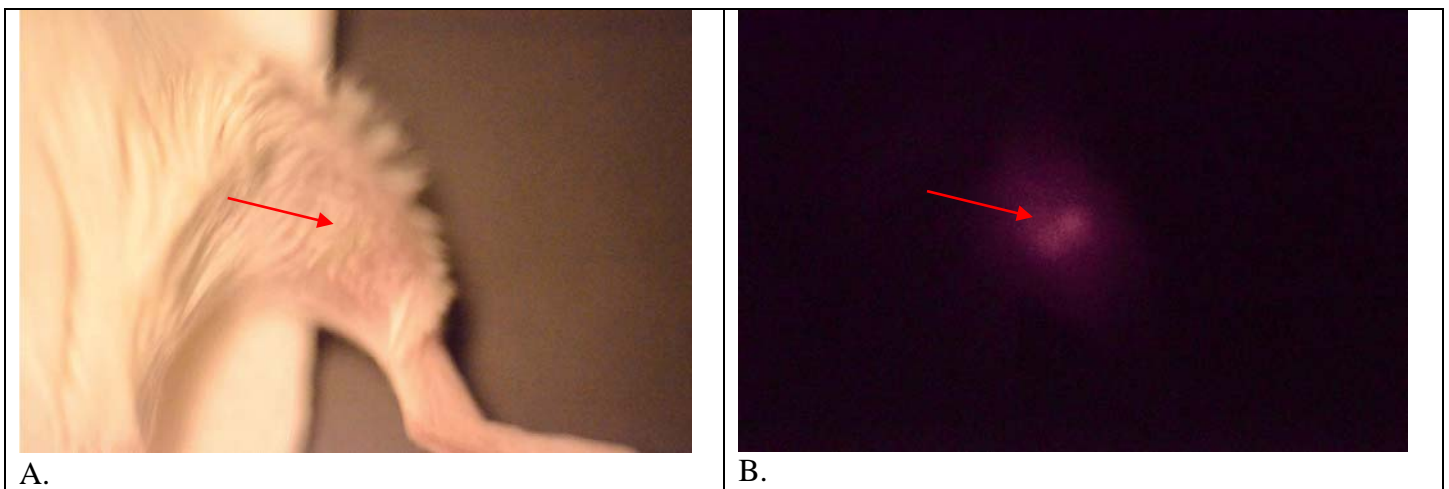


Figure 2. Image series shows mouse 8 days after intramuscular injection of AV006 microparticles. Photos taken in same location in A) normal view and B) Near-IR imaging modes. Red arrows indicates injection site.

Conclusion

The polymer PolyVivo AV006 (PLGA-FPI749) possesses fluorescence properties that allow for near-IR imaging in-vivo.