Young Innovators 2011

Formulation of Novel Particulate Breast Cancer Vaccines using Spray Drying and *In Vivo* Evaluation of Vaccine Efficacy

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Awards in Biotechnology

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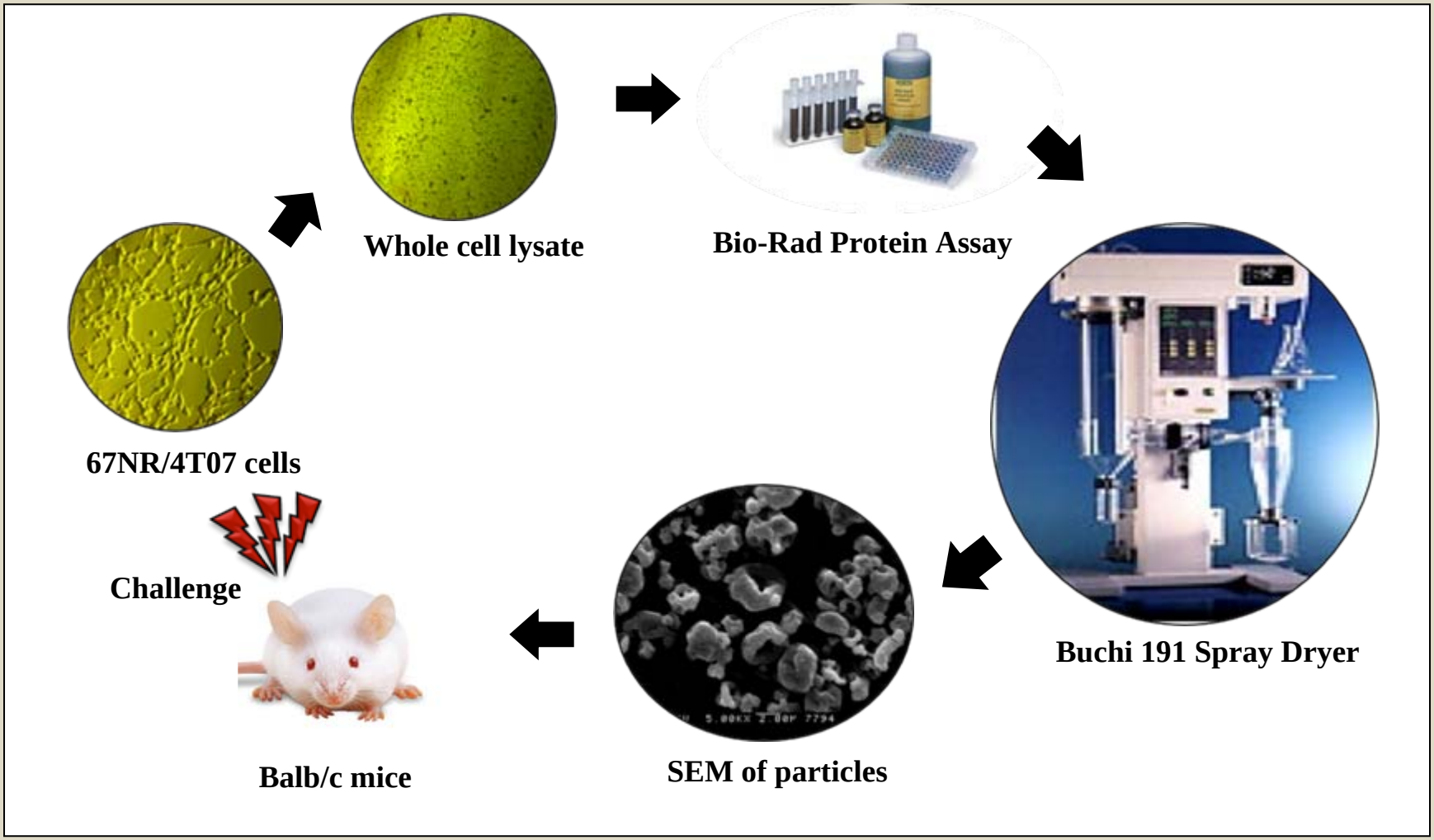
Abstract

This study aims to formulate and evaluate two particulate vaccines for breast cancer using murine breast cancer models. 67NR and 4T07 murine breast cancer cell lines were used to prepare whole cell lysate which served as the antigen source for the particulate vaccine. 67NR antigens were entrapped in an albumin matrix, while 4T07 antigens in a β-cyclodextrin matrix. Formulation matrices were spray dried using Buchi 191 spray dryer to obtain vaccine particles. The average particle size of both 4T07 β-cyclodextrin and 67NR albumin particles was 1.3-1.7µm with slight positive surface charge. Later the vaccine efficacy was evaluated in vivo in female Balb/c mice. Vaccine was delivered via oral, transdermal and subcutaneous routes and serum IgG response was measured during vaccination. Both vaccines could enhance the serum IgG levels significantly when compared to controls ($p<0.001$). Further the vaccine efficacy was tested by challenging the animals with live tumor cells. Vaccinated animals were protected from the challenge for significantly longer intervals than controls in both particulate vaccine studies ($p<0.001$) indicating the vaccine was efficient in generating protective immunity against murine breast cancer model.

Introduction

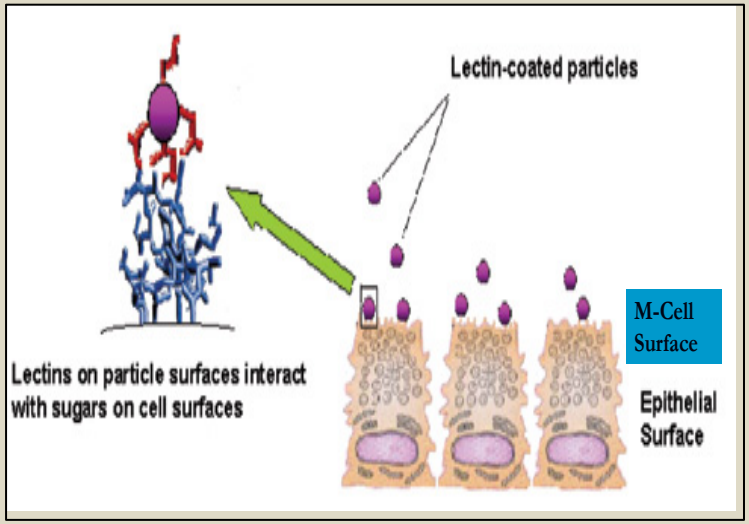
Breast Cancer is the major female specific cancer affecting the population in United States. Many therapies are being evaluated to combat this cancer such as chemotherapy, surgery, hormone therapy and radiation therapy. Most of these therapies are invasive and possess numerous adverse effects. Immunotherapy is being explored to provide a better treatment option to cancer patients. Various clinical trials are in progress utilizing this approach but so far no vaccine is available in the market. Currently our lab is investigating the efficacy of two whole cell lysate breast cancer vaccines of two murine models via two specialized microparticles which can be delivered orally, transdermally, and subcutaneously in a murine model.

Materials and Methods



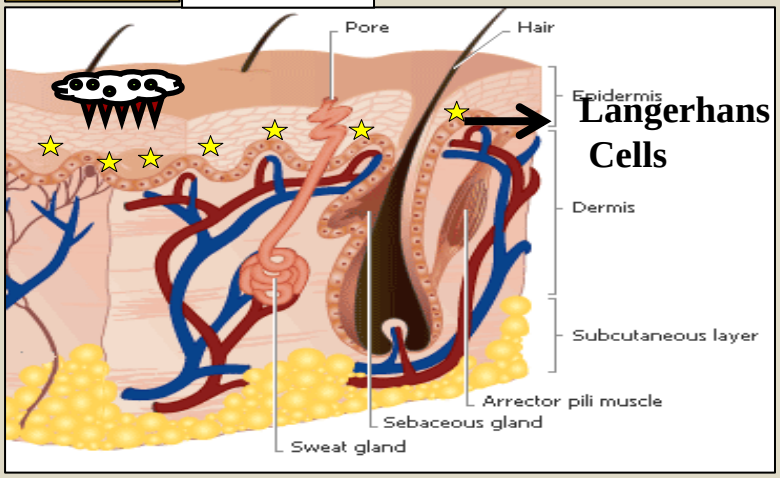
Materials and Methods

Oral Route



Transdermal Route

Admin Pen



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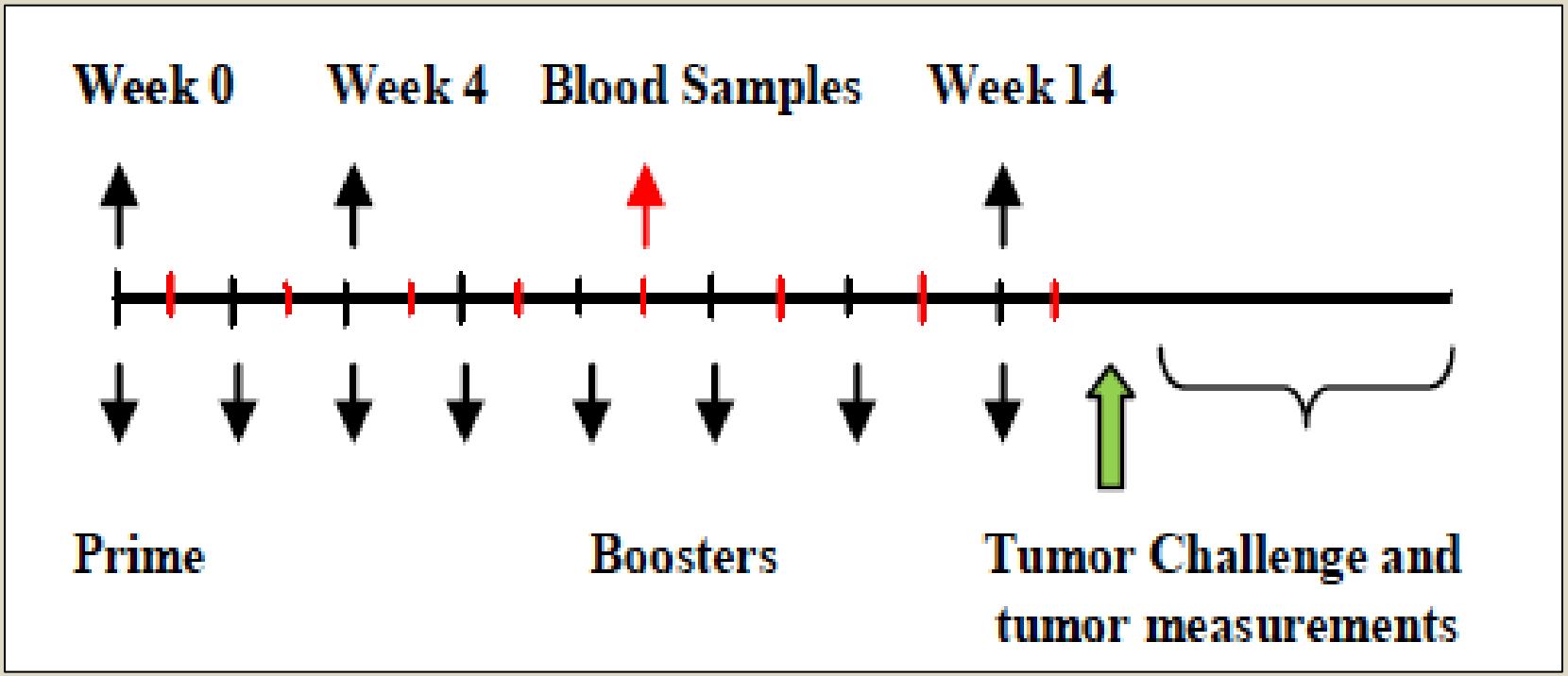
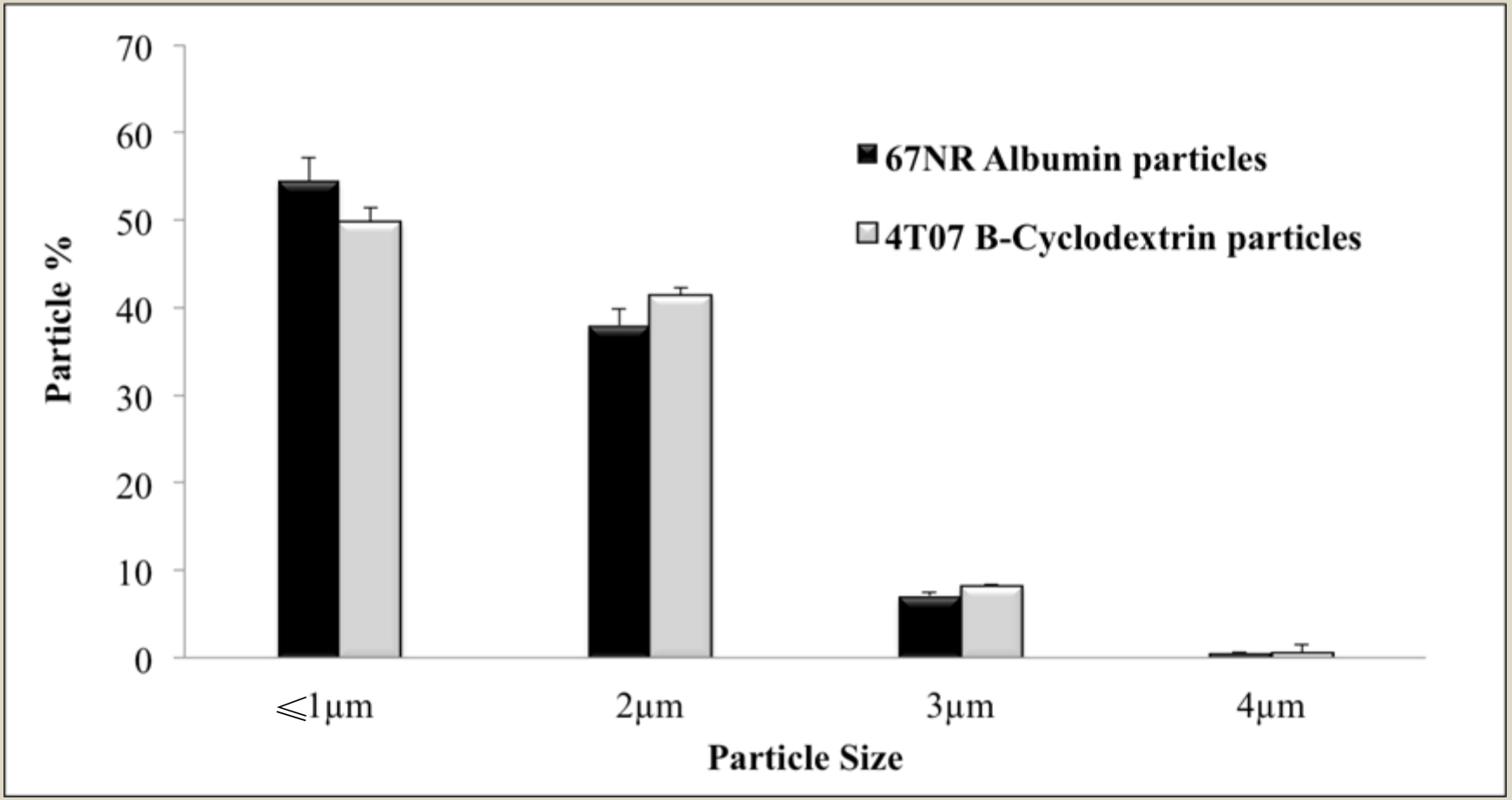
Materials and Methods

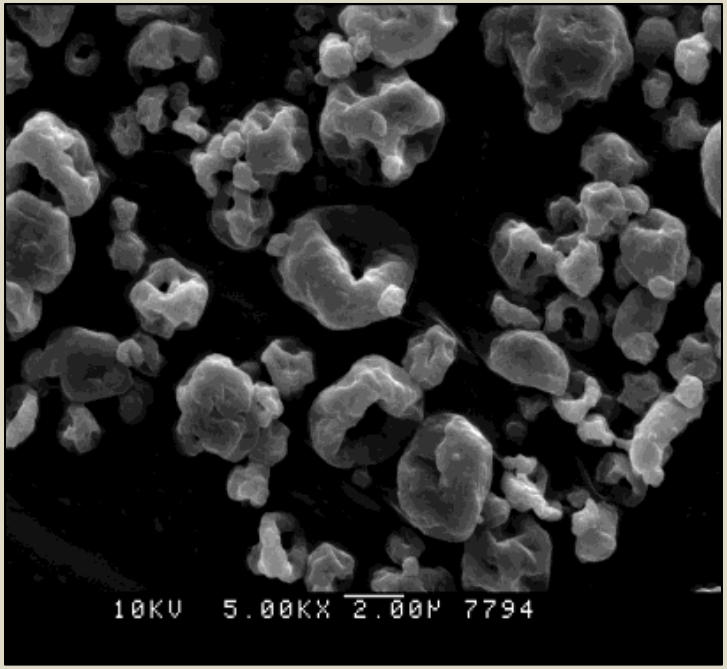


Immunization Scheme

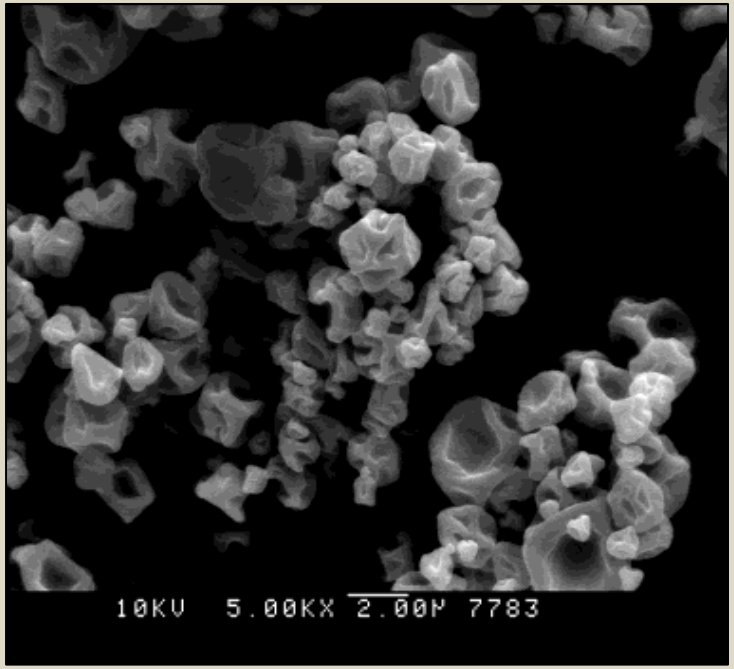
Results: Particle Size Distribution



Results: Particle Surface Morphology

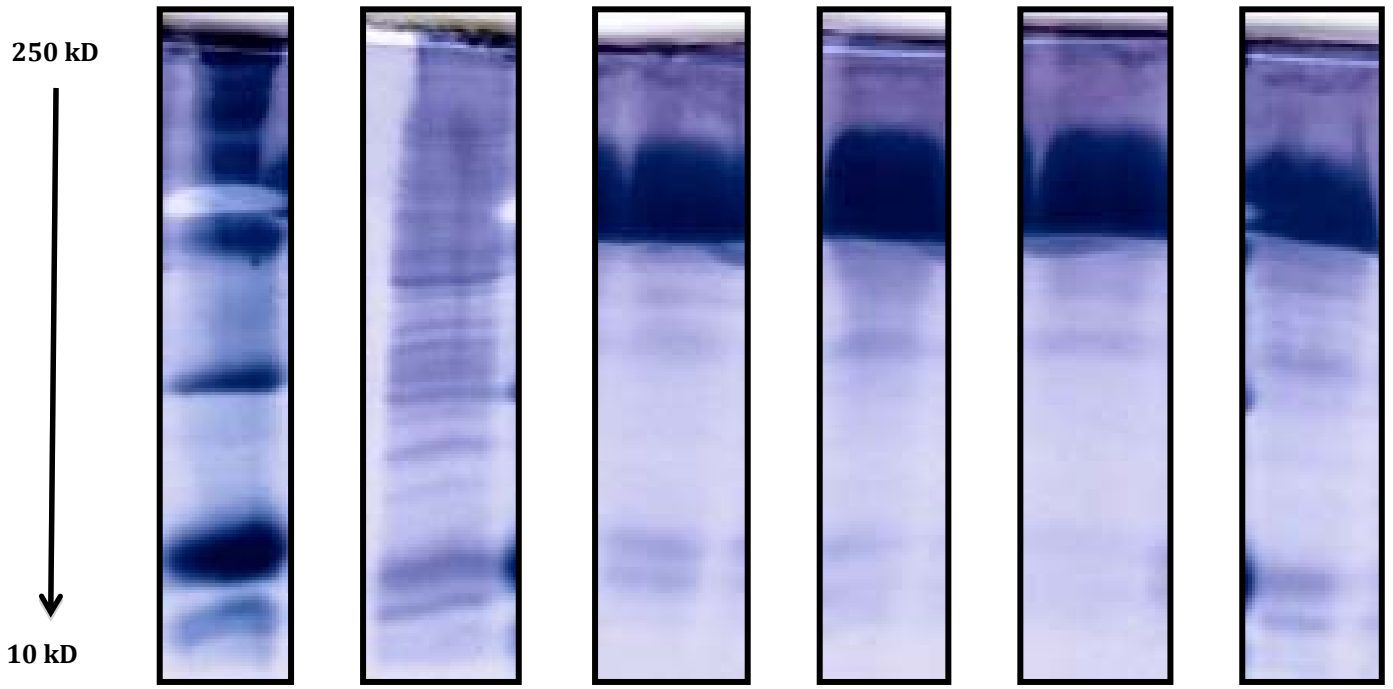


Scanning Electron Microscopy Image of 67NR vaccine particles



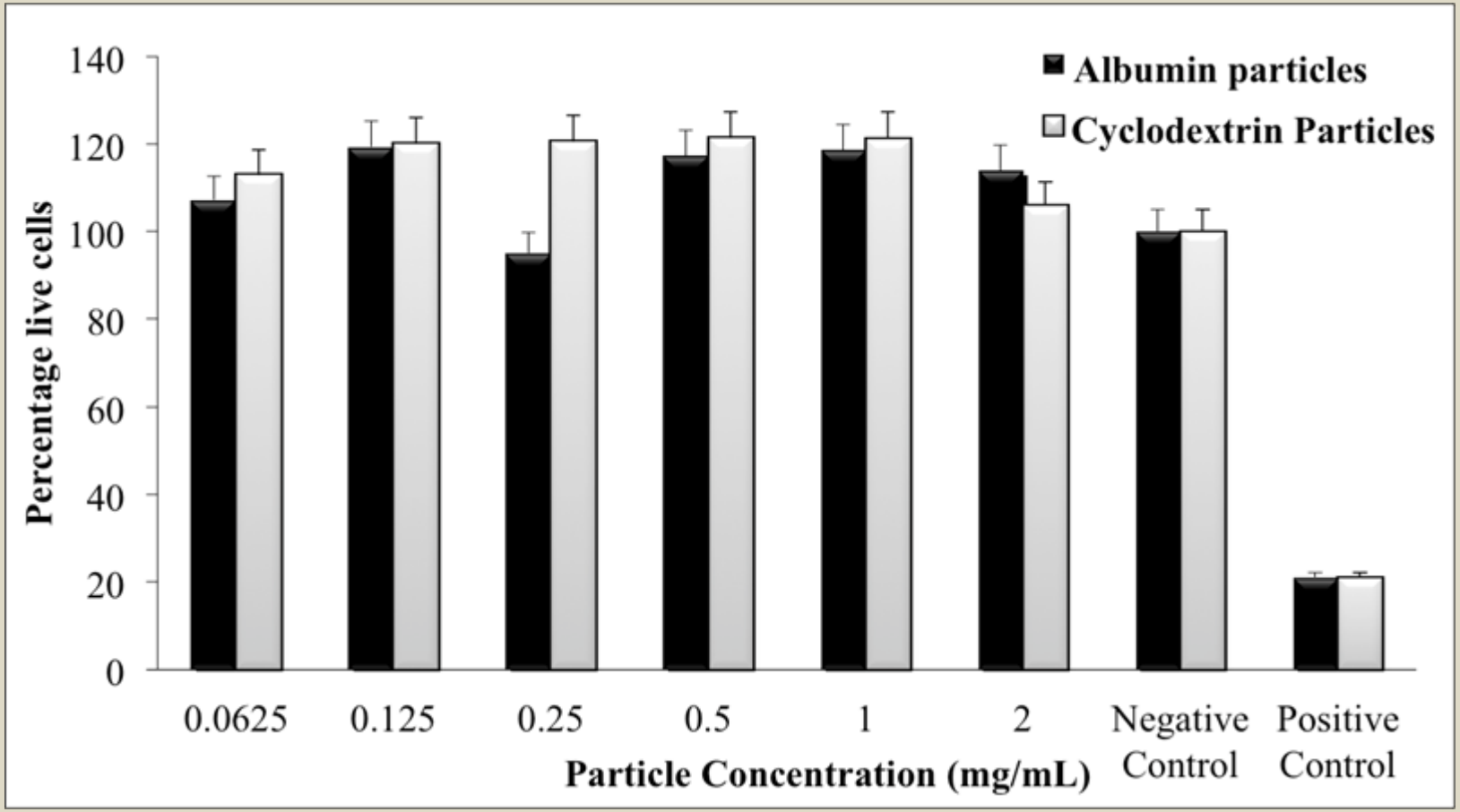
Scanning Electron Microscopy Image of 4T07 vaccine particles

Results: SDS PAGE Analysis

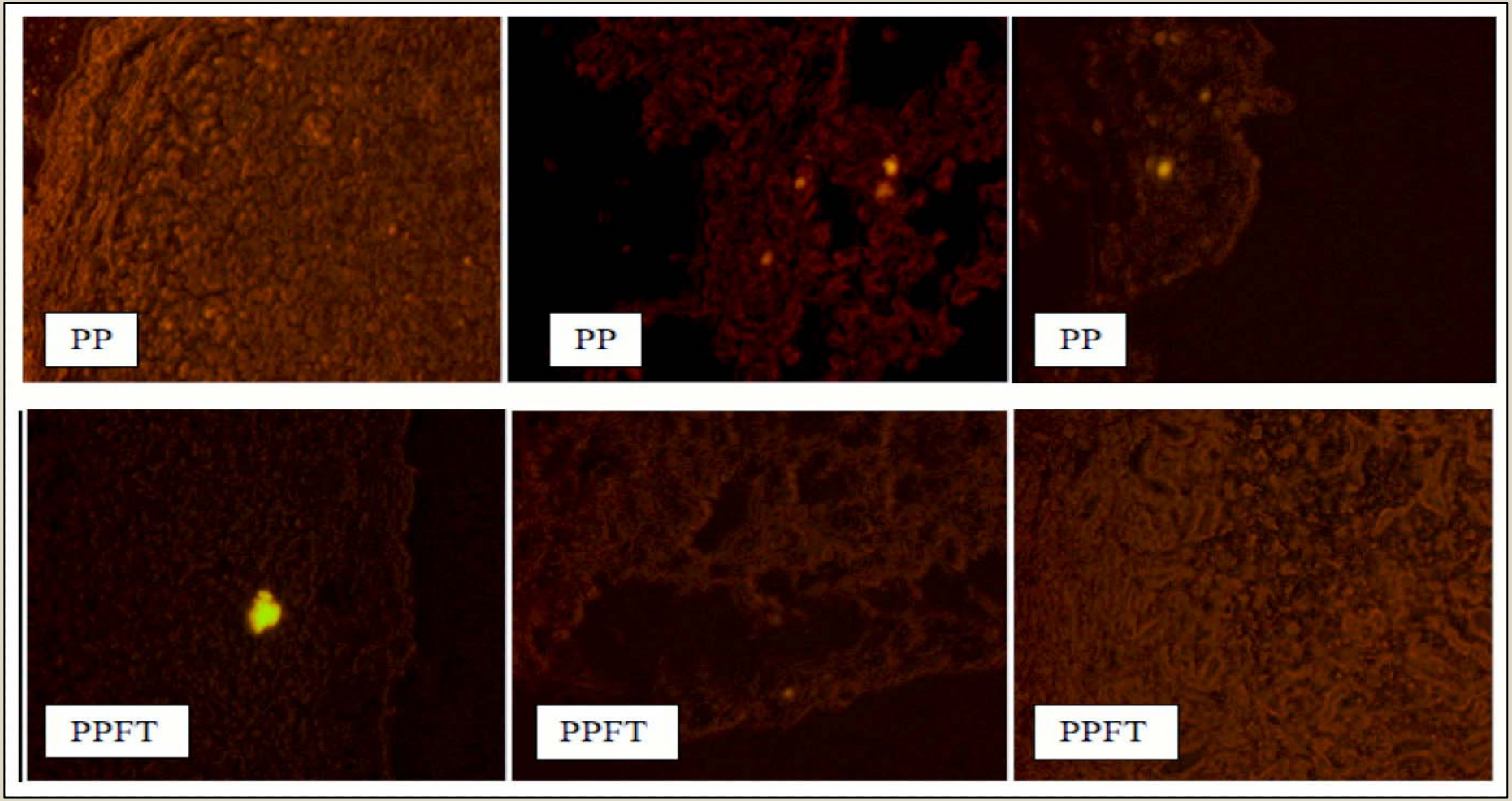


Standard	67NR Lysate	67NR Particles in pH 1.2	67NR Particles in pH 4.0	67NR Particles in pH 6.8	67NR Particles in pH 7.0

Results: MTS cell cyto-toxicity assay

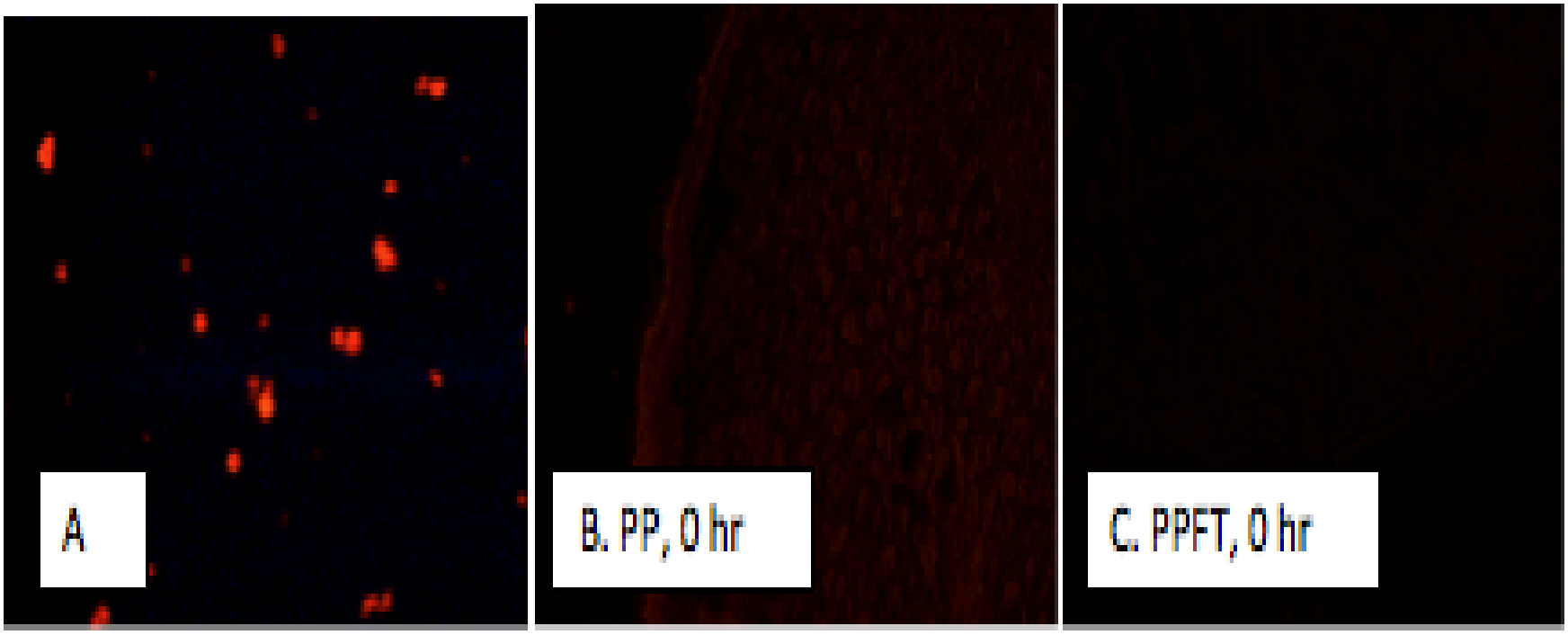


Results: In situ Particle Uptake Study



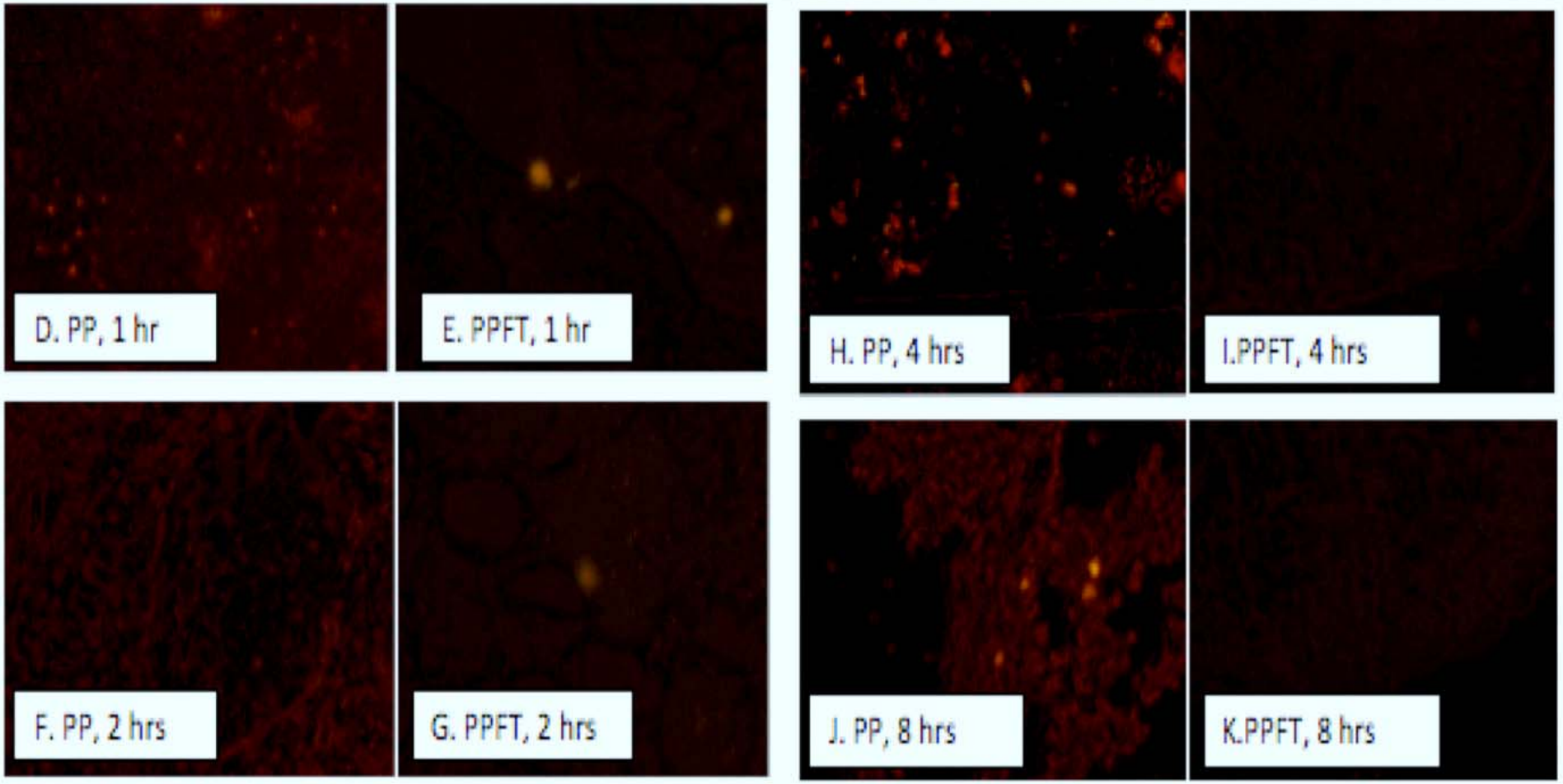
PP = Peyer's Patch; PPFT = Peyer's Patch Free Tissue

Results: In vivo Particle Uptake Study



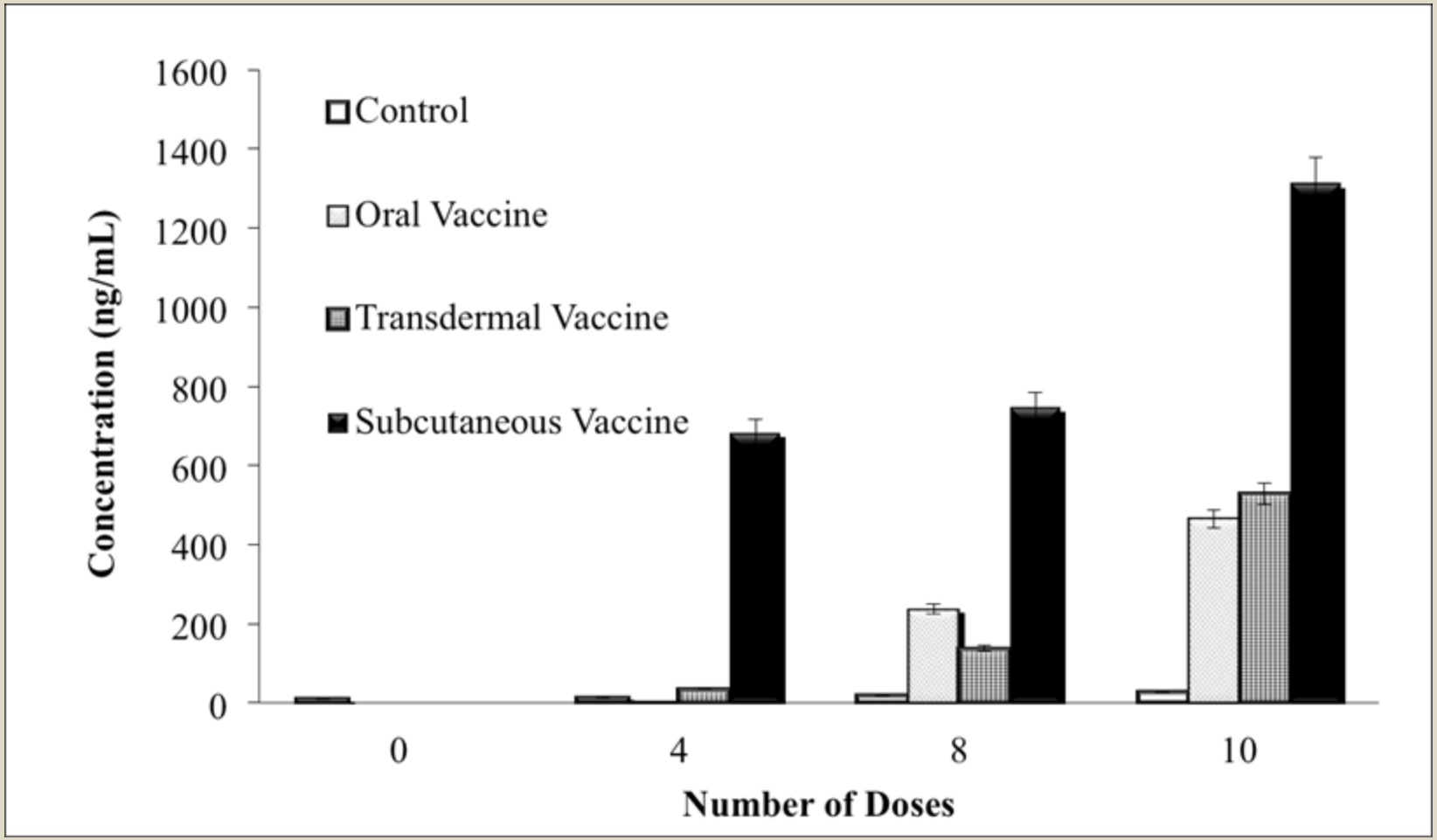
PP = Peyer's Patch; PPFT = Peyer's Patch Free Tissue

Results: In vivo Particle Uptake Study

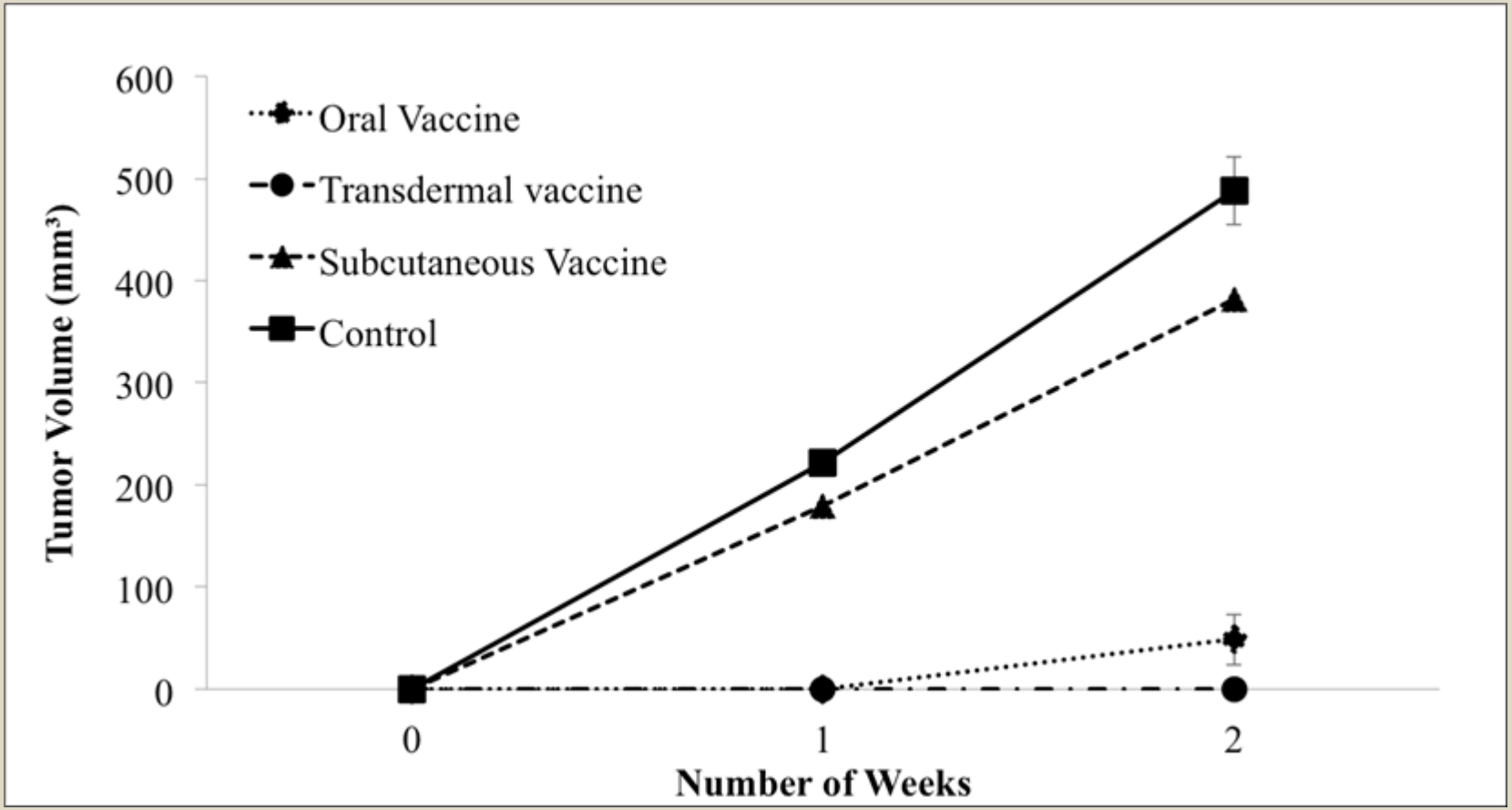


PP = Peyer's Patch; PPFT = Peyer's Patch Free Tissue

Results: Serum IgG Titers 67NR Vaccine



Results: Tumor Measurements 67NR Vaccine



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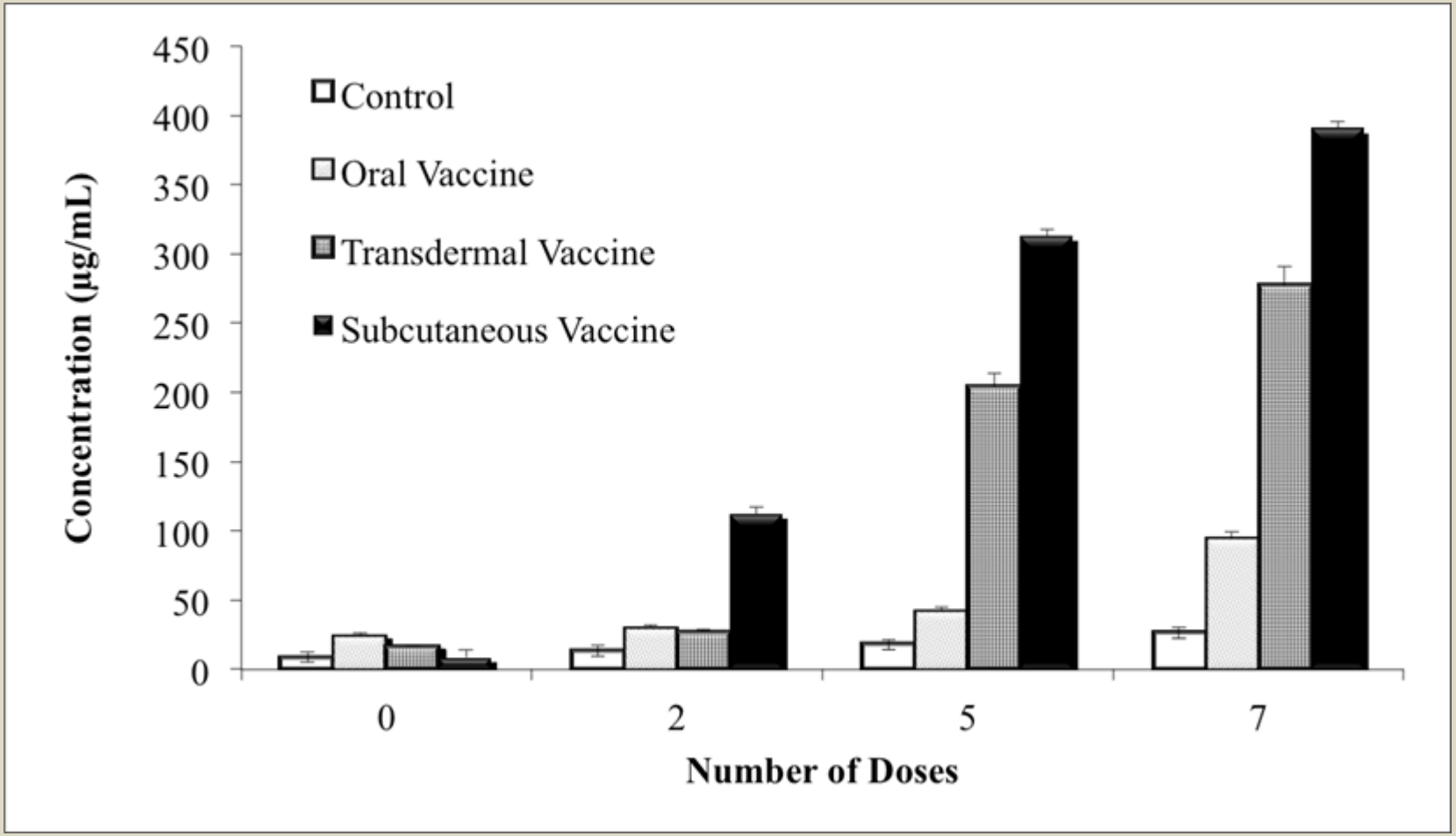
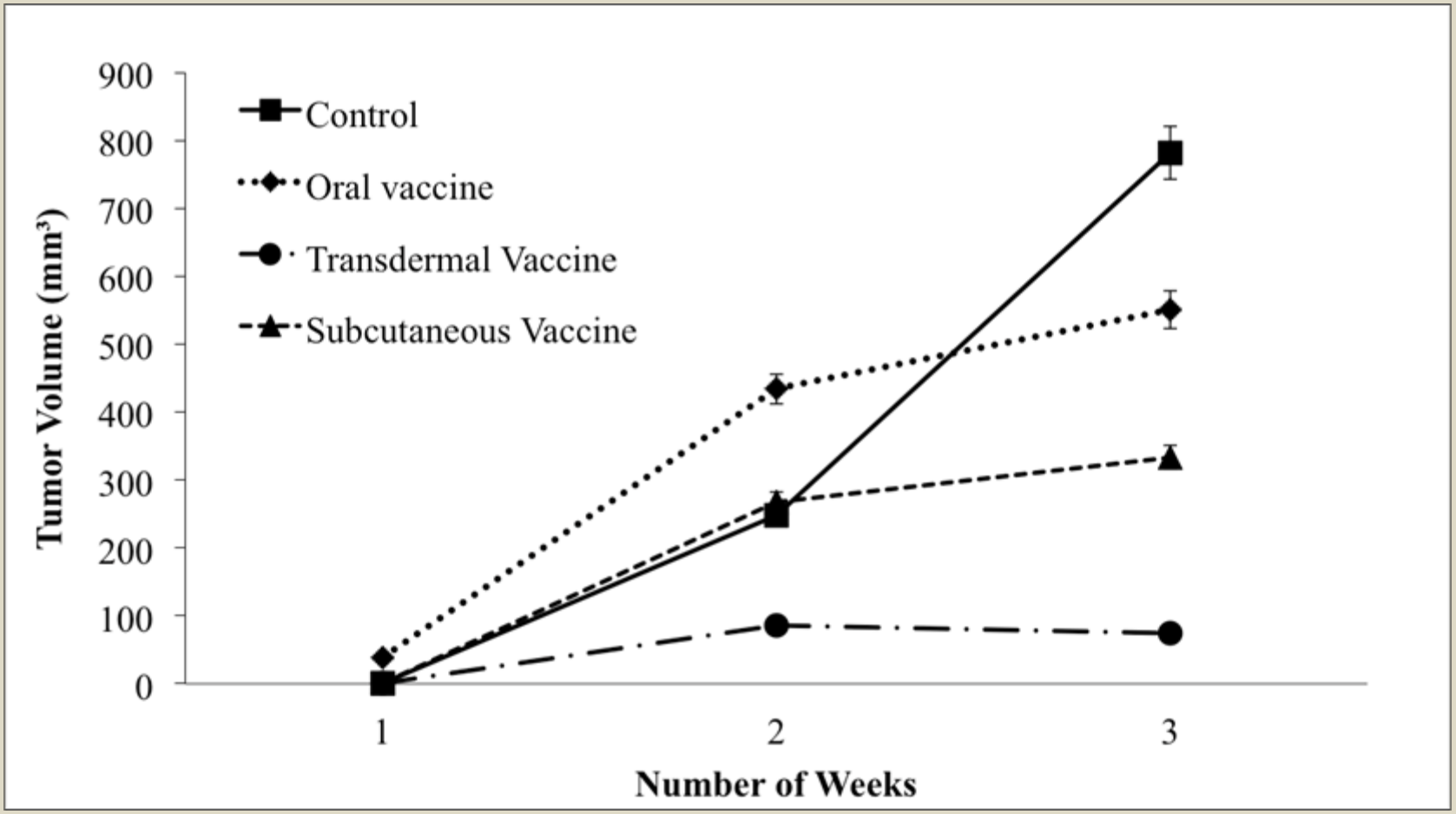
Results: Serum IgG Titers 4T07 Vaccine



Results: Tumor Measurements 4T07 Vaccine



Discussion

- Particle size of the formulation was important to achieve efficient particle uptake.
- The formulations were efficient in sustaining the release of antigen and provided enteric protection when given orally.
- Both 67NR and 4T07 oral particulate vaccines required multiple boosters to obtain significant antibody titers when compared to transdermal and subcutaneous particulate vaccines.
- Various studies are ongoing to understand the immune pathway followed by these vaccine particles. Flow cytometry based studies indicate role of cellular and humoral immune response that protected vaccinated animals longer than their control counterparts.

Conclusion

- The novel approach to formulate particulate breast cancer vaccines which can be efficiently delivered through oral, transdermal and/or subcutaneous routes proves to be promising mode of immunization against breast cancer as proven by two individual in vivo studies in this paper.

- This mode of immunization can be used as an individualized therapy, where tumor cells can be isolated from the patient and cell homogenate can be formulated into a particulate vaccine to avoid relapse and enhance immune response.

- Nanotechnology thus can serve as a savior for millions of patients suffering from this dreadful form of cancer.

Acknowledgments

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- **Dr. Ray Green**
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- **Family and friends**

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