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## ACUTE VASCULAR EFFECTS OF NANOPARTICLE INFUSION IN ISOLATED PERFUSED SKIN

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### Abstract

The majority of studies on the effect of nanomaterials on biological function involves either isolated in vitro cell systems or are concerned with in vivo effects after inhalational or dermal exposure. The present work reports on an intriguing observation of the vascular effects seen in an ex vivo perfused tissue preparation, the isolated perfused porcine skin flap (IPPSF), in studies conducted to assess nanomaterial biodistribution. Compared to a relatively large dataset involving organic chemical infusions (n=53), infusion of six different nanoparticles of diverse sizes and composition (silica or dextran coated Fe<sub>2</sub>O<sub>3</sub>, silica or citrate coated silver, PEG or carboxylated quantum dots (QD)) resulted in statistically significant post-infusion flap weight gain and an increase in arterial perfusion pressure (especially with QD-PEG). In contrast, infusion with nC<sub>60</sub> nanoparticles did not produce these effects. These observations suggest certain nanoparticle infusions may be associated with acute vascular physiological effects which merit further attention.

### Keywords

Nanoparticles; vascular toxicity; in vitro; biodistribution

### Background

A wide variety of engineered nanomaterials have been developed and explored for their application to varied disciplines. Safety has been a major concern affecting widespread adoption. Although there have been a number of in vivo inhalational and in vitro cellular toxicity studies (see Monteiro-Riviere and Tran, 2007 for comprehensive review), there is a paucity of work defining the physiological effects after systemic exposure secondary to injection for drug therapeutics or diagnostic applications or inadvertently post-absorption after inhalational, topical or oral exposure. There are limited studies specifically looking at the vascular effects of systemic nanomaterials of any composition.<sup>1,2,3</sup>

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Our laboratory developed an isolated perfused porcine skin flap (IPPSF) as an *ex vivo* model primarily to study dermal drug and chemical absorption and cytotoxicity.<sup>4,5</sup> This model has also been used to study drug distribution after intra-arterial infusion,<sup>6</sup> vascular effects of autonomic drugs<sup>7</sup> and more recently to model nanomaterial biodistribution after intra-arterial infusion.<sup>2,8</sup> In all experiments, data on arterial vascular resistance throughout the infusion and skin flap weight prior to and following infusions have been collected on some 3600 experiments. During recent studies involving intra-arterial infusion of various nanomaterials, we noticed that compared with organic chemical experiments, infusion of nanoparticles resulted in an increase in skin flap weight at the termination of an experiment and vascular resistance that tended to be greater than with studies involving organic small molecule infusions. These findings, although observational in nature, indicate a significant biological interaction is occurring whose mechanism and relevance to *in vivo* effects should be further explored *in vivo*.

## Methods

The IPPSF is a single-pedicle, axial pattern tubed skin flap obtained from the abdomen of female weanling Yorkshire pigs (*Sus scrofa*). Two flaps per animal, each lateral to the ventral midline, are created in a single surgical procedure. The procurement, care and use of animals were in accordance with the regulations and terms of the federal Animal Welfare Act and North Carolina State University's Institutional Animal Care and Use Committee guidelines. The procedure involves surgical creation of the flap with skin measuring 4 cm×12 cm and perfused primarily by the caudal superficial epigastric artery and its associated paired venae comitantes, followed by arterial cannulation and harvest in 48 hrs. The IPPSF is then transferred to a perfusion apparatus that is a custom designed temperature and humidity regulated chamber. Perfusion media consists of a modified Krebs-Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin. Normal perfusate flow-rate is maintained at 1 ml/min/flap (3–7 ml/min/100g) with a mean arterial pressure ranging from 30–70 mmHg, targets consistent with *in vivo* values reported in the literature. Viability for up to 24 hr has been confirmed through biochemical studies and extensive light and transmission electron microscopy studies. These techniques are fully described in the literature.<sup>4,5</sup>

Nanomaterials (sources listed in Table 1) study compounds are mixed into the arterial perfusate and concentrations measured in arterial and venous samples for biodistribution studies. These particles were characterized by transmission electron microscopy, dynamic light scattering (DLS) and Zeta potential and are listed in Table 1. A sample of organic chemical infusion flaps (n=53) previously conducted in our laboratory were selected for comparison. For the endpoints measured in the current work, skin flaps are weighed pre and post infusion and the weight change plotted. Perfusion pressure as an estimate of vascular resistance was measured in the arterial input line using an in-line pressure meter.

A one way analysis of variance was used to determine if the differences in the weight gain among the types of infusions was significant ( $p<.05$ ). A Dunnett's multiple comparison post hoc test was used to determine which nanoparticle infusion flaps were significantly different from the organic chemical infusions ( $p<0.05$ ). The mean and standard deviation of all perfusions for each type of nanoparticle were determined for each measured time point and plotted versus time to compare with the pressure during organic chemical perfusions. All statistical analysis and plots were generated with prism GraphPad (GraphPad Software, Inc., La Jolla, CA).

## Results and Discussion

The change in flap weight (mean  $\pm$  standard deviation) prior to and following the infusion experiments for each type of nanoparticle, compared with the chemical infusions, is shown in Figure 1. All nanoparticles except nC<sub>60</sub> resulted in significantly greater changes in the flap weight than have been observed previously with the organic chemical infusions. However, as seen in Figure 2, several of the nanoparticles appeared to result in an increase in pressure during the washout phase, only the infusions with the QD-PEG nanoparticles was elevated higher than the range of values observed for the organic chemical perfusions.

These studies imply that intra-arterial infusion of some nanoparticles result in modulating the vascular function as evidenced by changes in arterial pressure and skin flap weight gain well beyond control or drug infusion studies.<sup>7</sup> Increase in skin flap weight post infusion would be a marker of congestion and edema formation due to processes occurring at multiple sites in the flap vascular system. Praetner and co-workers<sup>1</sup> specifically associated anionic quantum dot association with capillary endothelium in in vivo mouse studies. Previously, we have shown capillary endothelium uptake of quantum dots in IPPSF studies which also demonstrated periodicity in arterial-venous extraction.<sup>2</sup> A number of in vitro endothelial cell studies and in vivo experiments have also hinted at nanoparticle effects on the vascular system.<sup>9,10,11</sup>

The present studies were not designed to probe the mechanism of these vascular effects but rather were an observation of abnormal vascular changes associated with nanoparticle studies conducted primarily for pharmacokinetic and biodistribution endpoints. Potential causes for such effects could be direct interaction of nanoparticles with vascular endothelial cell or agglomeration and precipitation in capillary beds resulting in an increase in vascular resistance and congestive edema, or local nanoparticle tissue deposition increasing osmotic pressure also resulting in skin flap weight gain post infusion. However, based on two decades of experimental data in this perfused tissue preparation, such effects are extraordinary and deserving of notation and further studies in other in vivo models. They also support the hint of cardiovascular toxicity seen with some nanomaterials and suggest this endpoint should be evaluated when in vivo toxicology studies are conducted.

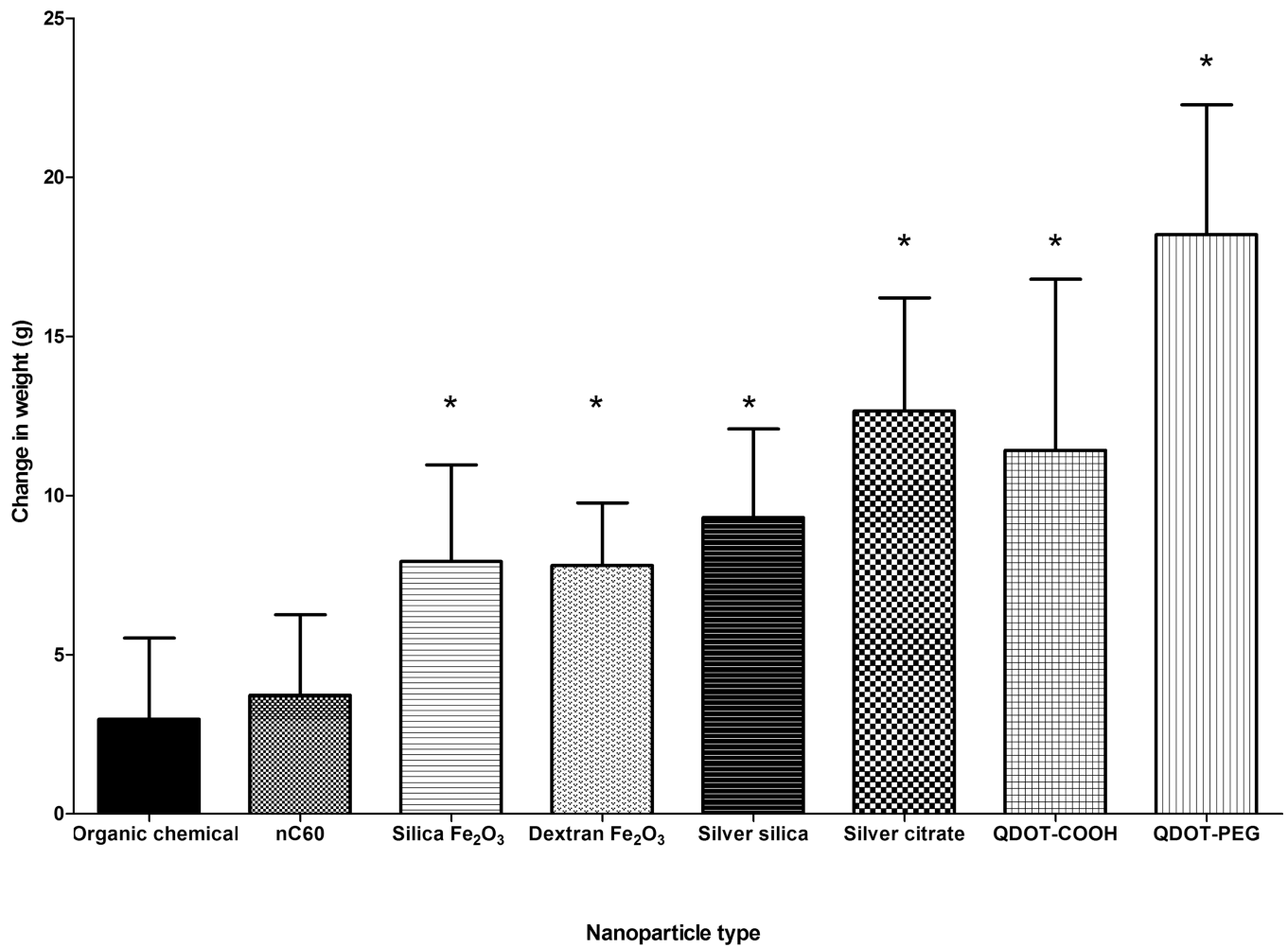
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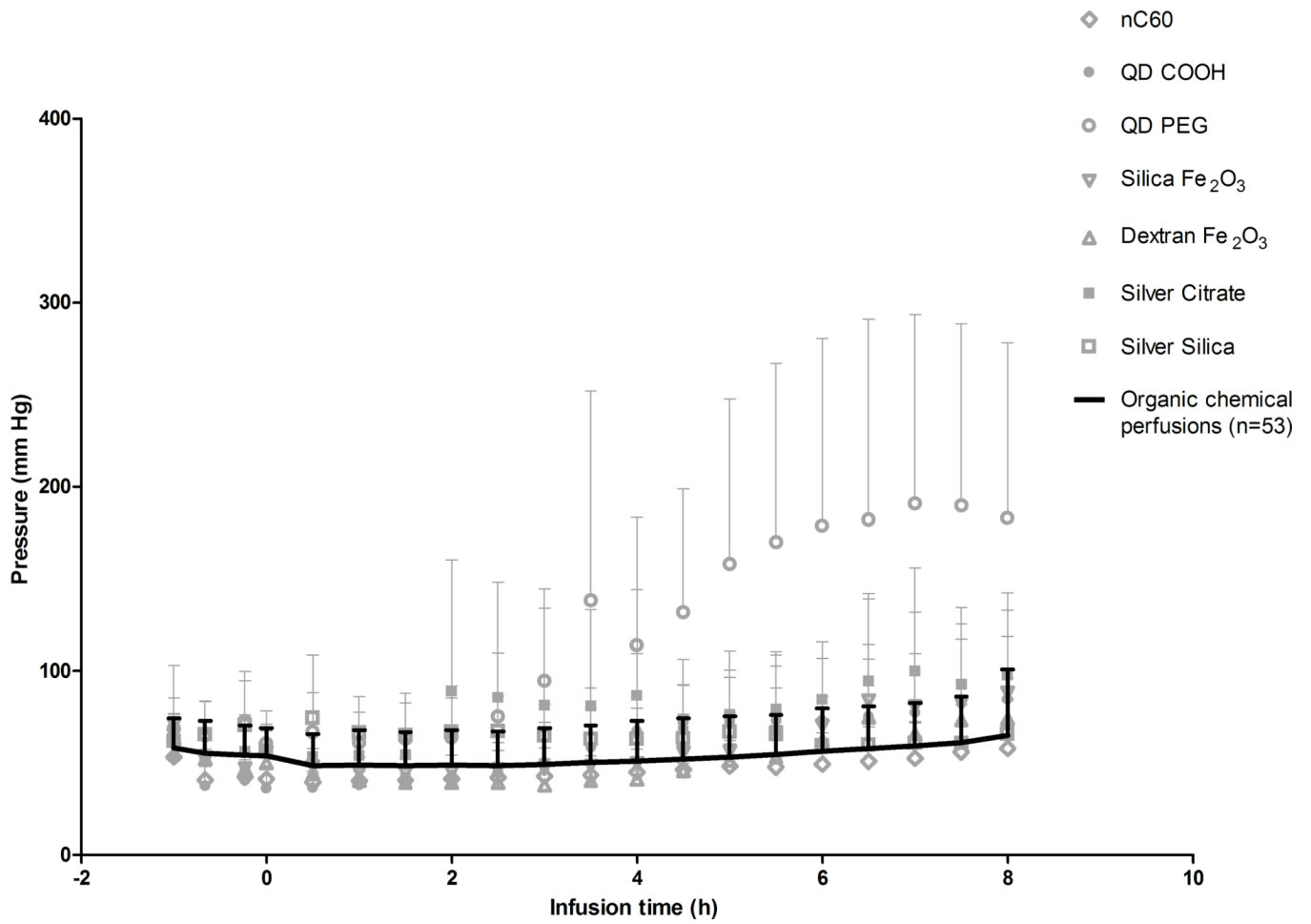
## References

1. Praetner M, Rehberg M, Bihari P, Lerchenberger M, Uhl B, Holzer M, Eichorn ME, Furst R, Perisic T, Reichel CA, Welsch U, Krombach F. The contribution of the capillary endothelium to blood clearance and tissue deposition of anionic quantum dots in vivo. *Biomaterials*. 2010; 31:6692–6700. [PubMed: 20619783]
2. Lee HA, Imran M, Monteiro-Riviere NA, Colvin VL, Wu W, Riviere JE. Biodistribution of quantum dot nanoparticles in perfused skin: evidence of coating dependency and periodicity in arterial extraction. *Nano Letters*. 2007; 7:2865–2870. [PubMed: 17685663]
3. Kreyling WG, Semmler-Behnke M, Mooler W. Health implications of nanoparticles. *J Nanoparticle Res*. 2006; 8:543–562.
4. Riviere JE, Bowman KF, Monteiro-Riviere NA, Dix LP, Carver MP. The isolated perfused porcine skin flap (IPPSF). I. a novel in vitro model for percutaneous absorption and cutaneous toxicology studies. *Fundam Appl Toxicol*. 1986; 7:444–453. [PubMed: 3781134]
5. Monteiro-Riviere, NA. Kempainen, BW.; Reifenrath, WG. *Methods for Skin Absorption*. CRC Press, Inc; 1990. Specialized technique: isolated perfused porcine skin flap; p. 175-189.

6. Williams PL, Riviere JE. Definition of a physiologic pharmacokinetic model of cutaneous drug distribution using the isolated perfused porcine skin flap (IPPSF). *J Pharm Sci.* 1989; 78:550–555. [PubMed: 2674404]
7. Rogers RA, Riviere JE. Pharmacologic modulation of the cutaneous vasculature in the isolated perfused porcine skin flap (IPPSF). *J Pharm Sci.* 1994; 83:1682–1689. [PubMed: 7891294]
8. Leavens TL, Xia XR, Lee HY, Monteiro-Riviere NA, Brooks JD, Riviere JE. Evaluation of perfused porcine skin as a model system to quantitate tissue distribution of fullerene nanoparticles. *Toxicol Letters.* 2010; 197:1–6.
9. Sommer AP. Cytotoxicity of calcium phosphate crystals and human-derived nanoparticles; an observed link. *Circ Res.* 2010; 106:e10. [PubMed: 20508196]
10. Moller P, Mikkelsen L, Vesterdal LK, Folkmann JK, Forchhammer L, Roursgaard M, Danielsen PH, Lofts S. Hazard identification of particulate exposure on vasomotor dysfunction and progression of atherosclerosis. *Crit Rev Toxicol.* 2011; 41:339–368. [PubMed: 21345153]
11. Monteiro-Riviere, NA.; Tran, L. *Nanotoxicology: Characterization, Dosing, and Health Effects.* New York: Informa Healthcare; 2007.



**Figure 1.** Comparison of the weight gains of skin flaps among types of IPPSF experiments. The weight gain varied significantly among infusion types (ANOVA,  $p < 0.05$ ) and all but nC<sub>60</sub> differed significantly ( $p < 0.05$ ) from the organic chemical infusions (\*).



**Figure 2.**

Arterial pressure (mean  $\pm$  standard deviation) versus time during both the 4-hr exposure and 4-hr washout phase for the various nanoparticles and organic chemicals.

Table 1

Characterization of nanoparticles infused into the IPPSF.

Nanomaterial	Concentration of Stock	Concentration Infused in IPPSF	Size (by TEM)	Size (by DL-S)	Zeta Potential	Nanomaterial Provided By
nC60	342 µg/ml	0.88 µg/ml	50 nm	94–352 nm	Not determined	1
621 QD-PEG	22.5µM	6.7µM, 3.3µM, 1.7µM, 0.8µM	5.8×8.4nm	37–40nm	Not provided	2
621 QD-COOH	22.5µM	6.7µM, 3.3µM, 1.7µM, 0.8µM	5.8×8.4nm	37–40nm	Not provided	2
Silver, Citrate BioPure	1.0mg/ml	0.9µg/ml	19nm	Not provided	-36.8mV	3
Silica-coated Silver	4.66mg/ml	0.5µg/ml	40nm	147nm	25.4mV	3
Dextran-coated (Fe <sub>2</sub> O <sub>3</sub> )	1.0mg/ml	1.3µg/ml	10nm	162nm	-20mV	4
Silica-coated (Fe <sub>2</sub> O <sub>3</sub> )	0.21mg/ml	1.2µg/ml	60nm	100nm	-36.7mV	5

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