

Physical Characteristics of 50 and 200 nm Magnetic Beads when Encapsulated with Branched Amphiphilic Peptides (BAP)

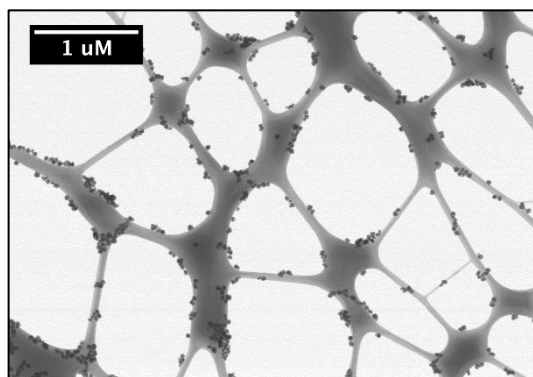
Branched Amphiphilic Peptide Capsule Magnetic Beads (BAPC-MB) are a new nanoparticle developed for use as in diagnostic platforms. The bases are Maleimide Super Mag Magnetic Beads which are dispersed in 75% ethanol and combined with our proprietary H₉Cys BAP. The result of the initial step is a magnetic bead with a monolayer of BAP which are washed to removed excess peptides through magnetic separation. The monolayer BAP magnetic beads are then dispersed with 100% tetrafluoroethylene and the H₉ BAP are added and mixed with sonication. The final step is diluting the mixture to more than 85% water. This synthesis results in the formation of BAPC-MB, which exhibit a peptide bilayer BAPC surrounding the individual magnetic beads.

Magnetic beads when encapsulated with BAP have the advantage of not forming aggerates following their suspension on Lacey Transmission Electron Microscopy (TEM) grids. This allows them to avoid clumping into a large mass and be able to remain distributed for delivery to cells.

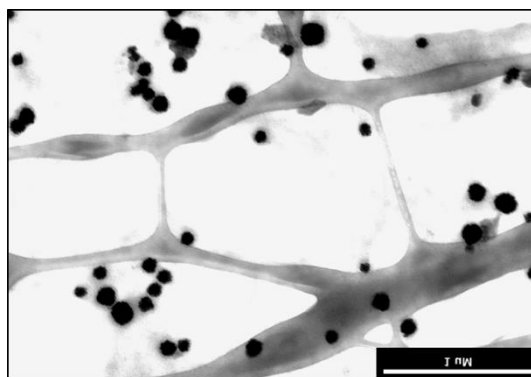
Encapsulating 50 and 200 nm magnetic beads results in BAPC-MB with final average diameters of 220 nm and 368 nm. As a reference point, the 50 nm magnetic bead has a starting size of 100 nm due to the dextran and maleimide coating on the surface of the magnetic beads.

The magnetic beads prior to BAP encapsulation have a zeta potential surface charge of -30, but when coated/encapsulated with BAP, the surface charge of the 50 and 200 nm magnetic beads are increased to +23 and + 38 mV respectively, which should increase cellular uptake.

50 nm BAPC-MB



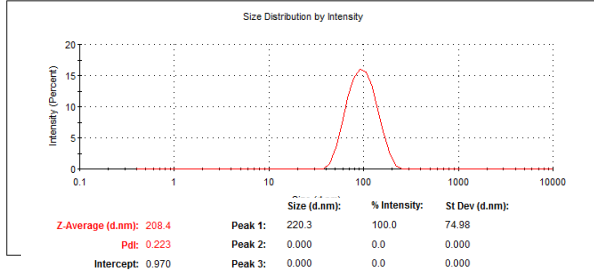
200 nm BAPC-MB



Transmission Electron Microscopy (TEM) images of 50 nm and 200 nm BAPC-MB on Lacey TEM grids shows well dispersed magnetic nanobeads coated/capped with a branched amphiphilic peptide bilayer.

Nanoparticle Size

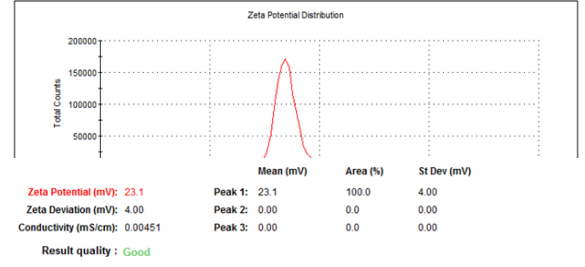
	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 89.98	Peak 1: 100.6	100.0	33.84
Pdi: 0.100	Peak 2: 0.000	0.0	0.000
Intercept: 0.948	Peak 3: 0.000	0.0	0.000
Result quality: Good			



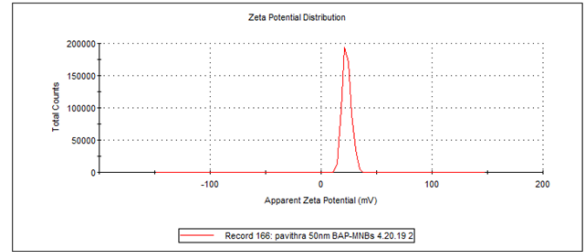
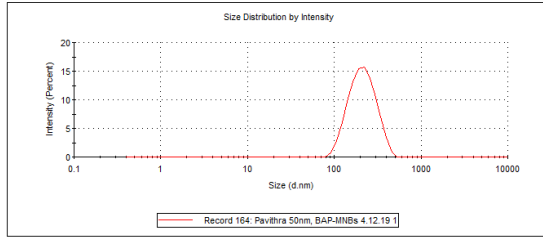
50 nm
Magnetic Beads

Nanoparticle Surface Charge

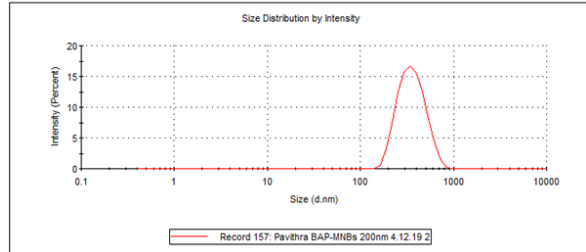
	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -30.9	Peak 1: -30.4	97.9	9.61
Zeta Deviation (mV): 10.2	Peak 2: -58.8	2.1	3.39
Conductivity (mS/cm): 0.00447	Peak 3: 0.00	0.0	0.00
Result quality: See result quality report			



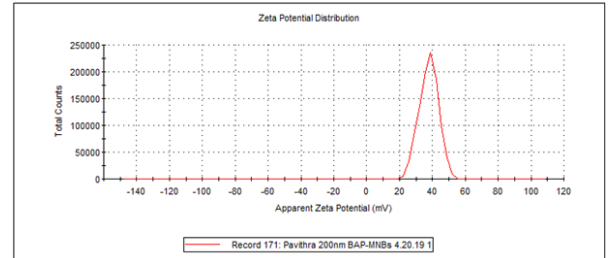
50 nm
BAPC-MB



	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 331.3	Peak 1: 368.0	100.0	120.4
Pdi: 0.125	Peak 2: 0.000	0.0	0.000
Intercept: 0.949	Peak 3: 0.000	0.0	0.000
Result quality: Good			



	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): 37.8	Peak 1: 37.8	100.0	5.80
Zeta Deviation (mV): 5.80	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.00608	Peak 3: 0.00	0.0	0.00
Result quality: Good			



200 nm
BAPC-MB

Rat Intestinal Epithelial Cells (IEC-18) Internalize Magnetic Nanobeads (MNBs) Encapsulated Within a Branched Amphipathic Peptide (BAP) Bilayer

To evaluate the effect of BAP encapsulation of magnetic nanobeads on both cellular uptake and cell viability, research was conducted with Branched Amphipathic Peptide Capsule Magnetic Beads (BAPC-MB) and rat intestinal epithelial cells (IEC-18). The study found that the percentage of IEC-18 cells taking up BAPC-MB was significantly higher when compared to magnetic beads alone. This is most likely due to the increased surface charge of the BAPC-MB (-30 to +23 mV) associated with the BAP encapsulation.

The IEC-18 cells also internalized approximately 50% more BAPC-MB in minimal media (OptiMEM®). Serum proteins present in media form a protein corona around BAPC-MB, further increasing their cellular uptake.

Before Separation

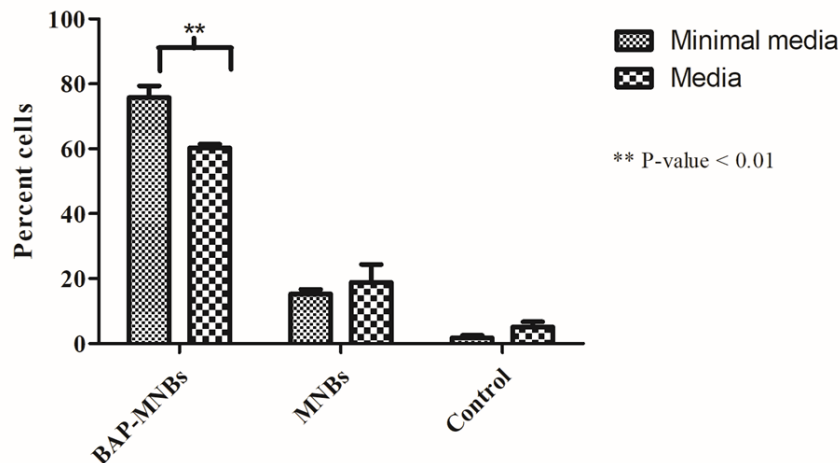


After Separation

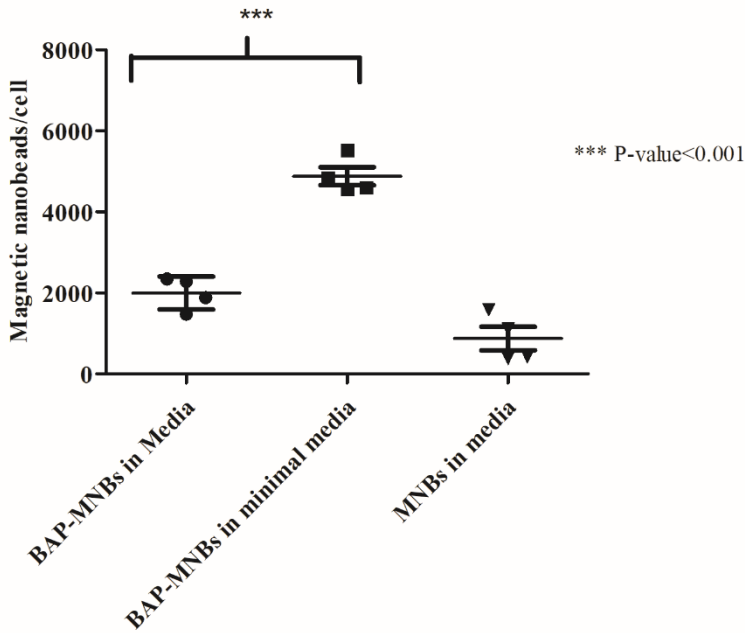


Cells that internalized the BAPC-MB can be effectively separated on a magnetic separator (T=0 oC, t= 25-30min).

Uptake of Magnetic nanobeads by Rat Intestinal Epithelial Cells (IEC-18) in media vs minimal media

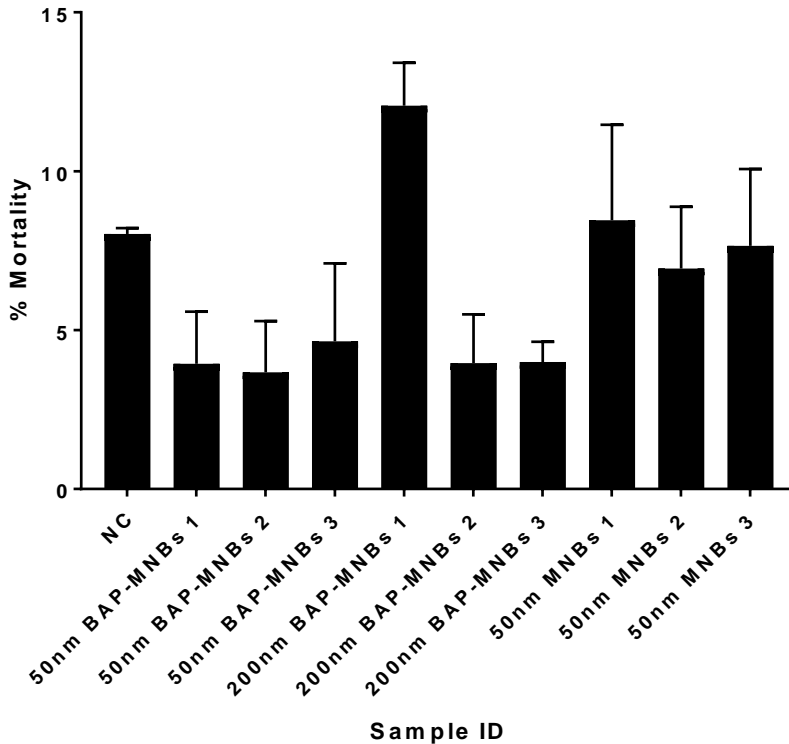


Quantitative analysis of Magnetic Nanobeads uptake by Rat Intestinal Epithelial (IEC-18) cells



The study found the viability of IEC-18 is not negatively affected upon internalization of 50 nm BAPC-MB up to 10^4 /cell. The higher concentration of 200 nm BAPC-MB (2.5×10^3 /cell) appears to have only a slight negative effect on viability when compared to controls.

MNBs toxicity data



NC: Negative control +7AAD

50nm BAP-MNB 1: 50nm BAP-MNBs 10^4 /cell

50nm BAP-MNB 2: 50nm BAP-MNBs 5×10^3 /cell

50nm BAP-MNB 3: 50nm BAP-MNBs 10^3 /cell

200nm BAP-MNBs 1: 200nm BAP-MNBs 2.5×10^3 /cell

200nm BAP-MNBs 2: 200nm BAP-MNBs 1.25×10^3 /cell

200nm BAP-MNBs 3: 200nm BAP-MNBs 2.5×10^2 /cell

50nm MNBs 1: 50nm MNBs 10^4 /cell

50nm MNBs 2: 50nm MNBs 5×10^3 /cell

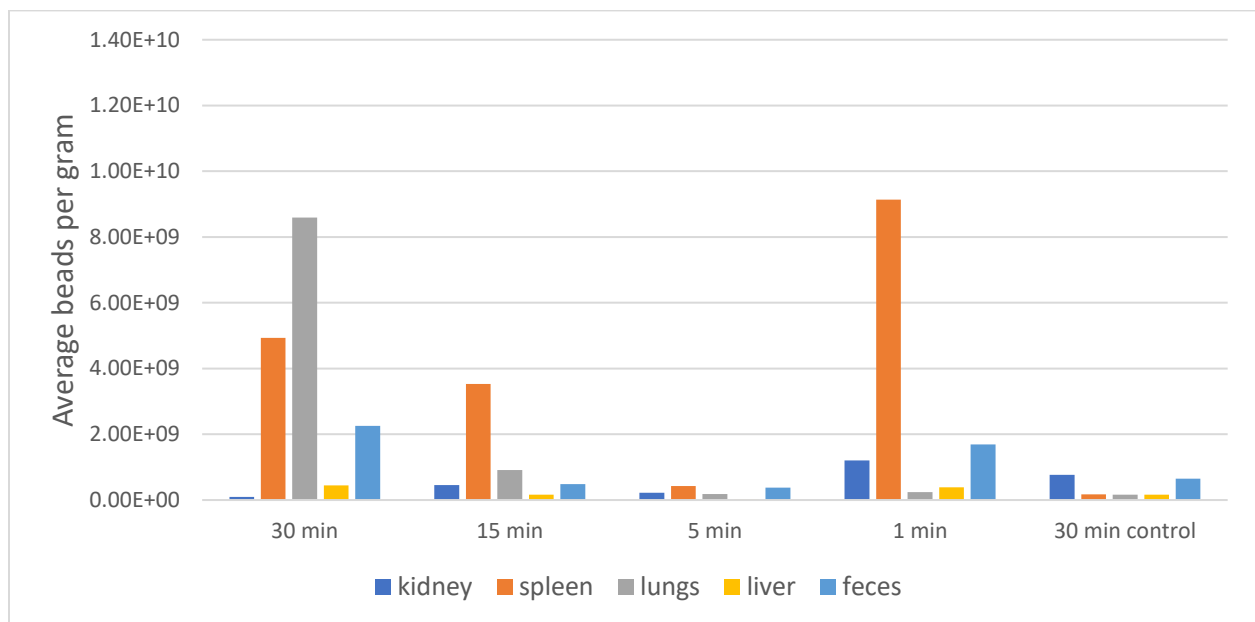
50nm MNBs 3: 50nm MNBs 10^3 /cell

Transdermal Delivery of Branched Amphipathic Peptide Capsule Magnetic (BAPC-MB) in Mice

To test the transdermal delivery capabilities of BAPC-MB, the tails of live mice were dipped in BAPC-MB solution for 1, 5, 15 and 30 minutes. After a 24-hour incubation period, the tissues were harvested for evaluation of tissue distribution.

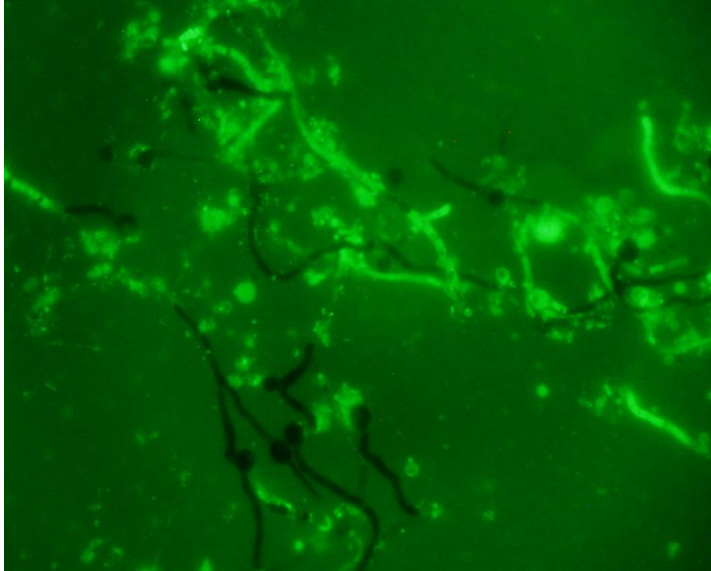
This initial proof-of-concept experiment demonstrates that 50 nm BAPC-MB are rapidly absorbed transdermally and distributed to several different tissues. Although limited in scope, this work found accumulation of the beads in the lungs and spleen, with apparent excretion in the feces. Additional studies are underway to evaluate distribution to additional tissues, as well as inclusion of both oral and intravenous routes of administration.

24-Hour Incubation Period



Branched Amphiphilic Peptide Capsule Uptake by *Aspergillus Nidulans*

The ability of branched amphiphilic peptide capsules (BAPC) to encapsulate and transport payloads into cells offers new approaches to deliver active ingredients (AIs). Until now, we have found that the BAPC were completely inert in previously tested eukaryotic cells. However, photographs have been collected that demonstrate *Aspergillus nidulans*, a common soil fungus, demonstrates intracellular uptake and subsequently break down the BAPC nanoparticles releasing the encapsulated dye inside the fungal cells.



BAPC are broken down in *Aspergillus nidulans*, releasing encapsulated dye inside the fungal cell.

Experiments were designed to optimize delivery of fungicides to this fungal species. In follow-up experiments, it was observed that BAPC can encapsulate and deliver the fungicide thiourea. When spotted on an *Aspergillus* spread milk plate, the BAPC-encapsulated thiourea showed a clear zone of inhibition. Thiourea is present in the isolated media of liquid cultures after exposing the fungi to BAPCs containing the AI. This result indicates that not only are the BAPC being opened, but the fungi are able to secrete BAPC-degrading proteases. This also demonstrates that common soil fungus will degrade BAPC, indicating that they will not accumulate in the environment.