Electronic Supplementary Material (ESI) for RSC Advances.

Enteric pH responsive cargo release from PDA and PEG coated mesoporous silica nanoparticles: A comparative study in *Drosophila melanogaster*

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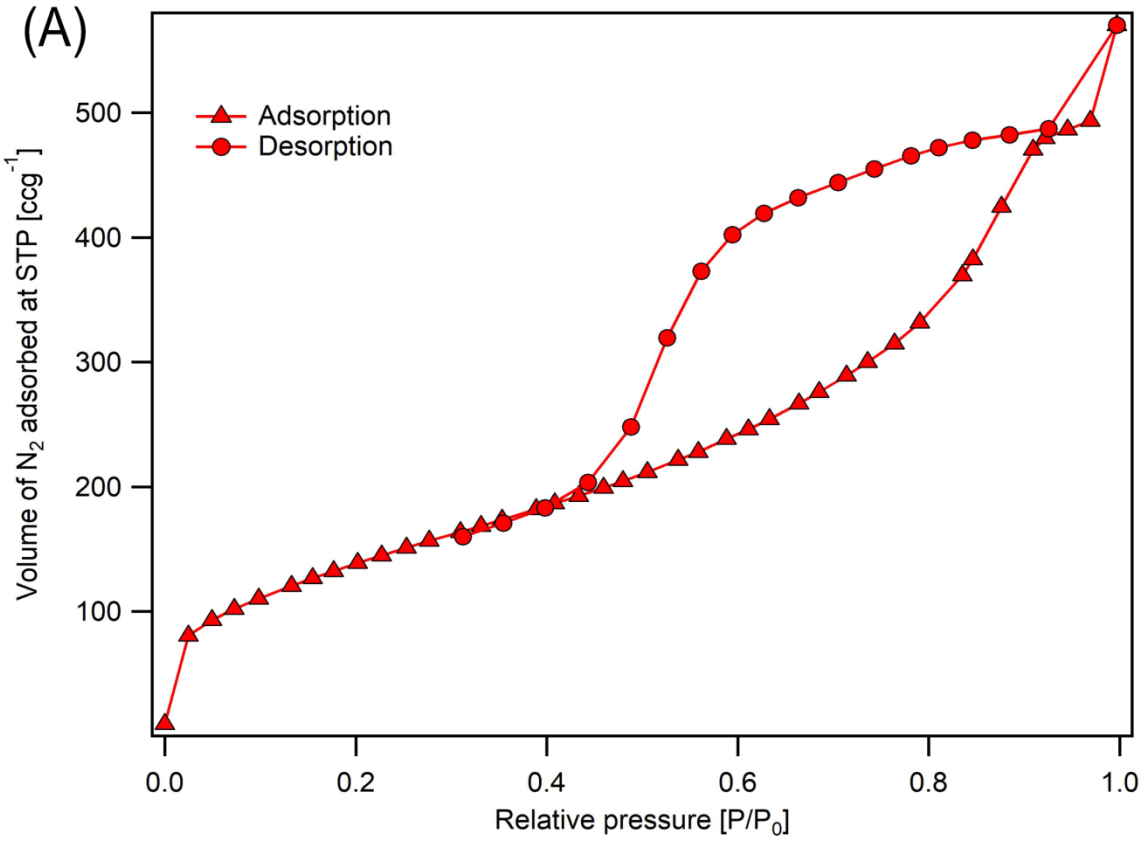
Surface area analysis

Nitrogen adsorption and desorption studies were carried out using Quadrasorb *SI* instrument. Before the nitrogen adsorption measurement, the samples were degassed overnight under vacuum using FloVac Degasser at 80 °C. Multi point BET surface area was obtained from adsorption isotherm from P/P_0 0.05-0.5 pore size distributions were calculated from adsorption isotherm using the BJH method.



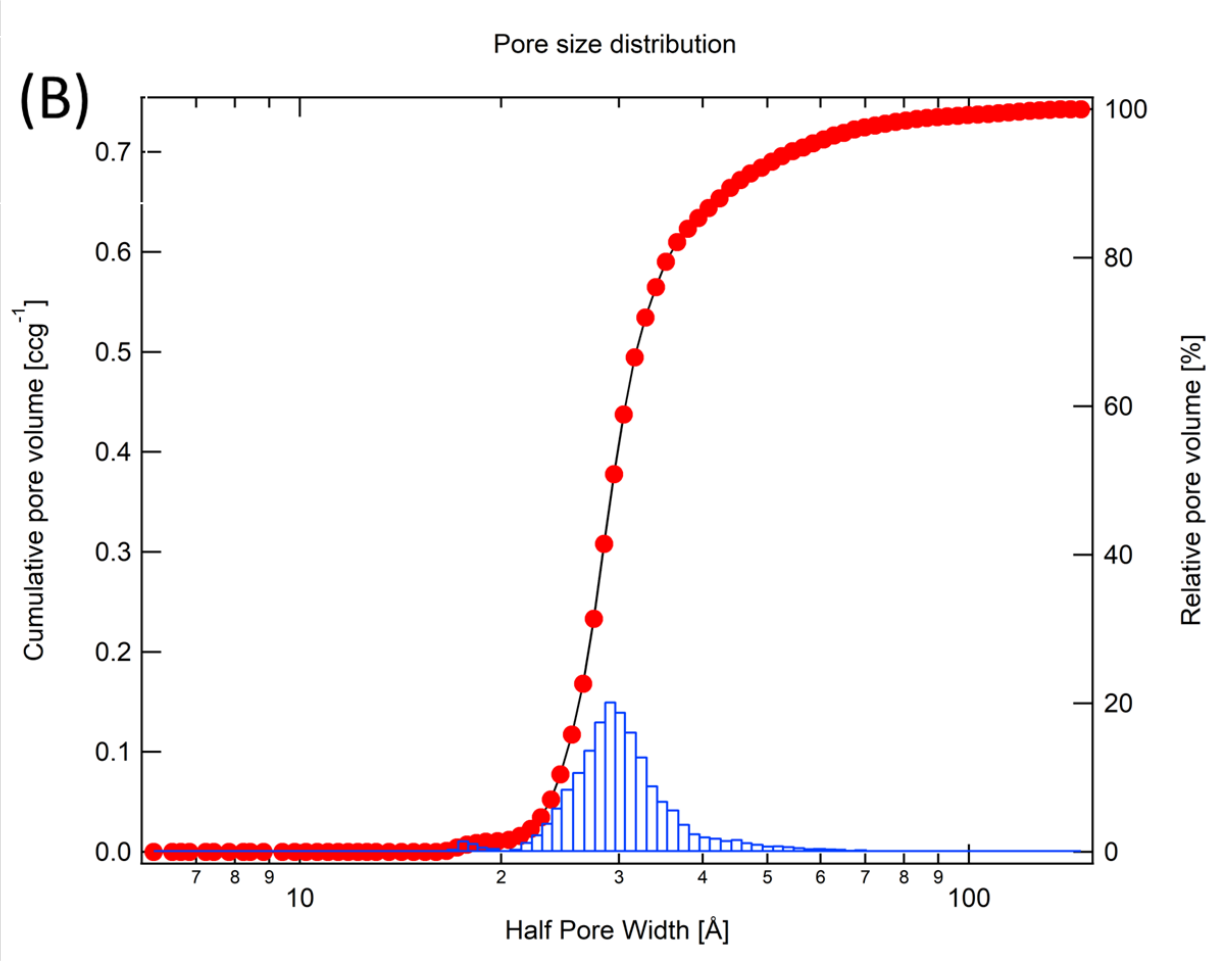
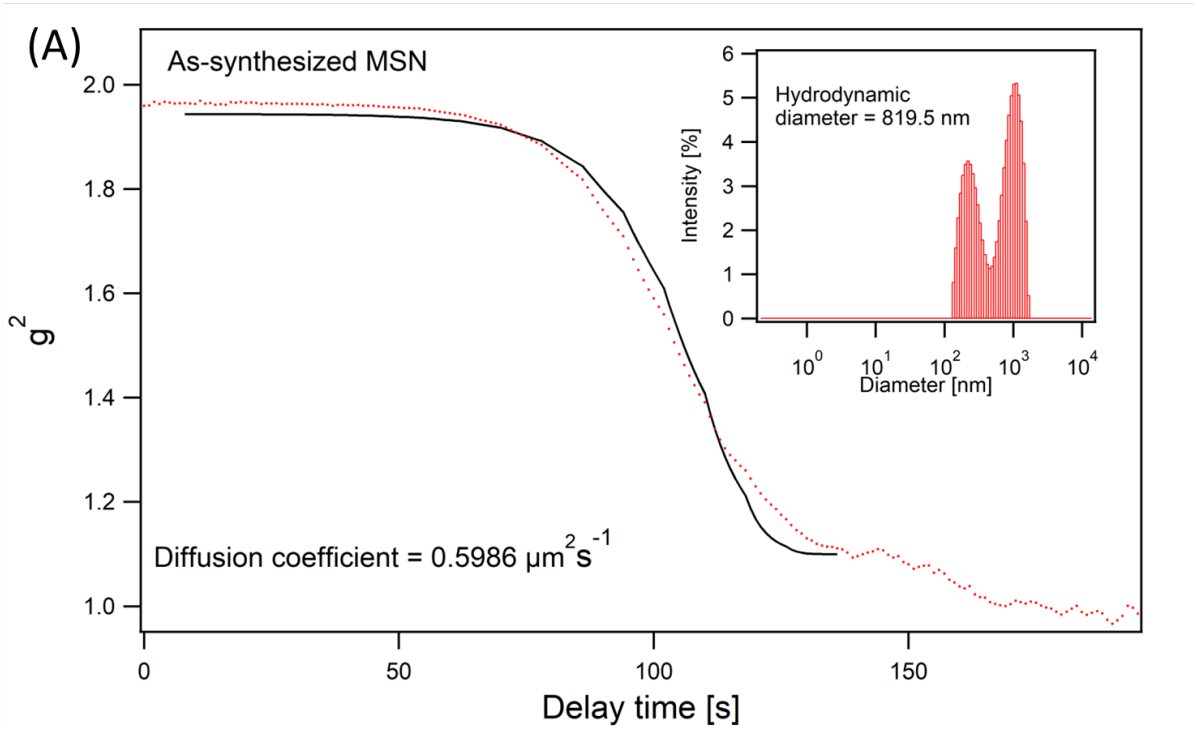
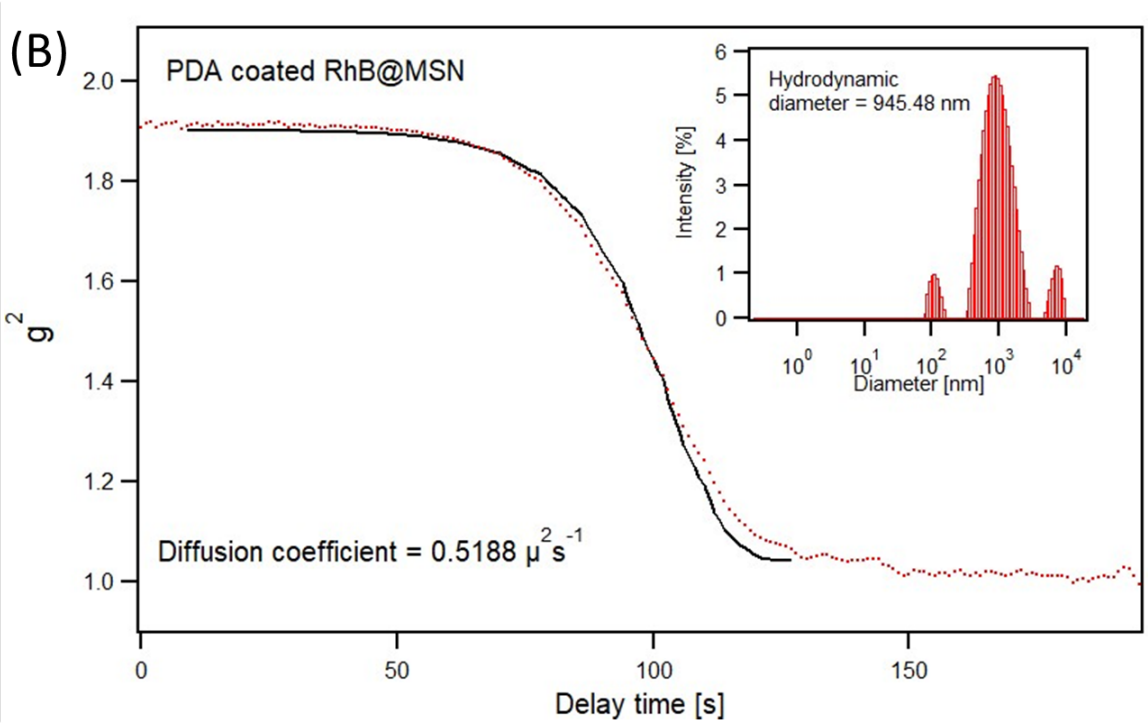


Fig: SI-1: (A) N_2 adsorption-desorption measurements of as-synthesized MSN, (B) Pore diameter and related pore volume of as-synthesized MSN calculated from multipoint BET-model.

Dynamic Light Scattering measurements

Dynamic light scattering (DLS) & zeta potential measurements were performed on Anton Paar Litesizer 500. For DLS measurements, a small amount of each solid sample was suspended in 10 mL of MilliQ® and sonicated for 10 min using a probe sonicator (Qsonica). A pulse of 1 min was applied at 40 eV with 10 secs relaxation time. The sample was cooled simultaneously in an ice bath. The samples were prepared in double filtered MilliQ®(first with 450 nm PVDF filter and a second time with 220 nm PVDF filter) water. The samples were measured directly without any pre-filtration. A quartz cell was used for measurement. The samples were measured for 1 min and auto fitting of the correlation function was performed using the Anton Paar DLS software Kalliope.





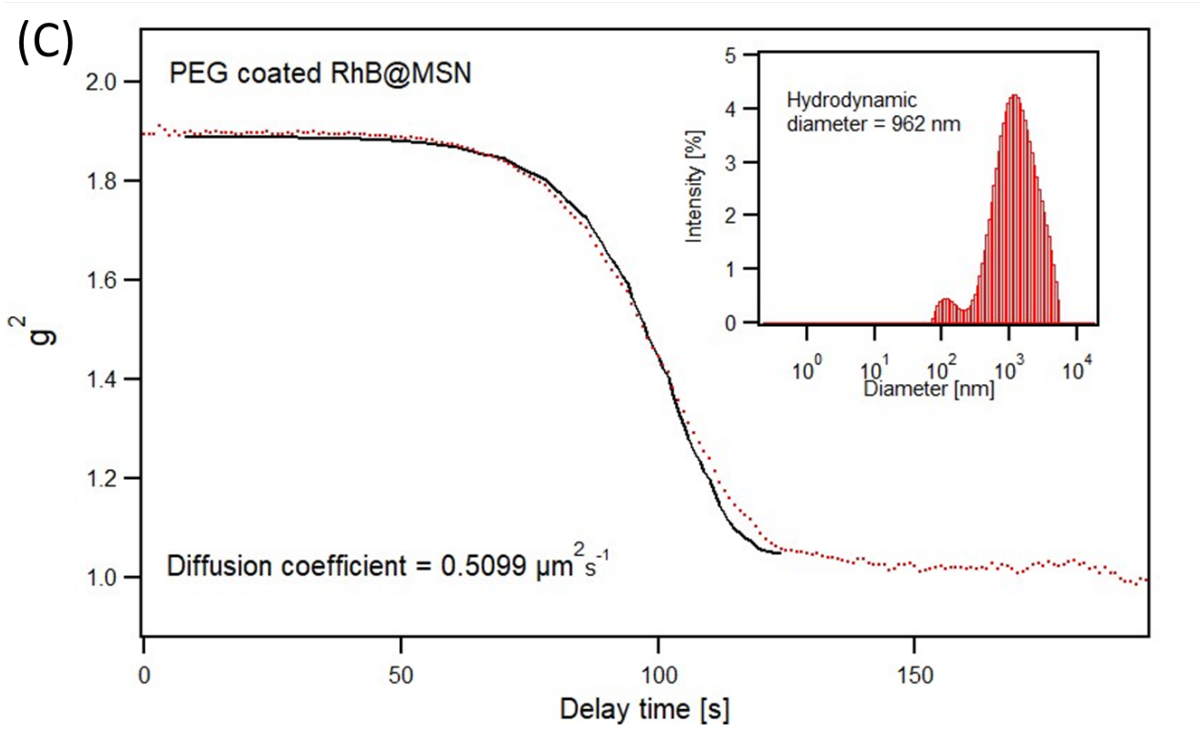


Fig: SI-2: Particle size distribution graphs for (A) As-synthesized MSN, (B) PDA coated RhB@MSN and (C) PEG coated RhB@MSN

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) measurements were performed on Carl Zeiss *EVO 18*. The powder samples were gold sputtered before measurement.

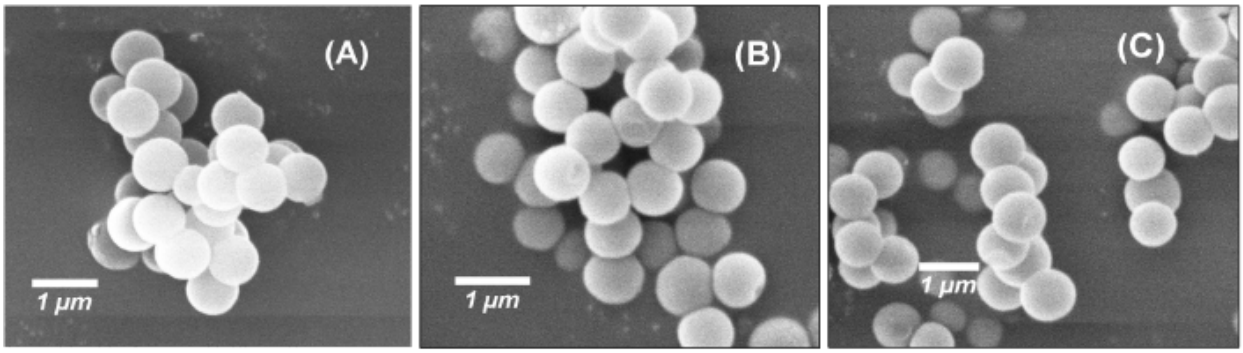


Fig: SI-3: Scanning Electron Microscope (SEM) images of (A) As-synthesized MSN, (B) PDA coated RhB@MSN and (C) PEG coated RhB@MSN.

Transmission Electron Microscopy (**TEM) analysis:**Transmission Electron Microscopy (TEM) measurements were performed on Philips CM200; operating voltage: 20-200 kv, resolution: 2.4 Å. The samples were suspended in water and loaded on a Cu grid and air dried before measurement. We measured particle size using ImageJ and statistics of the particle size was

performed for the analyzable particles on each image. A histogram of the obtained particle size distribution was then plotted for the analyzed images.

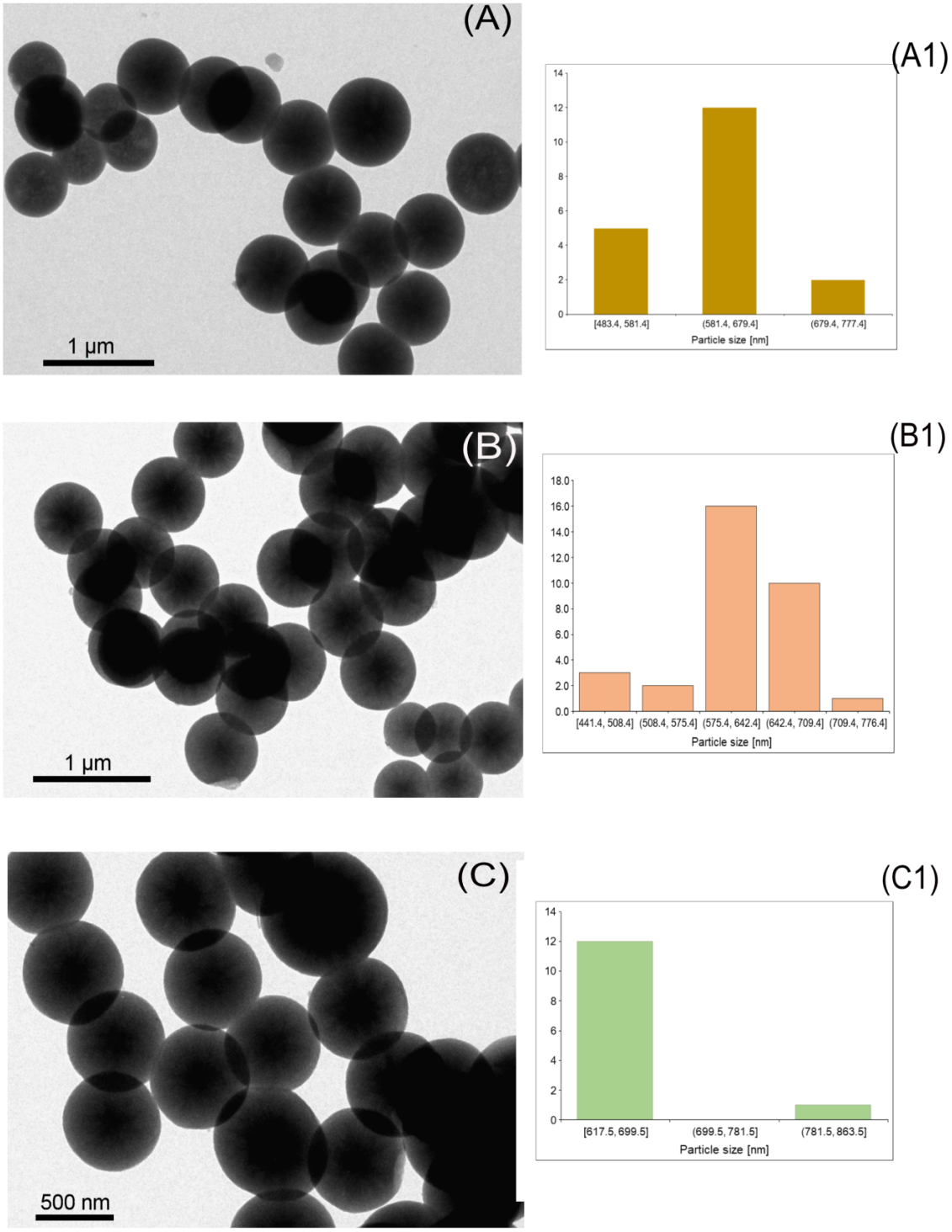


Fig: SI-4: Transmission Electron Microscope (TEM) images of (A) As-synthesized MSN, (A1) Particle size distribution of As-synthesized MSN, (B) PDA coated RhB@MSN, (B1) Particle size distribution graph of PDA coated RhB@MSN and (C) PEG coated RhB@MSN, (C1) Particle size distribution of PEG coated RhB@MSN.

Fourier Transform Infrared (FTIR) measurements

FTIR measurements were performed on a Perkin Elmer Spectrum-GX at resolution 4, Scan range from 4000-600 cm^{-1}, the total number of scans were16 and samples were scanned directly in Attenuated Total Reflection (ATR) mode with respect to air scanned as background. Presented FTIR spectrum for samples are ratio of sample and background scan. Peaks are labeled according to representative stretching/bending vibrations present in functional groups.

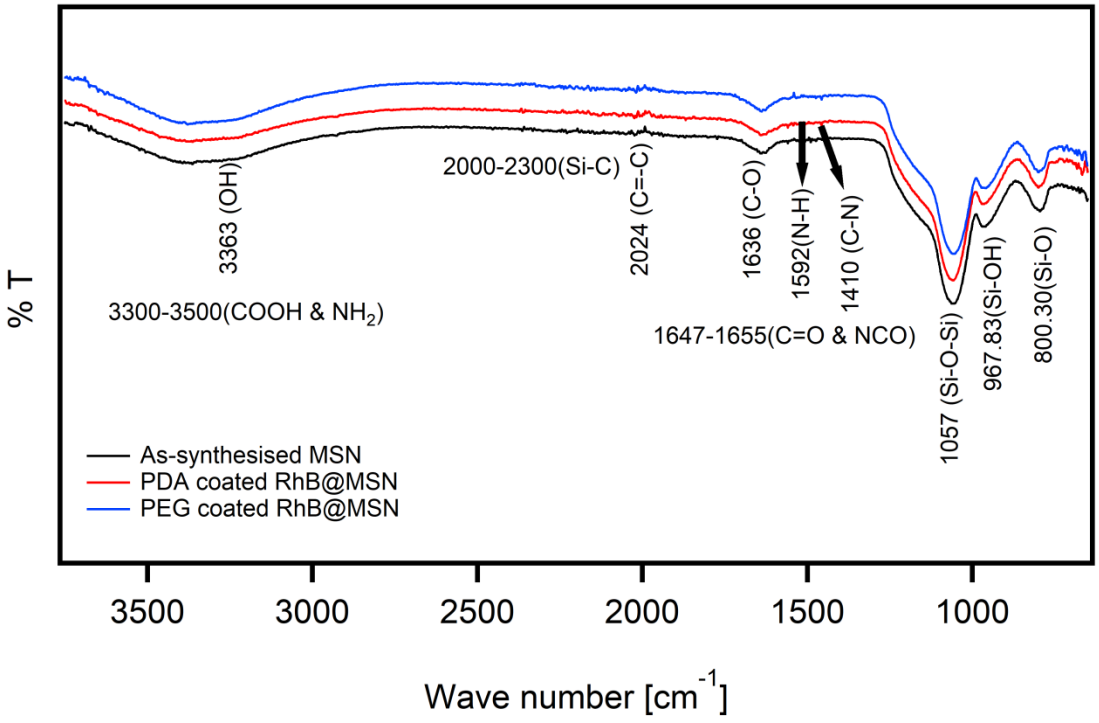
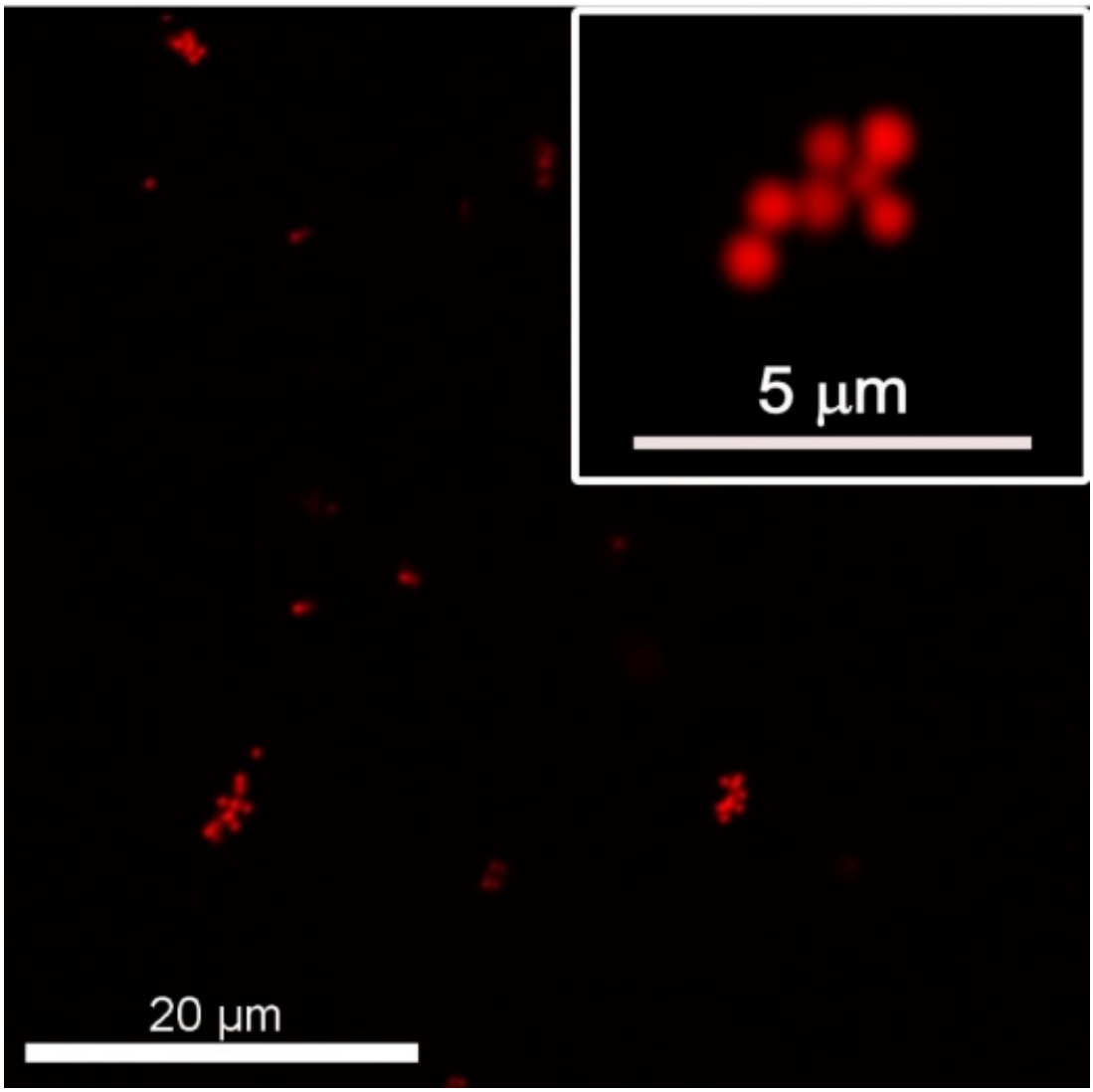


Fig: SI-5: Fourier Transform Infrared (FTIR) spectrum of as-synthesized MSN (Black), PDA coated RhB@MSN (Red) & PEG coated RhB@MSN (Blue).

FTIR spectrum was measured between the range of 600-4000 cm^{-1}. The peak at 3363 cm^{-1} indicates the presence of OH group ,the peak present in the range of 2000-2300 cm^{-1} indicates the presence of Si-C group followed by C-O group at peak position 1633.56 cm^{-1}, Si-O-Si group at peak position 1057 cm^{-1}, Si-OH group at peak position 967.83 cm^{-1} and Si-O group present at peak position 800.30 cm^{-1}.[1] Rhodamine B (RhB) dye was loaded in our particles as a cargo molecule. The peak present between the range of 3300-3500 cm^{-1} indicates the presence of COOH & NH_2 functional groups in RhB, peak in the range of 1647- 1655 cm^{-1} indicates the presence of C=C and NCO group in RhB.[2][3] The RhB loaded particles were also coated with two types of polymers PEG and PDA. Peaks observed at position 1592 cm^{-1} and 1442 cm^{-1} indicates the presence of N-H and C-N bonds of polymer coating. These peaks also represent the aromatic C=C bond of RhB dye. PEG coated RhB@MSN has functional groups similar to MSN and PDA coated MSN and hence it is difficult to analyze distinct groups present in the PEG coated RhB@MSN only by FTIR.

In vitro confocal images of different MSN particles

(A)



(B)

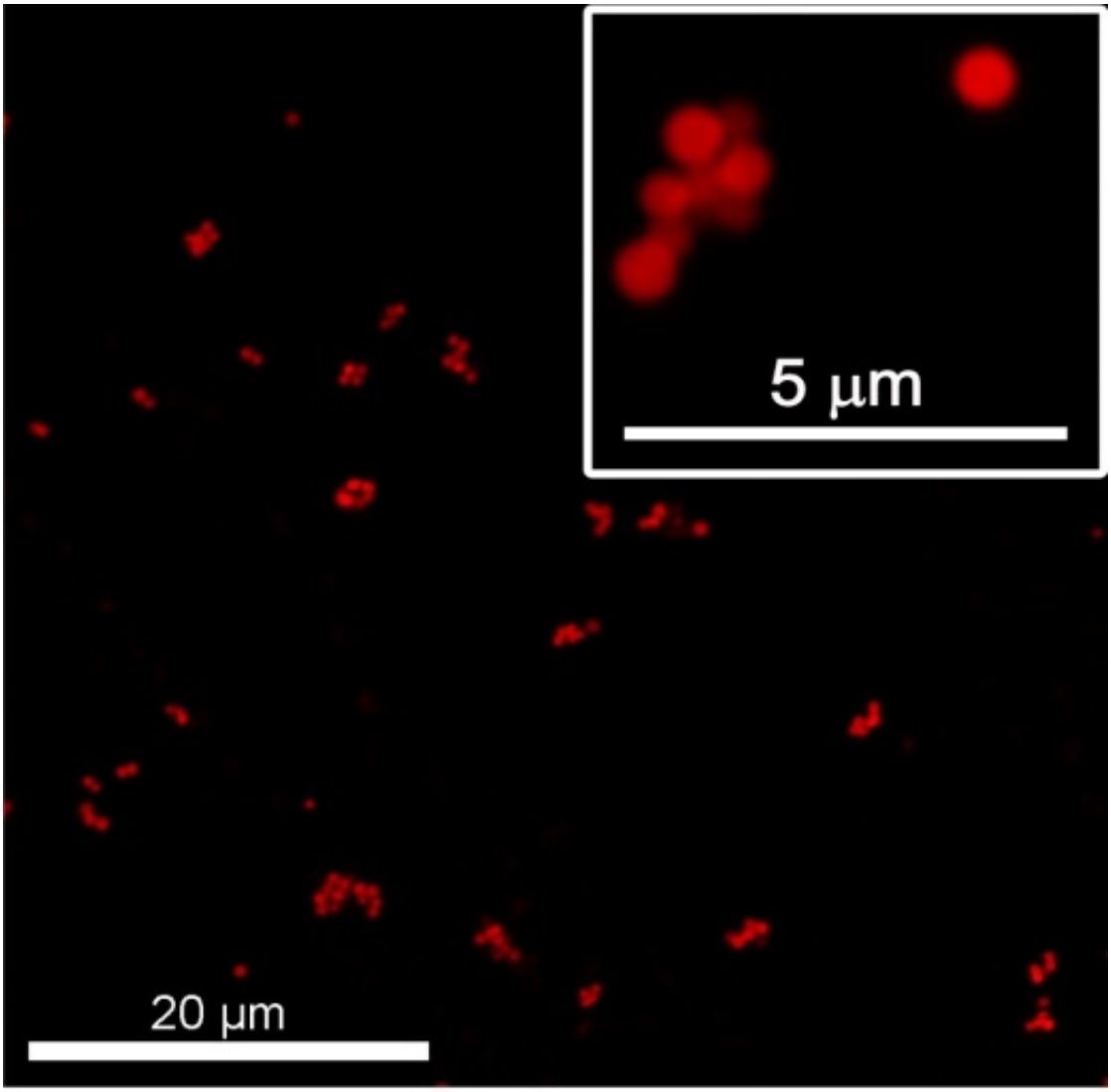
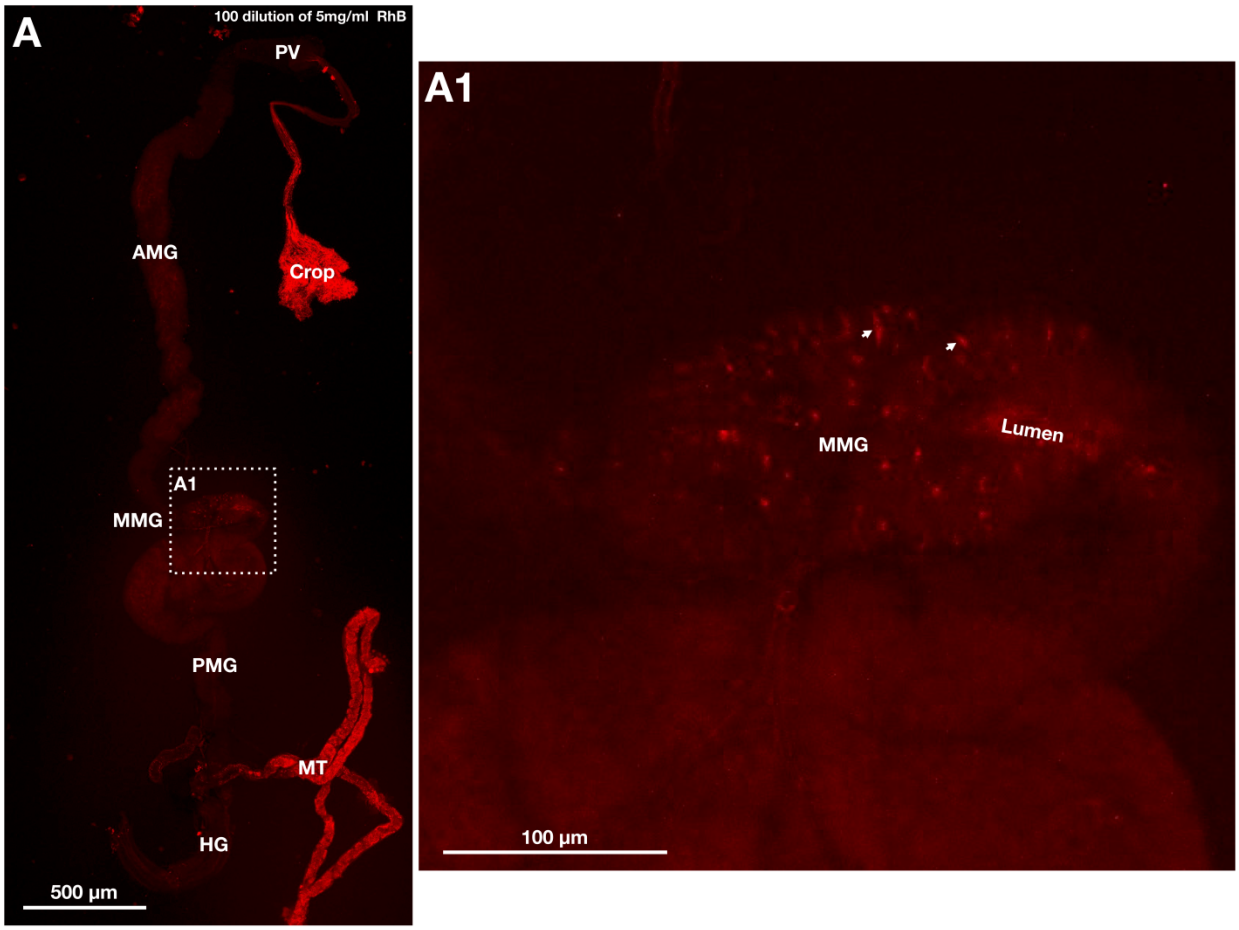


Fig: SI-6: Confocal images of (A) PDA coated RhB@MSN and (B) PEG coated RhB@MSN.

***In-vivo* imaging of Gut**



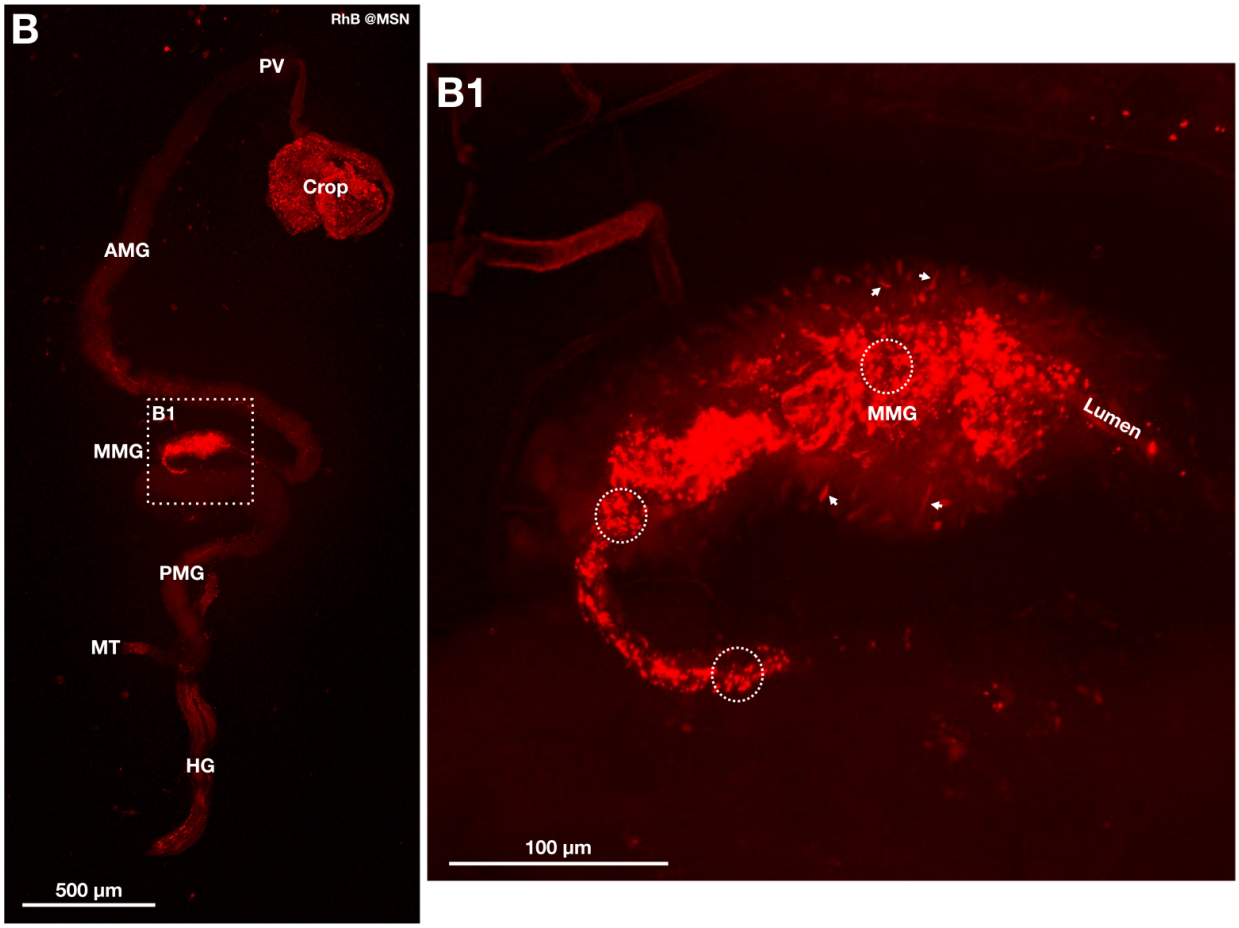


Fig: SI-7: (A) The confocal imaging of only RhB fed fly gut shows absorption of RhB molecule all over the gut. 100x dilution of 5 mg/100 mL RhB fed gut imaged in Zeiss LSM 880 confocal microscope. 20X tile image of RhB fed whole gut of female *Drosophila*. (A1) 20X image of middle midgut (MMG) of RHB fed female *Drosophila*. White arrows indicating paracellular transport of RhB. (B) Burst release of RhB@MSN particles was observed in the middle midgut (MMG) acidic zone. 0.1 mg/mL RhB@MSN fed gut imaged in Zeiss LSM 880 confocal microscope. 20X tile image of RhB@MSN fed whole Gut of female *Drosophila*. (B1) 20X image of middle midgut (MMG) of RHB@MSN fed female *Drosophila*. White arrows indicating paracellular transport of RhB. White dotted circles indicating agglomeration of particles in gut.

Fig: SI-8: CS-Q female flies immobilized on a glass slide using transparent nail polish. The wings are stuck on the side and their abdomens are facing upwards. This was done for feeding individual flies with the MSN nanoparticles. Fly guts were dissected for imaging 30 mins after feeding.

Survival analysis setup

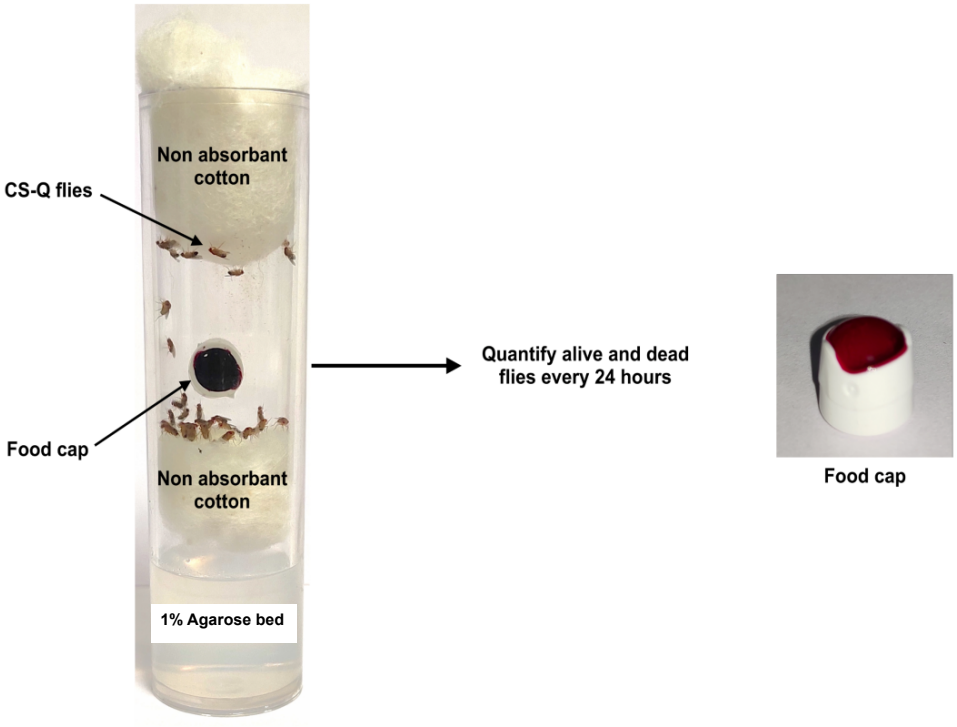


Fig: SI-9: Feeding vial for survival study with 1% agarose bed and food caps where MSN nanoparticles and controls were mixed in 1% low melting (LM) agarose and a dye to monitor feeding.

References

1. M. A. F. Robertson and K. A. Mauritz, *Journal of Polymer Science Part B: Polymer Physics*, 1998, **36(4)**, 595-606.
2. M. Pandurangappa and K. S. Kumar, *Analytical Methods*, 2011, ***3*(3)** ,715-723.
3. D. L. Postai, C. A. Demarchi , F. Zanatta , D. C. C. Melo and C. A. Rodrigues, *Alexandria Engineering Journal*, 2016, ***55*(2)** , 1713-1723.

Supplementary Information Table SI-1			
(Figure 3)Table Analyzed by Kruskal-Wallis test			
Gaussian Approximation P value			
Number of groups for all=3			
analyzed by Graph Pad Prism 5			
	UC-RhB@MSN, t=0	**PDA-RhB@MSN, t=0**	**PEG-RhB@MSN, t=0**
P value	0.0102	0.2908	0.0291
P value summary	*	ns	*
Do the medians vary signif. (P < 0.05)	Yes	No	Yes
Kruskal-Wallis statistic	9.17	2.47	7.073
	UC-RhB@MSN, t=1	**PDA-RhB@MSN, t=1**	**PEG-RhB@MSN, t=1**
P value	0.0072	0.1152	0.0183
P value summary	**	ns	*
Do the medians vary signif. (P < 0.05)	Yes	No	Yes
Kruskal-Wallis statistic	9.881	4.323	8
	UC-RhB@MSN, t=2	**PDA-RhB@MSN, t=2**	**PEG-RhB@MSN, t=2**
P value	0.0181	0.021	0.0228
P value summary	*	*	*
Do the medians vary signif. (P < 0.05)	Yes	Yes	Yes
Kruskal-Wallis statistic	8.028	7.731	7.565
	UC-RhB@MSN, t=3	**PDA-RhB@MSN, t=3**	**PEG-RhB@MSN, t=3**
P value	0.0164	0.0345	0.0137
P value summary	*	*	*
Do the medians vary signif. (P < 0.05)	Yes	Yes	Yes
Kruskal-Wallis statistic	8.221	6.731	8.578
	UC-RhB@MSN, t=4	**PDA-RhB@MSN, t=4**	**PEG-RhB@MSN, t=4**
P value	0.0207	0.0231	0.0073
P value summary	*	*	**
Do the medians vary signif. (P < 0.05)	Yes	Yes	Yes
Kruskal-Wallis statistic	7.758	7.538	9.846
	UC-RhB@MSN, t=5	**PDA-RhB@MSN, t=5**	**PEG-RhB@MSN, t=5**
P value	0.0073	0.0183	0.0073
P value summary	**	*	**
Do the medians vary signif. (P < 0.05)	Yes	Yes	Yes
Kruskal-Wallis statistic	9.846	8	9.846
(Figure 6) Comparison of Survival Curves			
Log-rank (Mantel-Cox) Test			
Chi square	3.073		
df	3		
P value	0.3806		
P value summary	ns		
Are the survival curves sig different?	No		
Logrank test for trend			
Chi square	1.281		
df	1		
P value	0.2577		
P value summary	ns		
Sig. trend?	No		