

Transport of Artificial Virus-like Nanocarriers (AVN) through intestinal monolayer via Microfold cells

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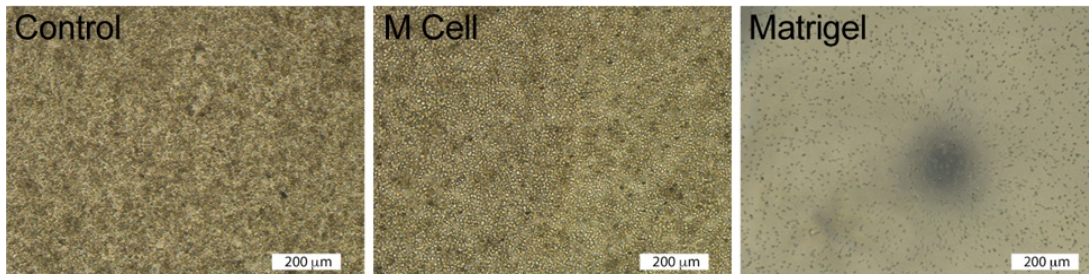
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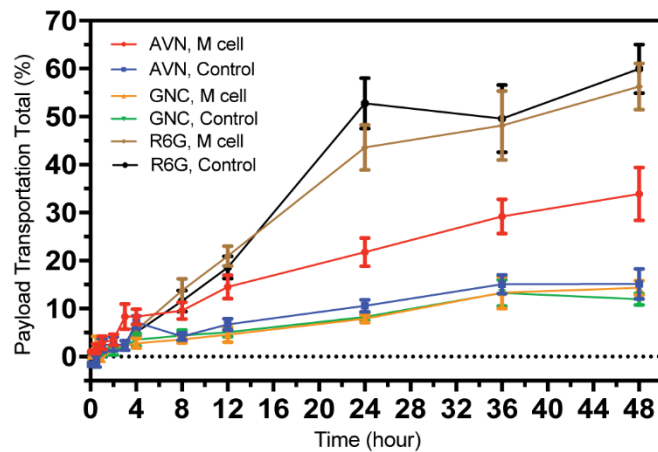
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Supplementary Fig. 1: Monolayer system bright field 20X images



Bright field images of control, M cell and Matrigel only transwells were taken 24 hours before experimentation. Images were taken at 20x magnification, and demonstrate that the control group and M cell group form a dense cell monolayer.

Supplementary Fig. 2: Monolayer test with free R6G dye data



We used Free dye (R6G) on monolayer w/o M cell as baseline for transport and also to check integrity of monolayer. If there are holes not covered by monolayer, R6G should not have gradually transport pattern it demonstrated in Supplementary Fig. 2

Supplementary Note 1: MRV transport total (%) on monolayer w/o M cell

MRV transport total (%) was calculated by dividing the total amount of MRV that passed through the monolayer at 2, 4, 6 and 12 hours post-infection (p.i.), by the total amount of MRV initially added (1.0×10^9 PFU). Each well began with 1200 μ l culture solution at bottom of monolayer and 400 μ l was removed at each time-point to measure the amount of MRV transport. After the first time-point sample was collected at 2 hours p.i., 400 μ l of culture solution was added into the bottom of monolayer to bring the volume back up to 1200 μ l. This was only done after the first time-point, therefore, each subsequent time-point resulted in a 400 μ l decrease in total volume of the bottom well. At 2 hour p.i., we utilized plaque assays to calculate the virus concentration in PFU/ml, represented by X_2 , we can get transport total for 2 hour as X_2 PFU/ml * 1.2 ml (the volume of the culture solution) / 1.0×10^9 PFU (total MRV added) * 100%. For 4 hour p.i., we need to consider the virus present in the 400 μ l we took out at 2 hour p.i. (X_2 PFU/ml * 0.4 ml), and add it to the virus transported at 4 hours p.i. (X_4 PFU/ml * 1.2 ml). Thus, transport total for 4 hour is $(X_2$ PFU/ml * 0.4 ml + X_4 PFU/ml * 1.2 ml) / (1.0×10^9 PFU) * 100%. For 6 hour, we need to add back 400 μ l we took out at 2 hour and 4 hour. Thus, transport total for 6 hour is $(X_2$ PFU/ml * 0.4 ml + X_4 PFU/ml * 0.4 ml + X_6 PFU/ml * 0.8 ml) / (1.0×10^9 PFU) * 100%. For 12 hour, we need to add 400 μ l we took out at 2 hour, 4 hour, and 6 hour. Thus, transport total for 12 hour is $(X_2$ PFU/ml * 0.4 ml + X_4 PFU/ml * 0.4 ml + X_6 PFU/ml * 0.4 ml + X_{12} PFU/ml * 0.4 ml) / (1.0×10^9 PFU) * 100%. For M cell and control, we have 6 sets at each timepoint (data shown in Table 1).

Table 1

Total Transport Total (%)	2 hour	4 hour	6 hour	12 hour
M cell	0.14%	2.16%	5.93%	9.11%
	0.04%	1.88%	5.81%	9.22%
	0.02%	2.09%	6.82%	10.70%
	0.02%	2.83%	1.47%	4.03%
	0.00%	0.01%	0.45%	3.17%
	0.02%	2.12%	1.07%	4.54%
Control	0.00%	0.08%	1.32%	1.03%
	0.00%	0.04%	0.72%	1.54%
	0.01%	0.22%	1.28%	0.99%
	0.01%	0.00%	0.11%	3.35%
	0.00%	0.00%	0.04%	2.37%
	0.00%	0.00%	0.04%	1.55%

Supplementary Note 2: AVN, GNC transport total on monolayer w/o M cell

In Fig. 5a and 5b 150 μ l of AVN with R6G, GNC with R6G, or free R6G was added on top of control or M cell monolayers and the total transport of R6G through the monolayer was measured at 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 36, and 48 hour post-treatment. Below the monolayer was 500 μ l culture solution in the bottom well, and at each time-point 50 μ l of the solution was removed to test R6G fluorescing intensity and 50 μ l of fresh solution was added back. Thus, for each time-point the dilution factor was 10 (500 μ l [total volume] / 50 μ l [volume removed]). Thus, we can calculate total R6G fluorescing value at each time-point the same way we did in Supplementary Note 1. For example, at 1 hour the total R6G fluorescing value should be $(X_1 * 10 \text{ [dilution factor]} + X_{0.5} + X_0)$. Then we took total fluorescing value and divided by initial fluorescing value * 100% to determine the transport total for each time-point (data shown in Table 1).

Table 1

0hour transport total (%)						
AVN+M cell	-0.57	0.26	1.26	-0.47	2.97	2.65
AVN+Control	0.74	-2.10	-1.57	-1.75	-2.50	-2.53
GNC+M cell	-1.21	0.80	-0.75	0.32	0.84	0.71
GNC+Control	-0.02	-0.11	0.28	-1.26	1.33	0.43
R6G+M cell	0.03	0.06	0.14	0.04	0.00	0.09
R6G+Control	0.09	0.05	0.06	0.00	0.02	0.09
0.5hour transport total (%)						
AVN+M cell	0.44	1.11	0.71	0.66	0.33	6.65
AVN+Control	1.66	0.02	-6.12	-1.83	2.21	-1.62
GNC+M cell	-1.43	3.77	4.81	-0.09	6.06	4.66
GNC+Control	2.01	0.03	0.06	-0.72	0.11	1.63
R6G+M cell	0.36	0.30	0.48	0.64	0.30	0.24
R6G+Control	0.40	0.14	0.24	0.39	0.05	0.23
1hour transport total (%)						
AVN+M cell	0.04	0.56	-0.26	4.36	4.27	8.63
AVN+Control	-0.65	0.09	-0.34	6.29	6.65	1.98
GNC+M cell	-0.15	-0.01	-0.46	0.32	0.42	-3.27
GNC+Control	-0.48	-0.62	-0.41	1.20	12.61	1.11
R6G+M cell	0.67	0.42	0.86	0.41	0.66	0.39
R6G+Control	0.80	0.73	0.54	0.38	0.20	0.88
2hour transport total(%)						
AVN+M cell	2.60	0.20	1.76	4.08	4.29	8.21
AVN+Control	0.40	0.95	1.42	8.54	5.55	-1.08
GNC+M cell	1.09	2.96	3.49	0.94	3.30	1.68
GNC+Control	0.71	0.51	1.74	-1.66	4.33	1.31
R6G+M cell	2.23	1.86	2.84	1.74	2.07	1.47
R6G+Control	1.95	3.07	1.97	0.82	0.90	0.75
3hour transport total (%)						
AVN+M cell	4.20	19.54	2.64	5.54	5.30	12.67
AVN+Control	0.89	1.51	2.13	0.71	7.43	1.43
GNC+M cell	1.31	0.98	1.69	2.47	1.99	5.35
GNC+Control	1.11	1.24	1.32	2.38	5.42	1.87
R6G+M cell	2.13	3.10	5.08	2.21	1.60	2.20
R6G+Control	2.63	4.06	2.73	1.71	2.30	4.28
4hour transport total (%)						
AVN+M cell	3.74	7.63	6.28	7.58	9.57	15.38
AVN+Control	3.88	5.23	12.08	9.42	4.09	8.27
GNC+M cell	2.42	2.96	1.83	0.65	1.03	7.67
GNC+Control	3.47	5.96	-0.99	0.33	7.37	5.13
R6G+M cell	6.59	4.45	10.49	5.03	2.82	3.77
R6G+Control	5.29	8.04	4.72	5.07	2.50	4.78

8hour transport total (%)						
AVN+M cell	10.86	5.36	3.71	14.24	9.80	13.55
AVN+Control	1.67	3.92	2.96	3.98	5.87	7.09
GNC+M cell	1.39	7.14	3.42	2.62	3.29	3.52
GNC+Control	4.71	0.11	2.74	4.51	8.45	5.99
R6G+M cell	24.48	11.40	13.59	6.44	12.17	14.58
R6G+Control	12.63	14.03	19.36	4.75	5.96	12.86
12hour transport total (%)						
AVN+M cell	11.91	8.36	8.14	16.22	22.42	20.23
AVN+Control	4.53	5.77	5.40	4.28	8.87	11.56
GNC+M cell	1.96	2.57	8.82	-0.12	4.73	9.65
GNC+Control	4.43	2.43	3.36	4.48	7.59	8.43
R6G+M cell	21.36	16.51	24.81	17.24	16.95	28.84
R6G+Control	9.44	24.72	23.40	15.70	16.95	21.18
24hour transport total (%)						
AVN+M cell	18.34	25.91	10.28	29.86	27.59	18.87
AVN+Control	14.39	8.91	12.09	10.83	5.49	11.80
GNC+M cell	9.51	10.74	5.85	6.42	8.68	6.19
GNC+Control	8.28	10.14	8.82	4.94	9.99	7.68
R6G+M cell	43.68	64.01	37.17	30.70	37.99	47.86
R6G+Control	39.84	38.02	62.98	56.95	48.63	70.19
36hour transport total (%)						
AVN+M cell	33.79	24.64	17.39	31.92	25.19	42.44
AVN+Control	20.35	20.44	16.44	13.12	10.05	10.04
GNC+M cell	9.30	23.90	22.16	6.61	4.30	13.92
GNC+Control	23.13	10.46	19.11	13.88	6.46	6.82
R6G+M cell	64.04	75.19	42.89	31.67	32.35	42.95
R6G+Control	31.88	81.55	49.57	43.16	39.68	51.58
48hour transport total (%)						
AVN+M cell	35.07	27.87	19.81	38.68	57.42	24.61
AVN+Control	11.58	10.41	14.95	8.35	29.80	15.90
GNC+M cell	14.73	15.94	19.20	10.08	9.46	16.47
GNC+Control	15.36	10.35	16.21	10.49	9.49	9.79
R6G+M cell	53.38	40.08	45.40	68.83	65.31	64.76
R6G+Control	49.78	56.17	58.11	63.08	49.54	83.19

Supplementary Note 3: AVN real transport total on monolayer with M cell incorporated

In Fig. 5c we only observe a GNC absorption peak in the AVN + M cell group indicating the fluorescing data we got from other three experimental groups involving the GNC or AVN are from R6G that unload from above the monolayer. Therefore, AVN + M cell fluorescing data, at each time-point, consists of two parts, the R6G that unloaded above the monolayer and the R6G payload that was transported through the monolayer with the AVN via M cells. Since both AVN and GNC has no coating outside, we assume R6G unloaded out of AVN/GNC at the same rate. At each time point the fluorescing data of GNC + M cell was subtracted from the AVN + M cell to provide the amount of R6G fluorescence that is payload within the AVN. This real payload fluorescing data was divided by initial fluorescing data to calculate real transport total for AVN on M cell incorporated monolayer (data shown in table 1).

Table 1

	0 hour	0.5 hour	1 hour	2 hour	3 hour	
AVN on M cell monolayer real transport total (%)	0.65	1.87	0.18	1.51	2.89	
	-0.54	-2.66	0.57	-2.76	18.56	
	2.02	-4.10	0.20	-1.73	0.95	
	-0.79	0.75	4.04	3.14	3.07	
	2.13	-5.73	3.85	0.99	3.31	
	1.94	2.00	11.90	6.53	7.32	
4 hour	8 hour	12 hour	24 hour	36 hour	48 hour	
	1.32	9.47	9.96	8.83	24.48	20.34
	4.66	-1.78	5.78	15.17	0.74	11.92
	4.45	0.29	-0.68	4.43	-4.77	0.61
	6.93	11.62	16.34	23.44	25.31	28.60
	8.54	6.51	17.69	18.92	20.89	47.95
	7.71	10.03	10.58	12.68	28.52	8.14

Supplementary Table 1: Multiple comparison (Turkey HSD) data for Fig. 5 (manuscript):

Transport behavior on monolayer platform

Time Point (Hour)	Group	Prob> t	Lower 95%	Upper 95%
0 hour	GNC + M cell	0.313	-1.85153	8.90344
	AVN + Control	0.2893	-1.43055	7.35085
	GNC + Control	0.075	-0.45620	13.42841
0.5 hour	GNC + M cell	0.7664	-3.14859	6.77467
	AVN + Control	0.3291	-1.44363	6.65868
	GNC + Control	0.2698	-1.98487	10.826
1 hour	GNC + M cell	0.1198	-0.7897	9.9477
	AVN + Control	0.9565	-5.2233	3.54376
	GNC + Control	0.4847	-3.1917	10.6702
2 hour	GNC + M cell	0.1466	-0.74750	7.35443
	AVN + Control	0.3384	-1.20136	5.41384
	GNC + Control	0.0401*	0.17993	10.63948
3 hour	GNC + M cell	0.0058*	1.3987	10.53987
	AVN + Control	0.0535	-0.04009	7.42365
	GNC + Control	0.0004*	3.76046	15.56167
4 hour	GNC + M cell	0.0017*	2.594	13.8155
	AVN + Control	0.372	-1.7731	7.38924
	GNC + Control	0.0010*	3.7693	18.25627
8 hour	GNC + M cell	0.4683	-2.9470	10.14318
	AVN + Control	0.8295	-3.6316	7.05646
	GNC + Control	0.3499	-3.1391	13.76018
12 hour	GNC + M cell	0.0074*	2.7117	22.51743
	AVN + Control	0.4036	-3.3029	12.86839
	GNC + Control	0.0038*	4.6127	30.18183
24 hour	GNC + M cell	0.0413*	0.3387	23.07378
	AVN + Control	0.6473	-5.1895	13.37354
	GNC + Control	0.0303*	1.1228	30.47366
36 hour	GNC + M cell	0.0277*	1.2036	27.95689
	AVN + Control	0.3835	-4.3132	17.53073
	GNC + Control	0.0105*	3.9198	38.45816
48 hour	GNC + M cell	0.0004*	6.4234	26.94318
	AVN + Control	0.1173	-1.2004	15.55398
	GNC + Control	<.0001*	10.6146	37.10556

This table shows the p values, determined from a Tukey HSD (honest significance difference) test, at every time-point to test significance between each experimental condition and AVN + M cell.