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Effect of Colonoscopy on Circulating Microvesicles

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Abstract

Circulating microvesicles (cMVs) are small membrane structures that are secreted by multiple cell types and have been found in blood, urine, saliva and other body fluids.
cMVs transfer information from cell to cell by transporting selected proteins, mRNA and microRNA and contain specific subsets of proteins that are likely to correlate to their origin.
Due to these specific proteins cMVs are of particular interest in discovering new tools to diagnose human diseases.
The numbers of cMVs shed by cells increases when the cell is biochemically stressed. To determine if the physical stress associated with bowel preparation and colonoscopy would result in an increase in the amount of colon cMVs shed into the vascular system, blood was collected prospectively from 27 individuals at five different time points and processed into plasma. The five time points were chosen for this study to establish the baseline level of colon cMVs, the effect of bowel preparation, the effect of colonoscopy, and then two time points after colonoscopy to determine when cMV levels returned to their baseline level.

Specifically, the five time points were:
1) before bowel preparation;
2) after bowel preparation and before colonoscopy;
3) one day post colonoscopy;
4) 3-5 days post colonoscopy;
5) 7-8 days post colonoscopy.

The cMV levels were profiled using a variety of protein markers that have been correlated to microvesicles and/or colon tissue in the literature.

Methods

Blood was collected in K2-EDTA tubes and centrifuged at room temperature to isolate the plasma layer. Plasma samples were then immediately frozen and stored at or below -20°C until tested.
For each sample the cMVs were enriched by a proprietary method and a Luminex sandwich immunoassay was used to detect cMVs. This assay is based on the antibody capture of cMVs and subsequent detection of the captured cMV by phycoerythrin labeled anti-tetraspanin antibodies.

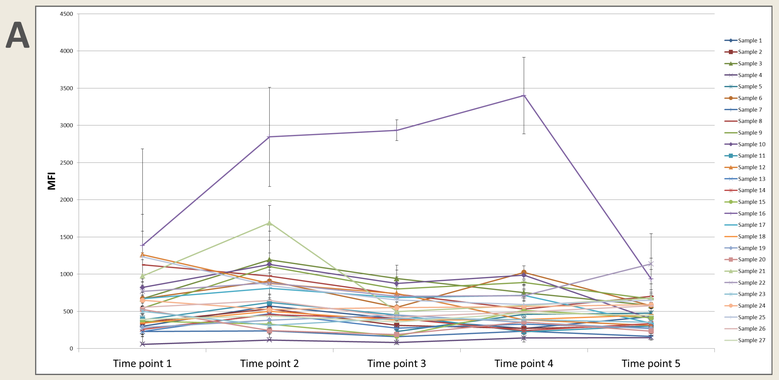
Results

No significant differences were observed among the tested time points for any of the protein markers tested.
Here we show the data for the tetraspanins CD9 (A), CD63 (C) and CD81 (D) and for CD10 (B) a membrane-bound metalloproteinase.

Conclusions

There was no statistical difference between any of the time points, suggesting that neither bowel preparation nor colonoscopy influence the secretion and composition of circulating microvesicles. Thus physical stress on the tissue does not appear to influence the secretion of cMVs in a comparable manner to biochemical stress.

A



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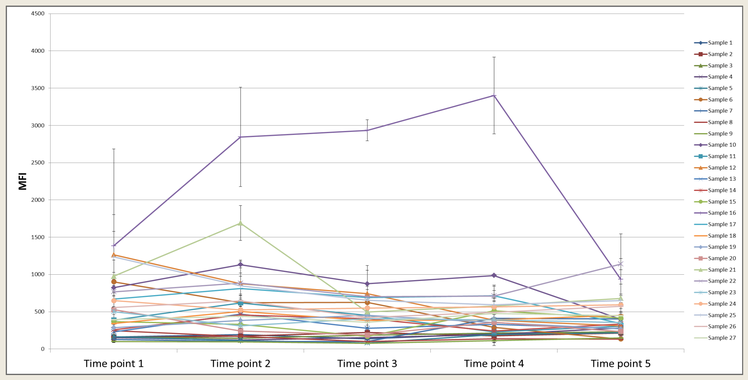

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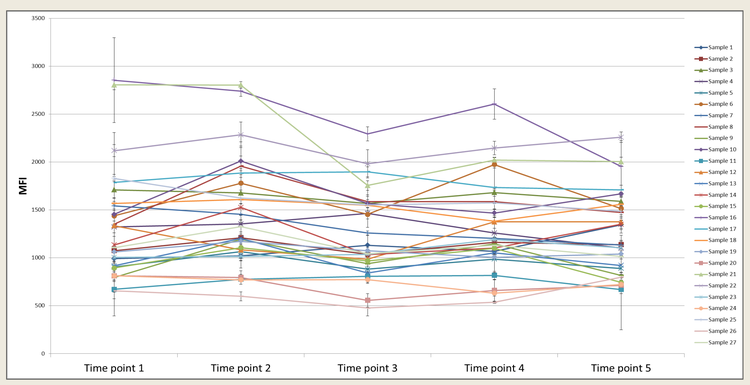

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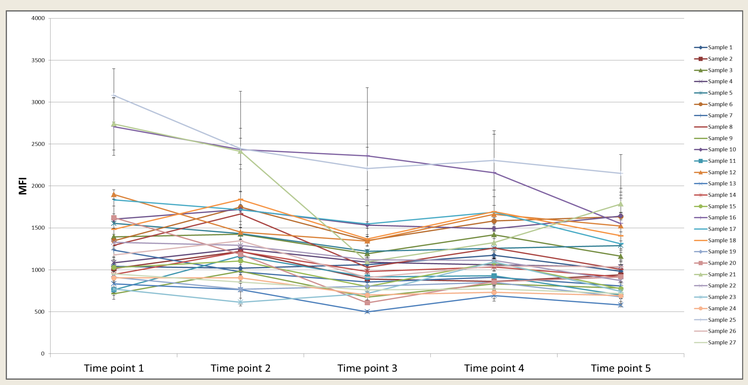

MFI values for 27 samples at 5 different time points for 4 protein markers used as captures in our assay: CD9 (**A**), CD10 (**B**), CD63(**C**) and CD81 (**D**).

E

CD9	Time point 2	Time point 3	Time point 4	Time point 5
Time point 1	8.17	34.18	37.37	5.69
Time point 2		8.67	13.80	0.73
Time point 3			33.15	15.29
Time point 4				11.95

CD10	Time point 2	Time point 3	Time point 4	Time point 5
Time point 1	23.62	31.35	38.76	7.52
Time point 2		19.45	25.39	5.18
Time point 3			34.06	19.54
Time point 4				15.72

CD63	Time point 2	Time point 3	Time point 4	Time point 5
Time point 1	18.57	20.85	37.16	24.87
Time point 2		5.83	15.12	7.49
Time point 3			21.74	34.27
Time point 4				26.28

CD81	Time point 2	Time point 3	Time point 4	Time point 5
Time point 1	37.68	2.35	10.60	2.10
Time point 2		1.56	9.39	1.32
Time point 3			12.29	39.99
Time point 4				11.50

(**E**) T-tests comparing pairs of time points for CD9, CD10, CD63 and CD81. After Bonferroni correction non of the p-values cross the threshold of significance (< 0.05).