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# Number of microvesicles in peripheral blood and ability of plasma to induce adhesion between phospholipid membranes in 19 patients with gastrointestinal diseases

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ABSTRACT

It was recently shown that the plasma protein-mediated attractive interaction between phospholipid membranes could in the budding process cause adhesion of the bud to the mother membrane [J. Urbanija, N. Tomšič, M. Lokar, A. Ambrožič, S. Čučnik, M. Kandušer, B. Rozman, A. Iglič, V. Kralj-Iglič, Coalescence of phospholipid membranes as a possible origin of anticoagulant effect of serum proteins, Chem. Phys. Lipids 150 (2007) 49–57]. Since in the *in vivo* conditions the budding of cell membranes leads to the release of microvesicles into the circulation, a hypothesis was put forward that the ability of plasma to cause adhesion between membranes supresses the microvesiculation process. In the present work, this hypothesis was tested in a population of 19 patients with gastrointestinal diseases. The number of microvesicles in peripheral blood of patients was determined by flow cytometry while the ability of plasma to cause adhesion between membranes was determined by adding patient's plasma to the suspension of giant phospholipid vesicles created by electroformation method, and measuring the average effective angle of contact between the adhered vesicles. Statistically significant negative correlations between the number of microvesicles and the average effective angle of contact (Pearson coefficient −0.50, $p=0.031$) and between the number of microvesicles per number of platelets and the average effective angle of contact (Pearson coefficient −0.64, $p=0.003$) were found, which is in favor of the above hypothesis. Patients with gastrointestinal cancer had larger number of microvesicles (difference 140%, statistical significance 0.033) and smaller average effective angle of contact (difference 20%, statistical significance 0.013) compared to patients with other gastrointestinal diseases.



## Introduction

Microvesicles (MVs) can be considered as extracellular organelles [2] which convey communication between distant cells. MVs found in circulation originate from different cells [3–6], but mostly from blood cells [7]. They are final products of a process starting with lateral redistribution of membrane components, proceeding with development of membrane buds and ending with pinching off the buds from the mother membrane [8]. Increased number of MVs was found in peripheral blood of patients suffering from cardiovascular disorders [3,4], infection [4,9–11], autoimmune diseases [12,13] and cancer [14–16]. Tumor-derived MVs are present also in other body fluids of patients with cancer [17].

Due to the importance of microvesiculation of membranes, the underlying mechanisms were studied in different systems. A simple system which could reveal physical mechanisms involved are giant phospholipid vesicles (GPVs). GPVs possess many important properties of cell membranes and can be observed live under an optical microscope. Vesiculation of GPVs can be induced by different mechanisms such as an increase of the temperature and manipulation of the solution in contact with the outer layer of the GPV membrane. In such studies it was found that the addition of plasma proteins (in particular, beta 2 glycoprotein I and antiphospholipid antibodies) into the suspension of GPVs may cause budding of GPVs [18], coalescence of GPVs [19] and adhesion of buds to the mother membrane [1], (Fig. 1). The accompanying theoretical studies on protein-mediated interactions between membranes indicate that plasma proteins can induce attractive interaction between membranes even when they are like-charged, due to positional and orientational distribution of protein molecules with spatially distributed charge [20].

It was suggested on the basis of the above experimental and theoretical results that plasma protein-mediated attractive interaction between membranes may prevent the release of MVs into the

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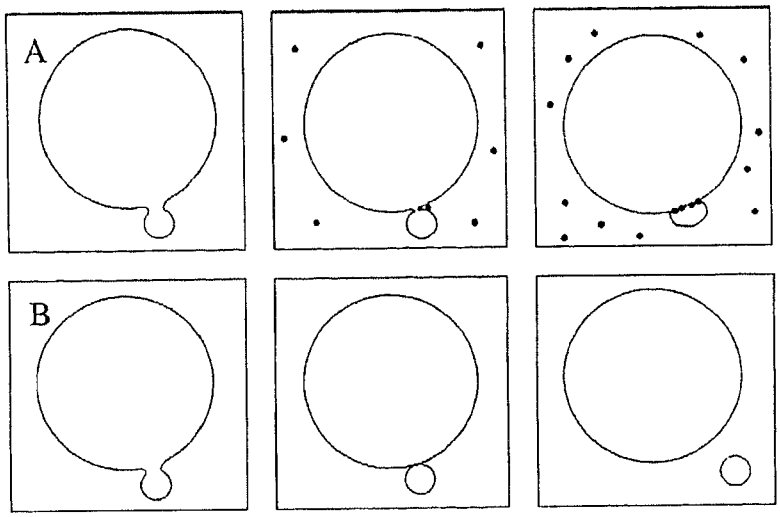



Fig. 1. Scheme of the budding and vesiculation of the membrane in the presence (A) and in the absence (B) of adhesion mediators. If adhesion mediators are present, the bud adheres to the mother cell membrane (A) while if adhesion mediators are absent the bud pinches off the mother membrane and becomes a microvesicle (B).

circulation [1]. A hypothesis was put forward that in subjects with plasma which induces a more pronounced adhesion between membranes the number of MVs in the peripheral blood will be smaller. A method for determination of the extent of plasma-induced adhesion between membranes was proposed by assessing the average effective angle of contact between GPVs which adhered due to the addition of plasma to the suspension of GPVs [21]. It was assumed that larger average effective angle of contact corresponds to a more pronounced adhesion [21]. The proposed trend was confirmed by a pilot study involving three healthy subjects [21]. However, in order to obtain decisive evidence regarding the validity, the hypothesis should be tested on different populations involving larger number of subjects. This work presents a study of a population of 19 subjects with various gastrointestinal diseases. At this point it is of interest to determine a general trend of the dependence of the number of MVs on the average effective angle of contact between GPVs. Therefore, it is preferable to include in the study the subjects within a wide range of the number of MVs, including patients with cancer, in whom the number of MVs is expected to be high. As among 19 of the included patients 5 of them were diagnosed with cancer we could also assess possible differences between the group formed by these patients and the group formed by all other patients, regarding the number of MVs and the average effective angle of contact between GPVs.

## Patients

We originally included in the study 21 patients from Department of Gastroenterology, University Medical Centre Ljubljana, who were admitted in 2007 due to various gastrointestinal diseases (Tables 1–3). Patients gave a written consent to participate in the study. Two experiments were performed (the first experiment included 15 patients and the second experiment included 6 patients). In two patients, the sample with GPVs was of insufficient quality to determine the average effective angle of contact, so these two patients were included only in the analysis of the number of MVs. To compare the patients with diagnosed gastrointestinal cancer with other patients we formed two respective groups. The group A (patients diagnosed with cancer) consisted of 5 patients and the group B (patients with other gastrointestinal diseases) consisted of 16 patients (for analysis of MVs) or 14 patients (for analysis of the average effective angles of contact between GPVs).

## Materials and methods

### *Preparation of human plasma samples and microvesicle isolation*

2 ml of venous blood was collected into vacutubes containing trisodium citrate and processed within 15 minutes (min). Following the centrifugation of blood (1500 ×g, 15 min, 20 °C, SIGMA 3K18 Centrifuge, Sigma), two plasma samples for each patient (250 μl each) were used for isolation of MVs according to the method described in Diamant et al. [7] while 300 μl of plasma was used for experiments with GPVs. To isolate MVs, 250 μl of plasma was centrifuged at 17,570 ×g for 30 min at 20 °C (SIGMA 3K30 Centrifuge, Sigma) and 225 μl of supernatant was removed. After adding 225 μl of phosphate buffer saline (136.9 mmol/l NaCl, 2.7 mmol/l KCl, 7.8 mmol/l $Na_2HPO_4 \times 2H_2O$ and 1.5 mmol/l $KH_2PO_4$, pH 7.4) containing 10.9 mmol/l trisodium citrate, centrifugation at 17,570 ×g and the removal of supernatant was repeated. For analysis by flow cytometer, the samples containing MVs were diluted 4 times: 975 μl of citrated phosphate buffer saline was added to 25 μl of the pellet. Isolation of MVs and experiments with GPVs were performed simultaneously, immediately after the separation of plasma from the cells.

### *Flow cytometry*

Samples containing isolated MVs were analyzed using a Coulter EPICS Altra flow cytometer (Beckman Coulter Electronics). 7000 events were recorded. The number of MVs was given relatively to the number of Flow-count fluorospheres ($1{,}05 \times 10^6$/ml in solution, Beckman Coulter). The samples for measurement of the number of MVs were prepared from 585 μl of diluted MVs and 15 μl of fluorospheres in solution.

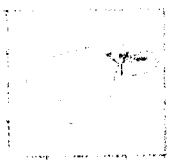
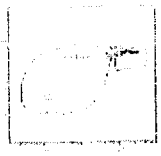
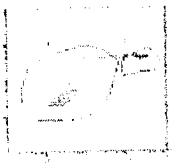
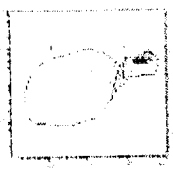
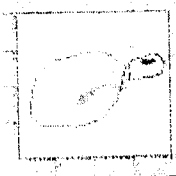
### *Platelet count*

For determination of the platelet count, 3 ml of venous blood was collected into vacutubes containing $K_3$EDTA anticoagulant. Platelets were counted by Siemens Advia 120 hematologic counter.

### *Preparation and observation of giant phospholipid vesicles*

GPVs were prepared by the modified electroformation method, originally proposed by Angelova et al. [22]. For neutral GPVs, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and cholesterol (Avanti

Table 1
Data on sex, age, forward scatter vs. side scatter distribution of MVs(I)/fluorospheres (J) as measured by flow cytometer, diagnosis, platelet concentration, number of MVs, number of MVs with respect to number of platelets and the average effective angle of contact between adhered GPVs, pertaining to the patients with gastrointestinal cancer (group A)

| # | Sex, year of birth | Forward scatter/side scatter of MVs (I) and flourospheres (J) | Diagnosis | Platelet concentration $\times 10^9$/l | MVs/fluoro spheres | MVs/fluoro spheres/ platelets ($10^{-9}$ l) | Average effective angle of contact (degrees) |
|---|---|---|---|---|---|---|---|
| 1 | F, 1963 |  | metastatic pancreatic cancer | 411 | 3.82 | 0.0271 | 84 |
| 2 | F, 1928 |  | locally advanced pancreatic cancer, hyperlipidemia | 349 | 3.64 | 0.0104 | 97 |
| 3 | M, 1922 |  | carcinoma papillae Vateri | 231 | 7.13 | 0.0308 | 103 |
| 4 | M, 1942 |  | initial hepatocellular carcinoma, liver cirrhosis | 130 | 0.4 | 0.0031 | 116 |
| 5 | M, 1949 |  | advanced hepatocellular carcinoma | 63 | 2.05 | 0.0325 | 80 |

Polar Lipids, Inc.), dissolved in a 2:1 chloroform/methanol mixture at concentration of 1 mg/ml, were combined in the proportion of 4:1 (v/v). Cholesterol was added to POPC to increase the longevity of vesicles.

20 µl of the lipid mixture was applied to each of the two platinum electrodes shaped as rods (approximate length 4 cm and diameter 1 mm). The electrodes were left in a low vacuum for 2 h for solvent to evaporate. The lipid-coated electrodes were thereafter placed into a microcentrifuge tube filled with 1.8 ml of 0.2 M sucrose solution to form an electroformation chamber. An AC electric current with an amplitude of 5 V and a frequency of 10 Hz was applied to the electrodes for 2 h, which was followed by 2.5 V and 5 Hz for 15 min, 2.5 V and 2.5 Hz for 15 min and finally 1 V and 1 Hz for 15 min. After the electroformation, 600 µl of sucrose solution containing GPVs and 1 ml of 0.2 M glucose solution were pipetted in three 2 ml plastic microcentrifuge tubes which were sealed with parafilm band to prevent entrance of air and to protect the solution from microorganisms. The vesicles were left for sedimentation under gravity in a low vacuum at room temperature for 1 day.

The GPVs were observed by an inverted microscope Nikon Eclipse T-300 with phase contrast optics. For the experiments 70 µl CoverWell™ Perfusion Chambers (Grace Bio-Labs), which allowed simultaneous performance of four experiments, were used. 7 µl of citrated plasma sample was added through a circular opening to the 63 µl of GPV suspension in the well of the perfusion chamber. For all experiments, the images of adhered GPVs were taken 30 min after the addition of plasma at 4 mm distance from the circular opening where plasma was introduced. The experiments were performed at room temperature.

*Determination of the mediating effect of plasma*

To assess the adhesion between GPVs after the addition of plasma, all clearly visible effective angles of contact between adhered GPVs from an image (see Figs. 2–4) were measured using Corel Draw software. An average from all the angles determined from a given frame was calculated for each patient.

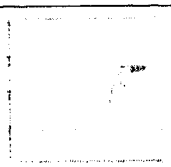
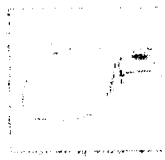
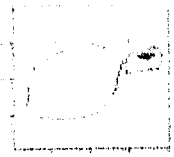
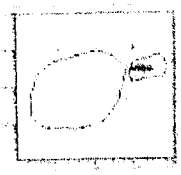
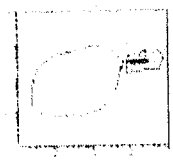
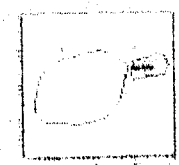
*Statistical analysis*

Methods of descriptive statistics were used. The statistical significance of the correlation was determined by the Pearson coefficient while the statistical significance of the difference between the two groups of patients was determined by the Student's *t*-test. Power analysis was performed by the Power and Precision V3 software.

## Results

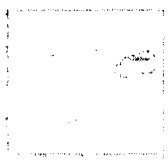
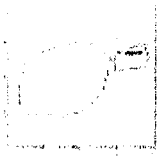
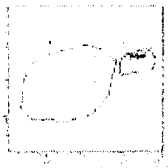
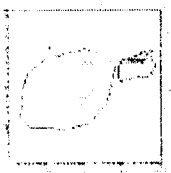
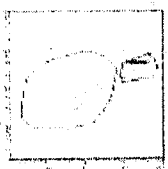
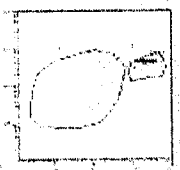
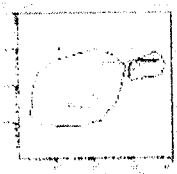
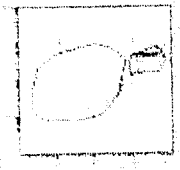
Fig. 2 shows the GPV samples 30 min after the addition of plasma pertaining to patients from group A (patients with gastrointestinal

**Table 2**
Data on sex, age, forward scatter vs. side scatter distribution of MVs(I)/fluorospheres (J) as measured by flow cytometer, diagnosis, platelet concentration, number of MVs, number of MVs with respect to number of platelets and the average effective angle of contact between adhered GPVs, pertaining to the patients from group B (patients marked by 6–11, 20 and 21)

| # | Sex, year of birth | Forward scatter/side scatter of MVs (I) and flourospheres (J) | Diagnosis | Platelet concentration × $10^9$/l | MVs/fluoro spheres | MVs/fluorospheres/ platelets ($10^{-9}$ l) | Average effective angle of contact (degrees) |
|---|---|---|---|---|---|---|---|
| 20 | F, 1945 |  | ulcus ventriculi | 389 | 0.62 | 0.0016 | |
| 21 | M, 1944 |  | acute pancreatitis, hyperlipidemia | 256 | 0.41 | 0.0016 | |
| 6 | M, 1919 |  | hemorrhagic anglodysplasia of the upper gut | 261 | 0.28 | 0.0011 | 95 |
| 7 | M, 1978 |  | ulcerative colitis | 1052 | 0.58 | 0.0005 | 108 |
| 8 | F, 1945 |  | liver cirrhosis | 338 | 1.34 | 0.0029 | 103 |
| 9 | M, 1932 |  | ulcus bulbi duodeni, infection | 668 | 1.14 | 0.0017 | 122 |
| 10 | M, 1958 |  | liver cirrhosis | 97 | 0.36 | 0.0037 | 110 |
| 11 | M, 1965 |  | liver cirrhosis | 137 | 0.56 | 0.0041 | 145 |

cancer). Correspondingly, Figs. 3 and 4 show the GPV–plasma samples pertaining to patients from group B (patients with other gastrointestinal diseases). The clearly visible effective angles of contact were measured as shown in Figs. 2,3 and 4 and the average for each patient was determined. Differences between GPV–plasma samples pertaining to different patients can be observed, such as the presence of "debris" in some samples and larger differences between refraction indexes of the vesicles and of the surrounding solution as exhibited in

Table 3
Data on sex, age, forward scatter vs. side scatter distribution of MVs(I)/fluorospheres (J) as measured by flow cytometer, diagnosis, platelet concentration, number of MVs, number of MVs with respect to number of platelets and the average effective angle of contact between adhered GPVs, pertaining to the patients from group B (patients marked by 12–19)

| # | Sex, year of birth | Forward scatter/side scatter of MVs (I) and flourospheres (J) | Diagnosis | Platelet concentration × $10^9$/l | MVs/fluoro spheres | MVs/fluoro spheres/ platelets ($10^{-9}$ l) | Average effective angle of contact (degrees) |
|---|---|---|---|---|---|---|---|
| 12 | M, 1951 |  | liver cirrhosis, infection | 78 | 0.27 | 0.0035 | 110 |
| 13 | F, 1941 |  | liver cirrhosis, acute cholecystitis | 100 | 0.44 | 0.0044 | 117 |
| 14 | F, 1940 |  | liver cirrhosis | 114 | 0.59 | 0.0051 | 118 |
| 15 | F, 1984 |  | autoimmune hepatitis | 105 | 0.66 | 0.0063 | 118 |
| 16 | M, 1934 |  | liver cirrhosis, infection | 174 | 0.53 | 0.0030 | 119 |
| 17 | M, 1955 |  | liver cirrhosis, hemoragic ulcer | 78 | 0.40 | 0.0051 | 128 |
| 18 | F, 1951 |  | acute pancreatitis, hyperlipidemia | 437 | 1.37 | 0.0031 | 135 |
| 19 | F, 1914 |  | hemorrhagic ulcer, infection | 396 | 0.35 | 0.0008 | 106 |

the halo effect. The observed samples contained different populations of GPVs with respect to their size and density.

We found a negative, statistically significant correlation (Pearson coefficient=−0.50, $p$=0.031) between the number of MVs in peripheral blood and the ability of plasma to induce coalescence between membranes – represented by the average effective angle of contact between adhered GPVs (Fig. 5A). Statistical significance of the correlation was even higher if the number of MVs was calculated

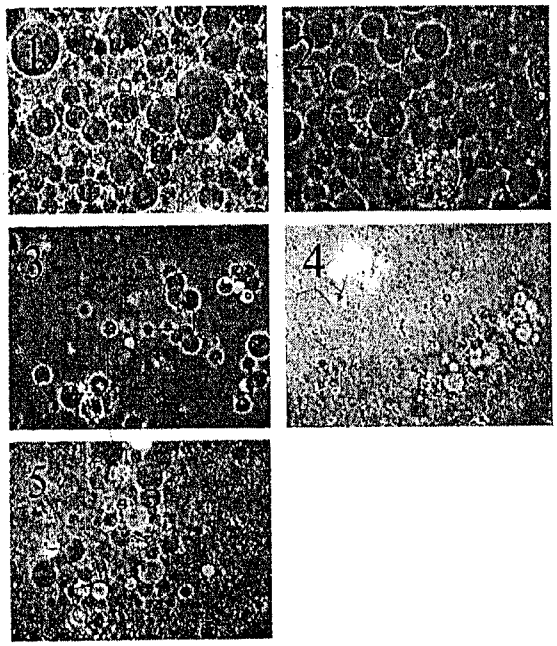


Fig. 2. Images of GPV samples 30 min after the addition of patients' plasma (group A, patients marked by 1–5). The effective angles of contact between the adhered GPVs are depicted. Small circles indicate the contribution of 360° by three adjacent effective angles of contact.

with respect to the number of platelets (Pearson coefficient=−0.64, $p$=0.003), (Fig. 5B). This result is in favor of the hypothesis that in subjects with plasma which induces a more pronounced adhesion

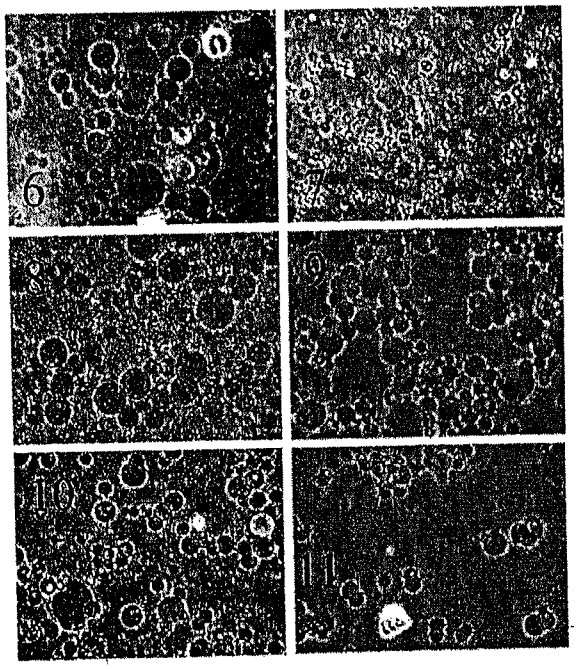


Fig. 3. Images of GPV samples 30 min after the addition of patients' plasma (group B, patients marked by 6–11). The effective angles of contact between the adhered GPVs are depicted. Small circles indicate the contribution of 360° by three adjacent effective angles of contact.

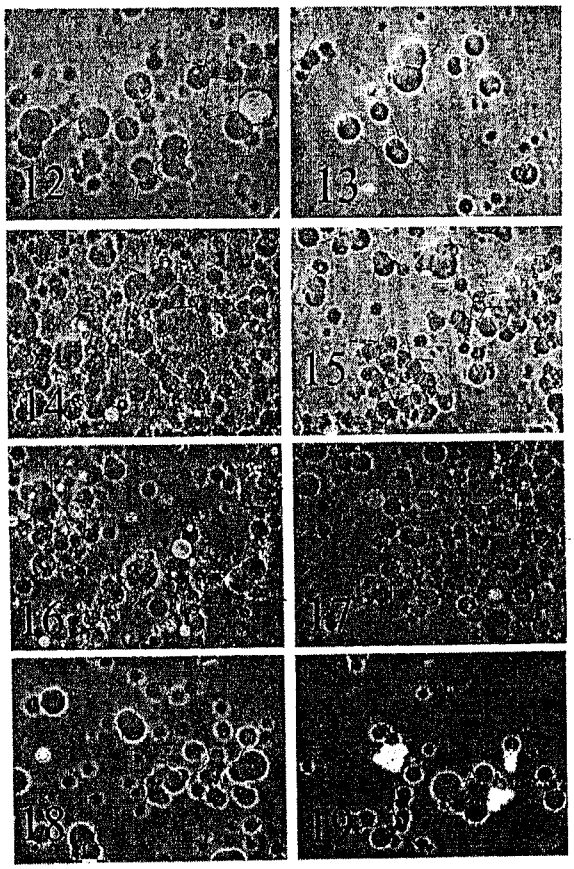


Fig. 4. Images of GPV samples 30 min after the addition of patients' plasma (group B, patients marked by 12–19). The effective angles of contact between the adhered GPVs are depicted. Small circles indicate the contribution of 360° by three adjacent effective angles of contact.

between membranes the number of MVs in the peripheral blood is smaller.

By comparing patients diagnosed with cancer (group A) with patients having other gastrointestinal diseases (group B) we found a large (140%) and statistically significant ($p$=0.033) difference between groups A and B regarding the number of MVs in peripheral blood (Table 4) while the difference between the two groups regarding the average effective angles of contact between GPVs was smaller than the difference in MVs, but still considerable (20%) and statistically significant ($p$=0.013) (Table 4). Further statistical analysis yielded power 100% for MVs at $\alpha$=0.05 while for the average effective angles of contact, the power at $\alpha$=0.05 was 90%, which is excellent regarding that there were only 5 subjects in group A. On the basis of these results we can conclude that considerable and statistically significant differences in the number of MVs and in the ability of plasma to cause adhesion of membranes between the two groups are indicated.

## Discussion

The main result of this work is the correlation between the number of MVs in peripheral blood of patients and the average effective angle of contact between GPVs after the addition of plasma to GPVs (Fig. 5). Our study presents the evidence in favor of the hypothesis that plasma which mediates attractive interaction between membranes may cause suppression of microvesiculation (Fig. 1) which results in a smaller number of MVs shed from the vesiculating pool. We think that the presented results are promising, however, further studies should be

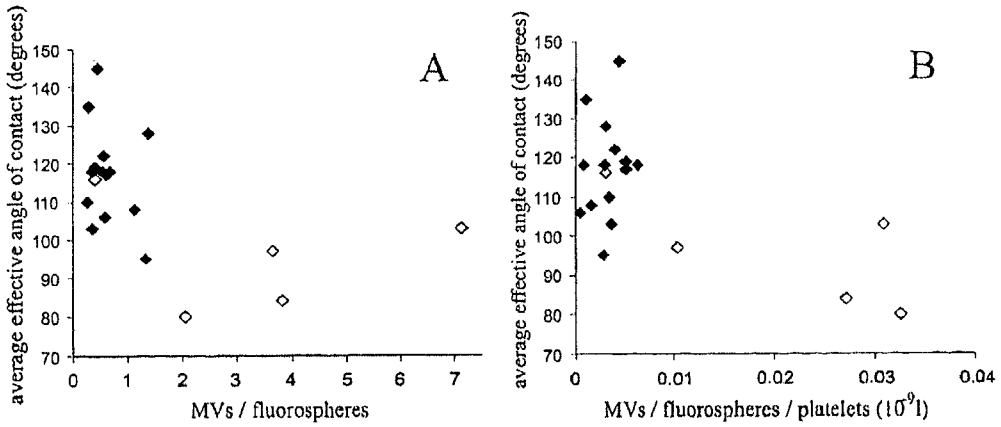



Fig. 5. Correlations between the average effective angle of contact between adhered GPVs and the number of MVs in peripheral blood (A) and between the average effective angle of contact between adhered GPVs and number of MVs per number of platelets (parameter *f*), for 19 patients with gastrointestinal diseases. Empty diamonds correspond to patients from group A (patients diagnosed with gastrointestinal cancer), while full diamonds correspond to patients from group B (patients with other gastrointestinal diseases).

made to validate the hypothesis. Also, some shortcomings of the method should be overcome.

We have improved the method for preparation of GPVs with respect to the previously reported study [21] where it was argued that the yield of GPVs was too low to analyse larger number of samples. In the modified protocol which was used in the present study, a larger part of lipid-coated electrodes was in contact with sucrose solution in the electroformation chamber, so that a larger lipid/solution interface is available in the electroformation process compared to previous protocol. During sedimentation samples were better protected against microorganisms. Also, according to the new protocol, the GPVs were rinsed from the electrodes by removing the electrodes from the solution and re-inserting them several times. In the previous protocol, the electrodes were rinsed by pouring the solution out of the chamber. By using the improved method of electroformation we can now test about 20 samples of plasma with the same yield of GPVs. In this respect, we could in principle analyze all samples from our study with the same yield of GPVs, however, we were limited by the limited number of blood samples that our centrifuge can process at the same time. Thus, the results presented were obtained from two experiments on two different days, whereby for each experiment, GPVs were produced a day before. In any case, even with the same yield, the population of GPVs is a heterogeneous one with respect to size and shape. Also, fluctuations in the density of GPVs in different samples are considerable (Figs. 2–4). Addition of plasma may cause bursting of GPVs, thereby diminishing their number in the sample. For each sample, we took the image at a position that was chosen previously for all samples – at the same distance from the insertion of plasma into the sample. These points may vary considerably in density of GPVs. In the future, further improvements of the method for production of GPVs and other possible methods for studying the ability of plasma to cause adhesion between membraneous structures should be considered.

We have found differences in the number of MVs and in the average effective angle of contact between patients diagnosed with gastrointestinal cancer (group A) and patients diagnosed with other gastrointestinal diseases (group B) (Table 4). Four out of five patients from group A form a separate group in Fig. 5 at large numbers of MVs and small average effective angles of contact between GPVs. In these patients the disease had advanced. The patient #1 died two weeks after the blood sample was taken, due to cardiac arrest following coma. In the fifth patient with cancer who had a small number of MVs and a large average effective angle of contact (and was with respect to these parameters indistinguishable from patients from group B) the tumor was smaller and the stage of the disease was described as initial. Also note that in diagrams showing the size and the forward scatter vs. side scatter distributions of MVs (Table 1) in patients with advanced cancer there is a pronounced region of small MVs while there is no pronounced region of small MVs in the patient in whom the disease was in the initial stage. Large difference in the number of MVs between the groups A and B (Table 4) would therefore be ascribed to the advanced stage of cancer in four out of five patients from group A. These results agree with the results of Kim et al. [16] who found an increased number of MVs in peripheral blood of patients with gastric cancer with respect to normal controls, the number of MVs increasing with the advanced stage of the disease.

Circulating MV concentration is influenced by the size of the pool of cells that are able to vesiculate, the intrinsic properties of cell membranes, the mediating effect of plasma to suppress microvesiculation and the ability of the clearance system to remove MVs from circulation. In order to better assess the effect of the ability of plasma to suppress microvesiculation, it was suggested [21] that the relevant parameter would be *f*=the amount of MVs in plasma/the size of the vesiculating pool, rather than the concentration of MVs. In healthy subjects, the major contribution of the vesiculating pool contributing to MVs in peripheral blood (around 80%) are platelets [7], therefore in

**Table 4**
Statistical analysis of the differences in the number of MVs and the ability of plasma to cause coalescence of membranes (represented by the average effective angle of contact between the adhered GPVs) between patients with gastrointestinal cancer (group A) and patients with other gastrointestinal diseases (group B)

| | Group A | Group B | Difference between groups | Number of subjects | Statistical significance of difference | Tails | Power at $\alpha$=005 |
|---|---|---|---|---|---|---|---|
| MVs/fluorospheres | 0.62 | 3.41 | −2.79 | 21 | 0.033 | 1 | 1.00 |
| st. dev. | 0.35 | 2.5 | | | | | |
| average effective angle of contact (degrees) | 117 | 96 | 21 | 19 | 0.013 | 1 | 0.90 |
| st. dev. (degrees) | 13 | 14 | | | | | |

the pilot study [21] the parameter $f$ was defined as the ratio between the concentration of MVs and the concentration of platelets. A better correspondence was found in the dependence of the parameter $f$ on the average effective angle of contact than in the dependence of MV concentration on the average effective angle of contact [21]. Similar results were obtained by applying this parameter to our population: the Pearson coefficient representing the strength of the correlation was higher for the parameter $f$ (Fig. 5B) than for the MV number (Fig. 5A, Table 4), mostly on account of the patients with diagnosed cancer. In line with the hypothesis, this indicates that also in these patients, platelets represent an important vesiculating pool. Indeed, it was found that an increased number of tissue factor-bearing MVs presumably derived from platelets was found in the blood of patients with Duke's D colorectal cancer [23]. It was suggested that the tissue factor might have been transported to the platelet membrane from monocytes and macrophages by means of MVs [24].

The tissue factor, an integral membrane protein of the vessel wall and the principal initiator of blood coagulation was found on circulating MVs [30–32]. It was reported that the tissue factor-bearing MVs originate from lipid rafts of the monocyte or macrophage membrane, the rafts being enriched in tissue factor and cholesterol [23,24,32], outlining the importance of redistribution of membrane constituents [33] and interactions between membrane constituents [34] in the budding process [8]. Moreover, tumor-released MVs of patients with cancer are likely to be involved in tumor progression [25–29]. In patients with cancer, MVs can therefore be considered as procoagulant and cancer-promoting.

Suppression of processes leading to the release of MVs into circulation may therefore be of benefit as to prevent or slow down the development of the above pathological processes.

In this work we address a physical mechanism that is common in all membranes, regardless to accompanying chemical processes which finally lead for example to blood clot formation or to protein synthesis. Suppression of the release of MVs by the mediating effect of proteins in solution adjacent to the membrane would apply to the budding of any membrane that is likely to shed MVs. Focusing on this mechanism, natural or artificial suppressors of microvesiculation would act simultaneously as anticoagulants and cancer invasive potential deccelerators. It would therefore be of interest to establish which plasma constituents can mediate the attractive interaction between membranes. In previous studies, such constituent was found to be the plasma protein beta 2 glycoprotein I [19,1]. In the future, the list of possible candidates should be expanded and their clinical relevance should be assessed.

## Conclusion

Results involving a population of 19 patients with gastrointestinal diseases support the hypothesis that in subjects with plasma which induces a more pronounced adhesion between membranes the number of MVs in the peripheral blood is smaller.

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