Isolated tumour microparticles induce endothelial microparticle release *in vitro*.

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Abstract

Cancer induces a hypercoagulable state, resulting in an increased risk of venous thromboembolism. One of the mechanisms driving this is tissue factor (TF) production by the tumour, released in small lipid bound microparticles. We have previously demonstrated that tumour cell line media induced procoagulant changes in HUVEC. The aim of this study was to investigate the effect of tumour microparticles and recombinant human TF (rhTF) on the endothelium. Procoagulant microparticles from the PANC-1 cell line were harvested by ultrafiltration. HUVEC were then incubated with these procoagulant microparticles or rhTF. Flow cytometry was used to investigate the effect of endothelial cell surface protein expression and microparticle release. Microparticles but not soluble TF was responsible for the procoagulant activity of cell-free tumour media. We also demonstrated an increase in endothelial microparticle release with exposure to tumour microparticles, with a positive linear relationship observed (R^2=0.6630, P<0.0001). rhTF did not induce any of the changes observed with microparticles. Here we demonstrate that procoagulant activity of tumour cell line media is dependent on microparticles, and that exposure of endothelial cells to these microparticles results in an increase in microparticle release from HUVEC. This suggests a mechanism of transfer of procoagulant potential from the cancer to the remote endothelium.

Introduction

Cancer is associated with a hypercoagulable state. The increased risk of venous thromboembolism as a result of this prothrombotic effect is a cause of significant morbidity and mortality in cancer patients [1] and confers poor prognosis. The risk of venous thromboembolism varies depending on the primary tumour cell origin, with pancreatic, ovarian and haematological cancers being among those associated with a higher risk and colorectal, breast and prostate among those associated with a lower risk [2]. The risk of thrombosis increases significantly in patients undergoing chemotherapy [3] .

The procoagulant state associated with malignancy is due to systemic activation of the coagulation system; a number of biological molecules have been implicated the most significant of which being tissue factor (TF) [4]. TF is a 47-kDa transmembrane glycoprotein receptor that acts as the main initiator of the extrinsic coagulation pathway. It binds to and activates Factor VIIa, the TF-VIIa complex then activates Factor X, leading to the generation of thrombin and thus activating the clotting cascade. TF is expressed by endothelial cells in response to injury, and is not expressed under normal physiological conditions. [5] Cancer cells have been found to express high levels of TF and cancer cell lines with higher cell surface expression of TF show a greater prothrombotic tendency *in vitro*. In addition, We have previously shown that blocking TF prevents coagulation of pancreatic cancer cells *in vitro* and demonstrated a linear relationship between cell surface TF expression and single cell clotting time calculated using clotting time as a measure of procoagulant activity [4].

TF is released in microparticles (MPs)- small phospholipid membrane bound vesicles which are shed from cells through blebbing of the cell membrane and contain proteins expressed on the surface of their cell of origin. Under physiological conditions they are released in response to activation, apoptosis and pathological conditions such as hypoxia [6] with the main tissues of origin being endothelial cells and platelets. MP release is increased in cancer patients [7] and constitute the main source of TF. These MPs have been demonstrated to display procoagulant activity both *in vitro* and *in vivo* [8] and in patients with pancreatic cancer higher levels of these MPs were found to be associated with a higher incidence of venous thrombosis [9]. TF has also been found to be linked to tumour stage

[10] and correlated with poor prognosis and tumour recurrence in cancer patients [11]. In addition, TF has been implicated in tumour growth, metastasis [12] and angiogenesis [2].

TF also exists as a soluble form dissolved in the plasma, whose role in cancer related thrombogenesis is currently under debate [13,14]. Soluble TF exists in two main isoforms: full length and alternatively spliced forms. Bogdanov et. al. found that the alternatively spliced soluble TF (asTF) exerted a prothrombotic effect when exposed to phospholipids through interaction with platelets. [14] However Censarek et. al. demonstrated no procoagulant activity of embryonic kidney cells with over expression asTF [13]. Hobbs et al showed that transfection of the pancreatic cancer cells with full length TF (flTF) significantly increased procoagulant activity but that this effect was not seen with asTF [15].

More recently other molecules have also been implicated in the procoagulant properties of cancer cells. We have previously demonstrated that blocking TF decreases but does not entirely remove the procoagulant activity of pancreatic cancer cells *in vitro* and demonstrated a role of phosphatidylserine (PS) and phosphatidylethanolamine (PE) in tumour procoagulant activity by demonstrating a dose dependent increase in cell clotting time when blocking the effects of these phospholipids with Annexin and Duramycin respectively [16]. In addition we demonstrated that necrosis reduces the procoagulant activity of cancer cells by reducing TF surface expression whereas apoptosis enhances the procoagulant activity of cancer cells through increased PS exposure [16] .

The procoagulant properties of MPs is well recognised; however little is known about their interaction with the endothelium. Activation of the endothelium and release of endothelial microparticles (EMP) has been demonstrated in patients with venous thromboembolism [17]. Previously we have shown an interaction between head and neck tumour MPs and endothelial cells *in vitro*, whereby following exposure of endothelial cells to tumour media MPs were shown to attach to a distinct population of endothelial cells. This conferred procoagulant activity to endothelial cells, demonstrated to be dependent TF [18]. If this interaction between endothelial cells and MPs is mediated via TF, it could be predicted that recombinant human TF (rhTF) may elicit a similar response. The aim of this work was therefore to investigate the addition of concentrated TF bearing MPs or rhTF to *in vitro* cultures

of HUVECs and analyse the response of the endothelial cells in terms of procoagulant activity, uptake of rhTF and release of EMP.

Methods

Cell Culture

Cells were incubated at 37°C in Dulbecco's Modified Eagle Medium (DMEM) (Lonza) supplemented with 10% Foetal Bovine Serum (FBS) (Labtech International Ltd), 1% penicillin/streptomycin (Lonza) and 1% L-glutamine (Lonza) and were stored at 37°ᶜ in a humidified atmosphere containing 5% CO_2. Cell counting was performed by light microscopy using a haemocytometer (Neubauer).

MIA-Pa-Ca2 are derived from a human pancreatic carcinoma taken from a 65-year-old Caucasian man. PANC-1 are derived from a human pancreatic carcinoma of ductal cell origin taken from a 56-year-old male. ES2 are derived from a human clear cell ovarian carcinoma from a 47-year-old female. A2780 are derived from an Ovarian endometroid adenocarcinoma. UM-SCC-81B (University of Michigan-Squamous Cell Carcinoma-81B) are derived from a tonsillar squamous cell carcinoma. UM-SCC-47 are derived from a tongue squamous cell carcinoma of a 53-year old male. SK-OV-3 are derived from the ascitic fluid of an ovarian serous cystadenocarcinoma of a 64-year old Caucasian female. OVCAR-3 are derived from the ascitic fluid of a high grade ovarian serous adenocarcinoma of a 60 year old female. U87 are derived from a primary glioblastoma.

Primary human umbilical vein endothelial cells (HUVEC; PromoCell, Heidelberg, Germany) were cultured in complete endothelial cell growth media (ECGM; PromoCell, Heidelberg, Germany) and incubated in 25cm^3 flasks at 37°C in 5% CO_2. HUVEC used were at passage 2 to 4.

Clotting analysis

Prothrombin time (PT) assays were used to assess procoagulant activity (PCA) using a Thrombotrack SOLO coagulometer (Alere, Stockport, UK). 100 µL of the sample to be analysed was incubated in 100µ 25mmol/l $CaCl_2$ at 37°C for two minutes. Coagulation was initiated by addition of 100µl of human normal plasma control (NormTrol plasma, Helena Biosciences, Gateshead, UK). A steel ball within the sample rotated due to a magnetic field and fibrin formation produced by the coagulation process prevents rotation of the steel ball, as measured by a change in the optical density. The time

taken for this to occur was defined as the clotting time. Samples that did not clot within 999.9 seconds (~17 minutes) were considered to be non-clotting.

Cell free media for assessment of coagulation was obtained by centrifuging 1ml of the sample at 400×*g* for 5 minutes to remove the cells. 300ml of the supernatant was removed and incubated at 37°C for 2 minutes and 100ml of this cell free media was then used in the clotting assay as described above.

Cells for assessment of coagulation were obtained by centrifuging cell solutions at 1000×*g* for 10 minutes to pellet the cells. The cell pellet was then resuspended in 1ml phosphate buffered saline (PBS) and cell counting was performed. 300ml of the solution was removed and incubated at 37°C for 2 minutes and 100ml of this cell solution was then used in the clotting assay as described above.

Flow cytometry

Flow cytometry for assessment of cell surface and MP expression from cancer cells lines and HUVEC was performed on a FACSCalibur (Becton Dickinson, Oxford, UK). For assessment of cell free media was obtained by centrifugation at 400*g* for 5 minutes to pellet cells. 25µl of this media was then incubated with 5µl of antibody (CD31:FITC, CD54:FITC, CD105:FITC, CD106:FITC or CD142:FITC) (Bio-Rad, Hemel Hempstead, UK) at room temperature in the dark for 30 minutes, when counting beads (Accucheck, Thermo-Fisher) and 150µl filtered PBS were added prior to assessment by flow cytometry using ISTH guidelines for enumeration of MP.

For assessment of cancer or HUVEC cells, 5µg of antihuman antibodies (CD31:FITC, CD54:FITC, CD105:FITC, CD106:FITC or CD142:FITC, Bio-Rad, Hemel Hempstead, UK) or an isotype matched negative control (IgG1:FITC, Bio-Rad) was then added to 50µl of cells ($2x10^5$) and incubated for 30 minutes, at room temperature in the dark. Cells were then washed in PBS and centrifuged. The pellet was then resuspended in 200µl filtered PBS and assessed by flow cytometry.

Ultrafiltration

In order to determine the component of cell media responsible for its procoagulant activity and to assess the role of rhTF versus MP-associated TF, MPs were harvested from tumour media and assessed for procoagulant activity. Cell free media from various cell lines was obtained by centrifuging cell media at 1000*g* for 10 minutes. 5ml of this media was then passed through either a 10,000 $_{MWCO}$ or 100,000 $_{MWCO}$ Vivaspin 6 centrifugal concentrator (Sartorius Stedim Biotech AG, Gottingen, Germany) concentrator and centrifuged at 1000*g* at 10 minutes time intervals until there was ~250μL fluid in the filter. The MP containing supernatant were then reconstituted in media to 5g using a balance. Clotting assays of the cell free media, reconstituted supernatant and filtrate were performed.

ELISA

ELISA was used to investigate TF expression of HUVEC and cancer cell media (Coagulation factor III Duoset, Bio-Techne, UK) according to the manufacturers' instructions using the reccommened ancillary reagents (Bio-Techne, UK).

Incubation of HUVEC in microparticles

In order to assess the effect of MPs on endothelial cells, MPs from the PANC-1 line were harvested by ultrafiltration and added to HUVEC media. PANC-1 cell free media (40mL) was concentrated using a 100,000 $_{MWCO}$ Vivaspin concentrator. The retained fraction was then suspended in 6.5ml endothelial growth media (Promocell, Germany). This MP containing solution was then further diluted in endothelial growth media at varying concentrations (0, 10, 20, 50 or 100%) and 1ml of MP solution was added to 9ml HUVEC media prior to incubation. HUVEC media and cells were then analysed at 2, 24 and 48 hours post incubation by flow cytometry.

Incubation of HUVEC in recombinant tissue factor

HUVEC were incubated in recombinant human TF (Innovin; Dade-Behring, Siemens, Camberley, UK) by addition of varying concentrations of 10mg/ml Innovin to the media prior to incubation.

HUVEC media and cells were then analysed at 24 hours by flow cytometry for cell surface markers or ELISA for soluble TF.

Addition of recombinant tissue factor to cancer cell lines

Pancreatic cancer cell lines (PANC-1 and Mia-Pa-Ca-2) were incubated in 20ml of media until they reached 90-100% confluency when 1ml of 10mg/ml Innovin was added to the media. Flow cytometry was used to assess TF expression: MP-TF expression was assessed at 4 and 24 hours and cell surface TF expression were assessed at 24 hours.

Statistics

Statistical tests were performed using GraphPad Prism version 8.01 for macOS, GraphPad Software, La Jolla California USA. Pearson correlation coefficient on linear regression analysis was used to investigate the relationships between groups. Analysis of variance was used to determine the statistical significance between groups. P values less than 0.05 were considered significant.

Results

Cancer cell and tumour media coagulation kinetics

Clotting assays of serial dilution of cancer cells or cell free tumour media showed clotting time was concentration dependent, with a strong negative linear relationship between cell number and clotting time on a log/log scale. This relationship was observed in both the cells and tumour media of all cancer cell lines tested, and was shown to be significant in all samples tested (Figure 1).

This gradient observed on a log/log scale showed a positive linear relationship (R^2=0.827) between cell dilution gradient and media dilution gradient (Figure 2). Cell mediated PCA was directly correlated with media PCA (Figure 3).

Ultrafiltration

In order to assess the component of the media responsible for coagulation, PANC-1 cell free media was passed through a $10{,}000_{MCW}$ or $100{,}000_{MCW}$ vivaspin concentrator. The filtrate was not observed to support clotting in any instance. Reconstituted supernatant was found to support coagulation after harvesting with either a $10{,}000_{MCW}$ or $100{,}000_{MCW}$ vivaspin filter. Reconstituted supernatant was found to have a similar but slightly longer clotting time than the original cell free media, attributed to small losses in microparticles. This effect was consistent and repeatable in PANC-1(n=12, pre-ultrafiltration PCA 433.7s, post reconstitution 444.4s) with an average loss of PCA of 4% ± 0.3%. Analysis of tissue factor concentration by ELISA showed the presence of soluble tissue factor in the filtrate, despite the fact that the filtrate was not found to support coagulation.

Incubation of HUVEC in microparticles

Incubation of HUVEC with PANC-1 microparticles showed that at 24 h TFMP detected in media was roughly proportional to the concentration of microparticle solution added (Fig. 4). No increase in the markers of endothelial cell activation were seen with addition of PANC-1 microparticles. To investigate the endothelial response to procoagulant microparticles, EMP release was plotted against microparticle-associated TF detected, using microparticle associated TF as a measure of procoagulant microparticle exposure. EMP release was determined by the average of EMP markers measured (CD54, CD105, CD106 and CD142) (Figure 5); we observed a positive linear correlation between the different EMP

markers (data not shown) indicating EMP markers measured were from a homogenous origin. We have previously described a positive association between endothelial markers in blood samples of patients with multiple myeloma [3].

Incubation of HUVEC in recombinant tissue factor

Flow cytometry showed that incubation of HUVEC in rhTF had no effect on EMP release (Figure 6) (R^2=0.008828 p=0.8595). No increase in endothelial surface expression of TF or markers of activation were seen with incubation in rhTF even at the highest concentrations.

Incubation of cancer cells in recombinant tissue factor

Clotting analysis of pancreatic cells incubated in rhTF at 24 hours showed no difference in the single cell clotting time of cells incubated in rhTF compared to the control cell group (p=0.9893) In addition, cell free media from pancreatic cells incubated in rhTF showed no difference compared to equivalent concentration of rhTF incubated for 24hours. Flow cytometry revealed no increase in MP-associated or cell surface TF expression following incubation in rhTF. This effect was observed in both PANC-1 and Mia-Pa-Ca-2 cell lines (data not shown). Analysis of TF concentration within cell culture media by ELISA showed no uptake of rhTF by pancreatic cells when compared to rhTF added to cell culture media alone and incubated for 24h (ANVOA p=0.9893) (Figure 7). However, the same general loss of rhTF concentration with time was seen in both the presence and absence of cells resulting in a half-life of rhTF in cell culture media of approximately 21.2 hours.

Discussion

Cancer cells have a procoagulant surface that is able to support coagulation *in vitro*. Cell free tumour media from those cell lines with TF surface expression were able to support clot formation *in vitro*, consistent with TF being a major factor within the hypercoagulant state associated with cancer. The power law has been found to underlie a variety of different metabolic pathways and we have previously demonstrated this relationship in the clotting of cancer cells [4] and tumour media [16]. Here we demonstrate this power relationship is consistently seen across a number of cell lines of different tumour sites including those derived from both primary tumours and metastases. There was also a positive relationship between the procoagulant activity of cells and corresponding media, providing evidence that tumour cell surface expression of procoagulant proteins governs the procoagulant activity of the media. The linear relationship observed suggests a similar rate of MP release between cell lines.

In order to assess the component of the media responsible for coagulation, MPs were harvested by ultrafiltration then reconstituted in media to assess the procoagulant activity of this solution and the filtrate. We showed that reconstitution of the supernatant to its original concentration restored the procoagulant effect of cell free media, demonstrating the procoagulant activity of tumour media is wholly dependent on substances larger than 100 KDa in size. This effect is presumably due to the presence of procoagulant MPs thus offering a new method of MP isolation. The filtrate was not found to support coagulation. Despite this, TF was detected in the filtrate by ELISA, provided evidence that MP bound TF but not soluble TF is responsible for the procoagulant effect of cell free media *in vitro*. We have previously demonstrated that filtration of cancer cell media through increasingly small filters progressively reduces its procoagulant properties, presumably due to removal of MPs [16].

The mechanism underlying activation of a hypercoagulant state in malignancy is yet to be fully elucidated. The role of TF in this phenomenon is well established, [2,19] and TF expression has been associated with procoagulant activity both *in vitro* [4] and *in vivo* [20,21]. Recent work has begun to focus on the interaction of TF with the endothelium. Incubation of endothelial cells with cancer cells was found to increased TF expression on endothelial cells *in vitro* [22] implying a potential role of the

endothelium. In addition, incubation of endothelial cells with monocytes derived MPs resulted in an increase in endothelial TF expression and production, *in vitro* [23] suggesting a role of MP in inducing a prothrombotic endothelial phenotype in thrombosis more widely, and not just limited to the hypercoagulable state seen in malignancy.

Here, we demonstrate increased endothelial MP release in response to exposure to increasing concentrations of tumour MP. MPs are known to enable communication between vascular endothelium and their cells of origin [24] and this may explain the formation of thromboembolism at sites distance from the cancer of origin in cancer patients, most commonly deep vein thrombosis and pulmonary embolism. EMP are considered a marker of endothelial dysfunction and are associated with procoagulant activity [25].

At the highest concentration used (10% v/v), the tumour MP added would have had a MP concentration equivalent to approximately two thirds of that of replacing endothelial media with tumour media previously described [18]. This may explain the response seen here and shows that this endothelial response is able to occur at low MP concentrations as endothelial cells display plasticity *in vivo* and are able to alter their gene expression in response to stimuli in their microenvironment [26].

No increase in endothelial cell surface TF expression was seen with addition of rhTF, with no increase in endothelial MP release detected suggesting the effect of TF on the endothelium is mediated through MP associated TF. Similarly, no effect on cells surface TF expression was seen with incubation of pancreatic cell lines with rhTF. Hobbs et al [15] showed that transfection of the Mia-Pa-Ca-2 pancreatic cell line with full length but not alternatively spliced TF increases the procoagulant activity of cancer cells. The Mia-Pa-Ca-2 cell line is a pancreatic cell line derived from a primary tumour with low expression of TF [16].

Internalisation of MPs by endothelial cells has been demonstrated [27,28] in a process dependent on lactadherin and αvβ3 integrin [29] before being transported to endosomes [30]. Ahron et al demonstrated increased TF mRNA production by endothelial cells following incubation with monocyte MPs [23] suggesting an internal process. There is also emerging evidence that

internalisation of TF by the endothelium occurs in vivo, and Kawamoto et al demonstrated the presence of MPs in the cytoplasm of dermal endothelial cells following xenograft transplantation of human melanoma cells in a mouse model and showed this process was dependent on endocytosis. [28].

The stability of rhTF in tissue culture was observed with half-life of 21.2 hours. The was no apparent difference in detectable rhTF in media with or without the culture of tumour cells suggesting no take up of rhTF by these cells. rhTF is commonly used to study the effect of TF in cell culture, owing to the scarcity of cell TF. Despite this it was recently found differences in the posttranslational modifications of natural and rhTF exist with greater phosphorylation of placental TF compared to rhTF, which was shown to result in differences in TF activity [31].

The association between MP associated TF and hypercoagulability is well established [2,19] but the role of soluble TF dissolved in the plasma remains to be determined. Here we demonstrate that the procoagulant activity of the media is dependent on MPs and that soluble TF is not able to support coagulation. Thus far, cell surface TF expression has been correlated with circulating levels in cancer patients, [2] and higher levels of TF have been demonstrate in the blood of cancer patients in a variety of different primaries including breast, [32] pancreatic [20] and ovarian [33]. TF expression has also been linked to an increased risk of venous thrombosis. [20,21] Increased endothelial expression of TF follow exposure to tumour media has also been demonstrated in an in vivo mouse model, following xenograft transplantation of human squamous carcinoma cells into a mouse model [34] and TF bearing MP have been shown to be active in clot formation [35]. Further studies are required to fully elucidate the mechanism of this phenomenon *in vivo*. If the mechanism described here exists in the more complex *in vivo* environment then there may be a relationship to be found between TF bearing MP and endothelial MP in the blood of cancer patients. This could lead to a more personalised anti-coagulant prophylaxis and other targeted therapies aimed at disruption of this interaction.

Conclusion

Cancer cell and tumour media procoagulant activities are linked. The component of tumour media responsible for the observed procoagulant activity is above 100 KDa, likely MP associated, with no procoagulant activity seen within the filtrate. Exposure of endothelial cells to MP but not soluble TF induced a proportional endothelial cell MP release.

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Conflicts of interest

None declared.

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Legends to Figures

Figure 1. The relationship between tumour media concentration (a) or cell number (b) and clotting time with logarithmic transformation in different cell lines. (47 (•), SK-OV3 (▲), 81b (□), Colo-320 (◇), PANC-1 (+), ES2 (×), OVCAR-3 (⊙), ASPC-1 (⊡), Mia-Pa-Ca-2 (⊗), A2780 (⊠)). Media: 47:R^2= 0.992p=<0.0001, SC-OV3: R^2= 0.9735 p=<0.0001, 81b: R^2= 0.9930 p=<0.0001 Colo-320 R^2=0.9913 p=0.0044, PANC-1 R^2= 0.9974 p=<0.0001, ES2: R^2=0.9787 p=0.0013, OVCAR-3 R^2= 0.9861 p=<0.0001, ASPC-1: R^2=0.9954 p=<0.0001.

Cell solutions: 47:R^2=0.9920p =<0.0001, SK-OV3: R^2=0.9936 p=<0.0001, 81b: R^2= 0.9990 p=<0.0001 Colo-320 R^2= 0.9987p=0.0044, PANC-1 R^2= 0.9791 p=<0.0001, ES2: R^2=0.9960

p=<0.0001, OVCAR-3 R^2= 0.9928 p=<0.0001, ASPC-1: R^2=0.9867 p=<0.0001, A2780: R^2=0.9867 p=<0.0001, MIA-Pa-Ca-2: R^2= 0.9936 p=<0.0001.

Effect observed in numerous repeats: (a) 47:8 SC-OV3: 4, 81b:12, Colo-320: 3, PANC-1: 5, ES2: 2, OVCAR-3: 2, ASPC-1: 3. (b): 47:2, SC-OV3: 4, 81b: 2, Colo-320: 3, PANC-1: 5, ES2: 2, OVCAR-3: 2, ASPC-1: 6, Mia-Pa-Ca-2: 9, A2780: 2.

Figure 2. The gradient of serial dilution of media PCA compared to PCA of cell dilution clotting assays. Each point represents a different cell line: PANC-1 (+), 81b (□), 47 (•), SC-OV-3 (▲), ES2 (×), OVCAR3 (⊙). Gradients were seen on a log/log scale and were taken as the average of multiple repeats. R^2=0.8267 p=0.03080.

Figure 3. PCA of cells and their respective cell free media at 48h incubation. Data represents an average clotting time of three clotting assays. Each point represents a different cell line: A2780 (⊠), PANC-1 (+), ASPC-1 (⊡), SCOV3 (▲), U87 (∗), ES2 (×). R^2=0.9866 p=<0.0001.

Figure 4. Tissue factor MPs detected by flow cytometry at 24 hours in response to increasing concentrations of PANC-1 microparticles as a percentage of original concentration. R^2= 0.8989 p=0.0141.

Figure 5. Endothelial microparticle release at 24 hours measured by flow cytometry in response to the concentration of microparticle associated tissue factor detected at 24 hours incubation with PANC-1 microparticle solution. R^2=0.6630 p=<0.0001.

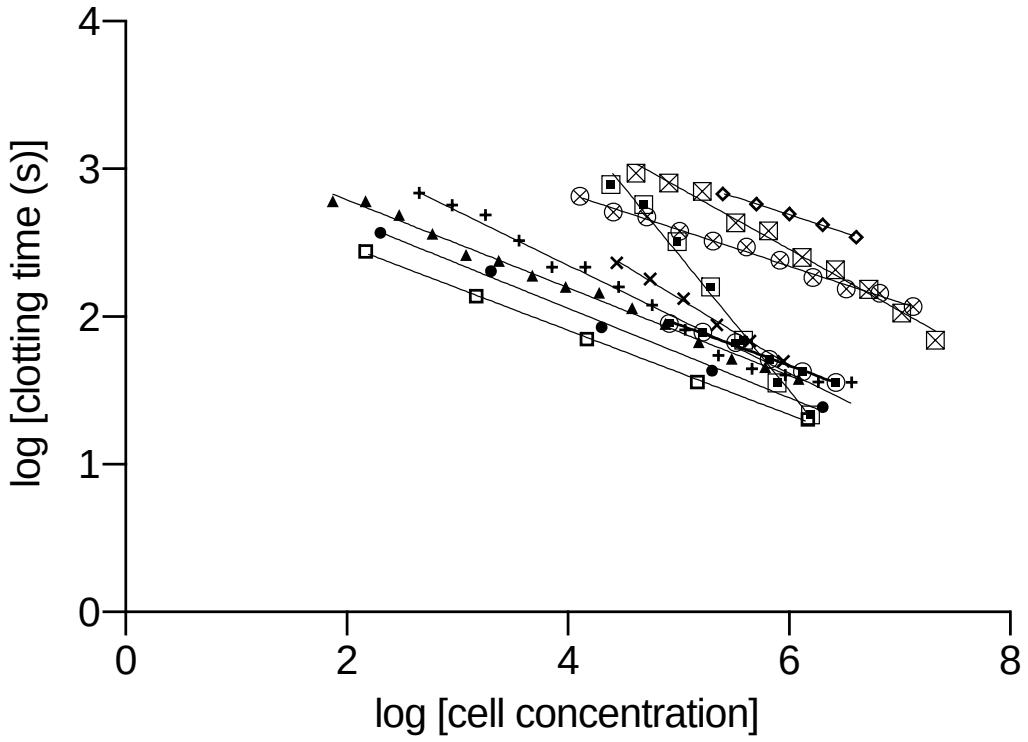
Figure 6. Endothelial microparticles in HUVEC media detected by flow cytometry with incubation of HUVEC in varying concentrations of recombinant tissue factor (1, 10, 20, 30, 60 or 120µl of 10mg/ml Innovin) added to 10ml HUVEC media at 24hours. R^2=0.008828 p=0.8595.

Figure 7. Tissue factor concentration (pg/ml) detected by ELISA in cell free media of pancreatic cancer cell lines (PANC-1(▲) and Mi-Pa-Ca-2(■)) and cell free control(•) following addition of different volumes of recombinant tissue factor added to 10ml cell media at 24 hours.

Figures

Figure 1

(a)



(b)

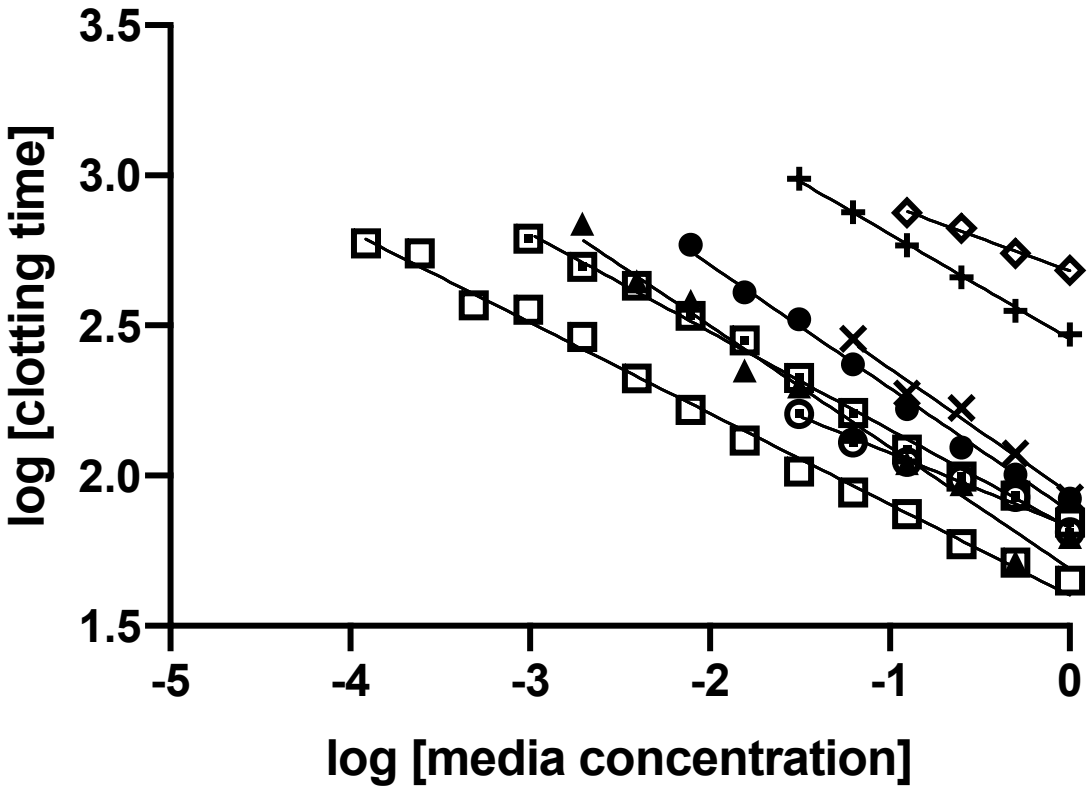


Figure 2

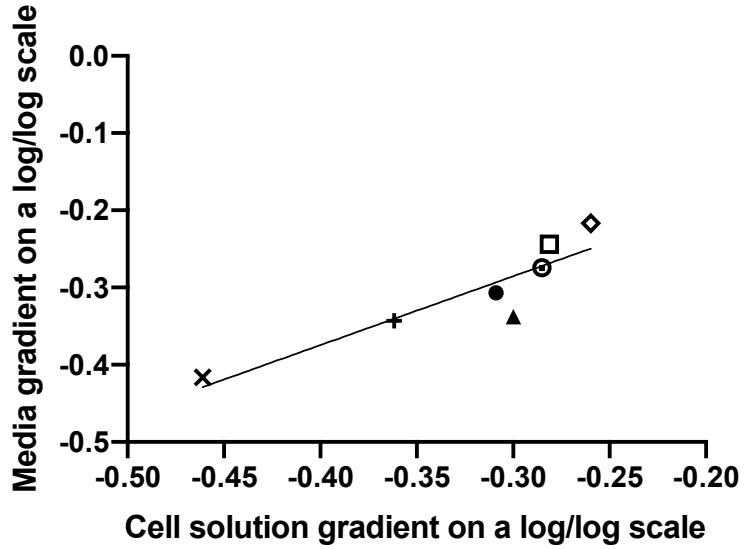


Figure 3

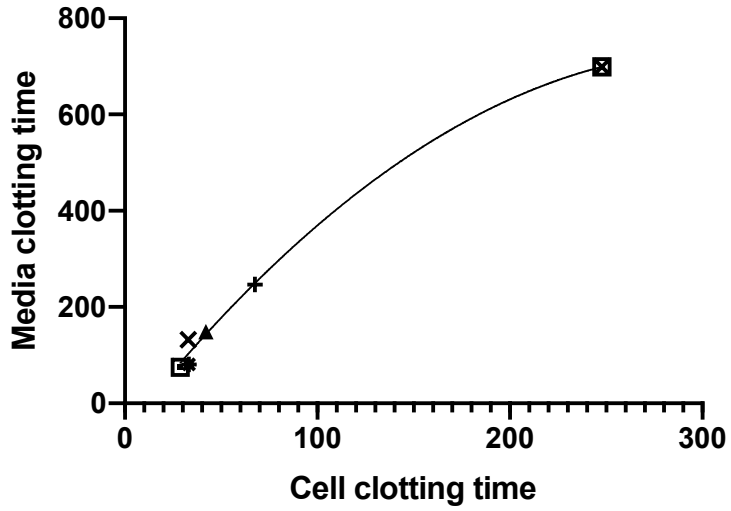


Figure 4

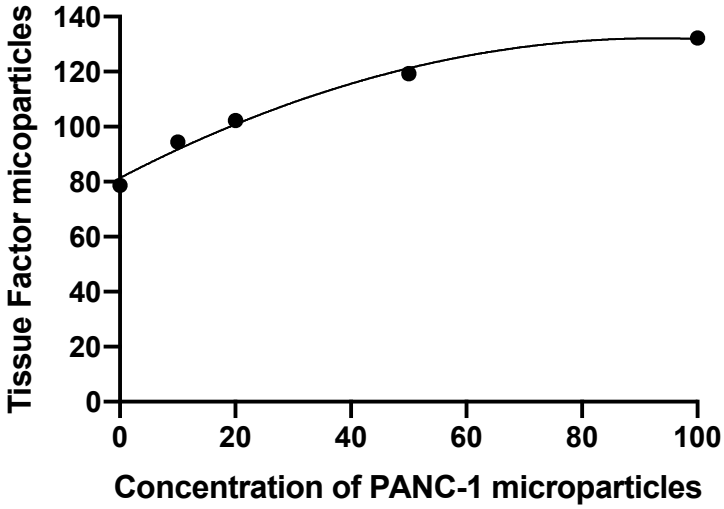


Figure 5

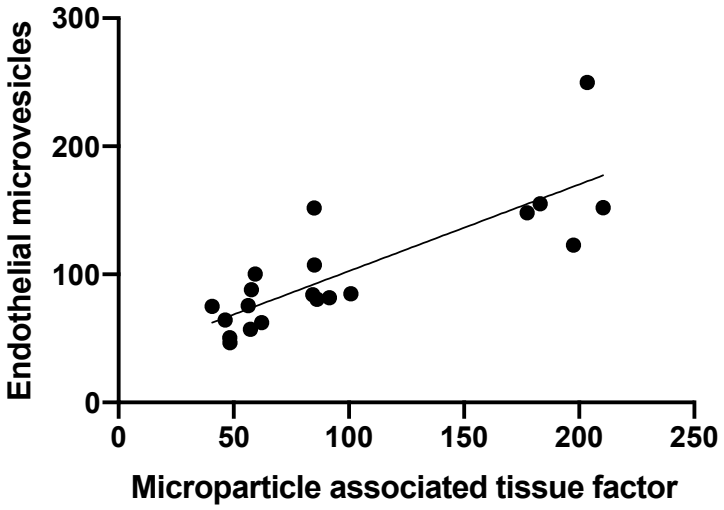


Figure 6

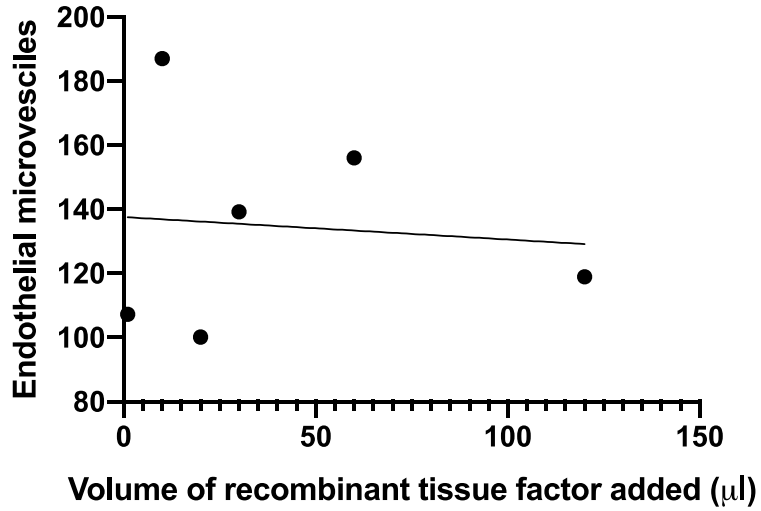


Figure 7