Phagocytic Uptake of Polymeric Particles by Immune cells Under Flow Conditions

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Abstract

Particles injected intravenously are thought to be cleared by macrophages residing in the liver and spleen, but they also encounter circulating immune cells. It remains to be established if the circulating cells can take up particles while flowing, and if the uptake capacity is similar under static and flow conditions. Here, we use an *in vitro* peristaltic pump setup that mimics pulsatile blood flow to determine if immune cells take up particles under constant fluidic flow. We use polystyrene particles of varying sizes as the model of a polymeric particle for these studies. Our results show that the immune cells do phagocytose under flow conditions. We demonstrate that cell lines representing myeloid cells, primary human neutrophils and monocytes take up sub-micrometer-sized particles at similar or better rates under flow compared to static conditions. Experiments with whole human blood show that even under the crowding effects of red blood cells, neutrophils and monocytes take up particles while flowing. Together, these data suggest that circulating immune cells are likely to phagocytose intravenously injected particulates, which has implications for design of particles to evade or target these cells.

Key Words: Neutrophils; Monocytes; Macrophages; Phagocytosis; Blood

Introduction

A relatively small proportion of nano- and sub-micro-sized particles administered through the intravenous route reach their target cell or organ due to clearance by immune cells[1–4]. The removal of particles from circulation is thought to occur primarily through the phagocytic action of macrophages residing in the liver and spleen[1,5]. Enhanced clearance in the liver appears to be due to the presence of large numbers of phagocytic macrophages and lower blood flow velocity (and hence nanoparticle flow velocity) in the liver sinusoids[6]. However, there are several phagocytic immune cells in circulation, and it remains unclear how these cells interact with the intravenously injected particles.

Phagocytosis of particles by immune cells has primarily been studied using models where the cells and particles are static[7–10], or when the cells are static, and the particles are flowing[11–13]. In comparison, uptake when both the cells and the particles are flowing remains poorly explored. Minasyan suggests that phagocytosis in the bloodstream is likely to be impossible due to the high velocity of blood flow, insufficient time for particulate capture and engulfment by immune cells, and crowding due to a large number of red blood cells[14], though experiments to prove this have not been conducted. Contrastingly, Tsoi et al.[6] have shown that peripheral blood mononuclear cells do phagocytose nanoparticles under flow conditions *in vivo*, but their phagocytic capacity may be lower than static conditions. Similarly, few other reports have also suggested that intravenously injected particles are associated with circulating immune cells. For example, Smith et al.[15] show that single walled carbon nanotubes are preferentially taken up by circulating monocytes. Eniola-Adefeso and colleagues have also shown 0.5 and 2 μm sized PLGA particles of varying shapes may be specifically taken up by neutrophils in circulation[16,17]. Recently, Zhang et al.[18] showed that 250-900 nm sized poly (lactic-co-glycolic acid) nanoparticles are internalized by monocytes and myeloid derived suppressor cells following in tumor bearing mice. Studies on quantum dots and gold nanoparticles have shown that smaller sized particles (<50 nm) generally

have higher circulation half-lives than larger sized particles, as they are less likely to be taken up macrophages in the liver and possibly circulating immune cells[19]. Intravenously injected bacteria and zymosan particles are also thought to be associated circulating immune cells[20–22]. While these reports suggest that uptake might occur under flow conditions, it does not prove it definitively. One possible alternate explanation is that the particles are taken up by stationary immune cells present in tissues (such as the liver or lung), which then enter circulation again[23]. Importantly, there has been no direct comparison of the phagocytic capacity of the same group of immune cells in static versus flow conditions, which might help to relatively quantify phagocytosis under flow conditions.

Herein, we sought to answer whether phagocytosis may occur when both cells and particles are flowing using an *in vitro* setup and directly comparing the phagocytic capacity of same cell-types when placed in static or flow conditions. We used a peristaltic pump to mimic the pulsatile nature of blood flow. Experiments on phagocytosis under flow conditions were conducted by varying flow rates from 0.1 to 10 ml/min, which are in the range of flow rates observed *in vivo*[24,25]. Our results show that phagocytic immune do take up particles under flow conditions and that the level of uptake depends on cell type, particle size, and flow rate.

Materials and Methods

Cell Lines

RAW 264.7 cells (Merck, USA) were cultured in DMEM complete media (DMEM (Cellclone and Lonza, India) containing 10% fetal bovine serum (FBS, Thermofisher, USA) and antibiotics). Cells were passaged when the flasks were about 80% confluent. The HL-60 cells (ATCC, USA) were cultured in IMDM complete media (IMDM (Merck) containing 20% FBS and antibiotics). Cells were passaged when the cells were at a concentration of 1 million cells in 1 ml.

Human Blood, Neutrophils and PBMCs

Peripheral venous blood samples were obtained from healthy volunteers following the norms laid out by the Indian Institute of Science's ethics committee for research with human samples (IHEC No: 5-15032017 and 2-31082018) and with informed consent. The blood was collected in EDTA-coated tubes for purified-cell experiments and citrate, or EDTA-coated tubes for whole blood experiments. For isolating immune cells, the blood sample was loaded onto histopaque (density 1.077 g/ml, Merck, USA) gently and was spun down at 400 RCF for 20 min at room temperature without brake. The buffy coat containing the PBMCs was carefully removed and subjected to RBC lysis for 3 minutes. The bottom-most layer containing the RBCs and neutrophils was subjected to RBC lysis for 9 minutes to obtain the neutrophils. The lysis was quenched with over two-fold volume of 1X PBS. Following quenching, the samples were spun down at 400 RCF for 4 minutes at 4ºC. The supernatant was discarded, and the cells were suspended in DMEM complete media. The cells were counted using a hemocytometer.

Particles

Polystyrene particles (Bangs Laboratories, USA) of varying sizes and labeled with different fluorophores were used as a model particle system. Volumetrically equivalent amounts of different particle sizes were used for the uptake studies. These approximately correspond to 1 particle (2.9 μm diameter) ≈ 200 particles (500 nm diameter) ≈ 3000 particles (200 nm diameter) per cell. For studies involving RAW 264.7 cells and 500 nm particles, the ratios were 50 particles per cell.

Uptake Studies under Static Conditions

The cells were seeded in a 24-well plate at a concentration of 0.1 or 0.2 million cells per well. Adherent cells (RAW) were allowed to adhere for a minimum time of 1 hour, while semi-adherent to non-adherent cells (HL-60, neutrophils and peripheral blood mononuclear cells) were allowed to remain in the plate for 20-30 min at 37°C. The peripheral blood mononuclear cells were used

as is, and hence contained monocytes, B cells, T cells and other mononuclear cells. Separately, polystyrene particles (varying numbers depending on the particle size) were sonicated for 5 min and then added to the cells. At the specific time points (2 hours or 4 hours), the media was removed, and cells collected from the wells were washed and stained with propidium iodide (2 µg/ml) to distinguish live from dead and run on a flow cytometer (BD FACSCelesta).

Uptake Studies under Flow Conditions:

A Masterflex L/S easy load peristaltic pump (Cole-Parmer, India) was used for the peristaltic flow experiments along with platinum-cured Silicone Tubing (Sani-tech STHT-C-062-2, 1/16" x 3/16", Saint-Gobain, India) (Suppl. Fig. 1). The tubing was passivated with a filter-sterilized 1% bovine serum albumin in PBS solution for 30 min each time the experiment was performed. The tubing was sanitized with a 70% ethanol wash and a water wash before and after each run, followed by autoclaving. Cells (RAW, HL-60, human neutrophils, and human PBMC) were suspended at a concentration of 1 million cells/ml in 1.2 ml of the appropriate type of media in test-tubes and were not allowed to adhere. Particles at the appropriate numbers (depending on size) were pipetted into this tube, and the cell and particle suspension was immediately (usually within 1 minute) subjected to flow in the peristaltic pump at the desired flow rate. For injection experiments, particles were injected into the tubing using an insulin syringe-needle. At pre-specified times, the cells flowing through the peristaltic pump were collected, washed, and suspended in 500 µl of 1X PBS. A small aliquot was taken for manual counting (using trypan blue to check for survival). The cell suspension was stained with propidium iodide (2 µg/ml) and run on the flow cytometer. To confirm that the particles had been internalized, trypan blue was added to the cell suspension (in addition to propidium iodide) containing buffer before running on the flow cytometer as trypan blue is known to quench the fluorescence signal of particles (with an excitation at 488 nm and emission at 510 nm wavelength) stuck to the membrane (and internalized particles are protected from the quenching).

Whole blood experiments

Heparin (20 IU/ml) was added to the blood sample collected in citrate or EDTA coated tubes within 20 min of collection. Whole blood (500 µl) was plated in a 24-well plate and placed in the incubator at 37ºC for 20 min. Polystyrene particles (500 nm) were sonicated for 5 min and then added to the cells at an approximate ratio of 1:200 (cells to particles) according to the combined theoretical neutrophil (5 million/ml) and PBMC (2 million/ml) counts in the blood. Whole blood and particle suspension was subjected to flow in the peristaltic pump at the desired flow rate for 2 hours. The cells were then collected and subjected to RBC lysis for 8 min. The cells were spun down and re-suspended in flow cytometry buffer (PBS containing 1% BSA and 4mM EDTA), and were stained with antibodies against CD45 (clone 2D1, BD Biosciences, USA), CD14 (clone M5E2, BD Biosciences) and CD15 (clone HI98, BD Biosciences) for 20 min at 4ºC. Finally, the cell suspension was stained with propidium iodide (2 µg/ml) and run on the flow cytometer. All flow cytometry data analysis was performed using FlowJo (Treestar, USA).

Statistics

The analysis of data and plotting of graphs was done using GraphPad Prism 8 (GraphPad Software, USA). Scatter plot data is reported through a single mean value with a standard deviation. For comparison of two groups, paired Student's 't' test was used (pairing was based on experiment's date when cells were from the same passage or individual, and particle additions were from the same stock solution). One-way ANOVA followed by post – hoc Tukey test was used to compare multiple groups.

Results

Blood flows at different rates (and velocity) in the arteries, veins, and capillaries[24]. To mimic flow rates in each of these circulatory system locations, we chose to perform experiments at three different flow rates – 0.1, 1, and 10 ml/min (~0.83 mm/s, ~8.3 mm/s, and ~83 mm/s). Under these conditions, we measured the uptake of polystyrene particles by immune cells and compared it to conditions where both cells and particles are stationary. Uptake was determined as the percentage of cells that have taken up particles (% uptake, a measure of how many cells are phagocytic) and the number of particles per cell (average values based on intracellular fluorescence intensities represented as median fluorescence intensities or MFI). Further, as the peristaltic setup could not be placed inside a 37ºC incubator, studies were performed at room temperature. We determined that for the short time-frame studies (~2 hours), uptake at room temperature (~25ºC) was slightly (and significantly) lower than 37ºC for the adherent macrophage (RAW 264.7) cell line and not significantly different for the suspension myeloid (HL-60) cell line (Suppl. Fig. 2).

Uptake by an adherent cell line

The adherent mouse macrophage cell line, RAW 264.7 (RAW), is highly phagocytic across particle sizes under static conditions[26]. To determine this cell's ability to take up particles while flowing, we tested uptake using polystyrene particles of three different diameters – 200 nm, 500 nm, and 2900 nm (Suppl. Fig. 3). These studies were initially done when cells and particles were co-incubated for 2 hours. We observed that uptake under flow conditions was dependent on both particle size and flow rate (Figure 1). The 200 nm particles were taken up equally well under both static and flow conditions, and no significant differences were observed across flow rates (Figure 1A and 1B). On the other hand, the percentage of cells that took up the 500 nm particles was significantly greater at 1 ml/min flow rate than the static condition and other flow rates (Figure 1C). However, the number of particles per cell was not different across flow rates and compared to the static condition (Figure 1D).

Further, we observed that under flow conditions, the percentage of cells taking up larger 2900 nm (2.9 μm) sized particles reduced significantly compared to static conditions but was not different across flow rates (Figure 1E). Nevertheless, the small number of cells that did take up 2900 nm particles under flow conditions could phagocytose a similar number of particles as static conditions (Figure 1F). Together, these data show that adherent macrophages' capacity to take up smaller particles (200 and 500 nm), but not larger particles (2900 nm), under flow conditions remains similar (if not greater) to static conditions. Additionally, flow rates do not appear to influence particle uptake, except for particles that are 500 nm sized, where the specific flow rate of 1 ml/min may be more suited for particle uptake as compared to other flow rates.

Uptake Kinetics

Having observed differences in uptake across flow rates at 2 hour post co-incubation, we next evaluated the kinetics of uptake under static and flow conditions. For these experiments, RAW macrophages were incubated with 500 nm polystyrene particles for 5 min, 15 min, 30 min, 60 min or 240 min at all 3 flow rates (and under static conditions). We observed that for all flow rates, the association of particles with cells was similar to (not statistically significant differences) or higher (statistically significant) under flow conditions compared to static conditions at 5 min, 15 min, 30 min and 60 min (Figure 2A and 2B). The kinetics of recognition and internalization of particles through the phagocytic process are suggested to be in the order of minutes[27,28], and hence at the early time points (5 and 15 min) we describe only association events under both static and flow conditions. By 30 min most of the particles were internalized by cells in flow and static conditions, which was confirmed using confocal microscopy (Suppl. Fig. 4) and measuring uptake through flow cytometry while using trypan blue to quench the signal from particles on the surface of a cell. Additionally, we observed that by 4 hours the uptake under static conditions has increased and become equal to or greater than uptake in flow conditions. These data suggest the kinetics of particle-cell interaction may be higher when cells and particles are both flowing, resulting in higher

number of cells associating with particles at early time points (5 – 30 min), but given enough time (2 and 4 hours) the overall uptake under both static and flow conditions is similar (Suppl. Fig. 5).

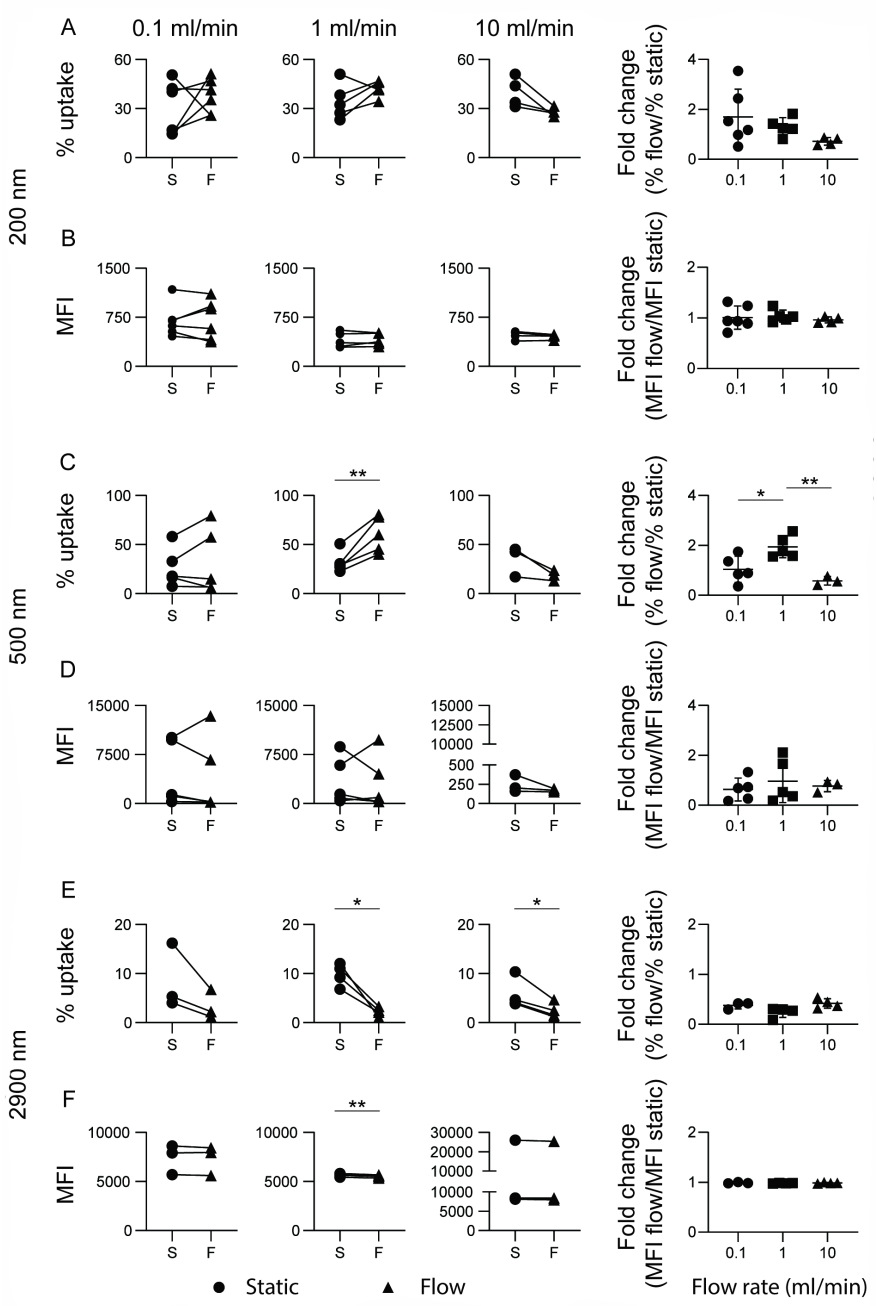
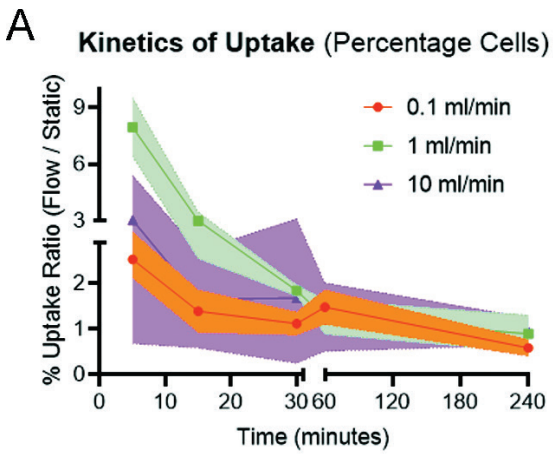
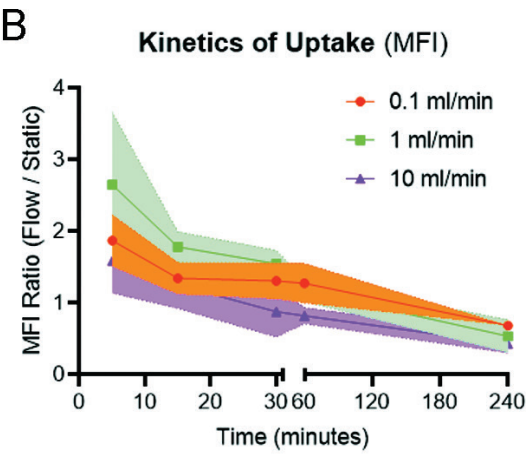


Figure 1: Phagocytosis by the adherent macrophage cell-line (RAW 264.7) at various flow rates. Uptake of 200 nm (**A and B**) or 500 nm (**C and D**) or 2900 nm (**E and F**) polystyrene particles plotted as either percentage uptake or particles per cell measured as median fluorescence intensity (MFI). Cell to particle ratio of 1:3000 (200 nm) or 1:50 (500 nm) or 1:1 (2900 nm) was used. Data represent results of n ≥ 3 independent experiments, and the connecting lines indicate paired experiments. A paired Student's 't' test was performed to determine statistical differences between two groups and a one-way ANOVA followed by post-hoc Tukey test was performed to determine statistical differences between multiple groups. * = $p < 0.05$, ** = $p < 0.01$, and non-significant when no notation.





	0.1 ml/min	1 ml/min	10 ml/min
5 min	0.009	0.064	0.035
15 min	0.276	0.052	0.294
30 min	0.417	0.017	0.786
60 min	0.590	0.327	0.335
240 min	0.043	0.120	0.925

	0.1 ml/min	1 ml/min	10 ml/min
5 min	0.0275	0.119	0.306
15 min	0.100	0.051	0.313
30 min	0.174	0.117	0.371
60 min	0.223	0.251	0.051
240 min	0.100	0.009	0.048

Figure 2: Kinetics of particle uptake. A – Ratio (flow/static) of percentage of cells with particles and **B** – ratio (flow/static) of particles per cell (denoted as median fluorescence intensity (MFI)) after 5, 15, 30, 60 and 240 minutes of co-incubation of polystyrene particles with cells. Symbols indicate the mean value of n ≥ 3 independent experiments and the shaded regions denote the standard deviation around those symbols. A paired 't' test was performed at each time point and p values are summarized in the table below the graph.

Uptake by a non-adherent cell line

Next, we sought to determine whether cells grown in suspension cultures would also behave similarly to an adherent cell line. We used HL-60 cells[29,30], a human promyeloblast cell line for these studies, and used a higher ratio of particles to cells as these cells are less phagocytic. We observed that uptake by HL-60 cells also changed based on the size of the particle and flow rate, but the data were different from RAW cell uptake. Among the smaller particles (200 nm and 500 nm), uptake under static and flow conditions were similar at lower flow rates (0.1 and 1 ml/min). However, uptake was significantly lower at the highest flow rate of 10 ml/min (Figure 3A, 3B, 3C and 3D). This data indicates that this cell line cannot engulf particles at flow rates that

approximately correspond to flow in large veins or smaller arteries. For the larger-sized particle (2900 nm), the percentage of cells with particles was low under both static and flow conditions (Suppl. Fig. 6), but the differences between the two were minimal (Figure 3E and 3F).

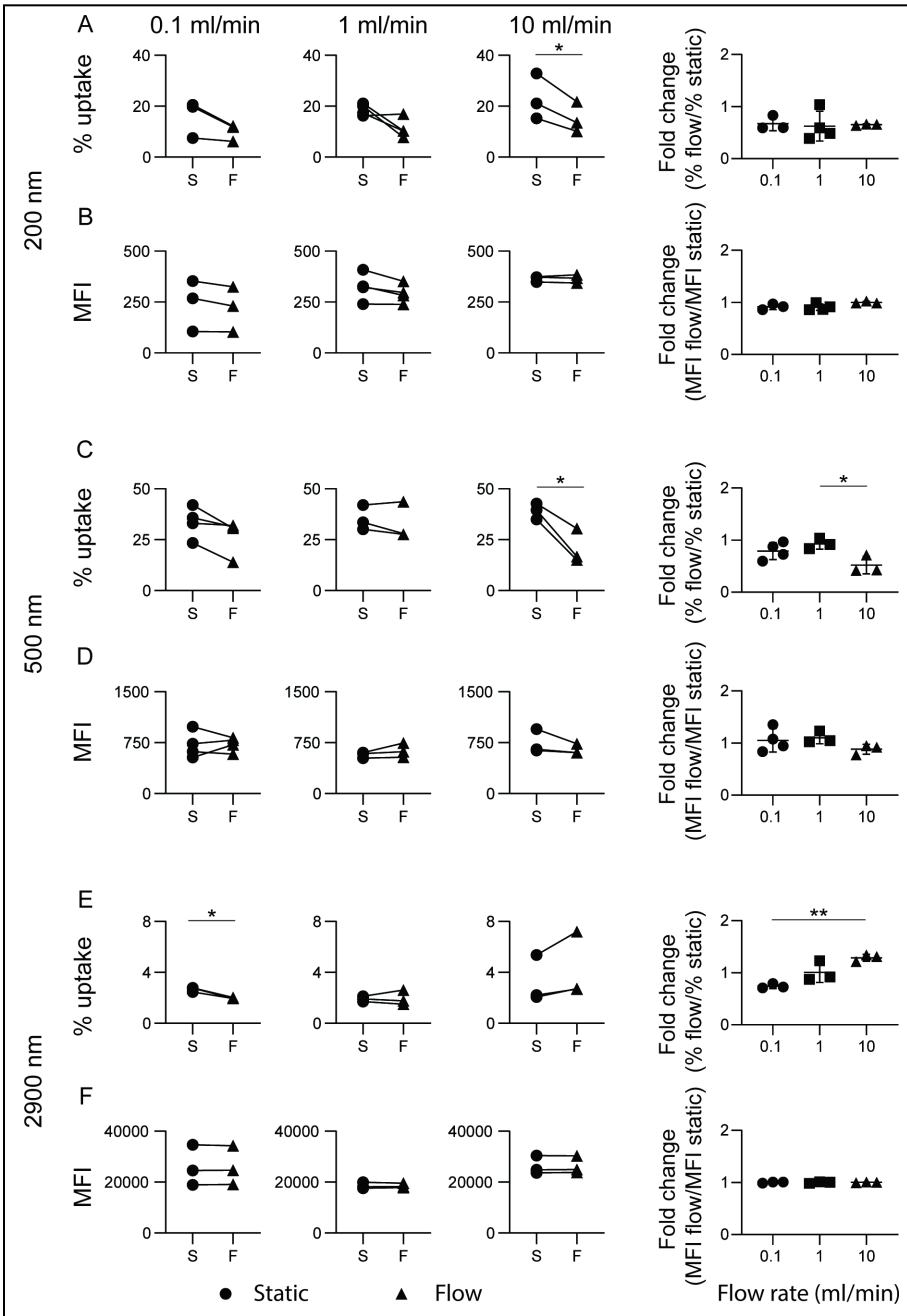


Figure 3: Phagocytosis by the suspension myeloid cell-line (HL-60) at various flow rates. Uptake of 200 nm (**A and B**) or 500 nm (**C and D**) or 2900 nm (**E and F**) polystyrene particles plotted as either percentage uptake or particles per cell measured as median fluorescence intensity (MFI). Cell to particle ratio of 1:9000 (200 nm) or 1:200 (500 nm) or 1:4 (2900 nm) was used. Data represent results of n ≥ 3 independent experiments, and the connecting lines indicate paired experiments. A paired Student's 't' test was performed to determine statistical differences between two groups and a one-way ANOVA followed by post-hoc Tukey test was performed to determine statistical differences between multiple groups. * = $p < 0.05$, ** = $p < 0.01$, and non-significant when no notation.

Uptake by primary cells

The cell line data suggest that particle internalization is possible under flow conditions, but cell lines may not entirely mimic primary cells[31]. Hence, we sought to determine if the observations held for cells isolated from human peripheral venous blood. For these studies, particles of one

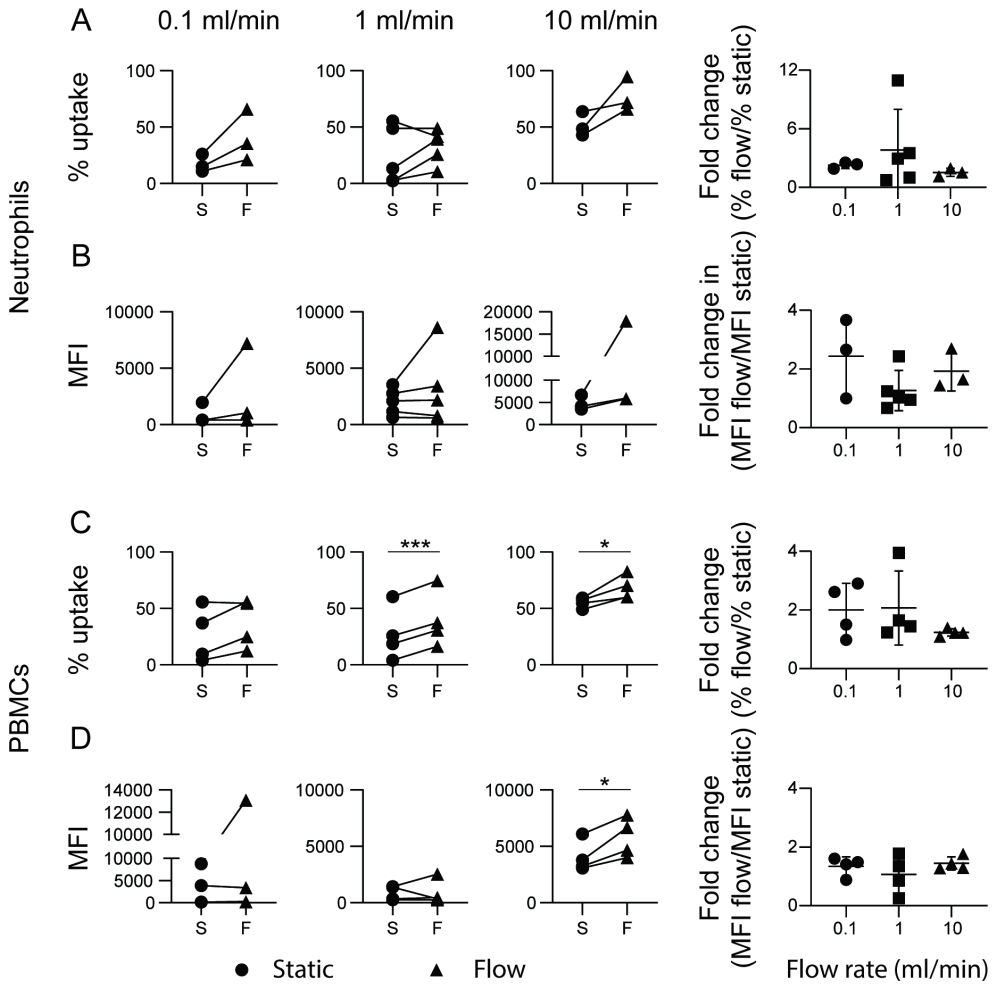


Figure 4: Phagocytosis by human neutrophils and PBMCs. Uptake of 500 nm polystyrene particles by neutrophils (**A and B**) or PBMCs (**C and D**) isolated from peripheral venous blood draws, plotted as either percentage uptake or particles per cell measured as median fluorescence intensity (MFI). Cell to particle ratio of 1:200 was used. Data represent results of n ≥ 3 independent experiments, and the connecting lines indicate paired experiments. A paired Student's 't' test was performed to determine statistical differences between two groups and a one-way ANOVA followed by post-hoc Tukey test was performed to determine statistical differences between multiple groups. * = $p < 0.05$, *** = $p < 0.001$, and non-significant when no notation.

size (500 nm) were used. Specific immune cells were purified using density gradient centrifugation, and isolated cell populations were subjected to phagocytosis studies under static and flow conditions. We observed that neutrophils took up particles slightly, but not significantly, better under flow than static conditions (Figure 4A, Suppl. Fig. 7). A similar trend was observed for the number of particles per cell (Figure 4B). On the other hand, peripheral blood mononuclear cells (PBMCs) showed a significantly increased percentage of cells with particles at 1 and 10 ml/min compared to static conditions (Figure 4C, Suppl. Fig. 7). Additionally, these cells also took up more particles per cell at the highest flow rate of 10 ml/min (Figure 4D). Nevertheless, the differences were not significant when comparing across flow rates (Figure 4C and 4D).

Whole blood experiments

While the data from purified primary cells suggests that uptake does occur under flow conditions, whole blood is a crowded environment where red blood cells outnumber the phagocytic immune cells by over a thousand to one. Such crowding affects the flow of cells and particulates[32], which in turn might affect phagocytosis. To determine if uptake occurs under crowded conditions, particles were added to whole blood, and uptake was measured under both flow and static conditions. After co-culture for two hours, cells were stained with antibodies to identify neutrophils and monocytes (Suppl. Fig. 8), and the uptake was measured. Interestingly, neutrophils from whole blood were better at taking up particles under flow conditions compared to static conditions at 0.1 ml/min (significantly better) and 1 ml/min (trending higher but no significant difference) flow rates (Figure 5A). At the higher flow rate of 10 ml/min, this difference was no longer observed, with neutrophils taking up particles equally well under static and flow conditions (Figure 5A). Similar to the observations in most cell line and purified primary cell studies, the total number of particles per cell did not appreciably change at any flow rate compared to static conditions (Figure 5B). Another observation from these studies was that the overall uptake of purified neutrophil

populations was higher than neutrophils in whole blood, under both flow and static conditions. Such lowering could be due to a reduced chance of a neutrophil encountering a particulate[31].

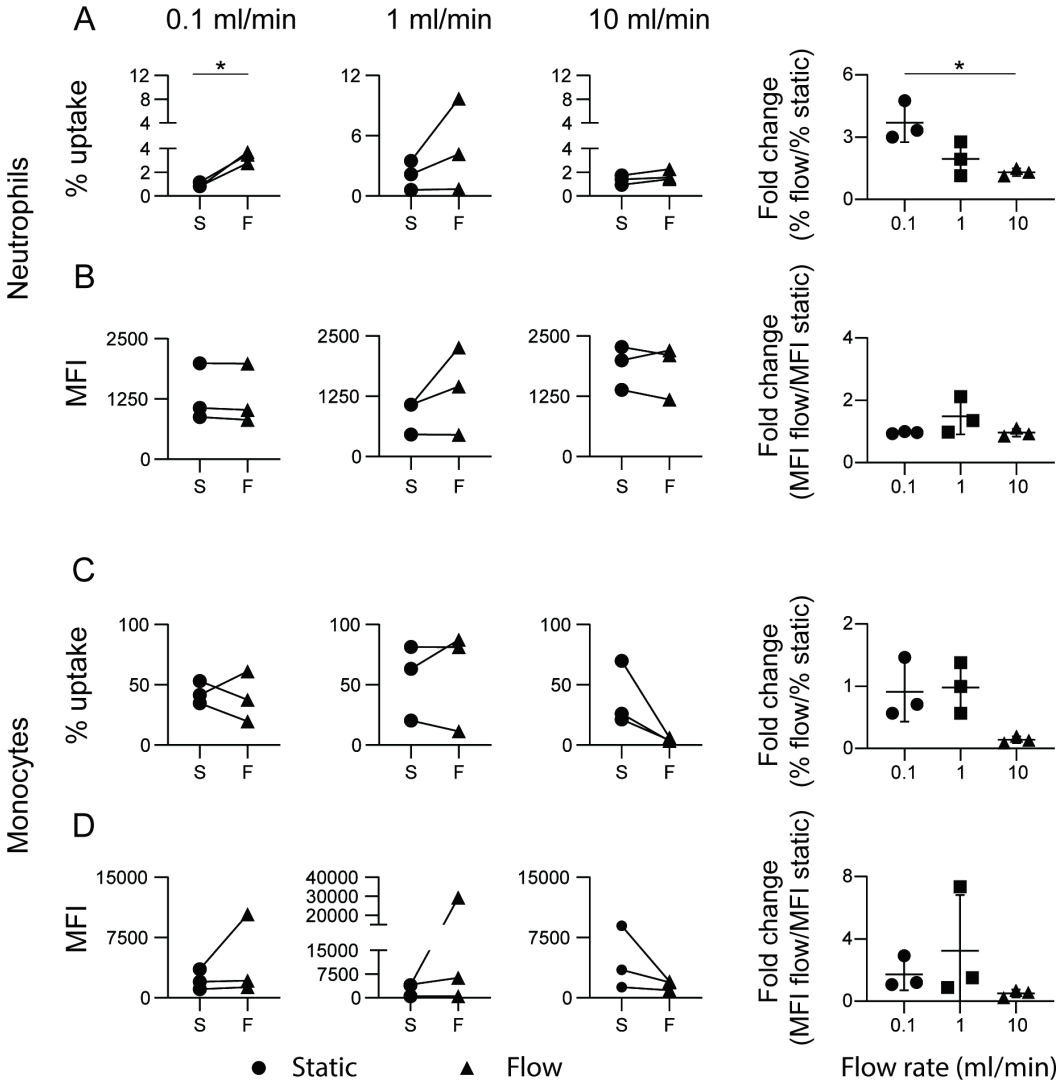


Figure 5: Phagocytosis by immune cells in whole blood. Uptake of 500 nm polystyrene particles by neutrophils (**A and B**) or monocytes (**C and D**) present in whole blood, plotted as either percentage uptake or particles per cell measured as median fluorescence intensity (MFI). Cell to particle ratio of 1:200 was used according to combined theoretical neutrophil and PBMC counts in the blood ($7x10^6$ cells/ml). Data represent results of n = 3 independent experiments for each flow rate, and the connecting lines indicate paired experiments. A paired Student's 't' test was performed to determine statistical differences between two groups and a one-way ANOVA followed by post-hoc Tukey test was performed to determine statistical differences between multiple groups. * = $p < 0.05$ and non-significant when no notation.

In comparison, monocytes from whole blood showed similar uptake at the lower flow rates of 0.1 and 1 ml/min and static conditions (Figure 5C). However, they did show a propensity to have lower uptake at the higher flow rate of 10 ml/min compared to static conditions, but these differences were not statistically different in our studies with a limited number of samples (Figure 5C). This trend continued for the number of particles per cell, with similar numbers observed at 0.1 and 1 ml/min but lower at 10 ml/min flow rate (Figure 5D).

Mimicking Clinical Conditions

Mixing of particles with cells prior to subjecting them to peristaltic flow (even if the mixing occurred only for a minute or so) does not accurately represent conditions similar to in vivo administration of particles in to the blood stream. In order to replicate such conditions, we began by subjecting RAW macrophages to peristaltic flow for 5 minutes, followed by injection of 500 nm polystyrene particles into the tubing in which cells were flowing. Uptake was measured 30 min following the injection of particles. Even in these experiments, we observed that the percentage of cells taking up particles under static and flow conditions were similar (Figure 6A) and while the number of particles per cell lower in flow conditions, the difference was not statistically significant (Figure 6B). This experiment further demonstrates that the uptake capacity of immune cells in flow is similar to that of static conditions.

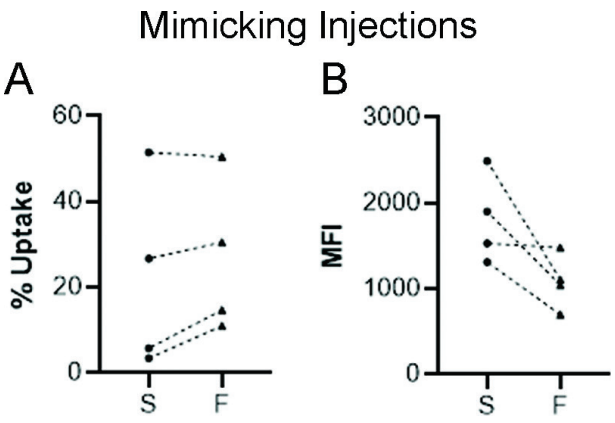


Figure 6: Phagocytosis following in vitro injection-like conditions. Uptake of 500 nm polystyrene particles by RAW macrophages following injection of the particles into the tubing were cells were flowing at 1 ml/min (flow conditions or "F") compared to static ("S") conditions, plotted as either percentage uptake (A) or particles per cell measured as median fluorescence intensity or MFI (B). Data represent results of n = 4 independent experiments. A paired Student's 't' test was performed to determine statistical differences, and data were non-significant.

Discussion

The uptake of particles via one of the endocytic processes occurs in a series of steps[33,34]. The first step involves immune cells coming in contact with the particle they intend to take up, which has been thought to occur when the cell crawls towards the particulate substance[35]. The next step is the formation of membrane extensions that engulf the target[28]. And the final steps are the internalization of the particle in an endosomal vesicle. It is believed that the processes of cell crawling and extending its membrane around a particle are more likely when there is minimal to no flow involved. Additionally, it has been suggested that these same cellular processes may also occur when the cell is stationary while the particle is flowing, with the particle's size and shape influencing the frequency of interactions[36]. However, it remains unclear if the particulate uptake processes mentioned above[28,35] apply to the condition where both cells and particles are flowing.

Our data shows that uptake does occur when both cells and particles are flowing. How this might happen in the light of the established models of endocytosis is to be determined. We speculate that flow conditions might increase the number of random interactions (collisions) between cells and particles. Preliminary evidence for this statement is our data on high particle-cell association at 5 and 15 minutes post interaction under flow conditions, and reduced uptake by neutrophils and monocytes under crowded conditions of whole blood (where productive collision events will be lower) than uptake levels of purified populations of the same cells.

Interactions form only the first step of the uptake process. In the next stage of engulfment, cells must extend their membrane around the particle. This step involves cytoskeletal rearrangements[37], and whether such changes occur under flow conditions needs to be explored further. Our data of equivalent or higher uptake of the smaller (200 and 500 nm) sized particles under flow conditions suggest that cytoskeletal rearrangement might occur in these conditions.

Similarly, lower uptake of the larger (2900 nm) particles might mean that even if such cytoskeletal reorganization occurs, they are likely limited and cannot engulf large particles.

Another aspect that affects uptake of particles is shear stress. Several studies have shown that shear stress is critical for cellular uptake of particles[13,38,39]. For example, Schubert and colleagues have shown that there is a positive correlation between shear stress and methacrylate nanoparticle uptake by adherent HUVEC cells[38]. Prina-Mello and colleagues have shown that the cellular uptake of particles is higher at low shear stress compared to static conditions[39]. Contrastingly, Ghandehari and colleagues have shown reduced uptake of silica nanoparticles by adherent RAW 264.7 macrophages under shear flow conditions compared to static conditions[13]. However, such shear stresses are only observed when the cells are stationary and bound to the culture surface, a condition common to all the aforementioned studies. In the work presented here, as both the particles and cells are flowing, and we have non-laminar flow, we expect the shear forces to be very different, but have not specifically measured them.

The protein corona on the particle surface has been shown to influence the ability of phagocytic cells to take up the particles[40]. It has also been shown that the type and quantity of proteins adsorbing on to nanoparticle surfaces under shear flow conditions are likely to be different[41,42]. In our studies, we incubate the particles with cells (under both static and flow conditions) in cell culture solution that contains fetal bovine serum. Hence, it is likely that the type and quantity of proteins adsorbing onto the particle surfaces are influencing the results we observe with regard to uptake under flow conditions.

Finally, the data presented here is for particles made of one material (polystyrene). The results of uptake by cells while flowing is hence limited to polystyrene particles and possibly other polymeric particles with similar densities and surface hardness[43]. Flow conditions are likely to be different

around metallic and inorganic particles that have different densities and surface hardness[43,44], which might affect their ability to interact with phagocytic cells[45]. Additionally, soft particles such as those made of lipids (liposomes) or other self-assembled materials are likely to have varying stabilities under flow conditions. Hence, it remains to be seen whether non-polymeric particles will be taken up by cells that are flowing. The particle shape also influences uptake[46], and we study particles of one shape (spherical). Future studies will need to examine how particle shape might alter their flow dynamics and affect uptake under fluid flow conditions.

Conclusion

In conclusion, we show that immune cells are capable of taking up polystyrene particles under flow conditions. The uptake capacity of all phagocytic immune cells tested is either similar or greater under flow than static conditions when presented with sub-micrometer-sized polystyrene particles. However, the uptake capacity is lower while flowing when the cells are presented with larger micro-sized (~3 μm) polystyrene particles. Additionally, we show that neutrophils and monocytes are also capable of taking up particles under the crowded conditions of whole blood. These studies reveal that circulating immune cells are likely to have the capacity to take up polymeric particulates injected intravenously, which has potential implications in our design of delivery vehicles that either seek to avoid or target these cells.

Acknowledgments

We thank G. K. Ananthasuresh for providing the peristaltic pump system. This work was supported by the Dr. Vijaya and Rajagaopal Rao funding for Biomedical Engineering research at the Centre for BioSystems Science and Engineering and the R. I. Mazumdar young investigator position (to SJ). This work was also supported in part by the DBT-Wellcome India Alliance

Intermediate Fellowship number IA/I/19/1/504265 (to SJ). MS and PS are in receipt of the Kishore Vajgyanik Protsahan Yojana and CSIR senior research fellowship, respectively.

Conflict of Interest

The authors declare no conflict of interest

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