# Effect of Adhesion and Substrate Elasticity on Neutrophil Extracellular Trap Formation

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**Abstract**

Neutrophils are the most abundant type of white blood cells. As part of their immune defense mechanisms they are able to decondense and release their chromatin as neutrophil extracellular traps (NETs). This process (NETosis) also plays an important role in many chronic and inflammatory diseases such as atherosclerosis, rheumatoid arthritis, diabetes and cancer. For this reason, much effort has been invested into understanding biochemical signaling pathways in NETosis. However, the impact of the mechanical micro-environment and adhesion on NETosis is not well understood.

Here, we study how adhesion and especially substrate elasticity affect NETosis. We employed polyacrylamide gels with varied elasticity (Young's modulus *E)* within the physiologically relevant range from 1 kPa to 128 kPa and coated the gels with integrin ligands (collagen I, fibrinogen). Neutrophils were cultured on these substrates and stimulated with chemical inducers of NETosis: phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharide (LPS). Interestingly, PMA-induced NETosis was neither affected by substrate elasticity nor by different integrin ligands. In contrast, for LPS stimulation, NETosis rates increased with increasing substrate elasticity ($E > 20$ kPa). Additionally, we show that LPS-induced NETosis correlates with the cell contact area indicating that adhesion signaling and NETosis pathways are connected. On the other hand, PMA-induced NETosis did not require adhesion signaling.

In summary, we show that LPS-induced NETosis depends on adhesion and substrate elasticity while PMA-induced NETosis is completely independent of adhesion.

## Introduction

Neutrophils are the most abundant cell type among circulating white blood cells. They are able to release neutrophil extracellular traps (NETs), web-like extracellular structures composed of decondensed chromatin, decorated with antimicrobial proteins in a process termed NETosis[1,2]. During NETosis, the chromatin inside the nucleus swells until it ruptures both the nuclear envelope and later the outer cell membrane[3]. NETosis is considered an important immune defense mechanism as neutrophils can bind and kill bacteria and other pathogens via NETs. Apart from physiological stimuli such as pathogens, cytokines (e.g. IL-8), activated platelets or urea crystals there are several additional NET-inducers like phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharides (LPS) which induce NETosis *in vitro*[4]. Even though NETosis was initially described as part of the innate immune defense system, we know today that dysregulated NETosis is also involved in a variety of chronic inflammatory and autoimmune diseases like atherosclerosis, systemic lupus erythematosus, preeclampsia and other malignant diseases[5–8]. Therefore, the question how NETosis is regulated *in vivo* and which environmental factors play a role for this process may bear great therapeutic consequences.

Mechanical properties of different tissues are environmental signals that are able to modulate functionality of surrounding cells. This has been demonstrated by a substantial amount of studies investigating the effect of physical environmental factors on cellular functions[9,10]. It has previously been shown that the phenotype and the functionality of immune cells such as macrophages and dendritic cells are affected by stiffness[11–13]. It has also been reported that substrate elasticity has an impact on neutrophil adhesion, migration and chemotaxis[14–16]. Transmigration of neutrophils through endothelium was also proven to be affected by sub-endothelial cell matrix stiffness[17]. Increasing tissue stiffness may be found in multiple pathological processes including, most prominently, atherosclerotic plaques[18] but also fibrosis[19] and cancer[20]. In general, cell adhesion is mediated through receptor-ligand interactions and the presentation of specific ligands on surfaces[21–24]. Integrin ligands have been previously shown to play an important role in leukocyte adhesion and migration[25,26,27]. Additionally, the ligand density on the surface affects adhesion and migration of neutrophils[27,28]. For example, neutrophils adhere *via* the integrin Mac-1 binding to the platelet receptor GPIb$\alpha$ and show the fastest adhesion maturation when ligand receptors where distributed upon the cells and/or surfaces with a medium distance of approximately 100nm[27]. Even though the role of integrins on neutrophil adhesion has been studied, there are contradictory studies concerning their role in NETosis[29,30]. Exemplarily, it has been shown that blocking of the integrin LFA-1 prevented NETosis[31]. However, the overall impact of substrate elasticity and adhesion-related processes on NETosis remain poorly understood.

In this paper, we explore the effect of substrate elasticity (Young's modulus $E$) and general adhesion on NETosis in human neutrophils. We use collagen I- and fibrinogen-coated polyacrylamide (PAA) substrates with stiffnesses within the physiologically relevant range ($E$ = 1 kPa to 128 kPa) and study the impact of elasticity and adhesion on NETosis, induced by different chemical stimuli (LPS, PMA).

## Material & Methods

### Polyacrylamide Substrate Preparation

PAA gels were prepared on round cover glass slides as previously reported[9,10]. In brief, the cover glasses were plasma cleaned, coated with 3-aminopropyltriethoxysilane (Sigma A3648) and afterwards incubated with 0.05 % of glutaraldehyde solution (Sigma G7651). Appropriate mixtures of acrylamide (Bio-Rad #161-0140) and bis-acrylamide (Bio-Rad #161-0140) diluted in Dulbecco's phosphate-buffered saline (Sigma-Aldrich) (PBS) were freshly prepared, stored at +4 °C and used within 2 months (see table in SI for details). Polymerization was initiated by addition of 1/1000 N,N,N,N-tetramethylethylenediamine (TEMED) and 1/100 ammonium persulfate solution (APS) . 35 µL of this solution was used per cover slip. A square hydrophobic cover glass was placed on top in order to equally distribute the solution on the bottom glass. Gels were polymerized for 60 minutes in a saturated water atmosphere to avoid evaporation and were approximately 70 µm thick. Young´s modulus *E* of PAA gels was quantitatively controlled by measurements on a bulk rheometer (MCR-501, Anton Paar, Austria). To prevent toxicity to cells, non-polymerized residues were thoroughly washed away. Substrates were treated with the photoactive cross-linker Sulfo-SANPAH (Thermo Scientific 22589) 0.4 mM in 50 mM HEPES buffer at pH 8, exposed to UV light ($\lambda$ = 365 nm) for 10 minutes and then either coated with collagen I (0.02 and 0.2 mg/mL) or fibrinogen (0.02 mg/mL, from human plasma) overnight at 4 $^{0}$C unless otherwise stated.

### Human neutrophil isolation

Neutrophils were isolated from healthy donors' venous EDTA blood. Donors were thoroughly informed about the possible consequences of the study and voluntary consent was obtained from each donor. Neutrophil isolation was performed according to published standard protocols[3,32]

Fresh blood from donors was collected with S-Monovettes KE 7.5 ml (Sarstedt). The blood was gently layered on top of some Histopaque 1119 (Sigma-Aldrich) in a 1:1 ratio and centrifuged at 1100 g for 21 minutes. Cells were separated by their density and different cell layers were formed. The third (transparent) and forth (pink) layer containing white blood cells were taken and mixed into Hank´s Balanced Salt Solution (HBSS) (without $Ca^{2+}$/$Mg^{2+}$, Thermo Fisher Scientific), followed by centrifugation at 400 g for 10 minutes. The supernatant was discarded and the pellet was resuspended in HBSS and then layered on top of a phosphate buffered percoll (GE Healthcare) gradient having the concentrations of 85%, 80%, 75%, 70% and 65% and centrifuged at 1100 g for 22 minutes. Neutrophils mainly concentrate within the 75% percoll layer wherefore we collected half of the 70% and 80% layers and the complete 75% layer. Afterwards the volume was replenished with HBSS and centrifuged at 400g for 10 minutes. The resulting pellet was resuspended in 1 mL of HBSS and cells were counted unsing a counting chamber. The desired cell number was solved in a medium containing Roswell Park Memorial Institute (RPMI) 1640 (Lonza), 0.5% fetal calf serum (FCS) (Biochrom GmbH). Neutrophil

purification was determined by a cytospin assay (Shanson, Cytospin 2 Centrifuge) followed by Diff Quick staining (Medion Diagnostics). In all experiments neutrophil purity was >95%.

**Neutrophil extracellular trap formation quantification**

Freshly isolated neutrophils were seeded (0.5 x $10^6$ cells/well) on PAA gels and collagen I- or fibrinogen- coated glass and incubated for 30 minutes at 37 °C, 5% $CO_2$ in order to allow the cells to adhere. NETosis was either stimulated with 5 nM Phorbol-12-myristate-13-acetate (PMA, Sigma-aldrich) or with 75 µg/mL lipopolysaccharide (LPS) from Pseudomonas aeruginosa (serotype 10.22, strain: ATCC 27316, Sigma-Aldrich) and incubated for 3 hours (37 °C, 5% $CO_2$). NETosis was stopped with 2% paraformaldehyde (PFA)(Roth) fixation overnight. The next day, the fixed neutrophil chromatin was stained using 1µg/ml Hoechst 33342,trihydrochloride,trihydrate (Hoechst) (life technologies) . Cells were imaged with a Axiovert 200 microscope (16x magnification, Zeiss) in the blue channel (Filter set49 DAPI shift free, 488049-9901-000, Zeiss) connected to a CoolSNAP ES camera (Photometrics). 6 images of different locations were taken per well. NETosis was quantified in a standardized blinded fashion as percentage of condensed and decondensed nuclei.

**Adhesion of neutrophils**

Freshly isolated neutrophils (0.5 x $10^6$ cells/well) were seeded on glass surfaces (coated with polyacrylamide (PAA) substrates or glass controls) coated either with collagen I or fibrinogen as stated above and incubated for 30 minutes allowing cell adhesion at 37 °C, 5% $CO_2$. Afterwards cells were fixed with 2% PFA overnight. Phase contrast images were taken for each setting and adhered cell area was calculated by using ImageJ 4.46r for at least 50 cells per condition.

**PI3K Inhibition**

Human neutrophils were treated with the potent PI3K inhibitor BAY 80-6946 (Copanlisib) for 20 minutes on ice and then seeded on PAA substrates (4 kPa, 128 kPa) and glass followed by a 30 minute-long incubation (37 °C, 5% $CO_2$). Afterwards cells were stimulated with 75 µg/ml LPS for 3 hours (37 °C, 5% $CO_2$). NETosis was stopped with 2% PFA fixation overnight. Cells were stained with Hoechst as described above and NETosis was quantified as mentioned earlier.

**Neutrophil extracellular trap formation on PLL and PLL-g-PEG coated glass surfaces**

Human neutrophils (10.000 cells/well) were seeded on glass surfaces coated with 0.5 mg/mL PLL (Sigma) or PLL-g-PEG (SuSoS Sturface Technology) and incubated for 30 minutes. As control, uncoated glass surfaces were used. Then cells were stimulated with either PMA (5 nM) or LPS (75 µg/mL) for 3 hours as described above. NETosis was stopped with 2% PFA fixation overnight. Cells were stained with Hoechst and NETosis was quantified as mentioned earlier.

## Results

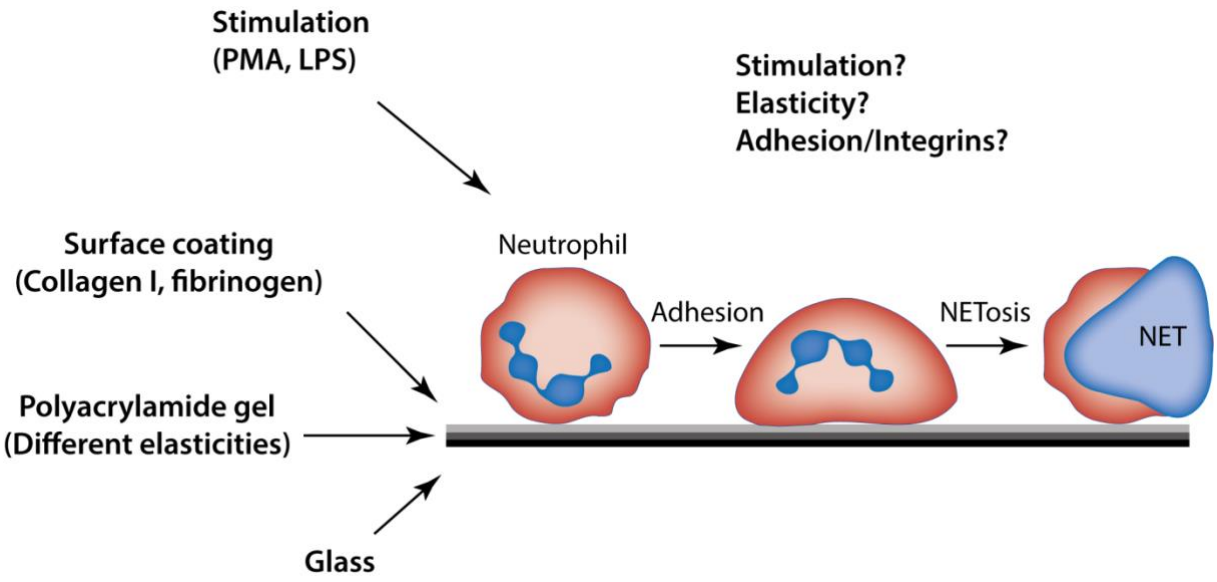


**Figure 1** ***Investigating the influence of substrate elasticity, surface coating and stimulation on NETosis.*** *Human neutrophils were cultured on polyacrylamide (PAA) gels of different elasticity and coating to control adhesion. Cells were stimulated with PMA or LPS for 3 hours and the effect of elasticity and surface coating on NETosis was assessed for the different types of stimulation.*

### Substrate elasticity affects NETosis in a stimulant-dependent manner

To investigate the effect of substrate elasticity on NETosis, freshly isolated human neutrophils were seeded on PAA gels coated with either collagen I or fibrinogen which are both known to interact with integrins on neutrophils[28,33]. The PAA substrate elasticity was varied within the physiological range (1 kPa, 2 kPa, 4 kPa, 8 kPa, 16 kPa, 20 kPa, 30 kPa, 64 kPa, 128 kPa). The prepared gels cover a wide range of physiological elasticities for example that of endothelial (14-16 kPa) or stromal tissue (30 kPa) etc.[9] In order to understand how substrate elasticity and the presence of certain integrin ligands affect NETosis, neutrophils were seeded and incubated for 30 minutes and then stimulated with either PMA or LPS for 3 hours **(figure 1)**. PMA is a well-known activator of protein kinase C (PKC) and frequently used to induce NETosis *in vitro*[34]. LPS on the other hand, induces NETosis in a receptor-mediated fashion[35].

NETosis was imaged and quantified on both surface coatings (collagen I and fibrinogen) for both ways of stimulation (PMA and LPS) **(figure 2)**. Representative images for 2 kPa, 16 kPa, 128 kPa and a glass control are shown in **figure 2**.

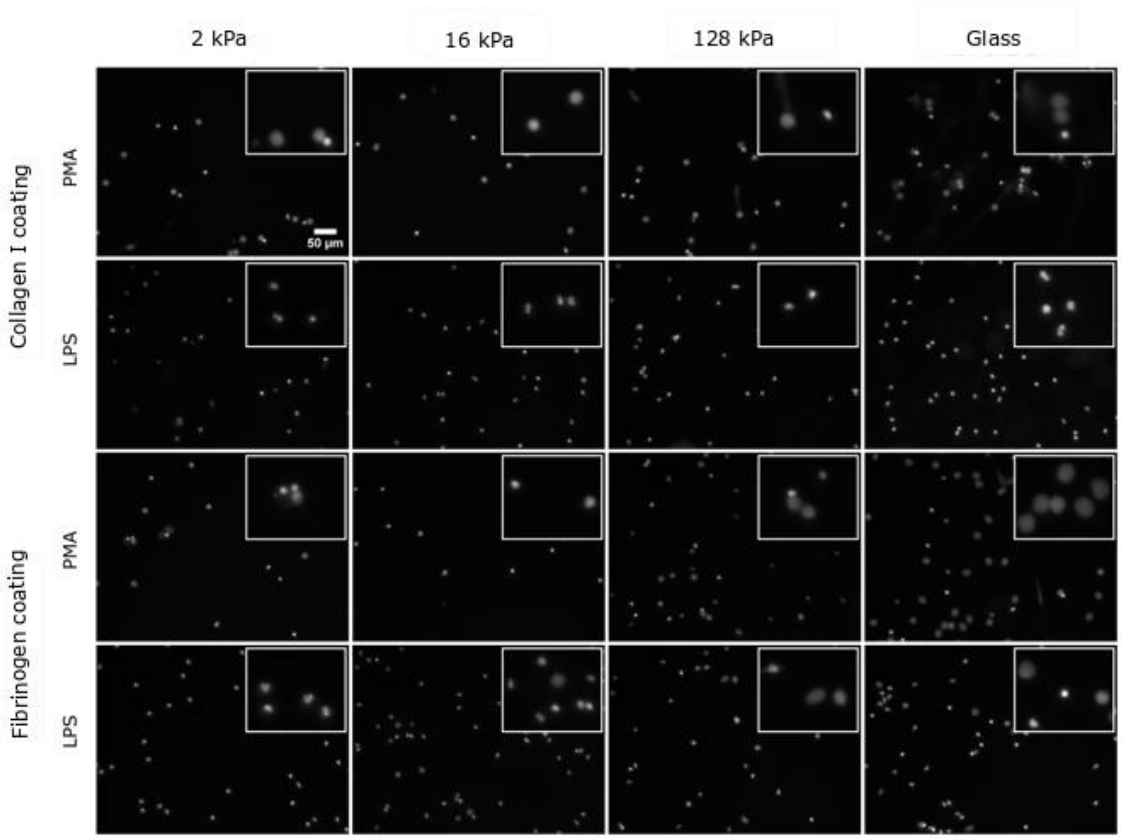


**Figure 2** ***NETosis is affected by substrate elasticity in a simulant-dependent manner***. *Human neutrophils were seeded on PAA gels and glass surfaces coated either with collagen I or fibrinogen. They were stimulated with either PMA (5 nM) or LPS (75 µg/mL) and incubated for 3h. The fluorescence images show the nuclei (Hoechst DNA/chromatin stain) of fixed cells. Insets show magnified cells/nuclei. The data shows that NETosis depends on all three parameters (substrate elasticity, stimulant, surface coating).*

Images of all other conditions are shown in the **supplementary figures S1 and S2**. PMA-stimulated NETosis was found to be independent of stiffness. On collagen I-coated substrates, the NETosis rate was similar among different stiffness values **(figure 3a)**. Similarly, on fibrinogen-coated substrates, PMA-induced NETosis **(figure 3c)** did not vary depending on the substrate stiffness. However, on glass surfaces NETosis was significantly higher compared to those on substrates with the following stiffnesses:8 kPa, 16 kPa, 20 kPa and 128 kPa. Therefore, we concluded that PMA induced NETosis is not affected in its outcome by stiffness.

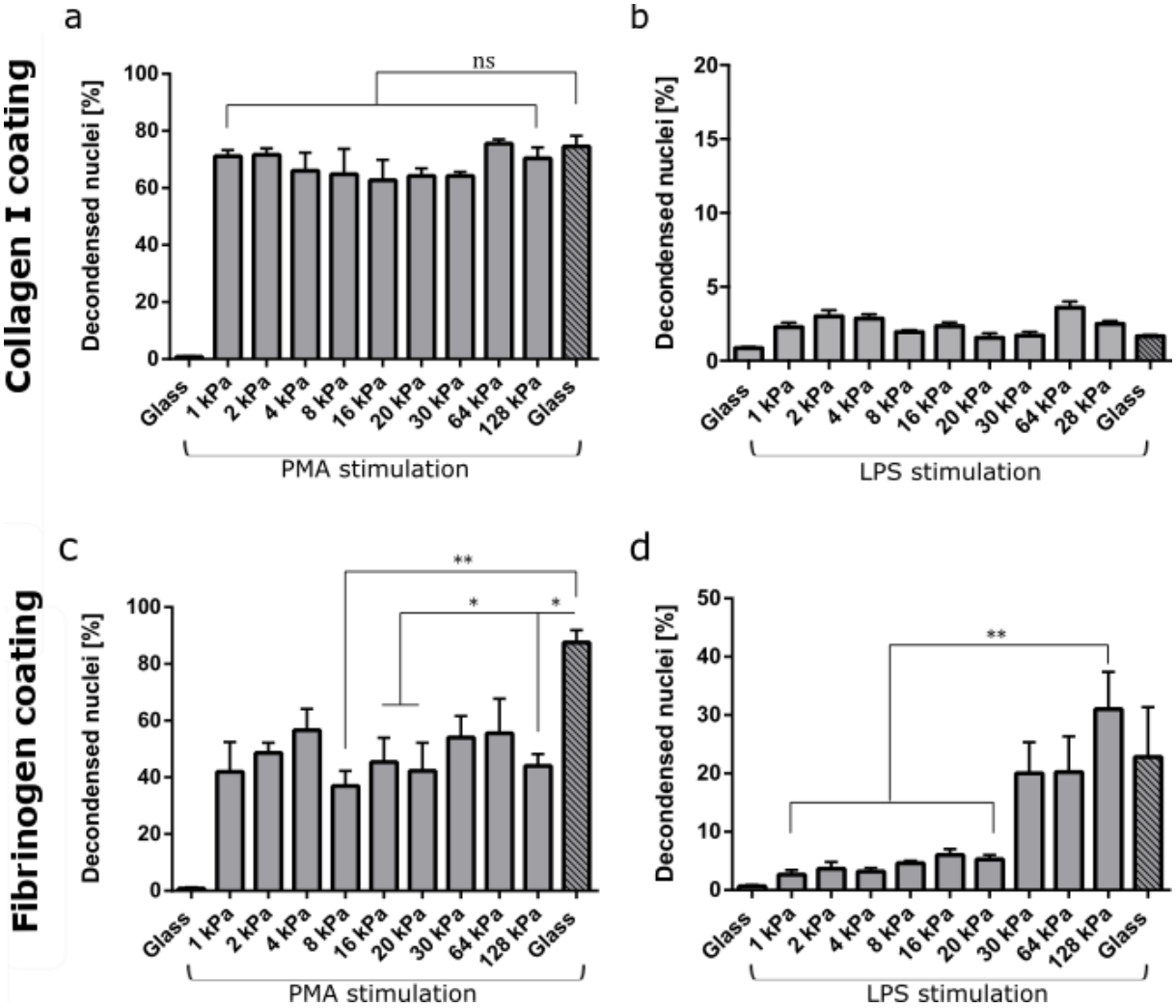


**Figure 3** ***NETosis depends on substrate elasticity, stimulant and surface chemistry.*** *Human neutrophils were seeded on PAA substrates and glass surfaces coated with either collagen I(a, b) or fibrinogen (c,d) and stimulated with either PMA (5 nM)(a, c) or LPS (75 µg/mL)(b, d) and incubated for 3h. n>500 cells for each condition. N=3 donors. Statistics: one-way ANOVA (Bonferroni's multiple comparisons test; * $p < 0.05$; ** $p < 0.01$; ns: not significant). Mean ± SEM.*

Interestingly, no activation was observed on collagen I-coated substrates upon LPS stimulation **(figure 3b).** However, NETosis was significantly affected by substrate elasticity under LPS stimulation on fibrinogen-coated surfaces **(figure 3d)**. We speculated that there could be an optimum density for NET formation on collagen, in accordance to previously published results that show an optimum ligand-receptor ratio for different biological functions including cell adhesion[27,36,24,37]. For that reason, we repeated the neutrophil stimulation assay on surfaces with a tenfold-reduced collagen amount. Interestingly, NET formation was now not only inducible on these surfaces but we also observed an increased spreading area of neutrophils and a correlating increase in NETosis rates **(figure 4)**. These observations corroborate our hypothesis that reducing the available amount of collagen on the surface may shift the biological response towards a more pro-adhesive phenotype and suggests that adhesion and NETosis are intertwined.

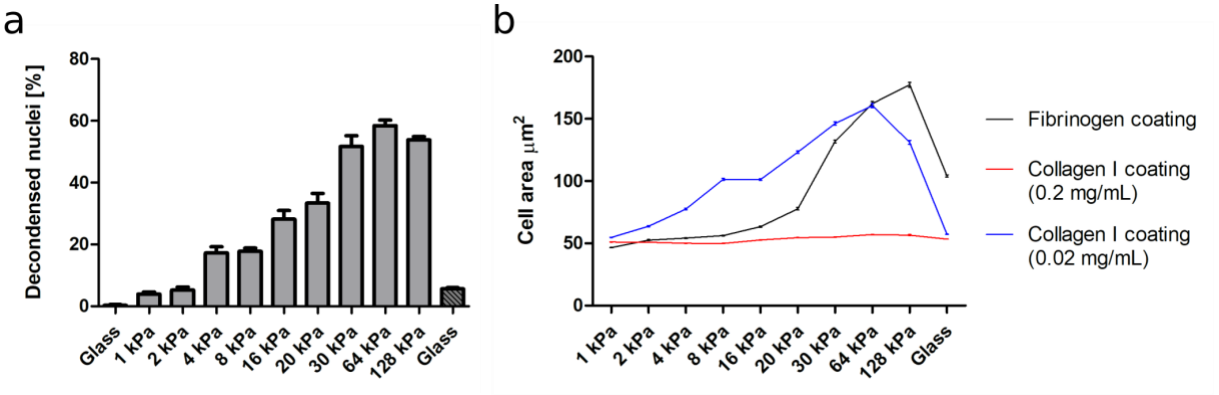


***Figure 4 NETosis on collagen coated gels depends on spreading area.*** *(a) Human neutrophils stimulated with 75 mg/mL LPS for 3 hours perform stiffness dependent NETosis on PAA gels (with lower collagen I concentration, 0.02 mg/mL). (b) NETosis rates correlate with spreading area. This finding explains why there is no NETosis on gels coated with higher collagen concentrations (corresponding to less cell spreading, see also figure 3). n>500 cells for each condition. N=3 donors. Mean ± SEM.*

**Correlation between neutrophil adhesion and NETosis**

To further investigate the connection between NETosis, adhesion and substrate elasticity we further assessed neutrophil adhesion on the different substrates. It is known that upon stimulation with PMA or LPS neutrophils initially start to adhere strongly to the provided surfaces before initiating the NETosis process[3], although it remains controversial whether this initial adhesion is necessary for the ensuing NETosis. During NETosis, the cells round up and the cytoskeleton is degraded. There have been contradictory studies on the role of integrin receptor engagement during NET formation[29,30] and it remains unclear to which extent NETosis and adhesion are correlated with one another.

Modifying the substrate stiffness most likely influences adhesion. Indeed, neutrophils have been reported to better adhere to stiff surfaces compared to soft surfaces[14,15]. Therefore, we next analyzed the spreading of human neutrophils on collagen I- and fibrinogen-coated substrates and strove to investigate the correlation between adhesion and NETosis. As a measure for adhesion we determined the cell contact area (*via* phase contrast microscopy). On fibrinogen-coated PAA substrates the cell contact area of neutrophils increased with higher stiffnesses **(figure 5a)** in a similar manner as the described increase in NETosis above. Next, a cross-correlation analysis for neutrophil spreading area versus NETosis on fibrinogen-coated surfaces was carried out for both PMA and LPS stimulation. While PMA-induced NETosis did not correlate with spreading area **(figure 5b)**, LPS-induced NETosis correlated with

spreading area **(figure 5c)**. Therefore, adhesion signaling appears to be highly relevant for LPS-induced NETosis.

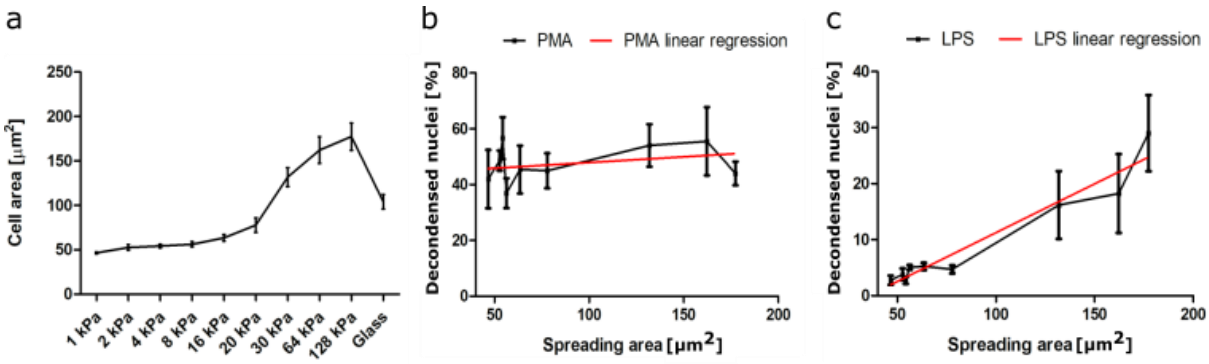


***Figure 5 Correlation of NETosis and cell spreading area.*** *Correlation between PMA-induced (a) and LPS-induced (b) NETosis rate on fibrinogen coated PAA gels and their corresponding mean spreading area. Black lines indicate NETosis rates (N=3 donors) for mean spreading area and red lines indicate a linear fit. Quantification of neutrophil adhesion on fibrinogen coated PAA gels and glass (c). Mean ± SEM.*

**Inhibition of stiffness-sensing by PI3K inhibition abrogates stiffness-dependent variations in NETosis**

PI3K activity has been shown to be important for neutrophil mechanosensing and enables these cells to distinguish between substrates of different stiffnesses[14]. To corroborate that the observed effects on LPS-induced NETosis could be attributed to variations in substrate stiffness, we treated neutrophils with a potent PI3K inhibitor, BAY 80-6946 (Copanlisib) and analyzed adhesion and NETosis. Fibrinogen coated 4 kPa and 128 kPa PA substrates were chosen as representative conditions for adhesion and NETosis quantification as they had revealed significant results in the experiments presented above. Neutrophil adhesion was impaired when neutrophils on stiff surfaces (128 kPa and glass) were pretreated with the inhibitor **(figure 6a)**. PI3K has been previously reported to be a signaling intermediate for PMA-induced NETosis, which is why moderate effects on PMA-induced NETosis were to be expected[35]. To investigate the connection between adhesion and NETosis we treated cells with PMA or LPS under PI3K inhibition. Inhibition of PI3K partially impaired PMA-induced NETosis **(figure 6b)**, however, completely abolished LPS-induced NETosis **(figure 6c)**.

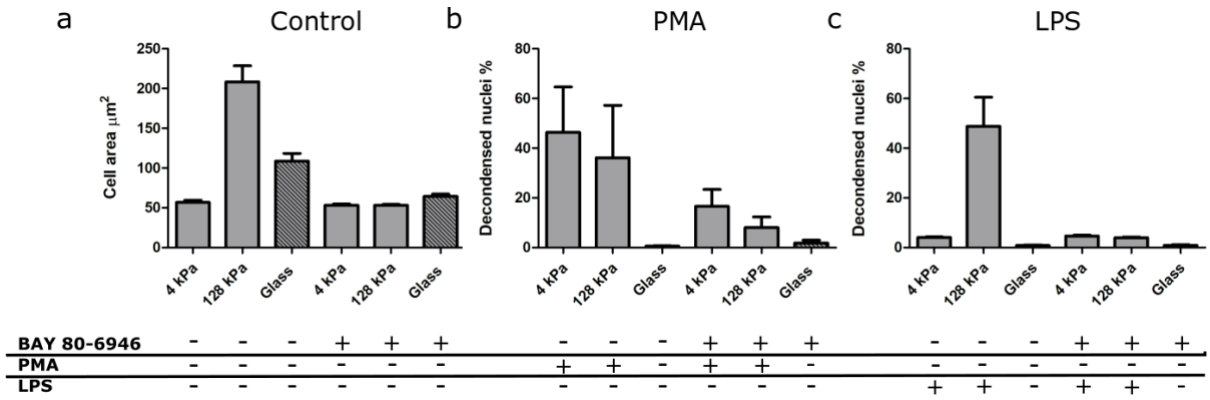


**Figure 6** ***PI3K inhibition impairs neutrophil adhesion and affects NETosis in a stimulant-dependent manner.*** *Human neutrophils were seeded on fibrinogen-coated PAA gels (4 kPa and 128 kPa) and glass (serving as a control) and the spreading area was quantified with and without PI3K inhibition after 30*

*minutes (a). PI3K inhibition leveled out the elasticity dependent differences in cell contact area. Then NETosis was induced by PMA (5 nM) (b) or LPS (75 µg/mL) (c)and incubated for 3 h and quantified. LPS-induced NETosis is completely abolished. In contrast, PMA-induced NETosis is only partially reduced by PI3K inhibition. n > 500 cells per condition. N=3 donors. Mean ± SEM.*

Then, to further prove the importance of adhesion for NETosis, we coated glass surfaces with poly-L-lysine (PLL) and poly-L-lysine-grafted-polyethylene glycol (PLL-g-PEG). PEG functionalization/passivation is often used to prevent unspecific adsorption of proteins and adhesion of cells[27]. This environment therefore does not provide any adhesive cues and can be used to test how adhesion affects NETosis. First, cells were seeded on glass, PLL-coated and PLL-g-PEG-coated surfaces and imaged by using reflection interference contrast microscopy (RICM). Dark regions in RICM images indicate close proximity between the cell and the substrate (i.e. adhesion), while bright regions indicate non-adhesive sitting of the cell on the substrate. Cells adhered to both glass and PLL-coated surfaces (**figure 7a, b**). However, they did not adhere to PLL-g-PEG coated surfaces as expected (**figure 7c)**. Cells were stimulated on these coatings and NETosis was found to be independent from adhesion under PMA stimulation as indicated previously[3] **(Figure 7d).** On the other hand, LPS-induced NETosis was completely inhibited on PLL-g-PEG.

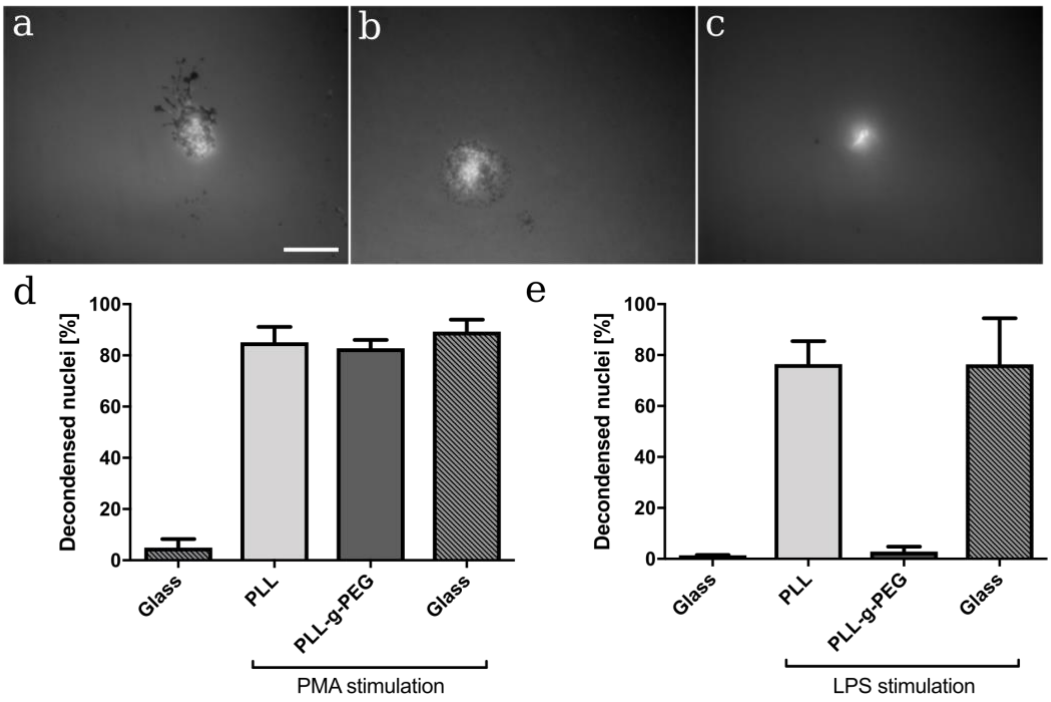


**Figure 7 LPS-induced NETosis but not PMA-induced *NETosis requires adhesion.*** *RICM images of fixed cells cultured on glass (a), PLL (b) and PLL-g-PEG (c) coated glass surfaces (Scale bar: 10 µm). Compared to glass and PLL surfaces, neutrophils cultured on PLL-g-PEG show no adhesion (a). NETosis on these different substrates was quantified for PMA (d) and LPS stimulation (e). PMA-induced NETosis also took place on the passivated non-adhesive PLL-g-PEG surfaces, while LPS-induced NETosis did not take place. N=2 donors. Mean ± SEM.*

## Discussion

Neutrophils are the most abundant leukocytes in humans. Being the first responders to inflammation, neutrophils infiltrate different kinds of tissue and subsequently face different mechanical and chemical environments. Substrate elasticity has been shown to influence important cellular functions such as adhesion, differentiation and migration of many different types of cells including neutrophils. [10,38–41]. Therefore, it is important to understand how NETosis is affected by adhesion in general and more specifically by substrate elasticity. Our results clearly indicate that LPS-mediated NETosis depends on the cells' adhesion and correlates with the adhesion/contact area.

In general, NETosis is initiated and transmitted *via* diverse pathways, highly depending on the respective stimulus. PI3K has been reported to be part of the intermediate signaling in PMA-induced and platelet-induced NETosis[35]. PI3K also plays an important role in neutrophil mechanosensing and has been shown to be required for neutrophils to sense substrates of higher stiffness[14]. In order to investigate the role of mechanosensing on neutrophil spreading as well as on NETosis, we tested a potent and highly selective PI3K inhibitor, BAY 80-6946 (copanlisib). Actually, PI3K inhibition impaired spreading of neutrophils and subsequently LPS-induced NETosis. It also inhibited PMA-induced NETosis to a certain degree, which is understandable as PI3-kinase is known to play a certain role in PMA-mediated activation of neutrophils. However, it did not abrogate NET formation completely as it did after LPS stimulation.

Another factor affecting cell adhesion is the available surface concentration of integrin ligands[21,36]. By using approaches from nanotechnology it is possible to control the exact distance and overall density of integrin ligands such as RGD and the Mac-1 ligand GPB1α or even link them to advanced near infrared fluorescent nanomaterials[27,42,43]. For neutrophils it has been shown that adhesion maturation and cellular functions such as spreading and migration depend on ligand density[27]. Here, we were able to show that on collagen, LPS-induced NETosis not only depends on substrate elasticity but also on the amount of available surface cues. On a substrate that provided a very high density of surface cues, NETosis did not take place in a relevant amount. However, a 10-fold reduction of the available collagen proteins enabled the neutrophils to undergo NETosis in a stiffness-dependent fashion under LPS stimulation. For PMA, NETosis was once again independent of surface cues, in this case density of surface receptors. Indeed, PMA-induced NETosis does not appear to require adhesion at all. These differences between the stimulants can be explained by the different involved receptor and signaling pathways for PMA- and LPS -stimulated NETosis. PMA is operating intracellularly and directly activates protein kinase C (PKC) and also triggers subsequent production of reactive oxygen species (ROS) which interact with MEK, ERK, PI3K, mTOR, MPO and NE[2,44,45]. LPS also activates PKC in neutrophils. It primarily acts through binding to the toll-like receptor 4 (TLR4)[46] found on neutrophils. Our results show that there is an active connection between adhesion and stiffness signaling and LPS-triggered NETosis, putatively by a connection between integrin and TLR4 signaling, which remains to be explored in depth in the context of NETosis. This hypothesis is also corroborated by the complete abolishment of LPS-induced NETosis through inhibition of phosphatidylinositol 3-kinase (PI3K). PMA-induced NETosis is also decreased after PI3K inhibition indicating a role of PI3K in PMA-signaling, however NETosis still took place albeit at a lower level.

Our results bear important medical and pharmacological as neutrophils and other cells of the immune system are continuously confronted with surfaces of different elasticity. One may speculate that reducing neutrophil responses on and in tissues with low substrate elasticity such as the brain, subcutaneous tissue or, most importantly, the blood itself may serve to keep aberrant immune responses in check. On the other hand, alterations of tissue stiffness as seen in arteriosclerotic vessels, tumor tissue or organ fibrosis might lead to an increase of inflammatory, NETosis-related processes. Indeed, NET-induced

inflammation might trigger tissue remodeling and lead to even more tissue stiffness, causing a pro-inflammatory, self-sustaining vicious circle. Our results may also be of importance to explain how NETosis can be triggered by implant materials[47], which tend to have a higher stiffness than "natural" tissues within the body. An improved understanding of the environmental factors that affect NETosis could therefore lead to novel therapeutic approaches for diseases that coincide with alterations of tissue elasticity such as arteriosclerosis, lung or liver fibrosis or cancer. They will also be of great importance for the design of future implant and exogenous materials which are designed to remain in the body for a certain time, such as catheters.

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