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TITLE: Quantitative analysis of clot density, fibrin fiber radius, and protofibril packing in acute phase myocardial infarction

SHORT TITLE: Clot ultrastructure in acute myocardial infarction

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ABSTRACT

Introduction. Coronary artery disease is associated with impaired clot structure. The aim of this study was to investigate acute phase myocardial infarction (AMI) and provide detailed quantitative analysis of clot ultrastructure.

Materials and Methods. Clot formation and breakdown, pore size, fiber density, fiber radius and protofibril packing were investigated in plasma clots from AMI patients. These data were compared to those from healthy controls.

Results. Analysis on clot formation using turbidity showed increased lag time, suggesting changes in protofibril packing and increased fiber size for AMI patients compared to healthy controls. Additionally, increased average rate of clotting and decreased time to maximum absorbance in AMI patients suggest that clots formed more quickly. Moreover, we observed increased time from max OD to max rate of lysis. Increased fibrinogen and decreased plasminogen in AMI patients were accounted for in represented significant differences. AMI samples showed increased time to 25% and 50% lysis, but no change in 75% lysis, representative of delayed lysis onset, but expediated lysis once initiated. These data suggest that AMI patients formed less porous clots made from more densely packed fibers with decreased numbers of protofibrils, which was confirmed using decreased permeation and increased fiber density, and decreased turbidimetry.

Conclusions. AMI plasma formed clots that were denser, less permeable, and lysed more slowly than healthy controls. These findings were confirmed by detailed analysis of clot ultrastructure, fiber size, and protofibril packing. Dense clot structures that are resistant to lysis may contribute to a prothrombotic milieu in AMI.

INTRODUCTION

Cardiovascular diseases are the most common cause of morbidity and mortality on a global scale, of which coronary artery disease (CAD) is one of the most prevalent. Myocardial infarction (MI) is the most devastating complication of CAD [1,2]. Blood clot structure has been associated with a number of thromboembolic diseases [3–5]. Altered plasma clot properties and structure evidenced by decreased clot permeability, were first demonstrated in patients with CAD [6]. Previously, we have demonstrated that male relatives of patients with premature onset of CAD form fibrin clots with a more prothrombotic phenotype: more rapid clot formation and thicker fibers, that were less permeable when compared to controls [7].

It was also demonstrated that fibrin clots taken from patients 3 months after the MI event had increased fibrin fiber numbers that were shorter in length, with decreased rate of fibrinolysis when compared to healthy controls [8]. Note that the study was not performed on clots taken from acute phase MI patients but on clots made from plasma taken from the patients 3 months after the event. Patients with acute myocardial infarction (AMI), when compared with chronic coronary syndromes, had denser fibrin clot networks, which were more resistant to lysis [9]. These features were associated with enhanced inflammatory status including raised C-reactive protein levels and oxidative stress [9]. Fibrin is one of the major components of the atherosclerotic plaque, and it was shown to promote plaque growth [10]. Additionally, fibrin clot properties were associated with CV complications during coronary angioplasty, namely stent thrombosis and no-reflow phenomenon [11,12]. Moreover, formation of denser plasma clots were associated with higher fibrin content assessed in the intracoronary thrombus aspirated from patients during acute phase ST-elevation MI [13].

Interestingly, a recent PLATO substudy — a large clinical trial with patients with acute coronary syndromes guideline-based therapies and followed for up to 1 year — showed that fibrin clot properties independently predicted the risk of spontaneous MI and CV death following the initial hospitalization [14]. Moreover, clot lysis time predicted worse outcome even after adjustment for other established cardiac biomarkers [14]. Despite the need for highly trained personnel to perform some of these tests, this study clearly showed a real-life significance and utility of fibrin clot properties assessment in AMI patients [14].

Here, we sought to determine whether clot ultrastructural and intrafibrillar fibrin arrangements in plasma clots taken from venous blood of patients with AMI differ from healthy volunteers. Specifically, we

performed extensive analyses based on scanning electron microscopy, confocal microscopy and light scattering methods, to understand detailed fibrin clot ultrastructure and protofibril arrangements in AMI patients, and compared these with healthy controls in order to see the magnitude of difference between patients and a healthy fibrinogen-level control range. To our knowledge, this is the first study in which these assessments have been made for clots formed from AMI patient plasma.

METHODS

Study population

Acute myocardial infarction patients

We assessed 23 patients hospitalized within 12 hours from the onset of the first clinical symptoms of AMI. We included both patients with ST-segment elevation MI (STEMI) and those with non–ST-segment elevation MI (NSTEMI). Both STEMI and NSTEMI diagnoses were established according to the Third Universal Definition of Myocardial Infarction [15]. The exclusion criteria included atrial fibrillation, venous thromboembolic events within previous 6 months, or any other disease treated with anticoagulant therapy, known cancer, signs of acute infection, chronic inflammatory disorders (e.g. rheumatoid arthritis), liver injury (alanine or asparagine transaminase >1.5 times above the upper limit of the reference range), estimated glomerular filtration rate (eGFR) <30 mL/min, and pregnancy.

Healthy controls

To establish a healthy control reference for each of the clot structure and function assays, we studied apparently healthy people (n=24) with a median age of 27.5 yrs, without a medical history of any disease and without any ongoing symptoms (healthy controls). The group did not receive any chronic medications prior to enrolment to the study.

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of Jagiellonian University (KBET/122.6120.271.2015), and the University of Leeds Research Ethics Committee (HSLTLM12045). Each study participant provided written informed consent.

Laboratory investigation

Blood samples from the interventional group were obtained on admission to the Emergency Department (AMI group), or between 8 and 10 a.m. (control group). The samples were processed 15–60 min after blood collection and stored at −70 °C until further analysis. Blood was taken from the antecubital vein with minimal stasis. In the AMI group, routine blood evaluation including complete blood count, lipid profile (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C] and triglycerides [TG]), and the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum creatinine, were analyzed by automated laboratory techniques.

Fibrinogen levels

Total amount of fibrinogen was measured using the Clauss method on a Start 4 hemostasis analyzer (Diagnostica Stago, Theale, UK) and the Fibri-prest automate kit (Diagnostica Stago, Theale, UK) following the manufacturer's instructions. All measurements were done in duplicate.

Clot formation and fibrinolysis

Plasma clot formation and breakdown were measured in 96-well plates (Greiner, Stonehouse, UK) with a powerwave microplate reader (λ = 340nm, Bio-Tek, Swindon, Sweden) as previously described [16]. Briefly, plasma was diluted 1:6 in Tris-buffered saline (TBS, 50 mmol/L Tris HCl, 100 mmol/L NaCl, pH 7.4). Clot formation and fibrinolysis was initiated by adding 0.1 U/mL of human α-thrombin and 10 mmol/L of $CaCl_2$ (final concentrations). Fibrinolysis was initiated by adding a final concentration of 30 ng/mL of tissue-type plasminogen activator. Measurements were made every 12 s for 3 h at a temperature of 37°C. Absorbance was measured in triplicate for each sample and average values were presented. Turbidity profiles were analyzed for lag time, maximum absorbance (Max OD), time to Max OD, and average rate of clot formation. Fibrinolysis profiles were analyzed for clot lysis time (CLT), time to 25%, 50%, and 75% lysis, and average rate of fibrinolysis. Experiments were performed in triplicate.

Clot permeability (K_s)

Permeability, a measure of the pore size for a fully formed clot, and the resulting permeation constant (Ks) were measured as described below. Plasma was diluted 1:4 in TBS and clotting was initiated by adding 0.1 U/mL of human α-thrombin and 10 mmol/L of $CaCl_2$ (final concentrations). The solution was immediately transferred to the well of a six-chamber Ibidi slide (Ibidi, GmbH, Martinsried, Germany) and allowed to clot for 2 h in a humidity chamber at room temperature. After two hours, 2.5 mL syringes without the inside plunger were added to one side of the Ibidi slide and slowly filled with TBS to a height of 4 cm taking care to not introduce any bubbles into the system. Clots were rinsed with TBS for 1 h whereby the TBS that flowed through the clot was collected at the opposite end of the channel and discarded. After 1 h, the amount of TBS that flowed through the chamber was collected, weighed and timed for calculation of flowrate every 5-10 min until 5 measurements were taken for each clot. The permeation constant, K_s, was calculated as described previously [17]. In short, $K_s = (Q \times L \times \eta)/(T \times A \times P)$, where Q is the liquid volume, L is the length of the clot, η is the viscosity, T is the time for the fluid to flow through the clot, A is the cross-sectional area of the clot, and P is the pressure drop. K_s gives an average pore size for the clot network of fibers. Experiments were performed in triplicate.

Laser scanning confocal microscopy (LSCM)

Clot fibrin fiber density was analysed using LSCM as described previously [16]. In short, plasma was spiked with a final concentration of 50 µg/mL AlexaFluor488-labeled fibrinogen and diluted 1:4 in TBS. Clotting was initiated with the addition of 0.1 U/mL of human α-thrombin and 10 mmol/L of $CaCl_2$ (final concentrations) and immediately transferred to six-chamber Ibidi slides. Clots were allowed to form for 2-4 h in a dark humidity chamber at room temperature after which they were imaged with a Zeiss LSM880 inverted microscope with a 40×oil immersion objective (Carl Zeiss, Ltd., Cambridge, UK). Three micrographs were taken for each clot and Z-stacks (30 slices, 20 µm total distance) were combined and flattened at maximum intensity (ImageJ, NIH, Bethesda, MD, USA).

Scanning electron microscopy (SEM)

Average fiber size was determined using high-resolution SEM. Briefly, clots were formed by adding 1 vol activation mix (5 mmol/L $CaCl_2$, 0.5 U/mL human α-thrombin, final concentrations) to 10 vol plasma. Immediately after mixing, the solution was transferred to the lid of a 0.6 mL Eppendorf tube (Eppendorf UK Limited, Stevenage, UK) and allowed to form for 2 h in a humidity chamber at room temperature. Fully formed clots were washed in saline solution (0.9 g/L) for at least 2 h and then placed in 2% glutaraldehyde solution (in saline) for fixation overnight. The next day, clots were further washed in sodium cacodylate buffer (67 mM $C_2H_6AsNaO_2$, pH 7.4) for 2 h, and then dehydrated in a series of increasing acetone concentrations (30-100%) until stored in 100% acetone. Fixed and dehydrated clots were critical point dried with CO_2, mounted onto stubs, and sputter coated with iridium using a Cressington 208 HR (Cressington Scientific Instruments, Watford, UK). Clots were imaged in 3 areas, at 3 different magnifications (5000×, 10,000×, and 20,000×) using a Hitachi SU8230 high-performance cold emission SEM (Chiyoda, Japan). Fiber size was measured by drawing an equally spaced x-y grid of lines on each 20,000× image. Fibers closest to the x-y crossing of each line were measured and the diameters were recorded. Diameters from three images were averaged for each sample and the average for each sample type (AMI and Healthy) are reported.

Turbidimetric analysis

Average fiber radius and protofibril packing were determined using previously validated light scattering methods [16,18]. To initiate clotting, plasma (diluted 1:6 in TBS) was mixed with an activation mixture of human α-thrombin and $CaCl_2$ (0.5 U/mL and 5 mmol/L, final concentrations) and immediately transferred to a UV-visible cuvette (Eppendorf Uvette, Sigma-Aldrich, Dorset, UK), sealed with parafilm to prevent dehydration, and allowed to clot at room temperature overnight. The following day, the fully formed clot was scanned over wavelengths between $500 < \lambda < 800$ nm in a Λ 35 UV-visible spectrophotometer (Perkin-Elmer, Cambridge, UK). Based on the absorption at each of these wavelengths, the average fiber radius, number of protofibrils, and protofibril spacing was determined. A detailed description of these calculations can be found in the supplemental section. Experiments were performed in triplicate.

Plasminogen levels

Plasminogen levels were measured using an enzyme linked immunosorbent assay (ELISA) for human plasminogen (ELH-PLG, RayBiotech, Norcross, GA, USA) following manufacturer's instructions. Plasma samples were diluted 1:50,000 for the concentrations values to fit within the provided standards range. Plasminogen concentrations were calculated by plotting each samples absorbance against the standard curve. Experiments were performed in triplicate.

Statistical analysis

Data are presented as mean ± standard deviation or median and interquartile range. Normality was assessed using the Shapiro-Wilk test. Differences between groups were determined using Student's t-test for normally distributed variables and the Mann-Whitney U-test for non-normally distributed variables. Adjustments for fibrinogen and plasminogen levels were done to correct for differences in these parameters between patients and controls. Adjustments for fibrinogen and plasminogen levels were done to correct for differences in these parameters between patients and controls using multiple regression modelling. Statistical analysis was performed in JMP 15.2 (SAS Institute Inc, Cary, NC, USA, 2020) and R 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria, 2021.

RESULTS

Patient Demographics

We analysed 23 patients with AMI and 24 healthy controls (32 ± 10.7 years; min-max: 19 - 64 years). Baseline characteristics of the study population including concomitant diseases and medications in AMI group are shown in Table 1. The AMI group had 30% higher concentrations of fibrinogen and 23.5% lower concentrations of plasminogen, when compared to healthy controls (Table 2). Taking these well-known characteristics of prothrombotic phenotype into account, we present all data where protein concentration (either fibrinogen, plasminogen, or both) was not already taken into account during the calculations (turbidimetry experiments) with p-values adjusted for either fibrinogen levels or fibrinogen and plasminogen levels using the regression analysis noted above (Table 2). Clinical characteristic data for the AMI group is available in Table 1.

Clot formation and breakdown

Changes in clot formation and breakdown are indicators of prothrombotic phenotype in patients with CAD [7]. To assess whether these changes are present in AMI patients, we compared clot formation in plasma samples from AMI patients to that of healthy controls. Though no significant differences in Max OD were found between groups prior to fibrinogen concentration adjustments, we found a significant decrease (18%) in Max OD for AMI samples after adjustment (Table 2). Furthermore, AMI clots had a significantly increased (24%) time to clot initiation (Lag Time, Figure 1A). Time to Max OD was significantly lower (27%) in AMI samples (Figure 1B). As a result, the average rate of clotting was also significantly increased in AMI clots (295%, Figure 1C). AMI samples had no significant difference in maximum rate of clotting (Vmax) compared to healthy controls (Figure 1D). Detailed data from clot formation taking into account fibrinogen concentration are presented in Table 2.

AMI plasma samples also showed different clot breakdown characteristics when compared with healthy controls. Considering the significant influence of fibrinogen and plasminogen concentrations on clot lysis and the significant difference between its concentration in studied groups, we present significant differences based on regression analysis as discussed previously (Table 2). The time from Max OD to maximum rate of lysis was significantly (3.5-fold) higher in AMI clots (Figure 2A). This can be further seen when comparing the time to 25, 50, and 75% lysis. Clots from AMI patients' samples had significantly increased time to 25% (6.1-fold) and 50% (2.8-fold) lysis (Figure 2B and 2C). At 75% lysis, we observed no difference between groups (Figure 2D). These results indicate that the major part of the clot in healthy controls can be lysed more quickly whereas the remaining part of the clot is being lysed much slower than in the AMI group.

Clot structure

In light of these differences in both clot formation and break down, the structure of fully formed AMI clots was investigated. Using LSCM, dramatic visual differences between AMI patients' samples and healthy controls were apparent. These differences were quantified and showed that AMI clots were made of 46% more fibers than healthy controls per 100 μm (Figure 3, Table 2). Permeation experiments showed the average size of the pores within a clot. AMI clots had a 51% lower average pore size (K_s), compared to healthy controls (Figure S1, Table 2). Together, these results show that AMI patients' samples form less porous, more densely packed clots than those from healthy controls.

To determine whether the differences found at the whole clot level were also found at the level of individual fibers, we examined individual fiber size using high resolution SEM. Visually representative images of AMI patients and healthy control plasma clots are shown in Figures 4A and 4B, respectively. Average fiber size was quantified for each plasma sample. AMI clots had a 13% larger fiber diameter than healthy controls (Figure 4C, Table 2). These data indicate significant changes at the individual fiber level for fibrin clots from AMI patients. To investigate fiber differences in further detail and under fully hydrated and physiological conditions, dynamic light scattering techniques (turbidimetry) were used to not only determine the average fiber radius but also the average number of protofibrils within each fiber. This analysis incorporates fibrinogen concentration into the calculations so no further adjustment (regression analysis), as in previous analyses, was performed. Using this technique, we found no difference in fiber radius between the groups (Figure 5A, Table 2), but a 13.7% decrease in the average number of protofibrils packed per individual fiber in AMI clots compared to those of healthy controls (Figure 5B, Table 2). In addition, there was no difference in mass/length ratio (Figure 5C, Table 2), but 10.2% increase in protofibril distance within AMI clot fibers compared to healthy controls (Figure 5D, Table 2). It should be noted that SEM and turbidimetry experiments are performed with dehydrated and fully hydrated clots, respectively. Taken together, our data indicate that major differences occur in the packing of protofibrils within individual fibrin fibers in clots from AMI patients when compared with healthy controls.

DISCUSSION

Extensive analysis using 1) high-resolution SEM imaging with assessment of fiber size; 2) turbidimetric analysis with evaluation of average fiber radius, protofibril packing and distance; and 3) LSCM imaging of fiber density in a plasma clot — has allowed us to present for the first time a detailed description of clot ultrastructure and intrafibrillar protofibril arrangements in plasma clots from AMI patients. Additionally, using clots from healthy individuals as a reference, our data indicate the magnitude of the differences between clot structure abnormalities in the AMI group in comparison with a healthy control range.

The purpose of this study was to assess the extent of the differences in clot ultrastructure between a healthy control range of each of these parameters and patients with AMI, which has not been assessed before. In a study performed by Undas *et al*. authors demonstrated a significant difference between fibrin

clot architecture between patients with AMI and with chronic coronary syndrome [9]. Furthermore, the observation that patients with AMI were characterized by more compact clots that were more resistant to lysis was associated with inflammatory markers such as elevated C-reactive protein, interleukin-6, or enhanced oxidative stress [9]. Similar to our healthy control range, patients with chronic coronary syndromes had significantly lower levels of fibrinogen when compared to AMI groups [9]. Interestingly, in the Undas *et al*. study patients with chronic coronary syndromes (controls) had also significantly lower concentrations of plasminogen, which was the opposite for our healthy control samples [9].

It is well established that increased concentrations of fibrinogen are adversely associated with the incidence of MI or stroke [19]. In addition, as discussed by Meltzer ME et al., plasminogen levels are a potential marker of inflammation, whereas high plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator (t-PA) levels may reflect increased lipid levels and to a lesser extent also inflammation [20]. Moreover, a reduced fibrinolytic capacity due to increased plasma plasminogen activator inhibitor concentrations has previously been associated with myocardial reinfarction [21]. Currently, increasing evidence suggests that the structure of the fibrin clot and its resistance to fibrinolysis could determine the risk of thrombotic vascular disease such as MI [14,22]. Interestingly, it was shown by Zalewski *et al.* that survivors of MI with a history of the no-reflow phenomenon after the percutaneous coronary intervention (PCI) were characterized by a more compact fibrin network and higher resistance to lysis [12]. However, these authors evaluated only patients after STEMI, and the PCI itself was performed 6 to 14 months before enrolment to the study. In our study the samples were collected in the acute phase of MI within a few hours from the symptom's onset. Our observations are in line with more recent data by Spinthakis *et al.* showing that a more compact and denser fibrin meshwork, with thinner fibrin fibres and smaller pores, is associated with increased lysis time, therefore less efficient fibrinolysis [23]. This phenomenon was also confirmed by assessment of mechanical properties of fibrin clot. Wei Li *et al.* demonstrated that thinner fibrin fibers have stronger Young's Modulus and therefore thin fibers can be 100 times stiffer than thick fibers [24]. It is interesting that in the same study the authors showed that fibrinogen glycation was not associated with the single-fiber modulus, therefore having negligible impact on fiber meshwork stiffness. Here we should note that some of these results may be due to relatively high concentrations of tPA (30 ng/ml) used for our experiments. For these data to be more accurate, clots should

297 be fully formed prior the onset of lysis. This would normally be done by lowering the tPA concentration to
298 values lower than 30 ng/ml and comparing the results to those found from experiments at higher tPA
299 concentrations. However, as we had only limited sample volumes, we were unable to repeat these
300 experiments at the desired lower tPA concentrations.

301 It has been suggested that factors other than MI itself could alter fibrin clot properties.
302 Hyperglycemia in acute coronary syndromes was associated with enhanced local thrombin generation and
303 platelet activation, and therefore unfavorably altered clot features in patients both with and without diabetes
304 [25]. A key previous study on AMI patients demonstrated denser networks which were more resistant to
305 lysis than patients from stable CAD cases [9]. It should be noted that these authors compared AMI patients
306 with stable CAD, but without a healthy reference range [9]. Additionally, these features were associated
307 with elevated inflammatory parameters such as CRP and oxidative stress [9]. Moreover, contrary to our
308 study, Undas *et al*. performed SEM analysis on only a subset of the whole study population [9]. It is worth
309 noting that in this study both patient groups received a relatively high dose of ASA (300 mg) [9], however
310 in our study a standard daily dose of 75 mg ASA was taken by all AMI patients (before the enrolment), and
311 a loading dose of ASA was administered after blood collection. It is well established that ASA has an effect
312 on structure, particularly at high concentrations [26–28]. In addition, a large fraction of patients with CAD
313 have recurrent MI, despite receiving standard daily dose of ASA (~ 75 mg daily), and are still at very-high
314 risk for future CV events [29].

315 Neergaard-Petersen *et al.* showed that assessing fibrin clot properties (increased clot area under
316 curve [AUC]) in patients on daily ASA dose could predict future CV complications[29]. Interestingly, neither
317 clot maximum absorbance nor lysis time showed significant association with future vascular events in this
318 study [29]. Multiple studies have shown that the addition of ASA, in either a purified fibrinogen system or in
319 plasma, increases fibrin fiber size and produces larger pores within the network in a dose dependent
320 manner [26–28,30]. These changes also contribute to increased fibrinolysis rates compared to low or no
321 dose ASA [27,30] Importantly, despite the use of ASA in all AMI patients, we still found that they
322 demonstrated a pronounced prothrombotic fibrin clot phenotype compared with the healthy reference range
323 based on subjects who were free of any medications.

Another study by Zalewski *et al.* demonstrated that in patients in acute phase MI the formation of denser plasma clots with higher resistance to lysis could be affected by fibrin content assessed in blood clots taken from intracoronary thrombi [13]. Furthermore, Silvain *et al.* found that in acute STEMI patients, the occlusive thrombus content (platelets and fibrin), taken similarly using thrombectomy, were dependent on ischemic time [31].

There is still a gap in knowledge pertaining to fibrin clot ultrastructure, and composition, especially in atherosclerotic cardiovascular diseases which are the leading cause of deaths worldwide [32]. Authors of the PLATO-substudy (a large clinical trial) assessed whether cardiovascular risk depended on fibrin clot properties using turbidimetric assays [14]. Each 50% increase in lysis time was associated with increased risk of CV death or spontaneous MI in this study. Similarly, each 50% increase in maximum turbidity was associated with an increased risk of CV death. Therefore, fibrin clots that were more resistant to lysis independently predicted adverse outcomes in patients with acute coronary syndromes. The authors commented that novel therapies targeting fibrin clot properties could be beneficial for improvement of the prognosis in patients with MIs.

Our SEM data suggests that clots from AMI patient plasma form larger fibers while data from turbidimetric analysis shows no change in fiber radius, but a significant decrease in protofibril numbers at an increased protofibril distance in for this same group. It should be noted that while turbidimetric analysis accounts for fibrinogen concentration inherently in the calculations, SEM analysis does not. The SEM results hold true when accounting for the differences in fibrinogen concentration. One possible explanation for these findings may be due to the differences in design between the two experiments. SEM experiments are done on dehydrated clots while turbidimetric experiments are done on fully hydrated clots. It has been shown previously that fibrin fibers are mostly made up of water [33]. In addition, several assumptions are made in turbidimetric analysis, including a lower limit on fiber size [34]. Turbidimetric experiments also average over likely hundreds or more fibers per sample. Since individual fibers are not measured in this analysis, small changes in individual fiber radius will not be seen due to averaging, only large differences would be apparent using this method. In SEM, individual fibers are measured and then averages are taken for 100-200 fibers per clot. While this results in local, small area fiber densities possibly not being representative of the overall clot, this can be accounted for by imaging and analysing multiple different

places on the overall clot and averaging the results. In short, SEM is likely more accurate on a local, small area average where each individual fiber measured holds more overall weight, while turbidimetric analysis is a more accurate assessment of the average fiber radius over the entire clot where local places with larger or smaller fibers will be averaged out over hundreds or more individual fibers.

Further investigation of the turbidimetric results show some interesting findings. While turbidimetric data showed no changes in fiber radius between AMI and healthy controls, the internal makeup of the individual fibers was different. For the same radius, healthy control fibers contained higher numbers of protofibrils and decreased protofibril distance or distance between adjacent protofibrils. These results, as noted previously, already adjust for changes in fibrinogen concentration and thus excludes the differing concentrations as the reason for these changes. These changes may cause changes in individual fibrin fiber strength and stability with the decreased number of protofibrils and increased protofibril distance contributing to less stable fibers in the AMI group. However, these mechanical properties were not tested in the current study and would require future investigation in the future. Taken together, our experimental results suggest that changes in individual fiber formation contributes to changes in overall clot structure on a compounding basis, likely leading to mechanical differences. Further studies on the formation the mechanical properties of individual fibers, similar to those done by the previously mentioned Wei Li *et al.,* in CAD and more specifically, AMI, should be investigated in future studies.

We have a few limitations to acknowledge. Firstly, this research was conducted as a case-control study, therefore the significant differences may be associated with confounding factors. Nevertheless, the aim of his study was to compare fibrin clot ultrastructure in AMI patients to healthy volunteers to show the magnitude of the difference that can be observed between pathology and physiology, which has not previously been shown. Secondly, all AMI patients had previously taken ASA, which was unavoidable in patients at high cardiovascular risk. It is well known that ASA causes differences in fibrin clot properties and this might reduce any differences between the two study groups, which was however not the case. Instead, we note that despite ASA use, which would normalise abnormal fibrin clot structure to a certain extent, the AMI group were characterised by a marked prothrombotic fibrin clot structure when compared with that in healthy controls free of any medication. Third, the number of patients and controls were limited. We suspect that the large spread in values for MaxOD, particularly in healthy controls, in combination with the limited

sample numbers, may explain why significance differences were found after adjustment for fibrinogen concentration. Finally, as noted above, our lysis data showed clots that may not have been fully formed prior to lysis, particularly in the healthy control samples, suggesting that the tPA concentration for lysis experiments could be repeated at lower concentrations. As we had limited sample volumes, we were unable to repeat these experiments at lower tPA concentrations which would allow for clots to fully form prior to the onset of lysis. Future studies should take this into account and adjust tPA concentrations to those lower than 30 ng/ml and compare the findings to those found in the current study to further confirm the drastic changes in lysis times, particularly during the initial onset of lysis. .

In conclusion, our study broadens our understanding of how the fibrin clot ultrastructure affects cardiovascular complications, using extensive analysis of fibrin clot ultrastructure by high-resolution SEM imaging, LSCM, and light scattering technique. We showed that clots from AMI patients demonstrate different ultrastructure and intrafibrillar protofibril arrangements, making such measurements useful tools for improving targeted therapies in patients with AMI.

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CONFLICT OF INTEREST: none declared

AUTHORS CONTRIBUTION

A.S. and S.R.B. collected samples, performed experiments, analyzed data, and wrote the manuscript. C.D. performed experiments and reviewed the manuscript. K.P.M, conducted statistical analysis; G.G. and J.N. collected samples, designed the inclusion criteria, and reviewed the manuscript. R.A.S.A designed the research and co-wrote the paper.

REFERENCES

[1] A.B. Wojtyniak, M. Gierlotka, G. Opolski, D. Rabczenko, Observed and relative survival and 5-year outcomes of patients discharged after acute myocardial infarction : the nationwide AMI-PL database, Kardiol. Pol. (2020). https://doi.org/doi: 10.33963/KP.15465.

[2] P. Feusette, M. Gierlotka, I. Krajewska-Redelbach, A. Kamińska-Kegel, S. Warzecha, L. Kalinowska, J. Szlachta, K. Kutkiewicz-Moroz, J. Sacha, A. Wojdyła-Hordyńska, R. Bryk, P. Jankowski, M. Gąsior, Comprehensive coordinated care after myocardial infarction (KOS-Zawał): A patient's perspective, Kardiol. Pol. 77 (2019) 568–570. https://doi.org/10.5603/KP.a2019.0038.

[3] A. Undas, R.A.S. Ariëns, Fibrin clot structure and function: A role in the pathophysiology of arterial and venous thromboembolic diseases, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 88–99. https://doi.org/10.1161/ATVBAHA.111.230631.

[4] Z. Bagoly, Altered fibrin clot phenotype as predictor of the risk of recurrent venous thromboembolism: Evidence is growing, Polish Arch. Intern. Med. 128 (2018) 569–571. https://doi.org/10.20452/pamw.4356.

[5] M. Ząbczyk, A. Undas, Plasma fibrin clot structure and thromboembolism: Clinical implications, Polish Arch. Intern. Med. 127 (2017) 873–881. https://doi.org/10.20452/pamw.4165.

[6] K. Fatah, A. Hamsten, B. Blombäck, M. Blombäck, Fibrin gel network characteristics and coronary heart disease: relations to plasma fibrinogen concentration, acute phase protein, serum lipoproteins and coronary atherosclerosis., Thromb. Haemost. 68 (1992) 130–5. https://pubmed.ncbi.nlm.nih.gov/1384157/ (accessed May 1, 2020).

[7] J.D. Mills, R.A.S. Ariëns, M.W. Mansfield, P.J. Grant, Altered Fibrin Clot Structure in the Healthy Relatives of Patients With Premature Coronary Artery Disease, Circulation. (2002) 1938–1942. https://doi.org/10.1161/CIR.0000033221.73082.06.

[8] J.P. Collet, Y. Allali, C. Lesty, M.L. Tanguy, J. Silvain, A. Ankri, B. Blanchet, R. Dumaine, J. Gianetti, L. Payot, J.W. Weisel, G. Montalescot, Altered Fibrin Architecture Is Associated With Hypofibrinolysis and Premature Coronary Atherothrombosis, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 2567–2573. https://doi.org/10.1161/01.ATV.0000241589.52950.4c.

[9] A. Undas, K. Szułdrzynski, E. Stepien, J. Zalewski, J. Godlewski, W. Tracz, M. Pasowicz, K.

Zmudka, Reduced clot permeability and susceptibility to lysis in patients with acute coronary syndrome: Effects of inflammation and oxidative stress, Atherosclerosis. 196 (2008) 551–557. https://doi.org/10.1016/j.atherosclerosis.2007.05.028.

[10] A. Bini, J.J. Fenoglio, R. Mesa-Tejada, B. Kudryk, K.L. Kaplan, Identification and distribution of fibrinogen, fibrin, and fibrin(ogen) degradation products in atherosclerosis. Use of monoclonal antibodies, Arteriosclerosis. 9 (1989) 109–121. https://doi.org/10.1161/01.atv.9.1.109.

[11] A. Undas, J. Zalewski, M. Krochin, Z. Siudak, M. Sadowski, J. Pregowski, D. Dudek, M. Janion, A. Witkowski, K. Zmudka, Altered plasma fibrin clot properties are associated with in-stent thrombosis, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 276–282. https://doi.org/10.1161/ATVBAHA.109.194936.

[12] J. Zalewski, A. Undas, J. Godlewski, E. Stepien, K. Zmudka, No-reflow phenomenon after acute myocardial infarction is associated with reduced clot permeability and susceptibility to lysis, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2258–2265. https://doi.org/10.1161/ATVBAHA.107.149633.

[13] J. Zalewski, J. Bogaert, M. Sadowski, O. Woznicka, K. Doulaptsis, M. Ntoumpanaki, M. Zabczyk, J. Nessler, A. Undas, Plasma fibrin clot phenotype independently affects intracoronary thrombus ultrastructure in patients with acute myocardial infarction, Thromb. Haemost. 113 (2015) 1258–1269. https://doi.org/10.1160/TH14-09-0801.

[14] W. Sumaya, L. Wallentin, S.K. James, A. Siegbahn, K. Gabrysch, M. Bertilsson, A. Himmelmann, R.A. Ajjan, R.F. Storey, Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: a PLATO substudy, Eur. Heart J. 39 (2018) 1078–1085. https://doi.org/10.1093/eurheartj/ehy013.

[15] K. Thygesen, J.S. Alpert, A.S. Jaffe, M.L. Simoons, B.R. Chaitman, H.D. White, H.A. Katus, F.S. Apple, B. Lindahl, D.A. Morrow, P.M. Clemmensen, P. Johanson, H. Hod, R. Underwood, J.J. Bax, R.O. Bonow, F. Pinto, R.J. Gibbons, K.A. Fox, D. Atar, L.K. Newby, M. Galvani, C.W. Hamm, B.F. Uretsky, P.G. Steg, W. Wijns, J.P. Bassand, P. Menasché, J. Ravkilde, E.M. Ohman, E.M. Antman, L.C. Wallentin, P.W. Armstrong, M.L. Simoon, J.L. Januzzi, M.S. Nieminen, M. Gheorghiade, G. Filippatos, R. V. Luepker, S.P. Fortmann, W.D. Rosamond, D. Levy, D. Wood,

S.C. Smith, D. Hu, J.L. Lopez-Sendon, R.M. Robertson, D. Weaver, M. Tendera, A.A. Bove, A.N. Parkhomenko, E.J. Vasilieva, S. Mendis, H. Baumgartner, C. Ceconi, V. Dean, C. Deaton, R. Fagard, C. Funck-Brentano, D. Hasdai, A. Hoes, P. Kirchhof, J. Knuuti, P. Kolh, T. McDonagh, C. Moulin, B.A. Popescu, Ž. Reiner, U. Sechtem, P.A. Sirnes, A. Torbicki, A. Vahanian, S. Windecker, J. Morais, C. Aguiar, W. Almahmeed, D.O. Arnar, F. Barili, K.D. Bloch, A.F. Bolger, H.E. Bøtker, B. Bozkurt, R. Bugiardini, C. Cannon, J. De Lemos, F.R. Eberli, E. Escobar, M. Hlatky, S. James, K.B. Kern, D.J. Moliterno, C. Mueller, A.N. Neskovic, B.M. Pieske, S.P. Schulman, R.F. Storey, K.A. Taubert, P. Vranckx, D.R. Wagner, Third universal definition of myocardial infarction, Circulation. 126 (2012) 2020–2035. https://doi.org/10.1161/CIR.0b013e31826e1058.

[16] S.R. Baker, M. Zabczyk, F.L. Macrae, C. Duval, A. Undas, R.A.S. Ariëns, Recurrent venous thromboembolism patients form clots with lower elastic modulus than those with non-recurrent disease, J. Thromb. Haemost. 17 (2019) 618–626. https://doi.org/10.1111/jth.14402.

[17] A.C. Bouman, H. McPherson, Y.W. Cheung, M. Ten Wolde, H. Ten Cate, R.A.S. Ariëns, A.J. Ten Cate-Hoek, Clot structure and fibrinolytic potential in patients with post thrombotic syndrome, Thromb. Res. 137 (2016) 85–91. https://doi.org/10.1016/j.thromres.2015.11.013.

[18] C. Dassi, L. Seyve, X. García, E. Bigo, R. Marlu, F. Caton, Fibrinography : A Multiwavelength Light-Scattering Assay of Fibrin Structure, Hemasphere. 3 (2019) 1–7.

[19] L. Wilhelmsen, K. Svärdsudd, K. Korsan-Bengtsen, B. Larsson, L. Welin, G. Tibblin, Fibrinogen as a risk factor for stroke and myocardial infarction, N Engl J Med. 311 (1984) 501–505. https://doi.org/10.1056/nejm198811243192103.

[20] M.E. Meltzer, C.J.M. Doggen, P.G. de Groot, F.R. Rosendaal, T. Lisman, Plasma levels of fibrinolytic proteins and the risk of myocardial infarction in men, Blood. 116 (2010) 529–536. https://doi.org/10.1182/blood-2010-01-263103.

[21] A. Hamsten, G. Walldius, A. Szamosi, M. Blombäck, U. Faire, G. Dahlén, C. Landou, B. Wiman, PLASMINOGEN ACTIVATOR INHIBITOR IN PLASMA: RISK FACTOR FOR RECURRENT MYOCARDIAL INFARCTION, Lancet. 330 (1987) 3–9. https://doi.org/10.1016/S0140-6736(87)93050-9.

[22] K.F. Standeven, R.A.S. Ariëns, P.J. Grant, The molecular physiology and pathology of fibrin structure/function, Blood Rev. 19 (2005) 275–288. https://doi.org/10.1016/j.blre.2005.01.003.

[23] N. Spinthakis, Y. Gue, M. Farag, G. Ren, M. Srinivasan, A. Baydoun, D.A. Gorog, Impaired endogenous fibrinolysis at high shear using a point-of-care test in STEMI is associated with alterations in clot architecture, J. Thromb. Thrombolysis. 47 (2019) 392–395.

[24] W. Li, J. Sigley, M. Pieters, C.C. Helms, C. Nagaswami, J.W. Weisel, M. Guthold, Fibrin fiber stiffness is strongly affected by fiber diameter, but not by fibrinogen glycation, Biophys. J. 110 (2016) 1400–1410.

[25] A. Undas, I. Wiek, E. Stêpień, K. Zmudka, W. Tracz, Hyperglycemia is associated with enhanced thrombin formation, platelet activation, and fibrin clot resistance to lysis in patients with acute coronary syndrome, Diabetes Care. 31 (2008) 1590–1595. https://doi.org/10.2337/dc08-0282.

[26] S. He, N. Bark, H. Wang, J. Svensson, M. Blomba, Effects of Acetylsalicylic Acid on Increase of Fibrin Network Porosity and the Consequent Upregulation of Fibrinolysis, J. Cardiovasc. Pharmacol. 53 (2009) 24–29. https://doi.org/10.1097/FJC.0b013e3181953e0f.

[27] R.A. Ajjan, K.F. Standeven, M. Khanbhai, F. Phoenix, K.C. Gersh, J.W. Weisel, M.T. Kearney, R.A.S. Ariëns, P.J. Grant, Effects of aspirin on clot structure and fibrinolysis using a novel in vitro cellular system, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 712–7. https://doi.org/10.1161/ATVBAHA.109.183707.

[28] S. He, M. Blombäck, G. Yoo, R. Sinha, A.H. Henschen-Edman, Modified Clotting Properties of Fibrinogen in the Presence of Acetylsalicylic Acid, Ann. N. Y. Acad. Sci. 936 (2001) 531–535. https://doi.org/10.1111/j.1749-6632.2001.tb03540.x.

[29] S. Neergaard-Petersen, S.B. Larsen, E.L. Grove, S.D. Kristensen, R.A. Ajjan, A.-M. Hvas, Imbalance between fibrin clot formation and fibrinolysis predicts cardiovascular events in patients with stable coronary artery disease, Thromb. Haemost. 120 (2020) 75–82.

[30] A. Antovic, C. Perneby, G.J. Ekman, H.N. Wallen, P. Hjemdahl, M. Blombäck, S. He, Marked increase of fibrin gel permeability with very low dose ASA treatment, Thromb. Res. 116 (2005) 509–17. https://doi.org/10.1016/j.thromres.2005.02.007.

[31] J. Silvain, J.P. Collet, C. Nagaswami, F. Beygui, K.E. Edmondson, A. Bellemain-Appaix, G. Cayla,

A. Pena, D. Brugier, O. Barthelemy, G. Montalescot, J.W. Weisel, Composition of coronary thrombus in acute myocardial infarction, J. Am. Coll. Cardiol. 57 (2011) 1359–1367. https://doi.org/10.1016/j.jacc.2010.09.077.

[32] World Health Organization, Cardiovascular diseases, Https://Www.Who.Int/Cardiovascular_diseases/About_cvd/En/. (n.d.).

[33] A.E.X. Brown, R.I. Litvinov, D.E. Discher, P.K. Purohit, J.W. Weisel, Multiscale Mechanics of Fibrin Polymer: Gel Stretching with Protein Unfolding and Loss of Water, Science (80-.). 325 (2009) 741–744. https://doi.org/10.1126/science.1172484.

[34] M.M. Domingues, F.L. Macrae, C. Duval, H.R. McPherson, K.I. Bridge, R.A. Ajjan, V.C. Ridger, S.D. Connell, H. Philippou, R.A.S. Ariens, Thrombin and fibrinogen ' impact clot structure by marked effects on intrafibrillar structure and protofibril packing, Blood. 127 (2016) 487–495. https://doi.org/10.1182/blood-2015-06-652214.

533 **Table 1: Characteristics of AMI patients and healthy controls.**

Variables	AMI group (n=23)	Healthy controls (n=24)
Male gender (%)	13 (56.5)	13 (54.2)
Age, yrs (IQR)	64.5 (57.3−78.8)	27.5 (25.3−39.3)
Max. hs TnT, ng/mL (IQR)	1.4 (0.07−4.9)	-
Glucose, mmol/L (IQR)	6.2 (5.6−11.6)	-
TC, mmol/L (SD)	5.2 (±1.2)	-
LDL, mmol/L (SD)	3.4 (±1.1)	-
HDL, mmol/L (SD)	1.5 (±0.5)	-
TG, mmol/L (SD)	1.3 (±0.6)	-
AST, U/L (IQR)	47.0 (29.0−132.0)	-
ALT, U/L (IQR)	28.0 (23.0−41.0)	-
hsCRP, mg/L (IQR)	4.9 (1.57−20.1)	-
Creatinine, umol/L (IQR)	80.5 (71.3−89.0)	-
eGFR, ml/min (SD)	75.7 (±21.4)	-
Lipid profile		
Total cholesterol, mmol/L	5.11 (4.46-5.70)	-

Low density lipoprotein cholesterol, mmol/L	3.44 (2.66-3.90)	-
High-density lipoprotein cholesterol, mmol/L	1.5 (1.08-1.85)	-
Triglycerides, mmol/L	1.1 (0.94-1.54)	-
Medical history		
Weight, kg (SD)	80.6 (±16.7)	-
Waist circumference, cm (SD)	99.9 (±16.4)	-
BMI, kg/m^2 (SD)	27.2 (25.2−30.7)	-
STEMI, n (%)	9 (39)	0 (0)
NSTEMI, n(%)	14 (61)	0 (0)
Previous MI, n (%)	7 (30.4)	0 (0)
Obesity, n (%)	12 (52.2)	-
Smoking, n (%)	8 (34.8)	0 (0)
Diabetes, n (%)	10 (43.5)	0 (0)
Chronic kidney disease, n (%)	4 (17.4)	0 (0)
EF, % (SD)	46.0 (±11.4)	-
SBP, mmHg (SD)	149.3 (±22.4)	-
DBP, mmHg (SD)	89.9 (±19.2)	-

Hypertension, n (%)	22 (95.7)	0 (0)
Hypercholesterolemia, n (%)	23 (100)	0 (0)
Hypertriglyceridemia, n (%)	2 (8.7)	0 (0)
Medications		
Beta-blocker, n (%)	21 (91.3)	0 (0)
ACEI, n (%)	20 (87)	0 (0)
ARB, n (%)	3 (13)	0 (0)
ASA, n (%)	23 (100)	0 (0)
Nitrates, n (%)	1 (4.3)	0 (0)
CCB, n (%)	5 (21.7)	0 (0)
Fibrates, n (%)	0 (0)	0 (0)
Loop diuretics, n (%)	7 (30.4)	0 (0)
Diuretic, n (%)	3 (13)	0 (0)
MRA, n (%)	9 (39.1)	0 (0)
Statin, n (%)	22 (95.7)	0 (0)
Fibrate, n (%)	0 (0)	0 (0)
Insulin, n (%)	5 (21.7)	0 (0)
Metformin, n (%)	4 (17.4)	0 (0)

Acarbose, n (%)	0 (0)	0 (0)
DPP-IV, n (%)	0 (0)	0 (0)
Sulfonylurea, n (%)	1 (4.3)	0 (0)

Data are given as mean (±SD), median (IQR) or number (%).

Abbreviations: hs TnT, high sensitive cardiac troponin T; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hs CRP, high sensitive C-reactive protein; eGFR, estimated glomerular filtration rate (CKD-EPI formula); STEMI, ST-elevation myocardial infarction; BMI, body mass index; MI, myocardial infarction; EF, ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; CCB, calcium channel blocker; MRA, mineralocorticoid receptor antagonists; DPP-IV, dipeptidyl peptidase IV inhibitors.

Table 2. The model for clot formation and breakdown.

Variable	Healthy Controls	AMI	P-value
Fibrinogen, g/L (SD)	2.8 (± 0.5)	4.0 (± 1.3)	<0.0001
Fibers per 100 µm (IQR)*	18.6 (18.3 – 20.3)	27.7 (24.5 – 28.5)	<0.0001
Fiber size, nm (SD)*	109.4 (± 7.6)	124.1 (± 14.9)	0.0050
Pore Size, (K_s) x 10^{-9} cm^2 (SD)*	76.0 (± 22.5)	37.0 (± 19.4)	<0.0001
Clot Formation			
Max OD, AU (SD)*	0.42 (± 0.09)	0.47 (± 0.16)	0.0002 #
Lag Time, s (IQR)*	256.2 (227.0 – 281.0)	318.3 (241.3 – 374.0)	0.0016
Time to Max OD, s (SD)*	6712.0 (± 1189.0)	4870.0 (± 835.6)	<0.0001

Average rate of Clotting, ΔOD/s (IQR)*	0.21 (0.17 – 0.36)	0.83 (0.73 – 1.21)	<0.0001
Maximum rate of Clotting, ΔOD/s (SD)*	0.91 (± 0.26)	1.14 (± 0.36)	0.6384
Clot Lysis			
Plasminogen, ug/mL (SD)	40.50 (± 12.94)	32.78 (± 8.30)	0.020
Time from Max OD to maximum rate of lysis, s (IQR)**	540.0 (424.0 – 1788.0)	4840.0 (4355.0 – 5352.0)	<0.0001
Time to 25% Lysis, s (IQR)**	628.0 (556.0 – 900.0)	3472.0 (2772.0 – 4104.0)	<0.0001
Time to 50% Lysis, s (IQR)**	1584.0 (1292.0 – 2372.0)	4302.0 (3548.0 – 4867.0)	<0.0001
Time to 75% Lysis, s (SD)**	4621.0 (± 1337.0)	5031.0 (± 1055.0)	0.4214
Fiber internal structure			
Fiber Radius, (Turbidimetry), nm (IQR)	99.3 (96.1 – 100.3)	100.0 (96.4 – 101.7)	0.232
Number of Protofibrils, N (SD)	211.2 (± 37.1)	182.2 (± 56.8)	0.034
Mass/ Length Ratio, Da/cm x 10^{13} (SD)	3.01 (± 0.71)	2.62 (± 0.82)	0.089
Protofibril Distance, nm (SD)	12.2 (± 1.1)	13.5 (± 2.1)	0.013

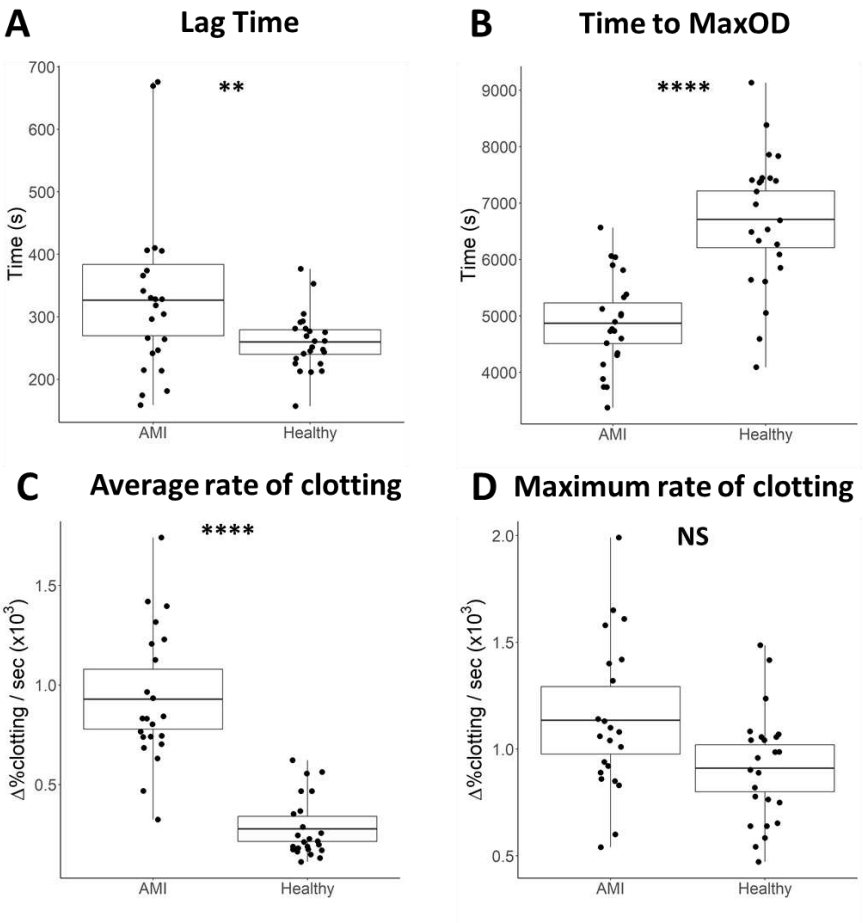
*** Raw data is shown, P-values are adjusted for differences on fibrinogen concentration.**

**** Raw data is shown, P-values are adjusted for differences in fibrinogen and plasminogen concentrations (**see *Statistical analysis* section)

 # After adjustment for fibrinogen concentration, MaxOD in AMI samples was significantly lower than healthy controls.

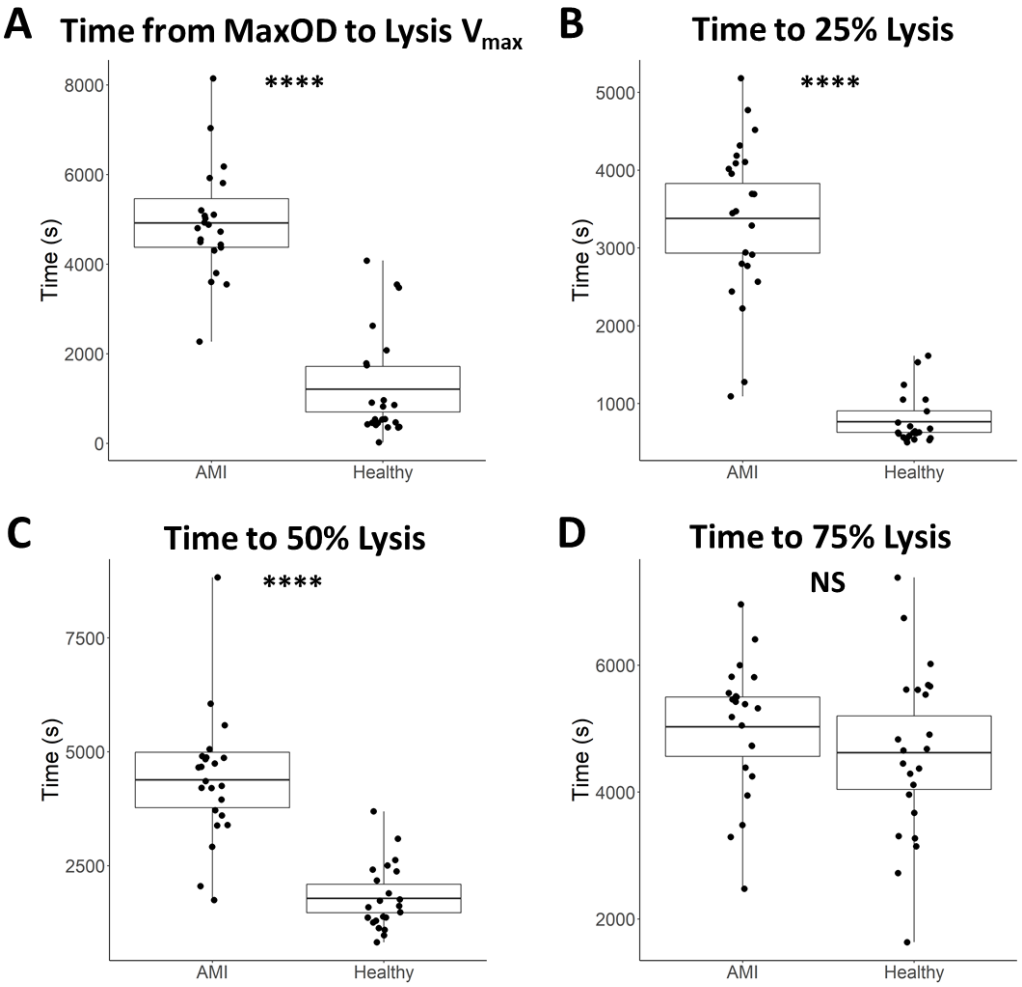
Abbreviations: AMI, acute myocardial infarction; FGN, fibrinogen; SD, standard deviation; IQR, interquartile range; Max OD, maximum absorbance.

552 **FIGURES**



553

554 **Figure 1: Turbidity for AMI plasma clots compared to healthy controls.** Lag time was significantly
555 higher for AMI clots than control clots (A). Healthy control samples had a prolonged time to Max OD (B)
556 and a reduced average rate of clotting (C). These results were consistent after adjusting for fibrinogen
557 concentration. No significant difference was found between groups when comparing maximum rate of
558 clotting (D). Data shown as mean with 95% CI (box) and min/max (whiskers). *** P<0.01, **** P< 0.0001.*



559

560 **Figure 2: Fibrinolysis for AMI plasma clots compared to healthy controls.** Plasma clots from AMI
561 samples had a prolonged time from maximum absorbance (Max OD) to maximum rate of lysis (A), when
562 compared to healthy controls. This was also reflected in the lysis profiles where AMI clots had prolonged
563 time to 25% (B) and 50% (C) lysis, when compared to healthy controls. Time to 75% lysis (D) values were
564 similar between groups. Data shown as mean with 95% CI (box) and min/max (whiskers). ***** P<0.0001.*

565

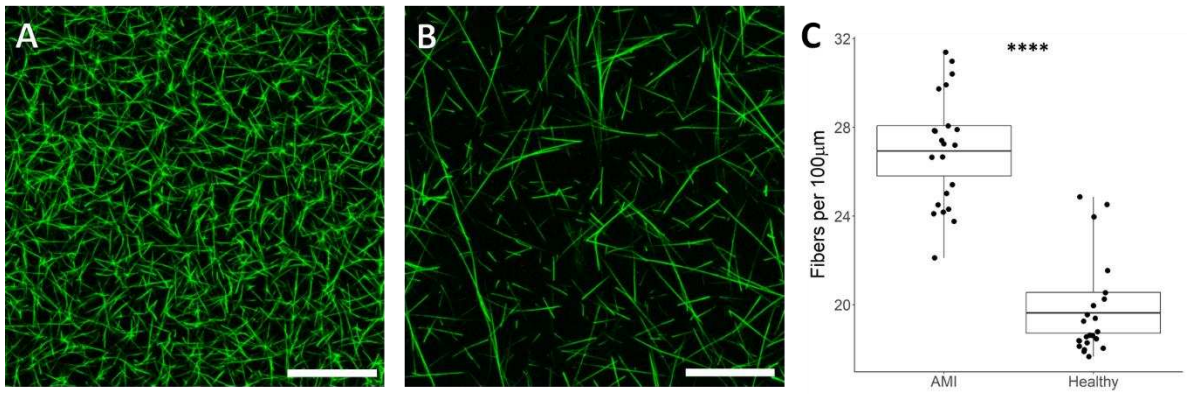
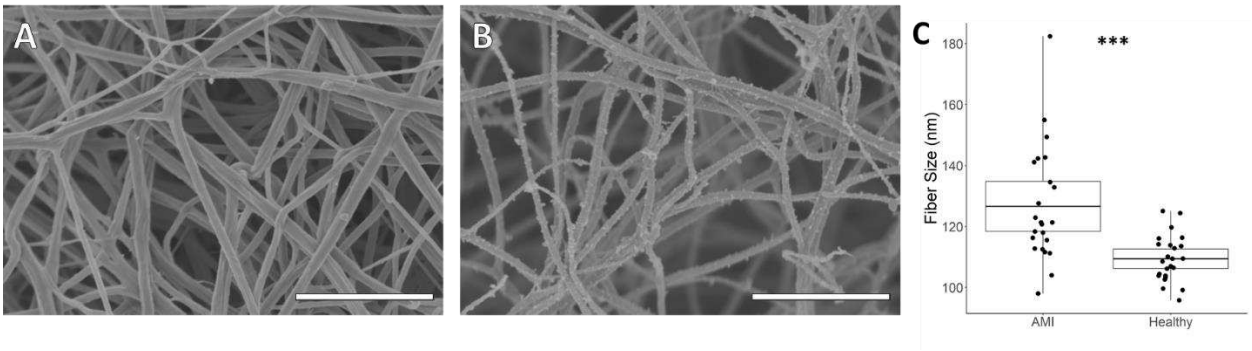


Figure 3: Comparison of final plasma clot structure using confocal microscopy. Representative images for clots from AMI patients (A) and healthy controls (B). AMI samples formed denser clots than those formed from healthy controls (C). Images (A, B) are represented as Z-stacks flattened at maximum intensity. Scale bar is 50 µm. Data shown as mean with 95% CI (box) and min/max (whiskers). **** *P<0.0001.*



572

573 **Figure 4: High resolution imaging comparison of fully formed plasma clots using scanning electron**
574 **microscopy.** Representative images for clots from AMI patients (A) and healthy controls (B). AMI samples
575 formed fibers that were larger in diameter than those formed from healthy (C). Scale bar (A, B) is 2 µm.
576 Data shown as mean with 95% CI (box) and min/max (whiskers). **** P<0.001.*

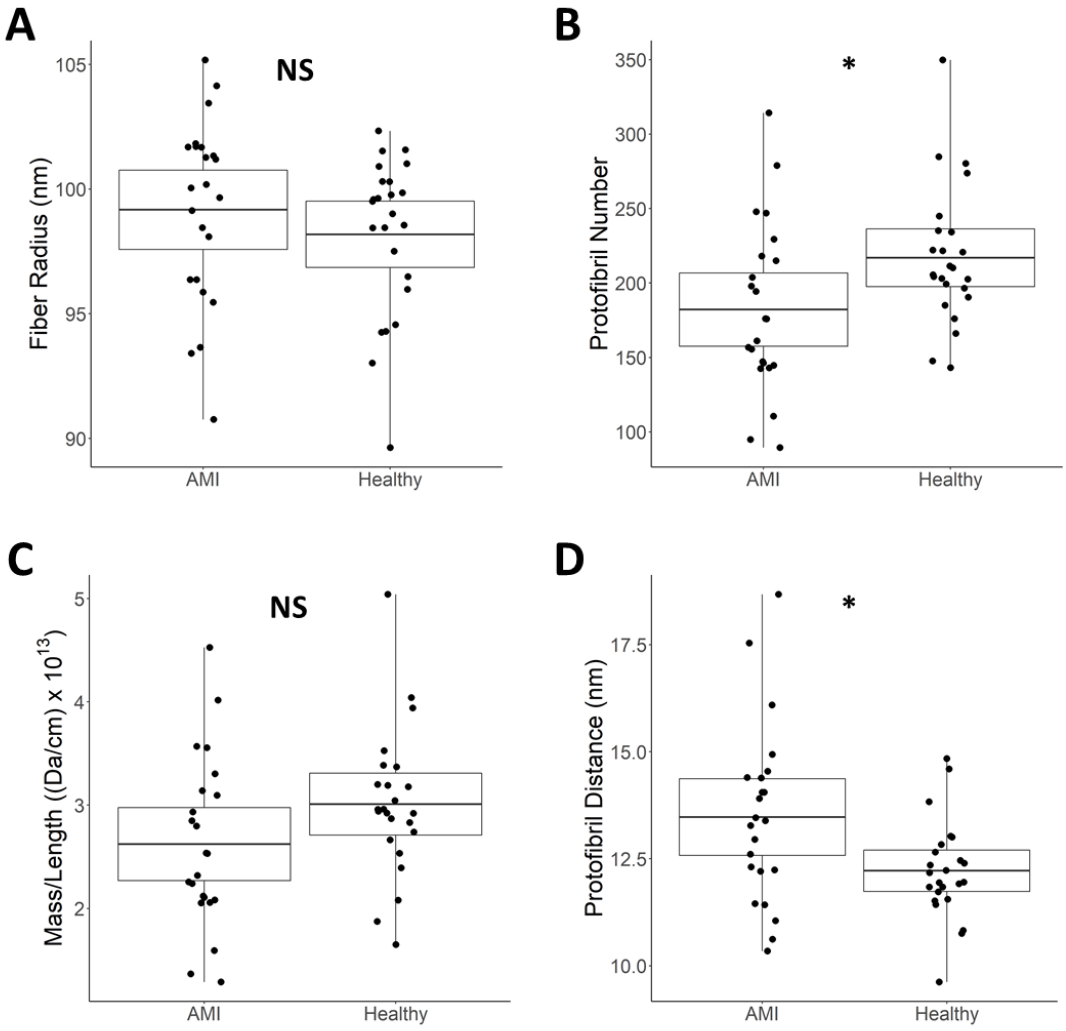


Figure 5: Average fiber radius and internal structure. AMI patients fibers formed clots with similar radius (A), but contained less protofibrils (B), than healthy controls. The mass/length ratio, the distribution of the protofibrils within a single fiber, was similar between the two groups (C). The distance between protofibrils was greater for fibers formed from AMI clots than for those from healthy controls (D). Data shown as mean with 95% CI (box) and min/max (whiskers). ** P<0.05*