

Morphological and poro-mechanical characterization of thrombi from aortic aneurysm

Leonet Joris, Baudouard Marc, Mariangela de Masi, Carine Guivier-Curien,

Eric Bertrand, Hubert Lepidi, Jerome Vicente, Valerie Deplano

▶ To cite this version:

Leonet Joris, Baudouard Marc, Mariangela de Masi, Carine Guivier-Curien, Eric Bertrand, et al.. Morphological and poro-mechanical characterization of thrombi from aortic aneurysm. pp.5186-5188, 2022. hal-03859503

HAL Id: hal-03859503 https://hal.science/hal-03859503

Submitted on 28 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Morphological and poro-mechanical characterization of thrombi from aortic aneurysm

J. Léonet^a, M. Baudouard^a, M. De Masi^{a,b}, C. Guivier-Curien^a, E. Bertrand^a, H. Lepidi^b, J. Vicente^c and V. Deplano^a

^aAix-Marseille Univ, CNRS, Ecole Centrale, IRPHE UMR7342, Marseille, France; ^bAix-Marseille Univ, AP-HM, Service de chirurgie vasculaire hôpital de la Timone, Marseille, France; ^cAix-Marseille Univ, IUSTI UMR7343, Marseille, France

1. Introduction

Intraluminal thrombus (ILT) is a fibrin structure with an aggregation of platelets, blood cells, blood proteins and cellular debris leading to a fluid-permeable structure by means of a network of canaliculi. ILT is organised in 3 distinct layers with different composition and structure depending on the level of thrombus ageing and leading to permeability gradients (Wilson 2013). ILT formation often occurs in aneurysmal aortas and its role in the pathology evolution is still debating. A part of the answer can be tackled through 3D thrombus morphology analysis and mechanical characterisation that are both not widely known. Only one experimental study investigated thrombus permeability from abdominal aortic aneurysm (AAA) (Boschetti 2007) considering the distinct thrombus layers. Histological studies can provide ILT compositions but only 2D morphology. Finally, only one work performed micro-computed tomography (μ CT) measurements, but no 3D microstructure was provided (Karagiannidis 2020). The objectives of this study are therefore to propose a new permeability experiment, μ CT measurements to obtain the 3D morphology of thrombus and histological study to link microstructure and permeability.

2. Methods

Four thrombi were removed from AAAs of human donors (average age 65 years). Fourteen disk samples, 16 slices and 10 cylindrical samples extracted from these 4 thrombi were used to performed permeability, histology and μ CT analysis, respectively.

2.1. Permeability

After removal, ILTs were placed in DMEM solution and stored at 4° C or frozen. The three layers of each of them were first separated and 14mm diameter disks were cut from each of these layers using a sharp punch. The 14 samples were divided into 9 luminal disks, 2 abluminal, 1 medial and 2 composed by the medial and abluminal layers because the transition between the layers was unclear. Each disk was placed into a homemade permeameter set-up. Agarose was used for few disks with diameter lesser than 14mm to compensate the permeameter cell. The permeability of samples was calculated according to the Darcy's law measuring the flowrate through the disks under constant hydrostatic pressure of 35mmHg and/or 100mmHg.

2.2. Histology

Samples from the same four ILTs were submitted to histological study. ILTs were fixed in 10% formalin solution, embedded in paraffin through conventional methods. Then, they were stained with Hematoxylin and Eosin (H&E) to highlight erythrocytes, leucocytes and fibrin. To bring out platelets, an immunohistology technique was performed using CD61 antibodies.

2.3. μ**CT**

Cylindrical samples of 3mm diameter were cut into the ILTs thickness keeping their three layers. Samples were first washed in distilled water to remove DMEM solution. They were then fixed in 4% formalin solution for 24h. After fixation, samples were subjected to a dehydration process, gradually stored in pure alcohol solution. Finally, specimens were immersed in 0.6% phosphotungstic acid (PTA) in 70% alcohol solution to improve tissue contrast. After the preparation, samples were immersed in pure alcohol and tested using a μ CT with 3μ m resolution. Porosity, interconnectivity and permeability were assessed using iMorph (Brun 2009).

3. Results and discussion

3.1. Permeability studies

Different values of permeability were obtained according to the tested layer. Permeabilities between $2.2.10^{-14}$ m² and $3.8.10^{-18}$ m² were measured for luminal disks depending on the use of agarose. No perfusion was reported for 4 luminal disks. Concerning medial and abluminal disks, none have perfused. These findings suggests that the luminal layer is fluid-permeable due to a canaliculi network. The study of Boschetti obtained approximatively the same permeability mean values for luminal, medial abluminal laver (3.10^{-17}m^2) 4.10^{-17}m^2 and $3.5.10^{-17}$ m², respectively). The result provided by



Figure 1. a) Overview of histological image. b) Abluminal layer. c) Medial layer (H&E). d) Luminal layer (CD61).



Figure 2. a) Vertical cut of ILT sample under μ CT. b) Luminal layer. c) Medial layer. d) Abluminal layer.

Boschetti is unexpected regarding the inhomogeneous ILT structure.

3.2. Histological analysis

Sixteen slides from the 4 ILTs were investigated by histological analysis. Figure 1 is an illustration of the results obtained. Luminal layer is mainly composed by erythrocytes and contains also leucocytes concentrated in loose fibrin network (not shown). The immunohistology reveals that a high number of platelets is present in the luminal layer (Figure 1d) contrary to the medial and abluminal layers where low concentration of platelets is observed. The porous medial layer shows agglomerated erythrocytes (Figure 1c). Finally, abluminal layer is composed by a condensed oriented fibrin network without porosity.

3.3. μ CT studies

Figure 2 is an illustration of the results obtained from μ CT. ILT shows a non-homogeneous structure through the thickness leading to porosity gradients (Figure 2a). The luminal layer is composed by loose fibrin networks. Considering the maximal representative elementary volume a mean pore diameter of 8.8μ m and a porosity of 7.4% are obtained (Figure 2b). The medial layer shows higher porosity with 8.2% and a mean pore diameter of 28.8µm (Figure 2c). Finally, abluminal layer is composed by dense fibrin fibers networks with lower porosity (4%) compared to the medial layer (Figure 2d). For both medial and abluminal layers, the average spacing between pores is high, 81µm and 150µm, respectively and they are not permeable at this scale. On the contrary, the pores are closer in the luminal layer with an average spacing of 54.8µm and few regions with connected pore clusters. The permeability of luminal layer is equal to $3.79.10^{-14}$ m².

3.4. Discussion

At macroscale different permeability values were obtained for the luminal layer. This difference may be explained using agarose and by the variability of the ILT composition. Even if only one measurement of permeability was performed using μ CT, macroscale and microscale values are comparable, and the structure of porous networks is consistent with these permeability measurements. Concerning both abluminal and medial layers macro and microscale permeability measurements agree. Finally, the microstructure obtained by μ CT is consistent with histology.

4. Conclusions

For the first time in the literature μ CT analysis on ILT allowed to obtain its 3D morphology with a resolution of 3μ m. This work is ongoing increasing the ILT sample number and improving resolution using a nanotube. Mechanical testing will be performed. These developments will allow to build a porous model for ILT combining data obtained at micro and macro scales to assess the role of the thrombus in pathology evolution.

Acknowledgements

Initiative d'Excellence d'Aix-Marseille Université- *MIDEX (AMX-19-IET-010).

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Boschetti F, Di Martino EM, Giota G. 2007. A poroviscoelastic model of intraluminal thrombus from abdominal aortic aneurysms. In: 2007 Summer bioengineering conference, Keystone, CO.
- Karagiannidis E, Konstantinidis NV, Sofidis G, et al. 2020. Rationale and design of a prospective, observational study for the quantitative estimation of thrombus burden in patients with ST-elevation myocardial infarction using

micro-computed tomography: the QUEST-STEMI trial. BMC Cardiovasc Disord. 20:1-8.

- Brun E, Vicente J, Topin F, et al. 2009. iMorph : an open source software for 3D structural and geometrical analysis of porous material. In: Proceedings of Metfoam. Bratislava, Slovakia.
- Wilson JS, L Virag, P. Di Achille, et al. 2013 Biochemomechanics of intraluminal thrombus in abdominal aortic aneurysms. J. Biomech. Eng. 135:021011.

KEYWORDS Intraluminal thrombus; aneurysmal pathology; micro-CT; permeability; histology

valerie.deplano@univ-amu.fr