Supplementary Information

Platelet packing density is an independent regulator of the hemostatic response to injury

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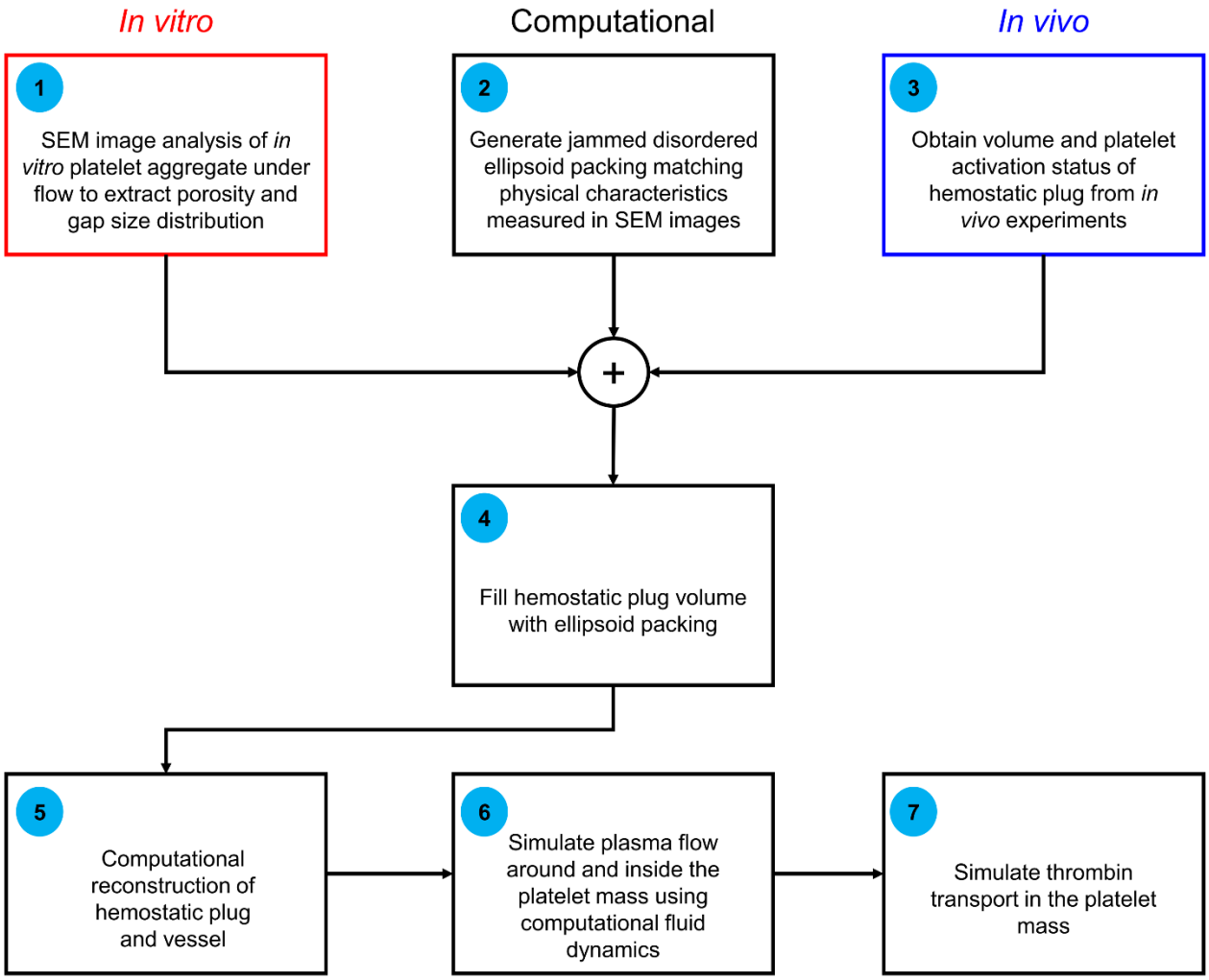


Figure S1. Overall approach flow chart.

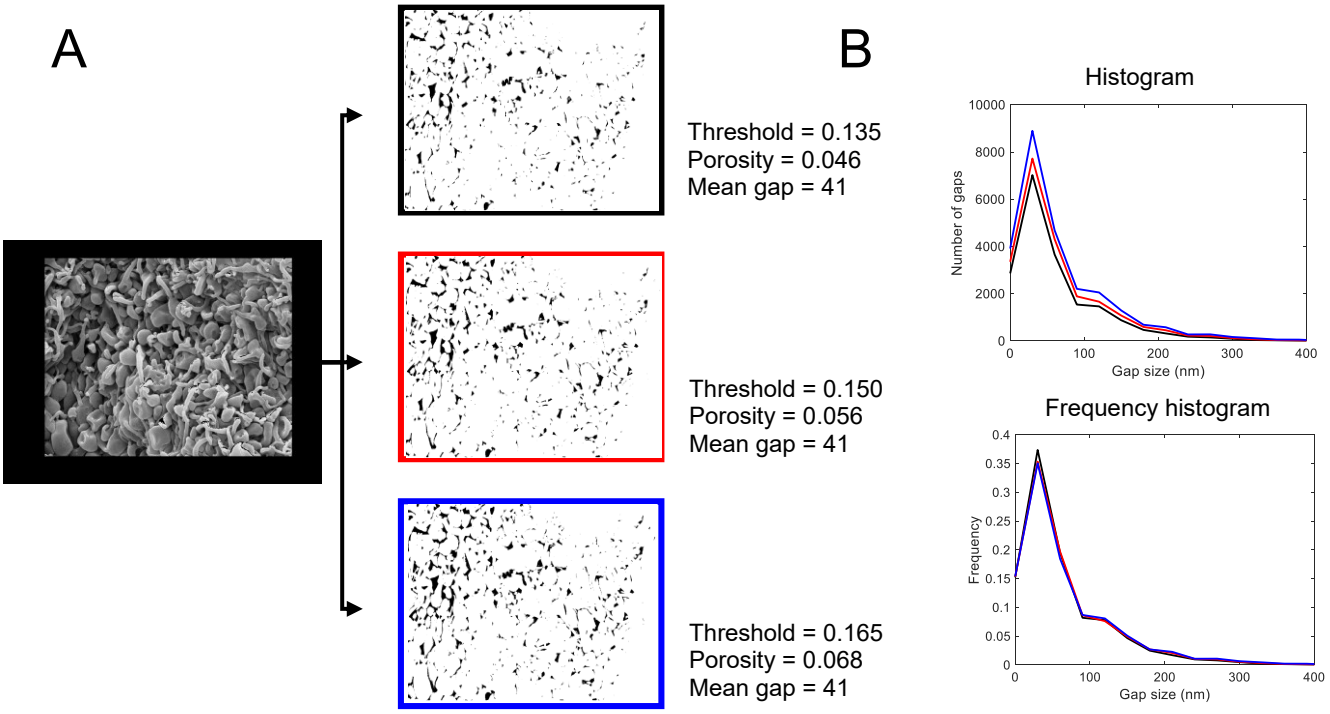


Figure S2. **Sensitivity analysis of SEM image analysis method. A)** We perturbed the value of the binary threshold by ±10% and evaluated the sensitivity index of the porosity and the mean gap size of the gap size distribution according to

$$s_{ij} = \partial \log X_i / \partial \log p_j = (dX_i / X_i) / (dp_j / p_j)$$

The local sensitivity s_{ij} reflects the relative change in the quantitative feature calculated for the model's *i*th output variable induced by a small relative change in the model's *j*th parameter. The sensitivity index for the porosity is 8.6 (± 1.2), while the sensitivity index for the mean gap size is 2.1 (± 2.1). These results indicate that the porosity changes approximately 8% with a 10% change in the threshold, while the mean gap size is not affected, indicating that our method is robust to small perturbations of the threshold value. **B)** The histogram and frequency histogram of the binary images illustrate that the gap size distributions obtained using the different thresholds are very similar.

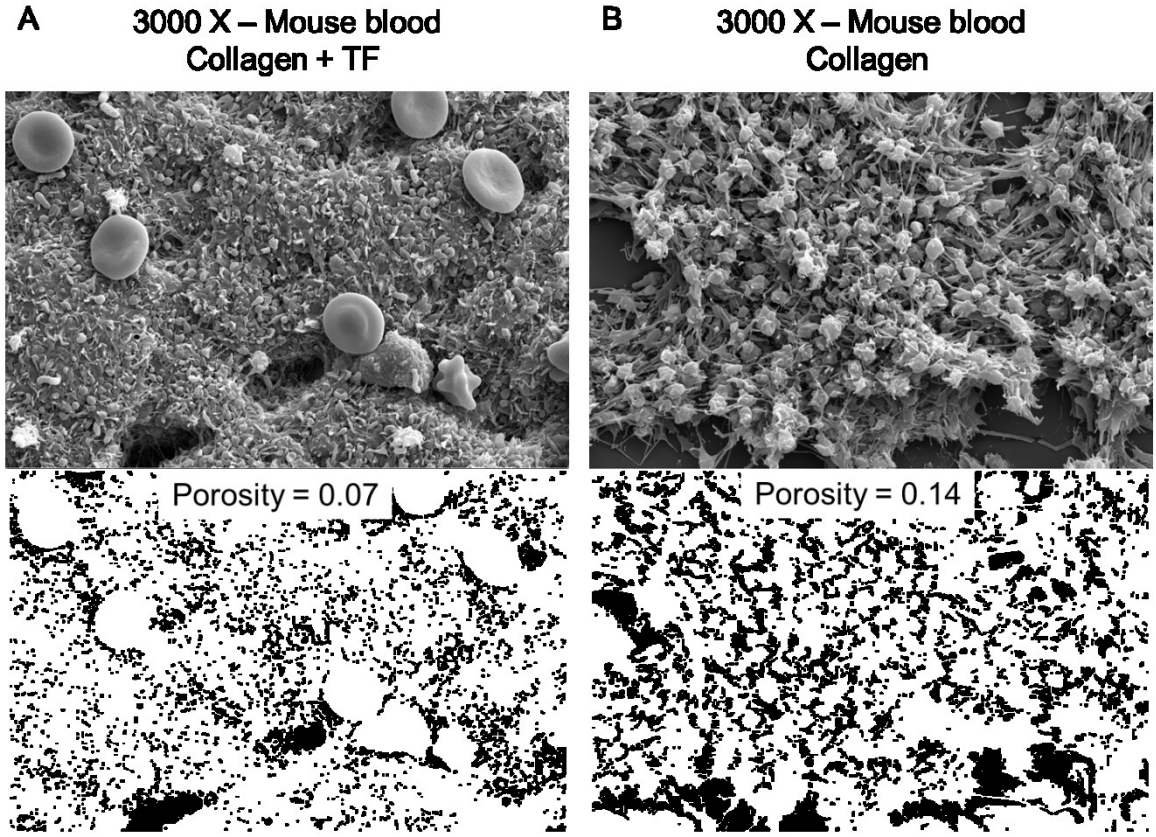


Figure S3. Platelet aggregate microenvironment on different procoagulant surfaces. SEM image (top) and corresponding binarized version (bottom) of a platelet aggregate flowed at venous shear rate over collagen plus tissue factor (**A**) or collagen alone (**B**). The extracted 2D porosities recapitulate the visual differences between the two treatments.

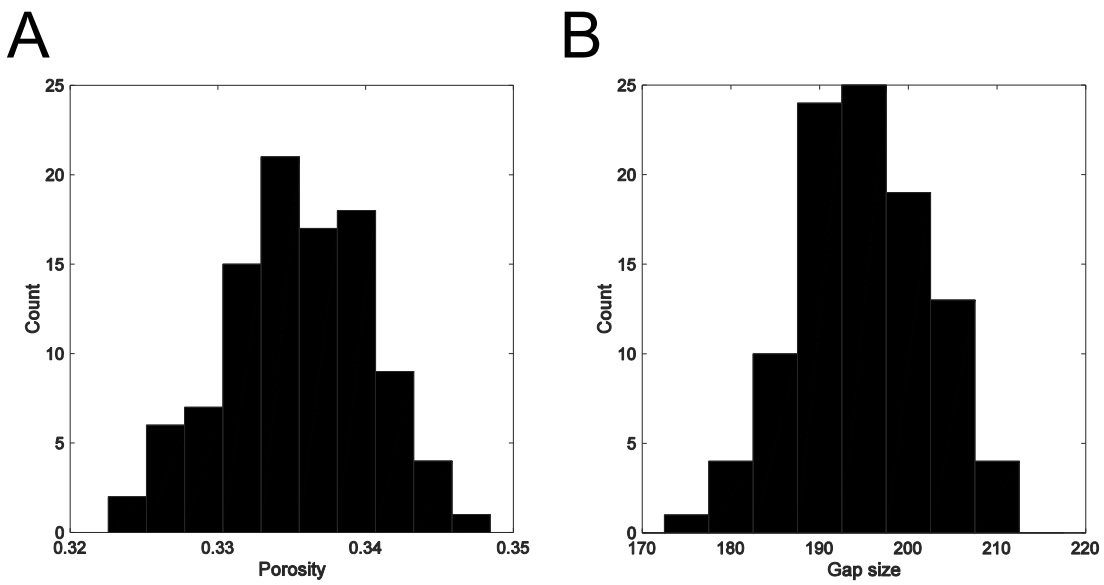


Figure S4. **Reproducibility of the packing algorithm**. To illustrate the robustness of the packing algorithm 100 runs with the 400 identical ellipsoids. The results show that there is minor variation in porosity (Mean = 0.335, Standard deviation = 0.004) (**A**) and mean gap size (Mean = 195, Standard deviation = 7.4) (**B**).

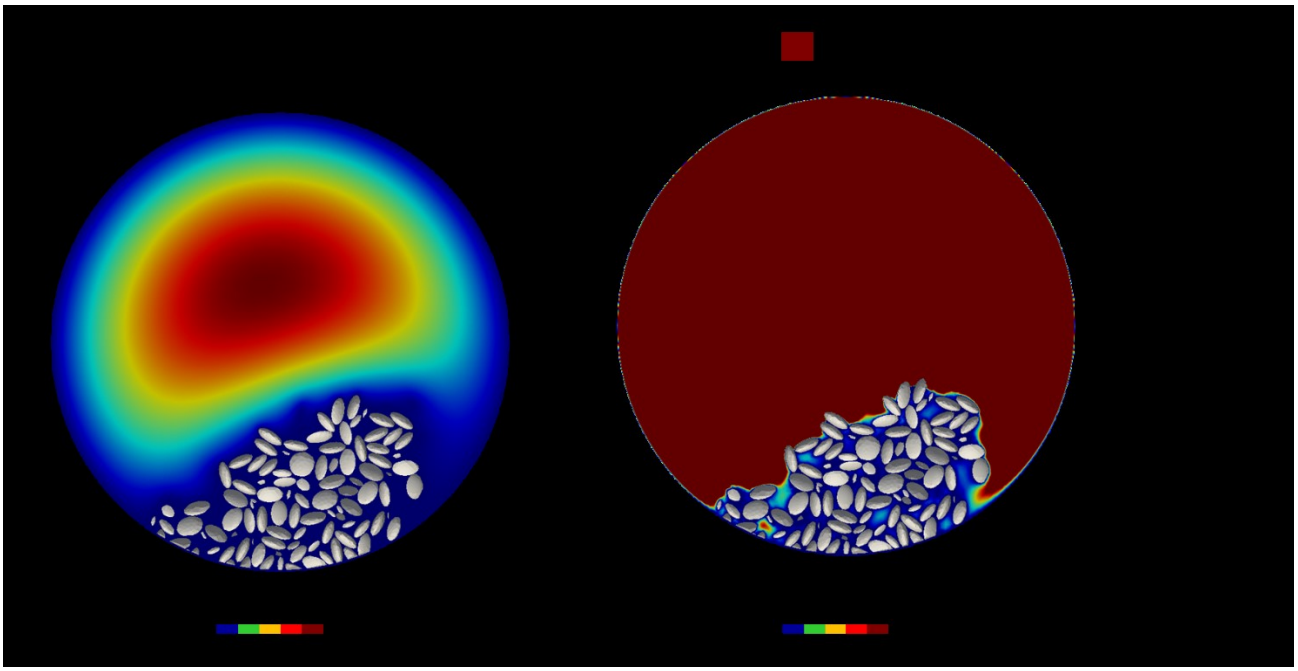
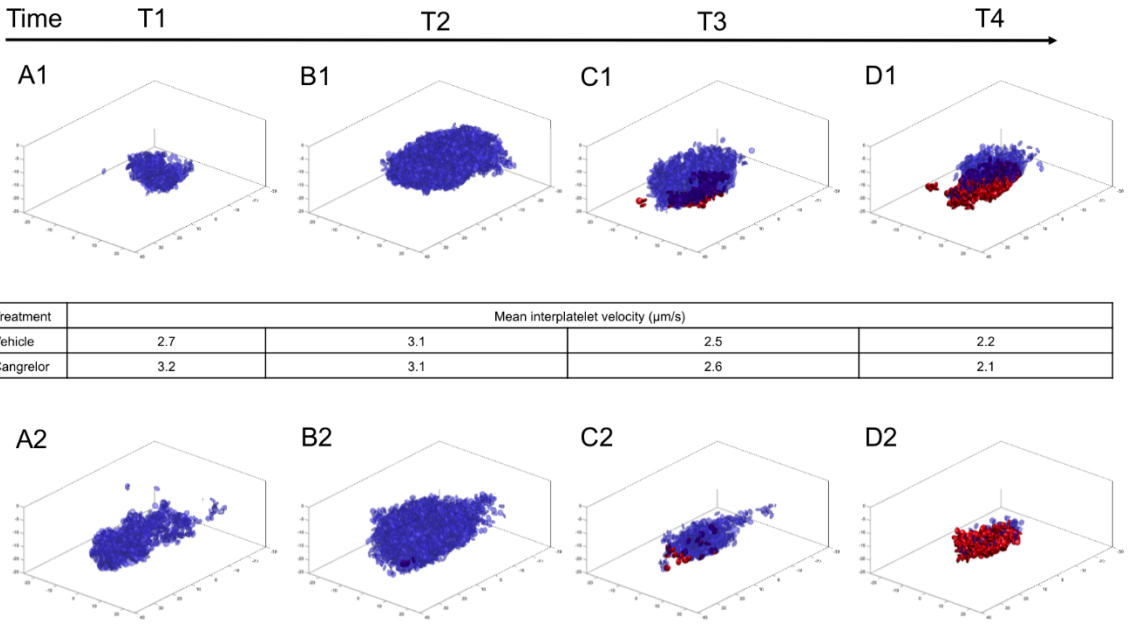


Figure S5. **Interplatelet plasma velocity**. Plasma velocity visualized in a cross section of a reconstructed hemostatic platelet plug. The two images are displayed with different velocity scales to better illustrate the reduction of plasma velocity in the platelet mass.



Treatment	Mean interplatelet velocity (μm/s)			
Vehicle	2.7	3.1	2.5	2.2
Cangrelor	3.2	3.1	2.6	2.1

Figure S6. Interplatelet plasma velocity during hemostatic plug evolution. A hemostatic plug captured during its evolution in vehicle (**A1-D1**) and cangrelor (**A2-D2**) treated mice. The four different temporal stages correspond to the initial platelet recruitment (T1), maximum volume (T2), initial core detection (T3), stable configuration at 20 mins post-injury (T4). The interplatelet plasma velocity is minimally affected by either the stage or the treatment.

2. Supplementary tables

Table S1 – effect of ellipsoids aspect ratio

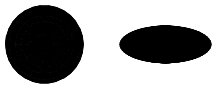
Semi-axis ratio	2D shape projections	Porosity	Mean gap size (nm)	Mean interplatelet velocity (μm/s)
1-1-0.4		0.33	195	2.52
1-1-1		0.36	250	2.45
1-1-0.25		0.39	204	2.72

Table S2 – interplatelet plasma velocity sampled along the flow direction

Interplatelet velocity (μm/s)	Front	Middle	Back
Core	0.789	0.568	0.749
Shell	1.053	0.729	0.845

Table S3 – relative thrombin concentration sampled along the flow direction

Relative thrombin concentration	Front	Middle	Back
Core	0.7	0.9	0.6
Shell	0.2	0.3	0.2

3. Computing the gap size distribution and the porosity

Gap size distribution Matlab script

```
function cl = chordLengthDistribution2D(binImage,pixelSize)

% lines preceded by % signs are comments and are not read by the program
% binImage is a binary version of an image, 0 is an empty spaces, 1 is a space occupied by a platelet (or fibrin)
% pixelSize is the size of the pixel in nm

% empty vector to store all chords
cl = [];

% procedure across rows
for i=1:size(binImage,1),

   ccl = 0;

   % when a zero is encountered the algorithm starts recording a gap
   % it proceeds to elongate the gap until a 1 is encountered
   for k=1:size(binImage,2),
      if(binImage(i,k)==0)
         ccl = ccl + pixelSize;
      % when a 1 is encountered, the algorithm stores the length of the gap and resets it to zero
      elseif(binImage(i,k)==1)
         cl = [cl,ccl];
         ccl = 0;
      end
   end
end

% same procedure as above across columns
for i=1:size(binImage,2),

   ccl = 0;

   for k=1:size(binImage,1),
      if(binImage(k,i)==0)
         ccl = ccl + pixelSize;
      elseif(binImage(k,i)==1)
         cl = [cl,ccl];
         ccl = 0;
      end
   end
end

% retains all non zero gaps
cl = cl(cl>0);

% end of file
```

This Matlab script reads the binary image supplied line by line first and column by column after that. If an empty space is encountered (represented by a 0) the start of a gap is recorded. The gap will keep elongating until a space occupied by a platelet is found (represented by a 1). The next gap will start when the next empty space is found. At the end all the zero-length gaps are eliminated from the analysis. The result is a vector containing all the horizontal and vertical gaps in the supplied image. Given a vector of bin sizes a histogram of gap sizes is computed.

Porosity measurements

The porosity is calculated from a binary image by counting the sum of all empty spaces divided by the size of the image

$$\varepsilon = \frac{N(pixels = 0)}{Total\ pixels}$$

The porosity value measured from the SEM images represents a 2D porosity. The ellipsoid packings are in 3D. To appropriately compare the 2D porosity computed from the experimental data with the computationally reconstructed ellipsoid packings we obtain cut planes spaced 10 nm apart in each of the three dimensions of the 3D packings and compute the 2D porosity. The end result is a distribution of 2D porosity values from which an average porosity is obtained. This way a 2D surface porosity measured from the data can be compared directly with an appropriately derived 2D porosity value, and indirectly with the 3D porosity, of the computational counterpart.