Microfluidic systems with vascular biomimetic surfaces for assessment of platelets and fibrin components of hemostasis: comparison with a classic annular device in the evaluation of the antithrombotic effects of apixaban

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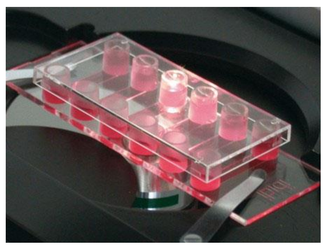
- **Background:** Blood flow devices have improved our understanding on the mechanisms of bleeding and thrombotic disorders. The main inconvenient is that use of the classic devices is not standardized between different laboratories. New microfluidic technologies have been widespread implemented by reducing blood volumes and simplifying the evaluation process. Studies comparing the performance of microfluidic and classic devices are needed.
- **Aims:** Compare the results of a microfluidic model using a biomimetic vascular surface vs. a classic annular device exposing genuine subendothelial components. Validate their performance evaluating the in vitro antithrombotic effects of apixaban (APIX).

METHODS

Citrated-recalcified blood aliquots were exposed to thrombogenic surfaces under flow conditions (5 min, arterial-like shear rate 800/s).

1) Compare microfluidic *vs*. classic annular perfusion device
2) Evaluate platelet and fibrin deposition onto perfused surfaces.
3) We tested the effect of different APIX concentrations (0, 10, 40 and 160 ng/mL) incubated for 30 min (at 37ºC)

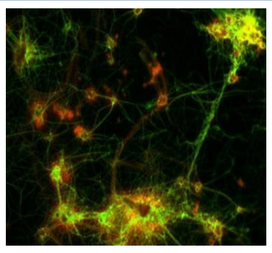
Annular flow chamber with damaged arterial vessel



IBIDI µ-Slide VI [0.4]

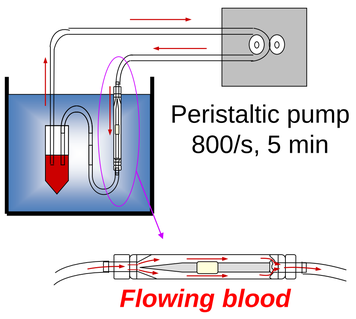
Blood volume *per* condition: 0,3 mL (not-recirculated)

- Fixation
- Immunofluorescence
  - Block
  - Primary antibodies
  - Secondary flourescent antibodies

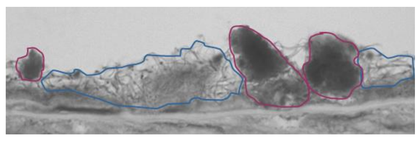


Parameters
- **% platelet coverage**
- **% fibrin coverage**

Annular flow chamber with damaged arterial vessel



- Thin cross-sections
- Toluidine blue staining



Platelet Coverage
Fibrin Deposition

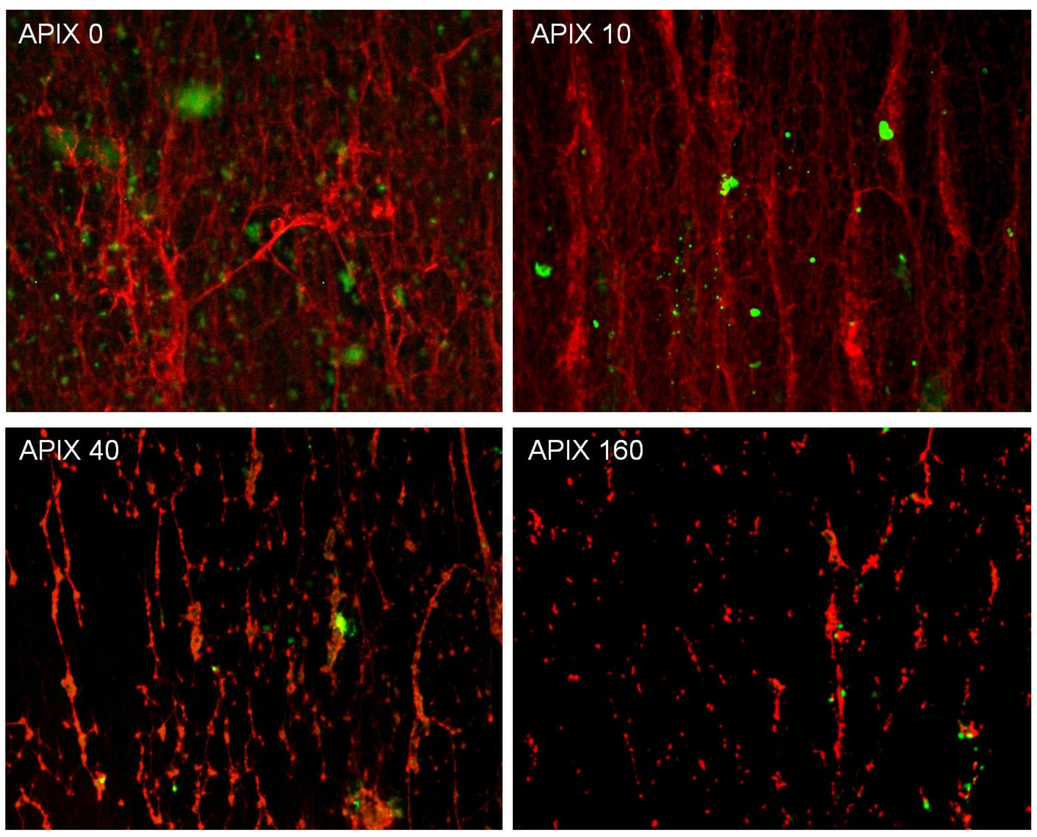
- Fixation
- EtOH dehydratation
- Resin inclusion

Parameters
- **% platelet coverage**
- **% fibrin coverage**

Blood volume *per* condition: 22 mL (recirculated)

1. RESULTS: MICROFLUIDIC DEVICE

Thrombogenic surface: biomimetic combination with type-I fibrillar collagen and traces of tissue factor *(Lopez-Vilchez et al. Coron Artery Dis 2009)*

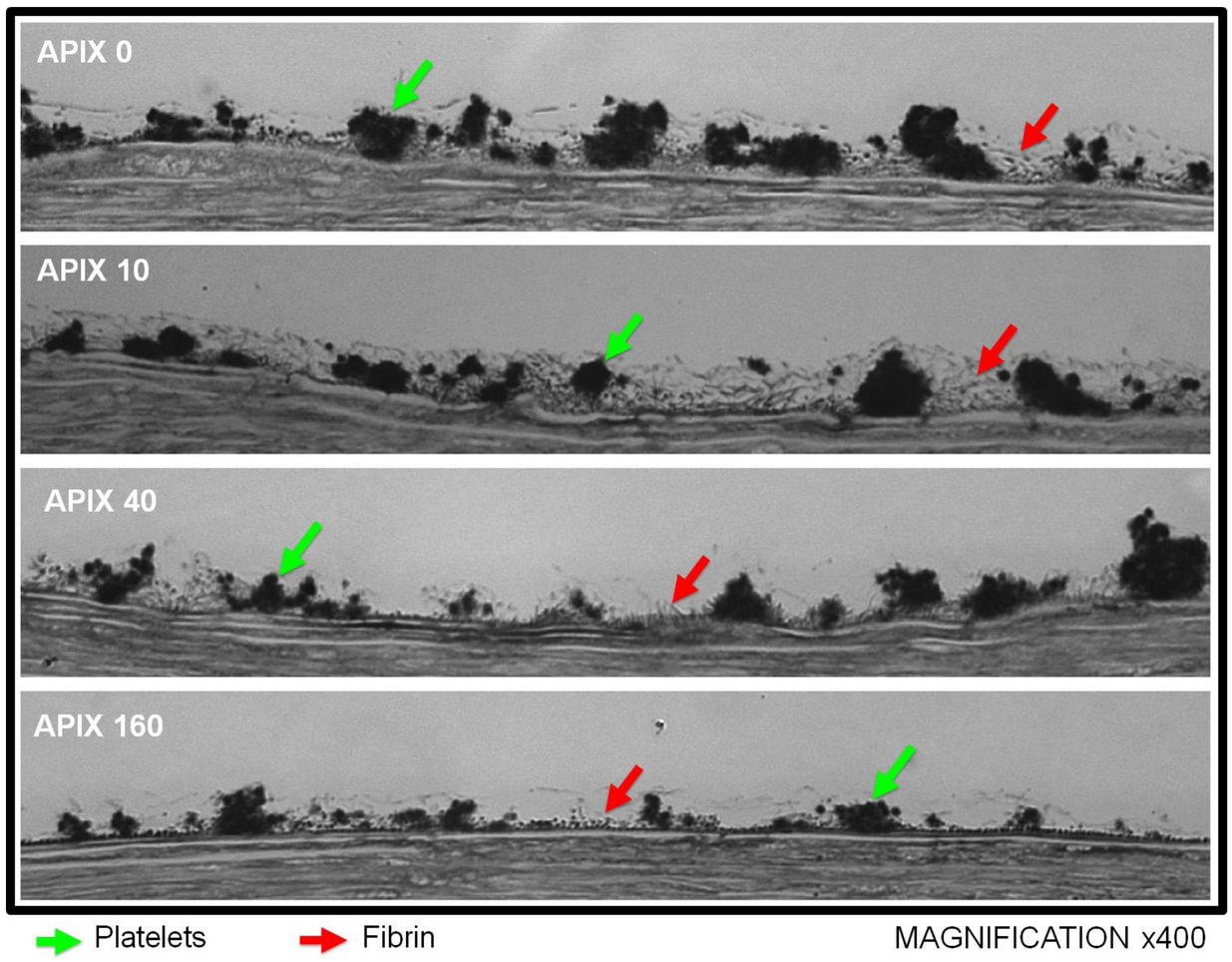


Platelets (anti-CD36 Alexa Fluor 488) Fibrin (anti-fibrin(ogen) Alexa Fluor 594)

MAGNIFICATION x200

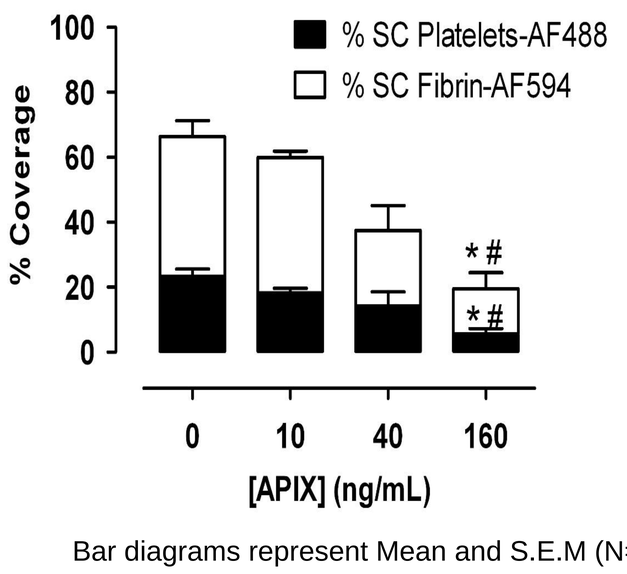
2. RESULTS: CLASSIC ANNULAR PERFUSION MODEL

Thrombogenic surface: Damaged rabbit aorta *(Pujadas-Mestres et al. PLoS One. 2017)*

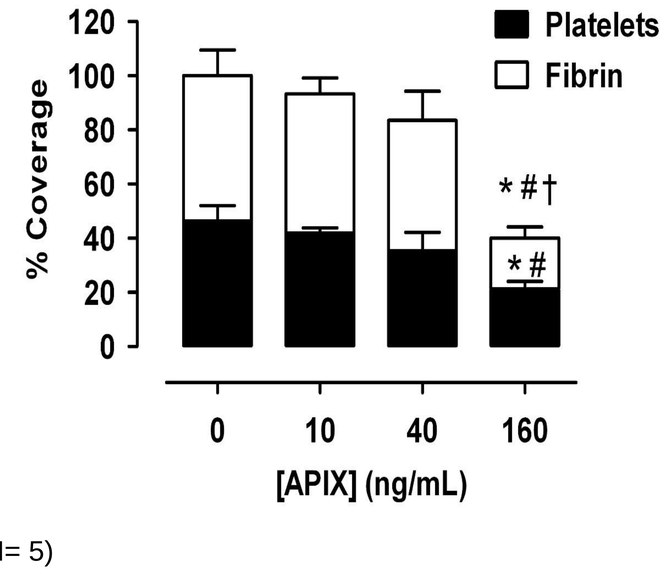


3. RESULTS: COMPARISON OF MORPHOMETRIC EVALUATIONS

MICROFLUIDIC CHANNEL DEVICE



CLASSIC ANNULAR DEVICE



Bar diagrams represent Mean and S.E.M (N= 5)

* p<0.05 vs. APIX 0 # p<0.05 vs. APIX 10 † p<0.05 vs. APIX 40

Conclusions

- Platelet and fibrin interactions with biomimetic vascular components in the microfluidic model, show a high level of correspondence with those observed in the classic annular perfusion device.
- The microfluidic model explored offers a reliable alternative to classical flow-based systems.
- Moreover, this microfluidic approach may be useful in the evaluation of drugs that interfere with hemostasis.

- APIX caused a dose-dependent reduction in platelet and fibrin interactions onto the different thrombogenic surfaces, with similar results and statistical trends in both flow devices
- APIX at 40 ng/mL statistically reduced platelet coverage; this effect was further increased at 160 ng/mL, also affecting the fibrin deposition.

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