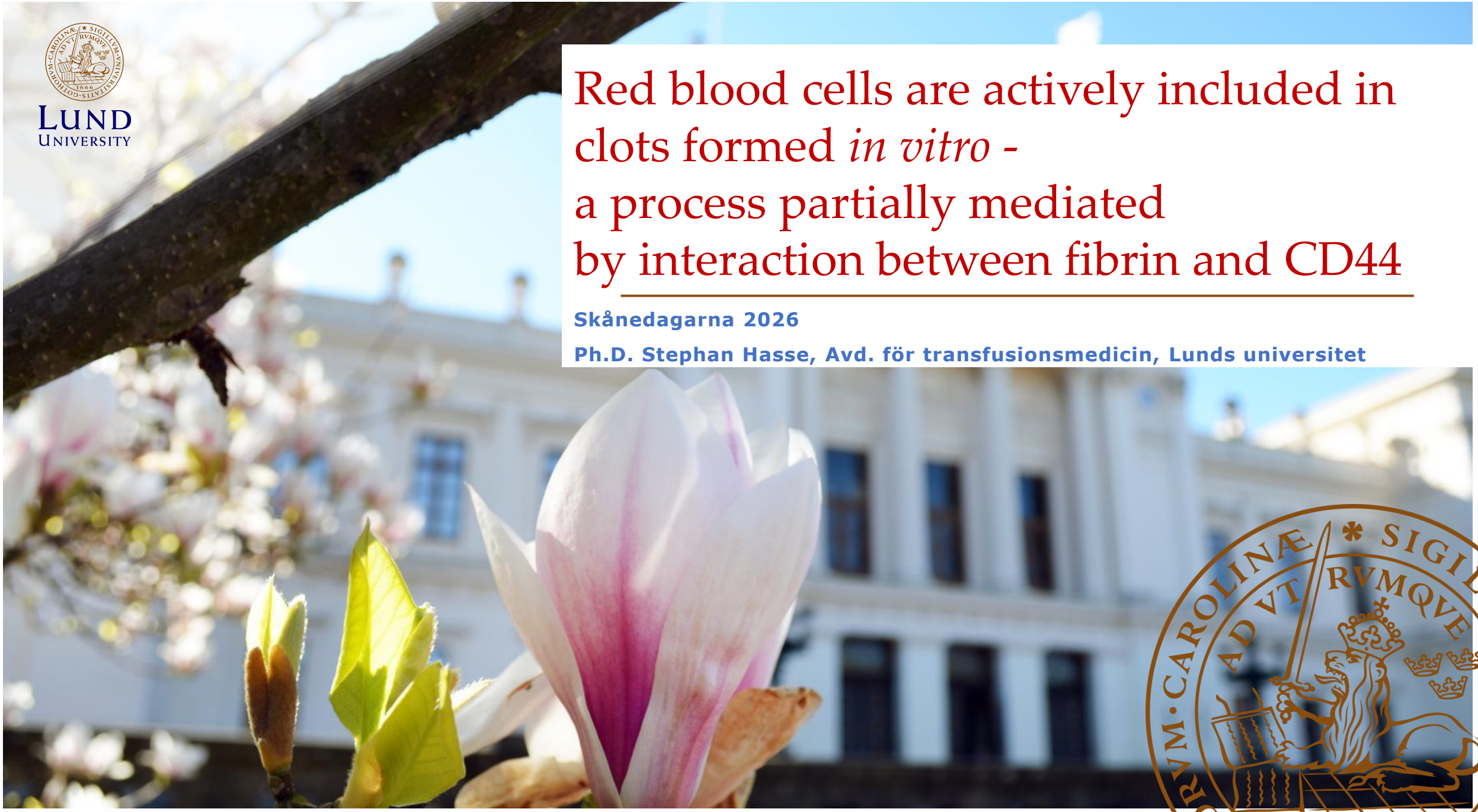
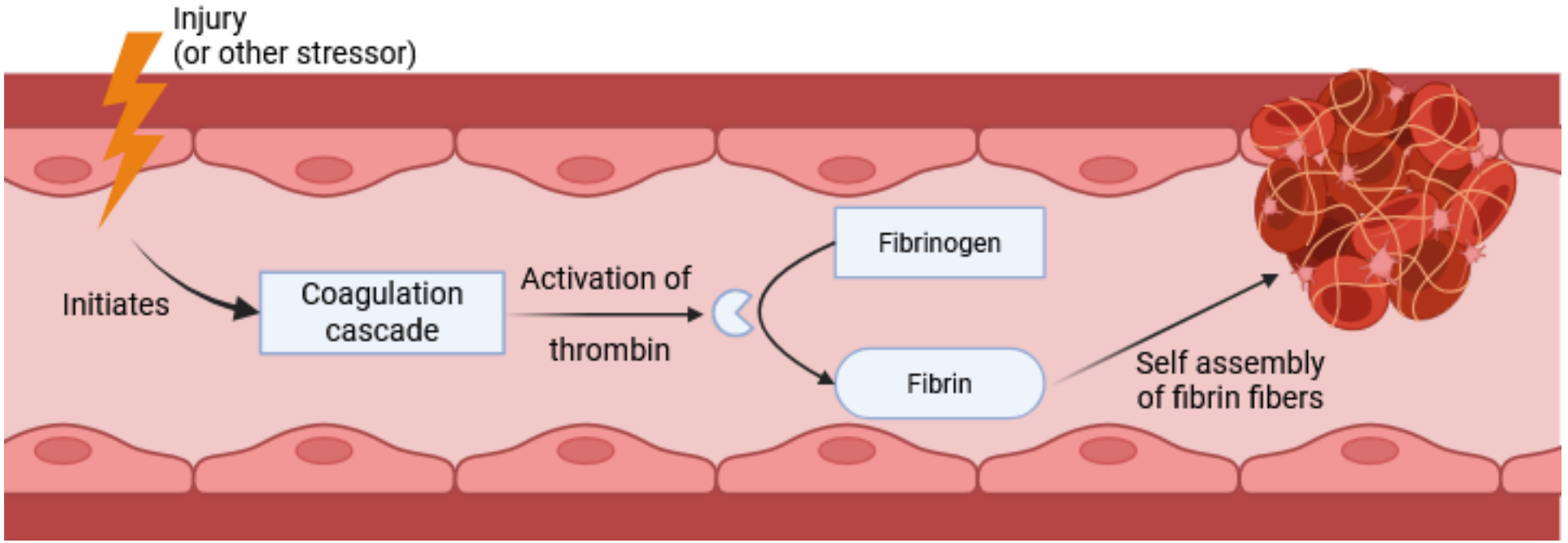


# Red blood cells are actively included in clots formed *in vitro* - a process partially mediated by interaction between fibrin and CD44

Skånedagarna 2026

Ph.D. Stephan Hasse, Avd. för transfusionsmedicin, Lunds universitet

# Hemostasis: quick and simplified rundown




Platelets drive the whole process

RBCs are widely considered as passive bystanders…

# RBCs are passive bystanders in hemostasis, or are they?

RBCs bind to platelets
affect platelet activation and clot contraction

(B. Walker *et al.*, Am J Physio Cell Phyiol, 2014; B.L. Walton *et al*., Blood, 2017; N.G. Evtugina *et al*., J Thromb Haemost, 2023)

RBCs and their extracellular vesicles participate in thrombin generation

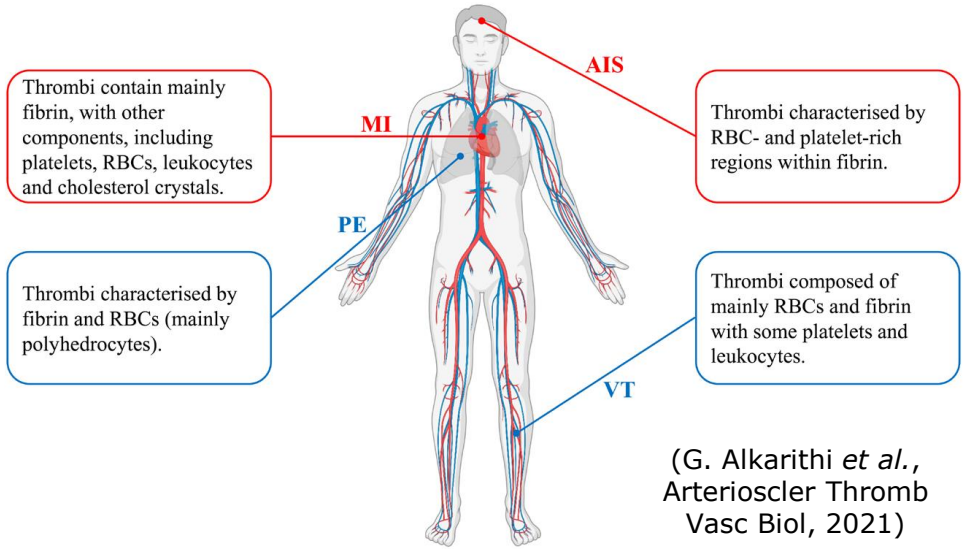
(M.F. Whelihan *et al.*, Blood, 2012; C. Klatt *et al.*, J Clin Invest, 2018; S. Sun *et al*., Blood Adv, 2023)

RBC inclusion in clots increases resistance to fibrinolysis
increases clot volume
decreases clot permeability

(K.C. Gersh *et al*., Thromb Haemost, 2009; N. Wohner *et al*., Arterioscler Thromb Vasc Biol, 2011)

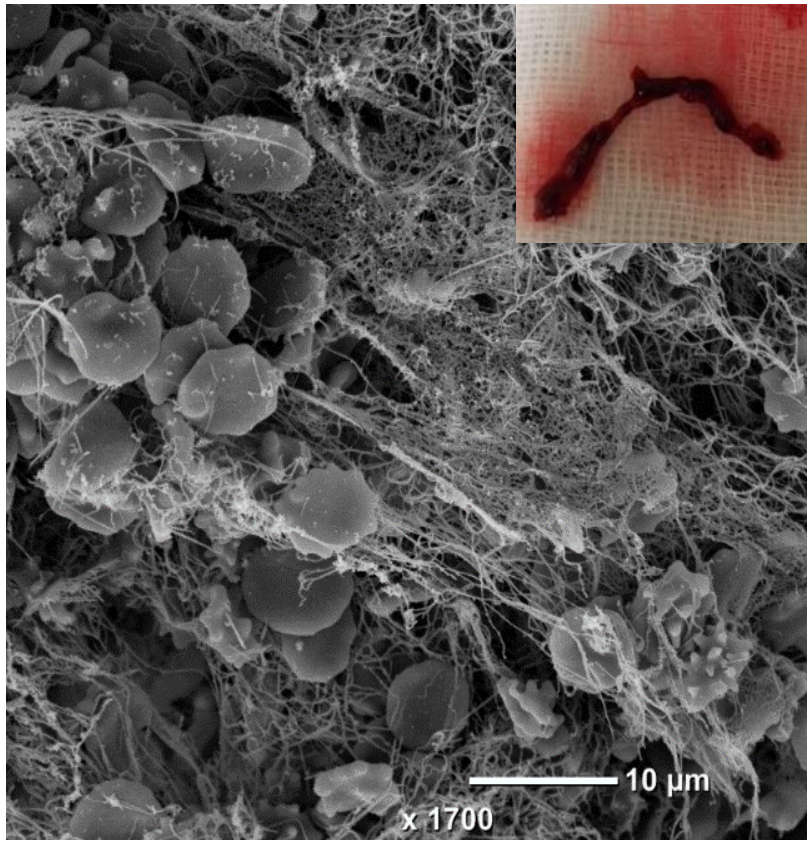
Clot composition varies across clots
depends on where the clot is formed

(G. Alkarithi *et al.,* Arterioscler Thromb Vasc Biol, 2021)




(G. Alkarithi *et al.*, Arterioscler Thromb Vasc Biol, 2021)

# How RBCs get included in clots? Passively or actively?



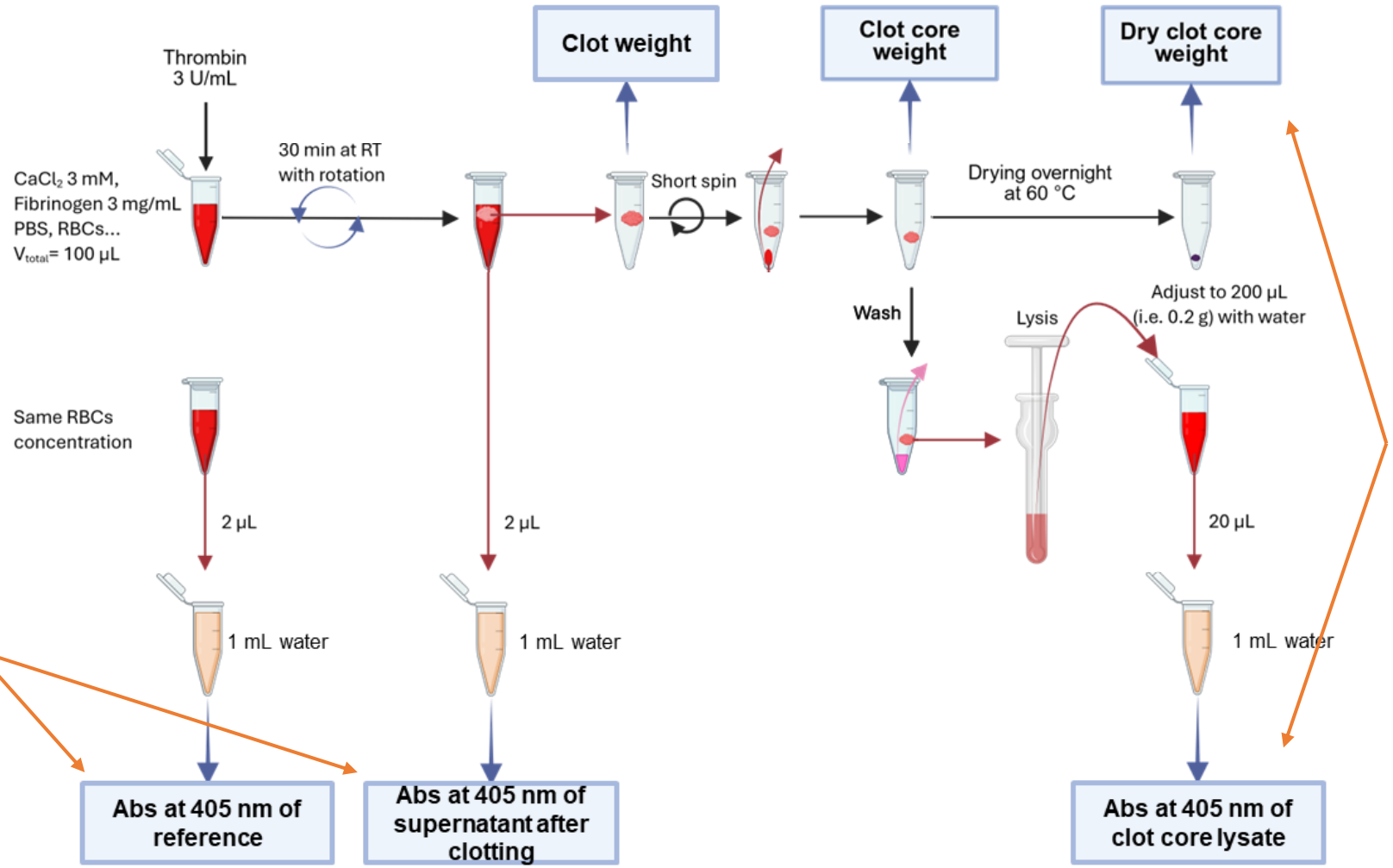

Vidovic & Olsson: unpublished
Hasse, Storry, Olsson: Manuscript in preparation

Our aim is to determine if

ligand-specific RBC interactions with coagulation actors participate in RBC inclusion in blood clots.

What model to investigate RBC inclusion in clots?

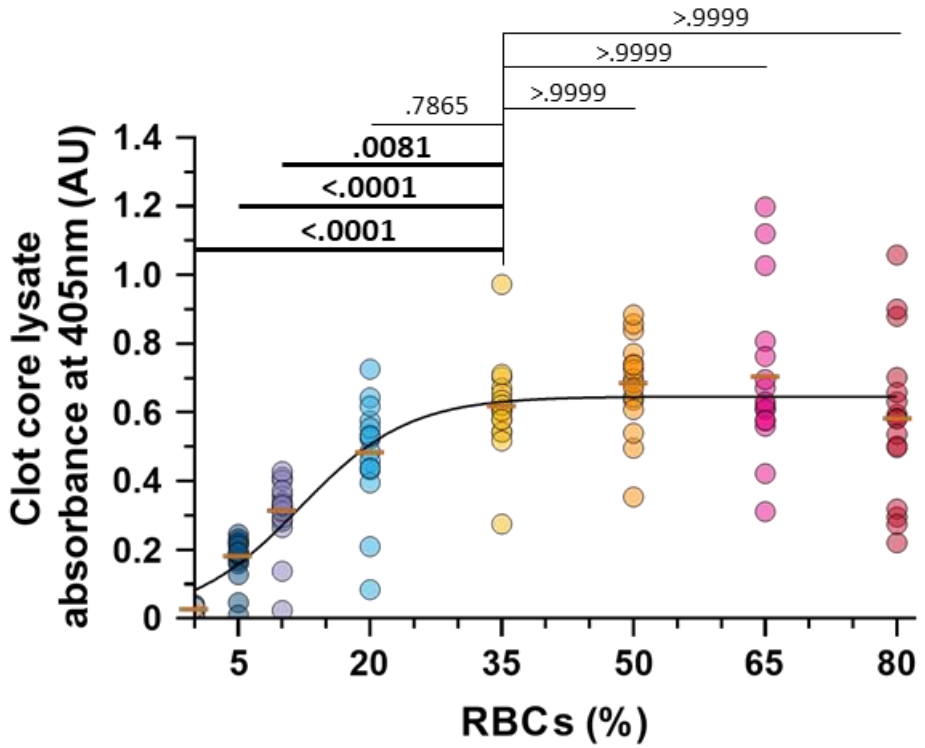
# In vitro clot model & measurements:

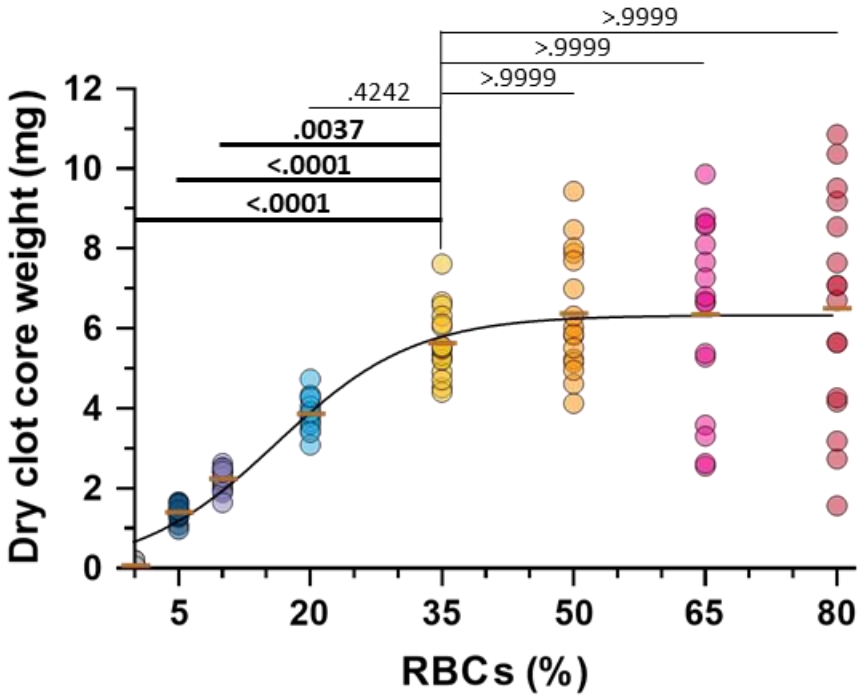



RBC concentrations in supernatant

RBC total quantities in clot cores

# Is RBC inclusion in clot a passive process?




RBC quantities in clot core do not follow the hematocrit but appear to reach a plateau from around 35%…

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*Friedman test with Dunn's multiple comparisons test, comparisons only to 35% RBCs; the mean of the populations and non-linear regression (Gompertz growth) are shown, n=16*

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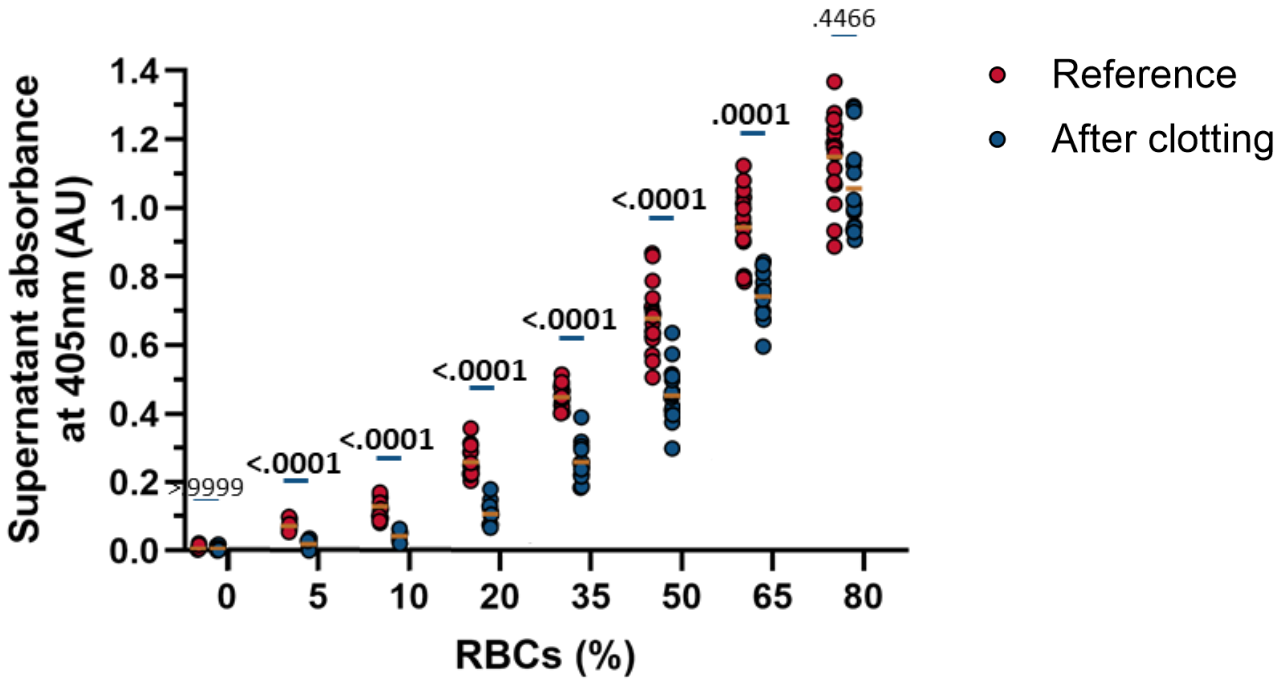
# Is RBC inclusion in clot a passive process?




RBC concentrations in the supernatant dropped after clotting, which also suggests uptake of RBCs by the clot.

*RM 2-way ANOVA with Geisser-Greenhouse correction and Šidák's multiple comparisons test;*
*All relevant comparisons and the mean of populations are shown, n=16.*

# Is RBC inclusion in clot a passive process?

| | |
|---|---|
| RBC quantities in clot core | did not correlate with the hematocrit |
| RBC concentration in supernatant | decreased after clotting<br>because of accumulation in the clot core |

Therefore, RBC inclusion in clot cores appears to be an active process.

If something makes RBCs stay in clot cores,
then it should be possible to compete with it.

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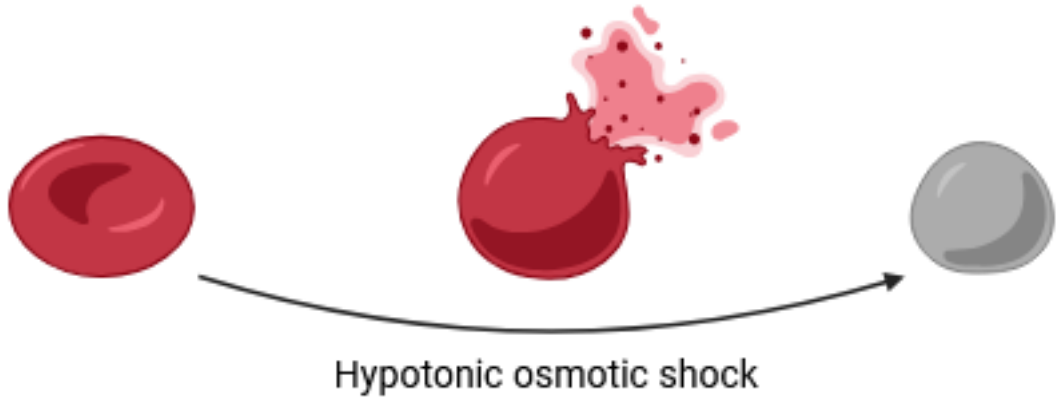
# How to confirm active inclusion of RBCs in clots?

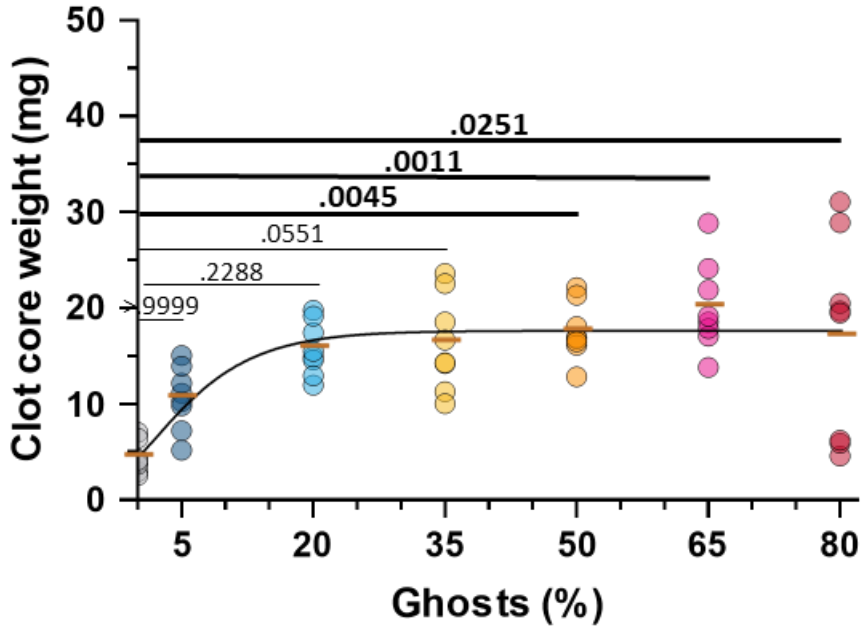
**Use of ghosts:**




low dry weight

no absorbance at 405 nm

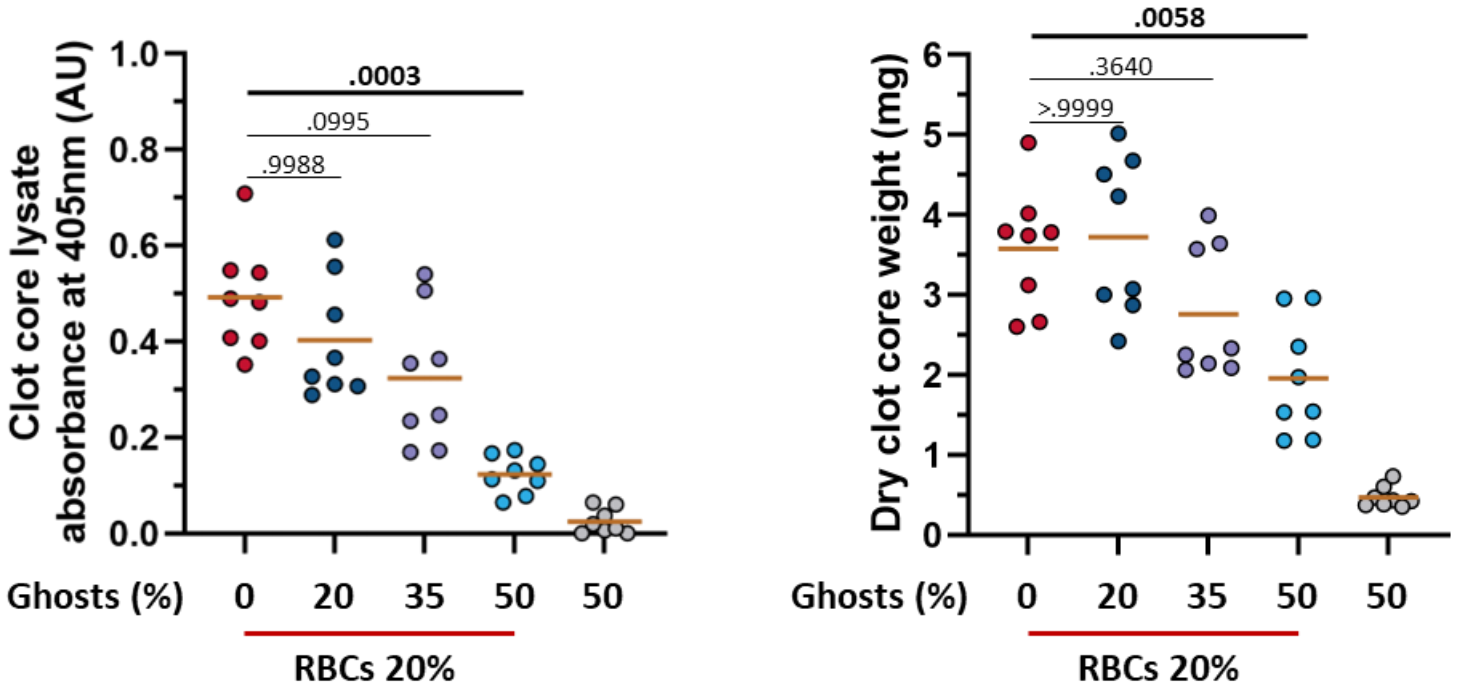
are retained similarly to RBCs in clot cores




Can ghosts compete with RBCs for inclusion in clot core?

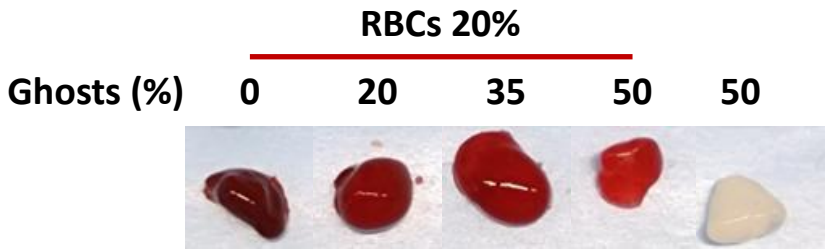
*Friedman test with Dunn's multiple comparisons test, comparisons only to 0% ghosts; the mean of the populations and non-linear regression (Gompertz growth) are shown, n=8*

# Can ghosts compete with RBCs?










Ghosts, indeed, compete with RBC inclusion.
It confirms that RBCs are actively included in clot cores.



*Friedman test with Dunn's multiple comparisons test;*
*All tested comparisons and the mean of populations are shown, n=8.*

# What causes RBC inclusion in clot cores?

RBCs can bind fibrin(ogen)
(D. Lominadze *et al.*, FEBS letter, 2002; M. Brust *et al.*, Sci Rep, 2014)

Reportedly by an interaction with: RGD integrins
(D. Lominadze *et al.*, FEBS letter, 2002; F.A. Carnavlho *et al.*, ACS Nano, 2010)
CD47
(S. De Oliveira *et al.*, Biochim Biophys Acta, 2012)

**Targeting them did not impact RBC inclusion in clot cores**

CD44 (Indian blood group) binds fibrin via the same epitope as Hyaluronic Acid (HA)

(C.S. Alves *et al.*, Am J Physio Cell Phyiol, 2008; P.S. Raman *et al.*, J Cell Sci, 2011; C. Wang *et al.*, Blood Adv, 2022)

Does targeting CD44 impact RBC inclusion?

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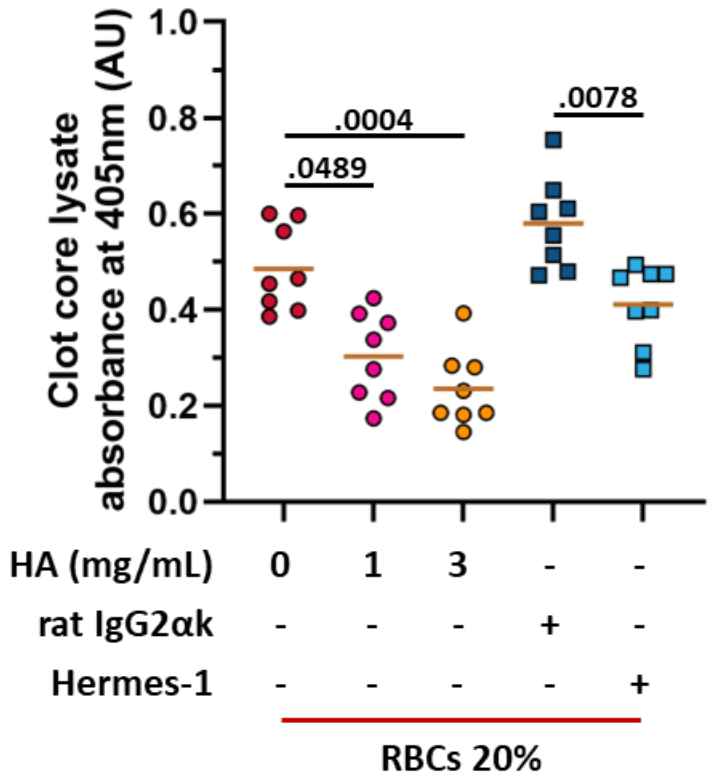
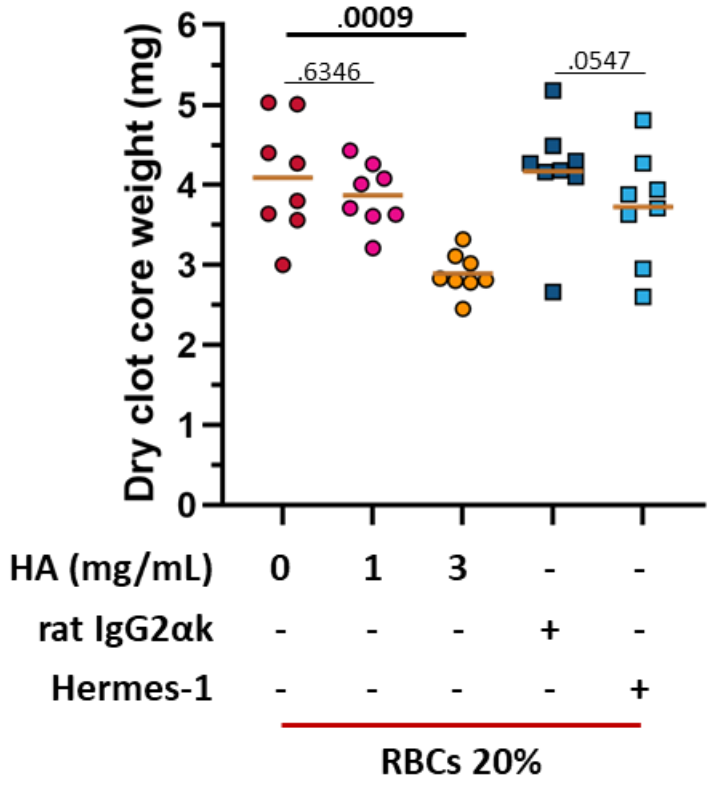
# Is RBC inclusion mediated by CD44?

Competition with hyaluronic acid (HA,●) or CD44 neutralizing antibody Hermes-1 (□)




CD44 partly explains the active inclusion of RBCs in clot cores.

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*Friedman test with Dunn's multiple comparisons test (HA) or Wilcoxon test (Hermes-1);*
*All tested comparisons and the mean of populations are shown, n=8.*

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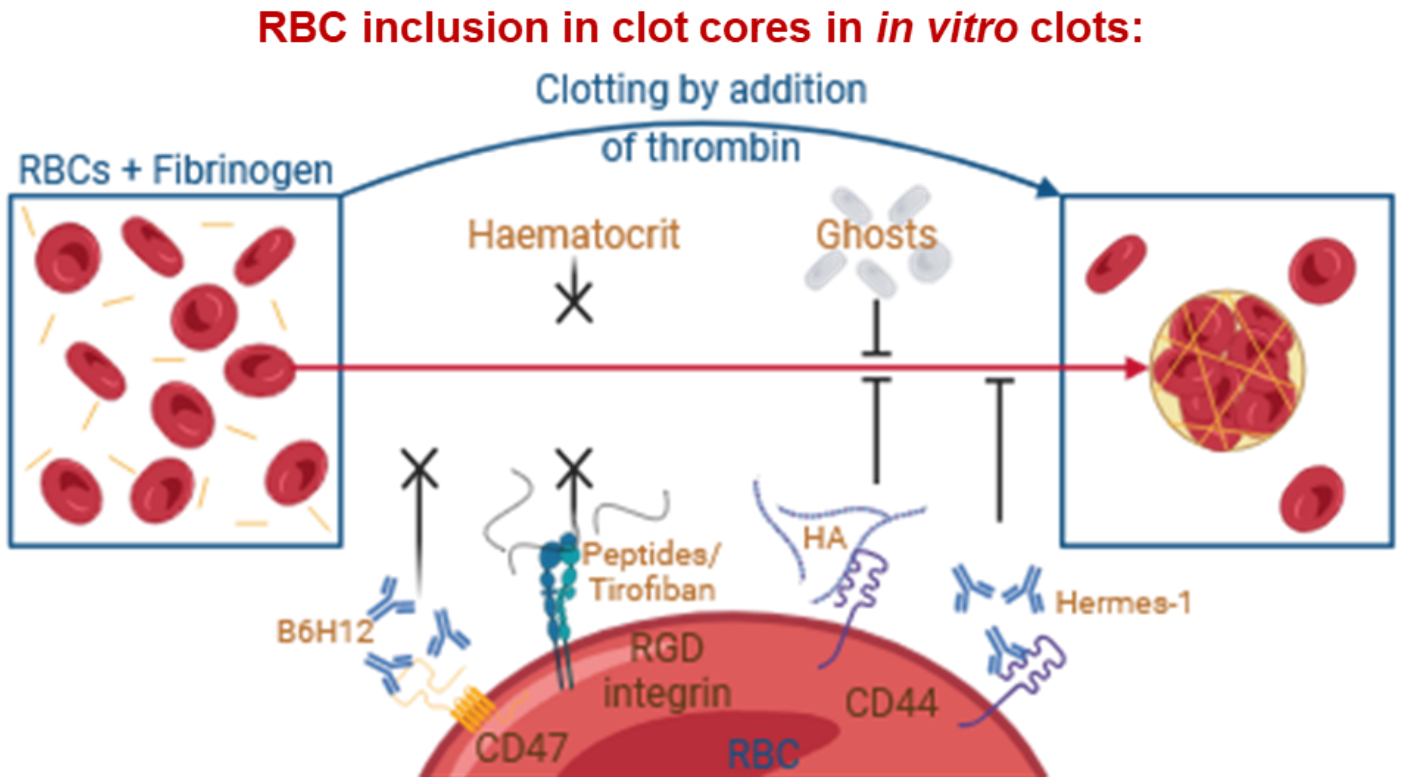
# Conclusions:

1. RBCs are actively included in clot core

2. It is partly mediated by CD44-fibrin binding



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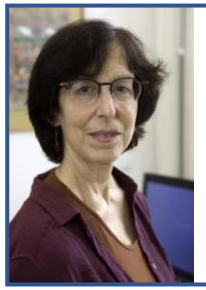

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