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Faculty of Health Sciences

Fakulteit Gesondheidswetenskappe
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Can viscoelastic and ultramicroscopy techniques be a potential tool in a precision medicine approach for type II diabetes patients?

https://researchgate.net/profile/Etheresia_Pretorius/

Synopsis of talk

- Background – are we winning the war against type II diabetes?
- Diabetes/ metabolic syndrome and inflammation
- Inflammation and the coagulation system
- The diabetic coagulation system
- Conclusions and suggestions: a *precision medicine approach using individualized patient tracking*

Diabetes prevalence

- The prevalence for all age-groups worldwide was estimated to be 2.8% in 2000
- Predicted to increase to 4.4% in 2030
- Among adults in the US, the prevalence of undiagnosed diabetes is currently 4.1% and **prediabetes is at a staggering 35.6%**
- Type II diabetes is associated with glycemic disorders, and also (frequently) comorbidities including dyslipidemia and hypertension

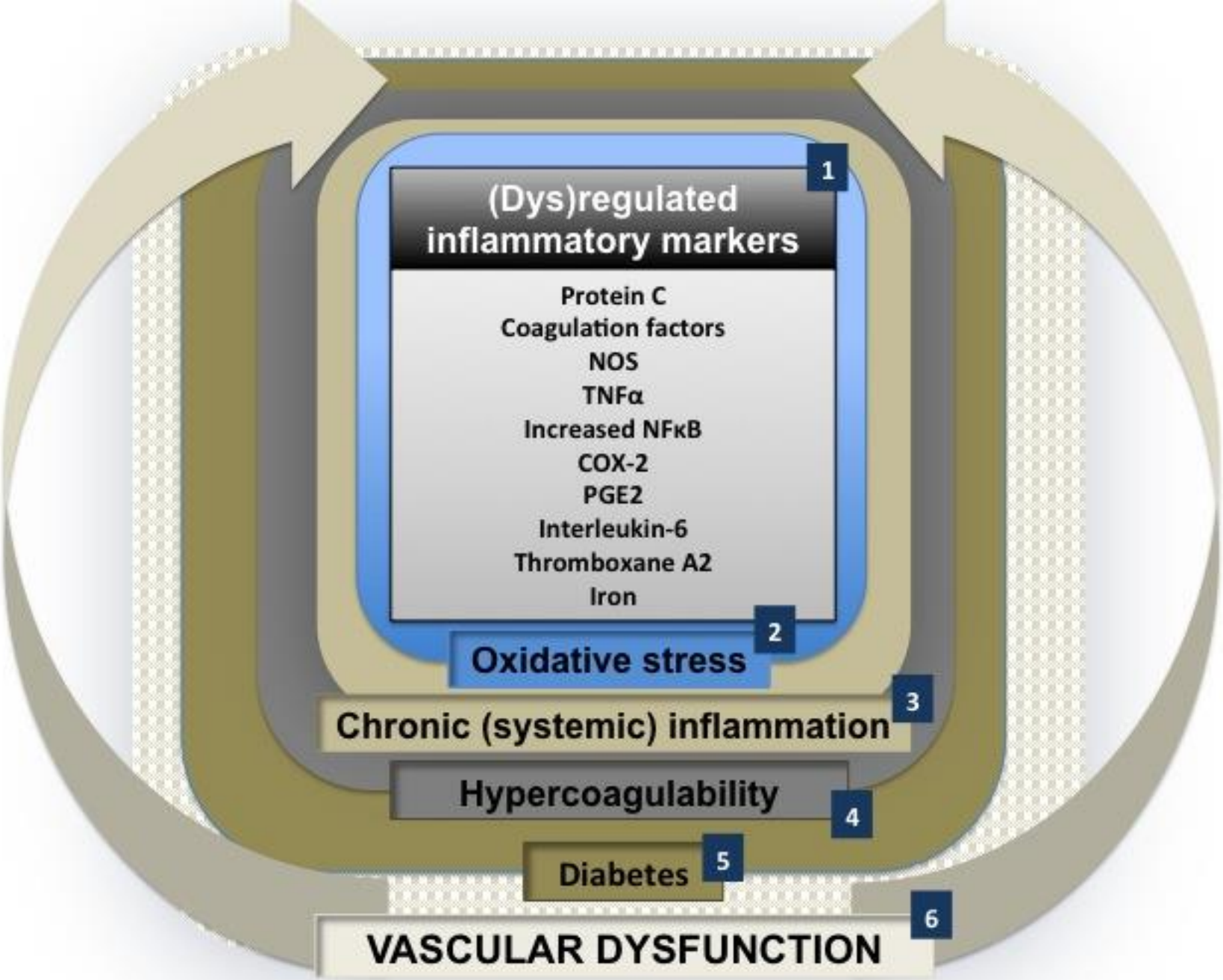
Cardiovascular risk factors

- Compared with individuals without diabetes, patients with type II diabetes have a considerably higher risk of cardiovascular morbidity and mortality

- Cardiovascular risk versus inflammation

Selected references showing a changed inflammatory marker profile in diabetes type II individuals

Inflammatory marker	Selected references
Low Protein C	(Kitada et al., 2010, Kim et al., 2014)
High levels of coagulation factors (II, V, VIII, X and von Willebrandt factor)	(Cucuianu and Coca, 2012, Kim et al., 2014, Goldberg, 2009, Tousoulis et al., 2013, Madan et al., 2010)
Increased NOS	(Khanna et al., 2014, Dellamea et al., 2014, Dzugkoev et al., 2013, Schneider et al., 2013, Cohen and Tong, 2010)
Increased TNFα	(Mandosi et al., 2010, Prasad et al., 2007, Chacón et al., 2007, Kampoli et al., 2011)
Increased NFκB	(Moraes et al., 2014, Kassan et al., 2014, Patel and Santani, 2009, Andreasen et al., 2011, Kassan et al., 2013)
Increased COX-2	(Kassan et al., 2013, Wong et al., 2013b, Rudock et al., 2009, Kellogg et al., 2008, Bagi et al., 2006)
Increased PGE2	(Schoenberger et al., 2012, Salvi et al., 1998)
Iron (increased serum ferritin levels)	(Salonen et al., 1998, Ford and Cogswell, 1999, Wilson et al., 2003, Jiang et al., 2004, Mert et al., 2005, Acton et al., 2006, Alissa et al., 2007, Forouhi et al., 2007, Jehn et al., 2007, Sun et al., 2008, Kolberg et al., 2009, Rajpathak et al., 2009, Kim et al., 2011, Lee et al., 2011, Ryoo et al., 2011, Bao et al., 2012, Lyssenko et al., 2012, Montonen et al., 2012, Zhao et al., 2012, Aregbesola et al., 2013, Guo et al., 2013, Jung et al., 2013, Kundu et al., 2013, Kunutsor et al., 2013, Ponikowska et al., 2013, White and Collinson, 2013).
Increased interleukin-6	(Schoenberger et al., 2012, Goldberg, 2009, Tousoulis et al., 2013, Wang et al., 2013, Kampoli et al., 2011)
Increased thromboxane A2	(Zuccollo et al., 2005, Winocour, 1994)


1
(Dys)regulated inflammatory markers
Protein C
Coagulation factors
NOS
TNFα
Increased NFκB
COX-2
PGE2
Interleukin-6
Thromboxane A2
Iron
2
Oxidative stress
3
Chronic (systemic) inflammation
Hypercoagulability
4
5
Diabetes
6
VASCULAR DYSFUNCTION

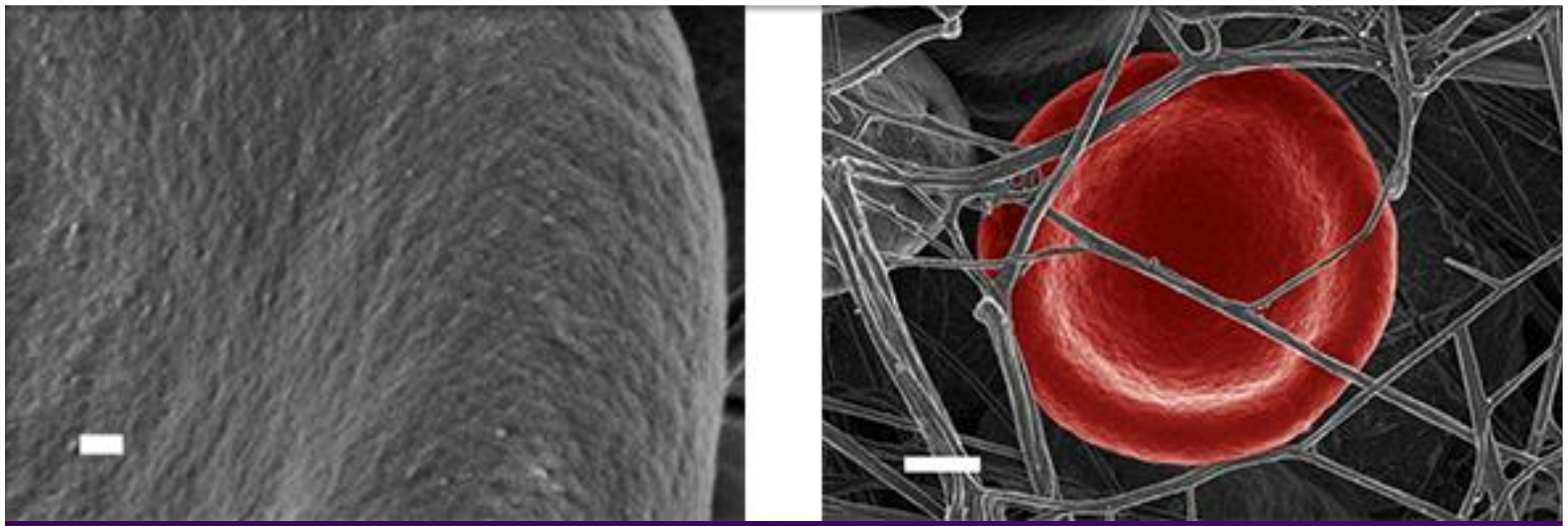
Inflammation and the coagulation system

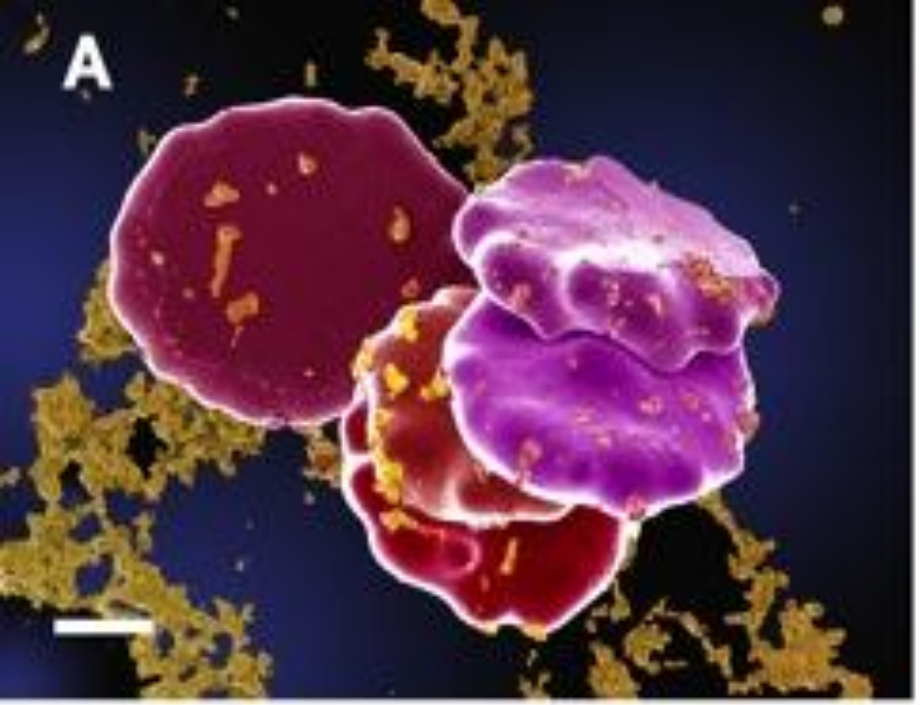
- Erythrocytes (RBCs), platelets and atypical fibrin fiber formation or an altered fibrin structure are a hallmark of inflammation

- Selected examples showing the effect of inflammation on RBCs and fibrin fiber structure

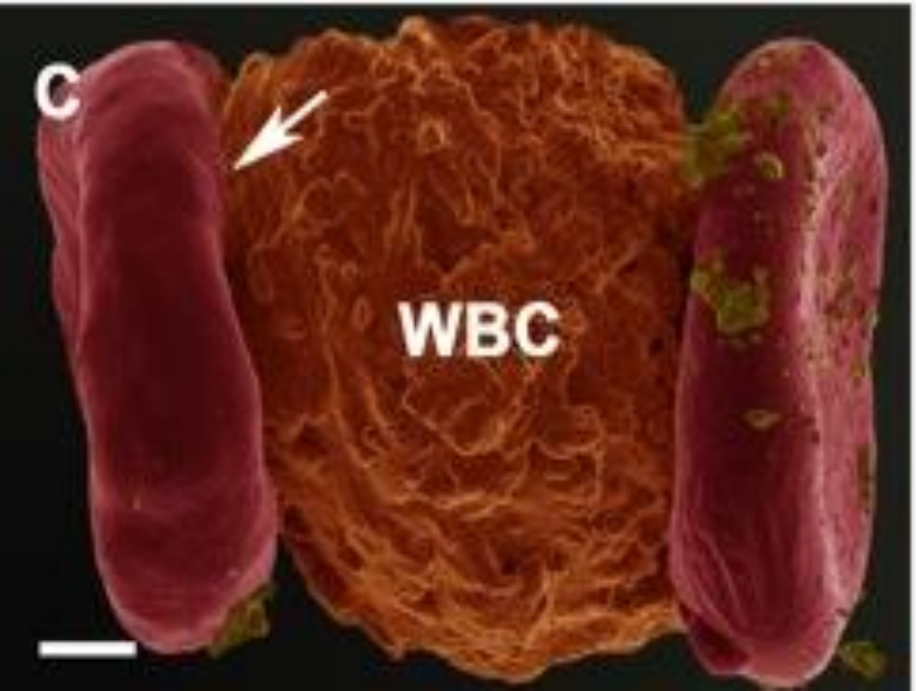

1 µm

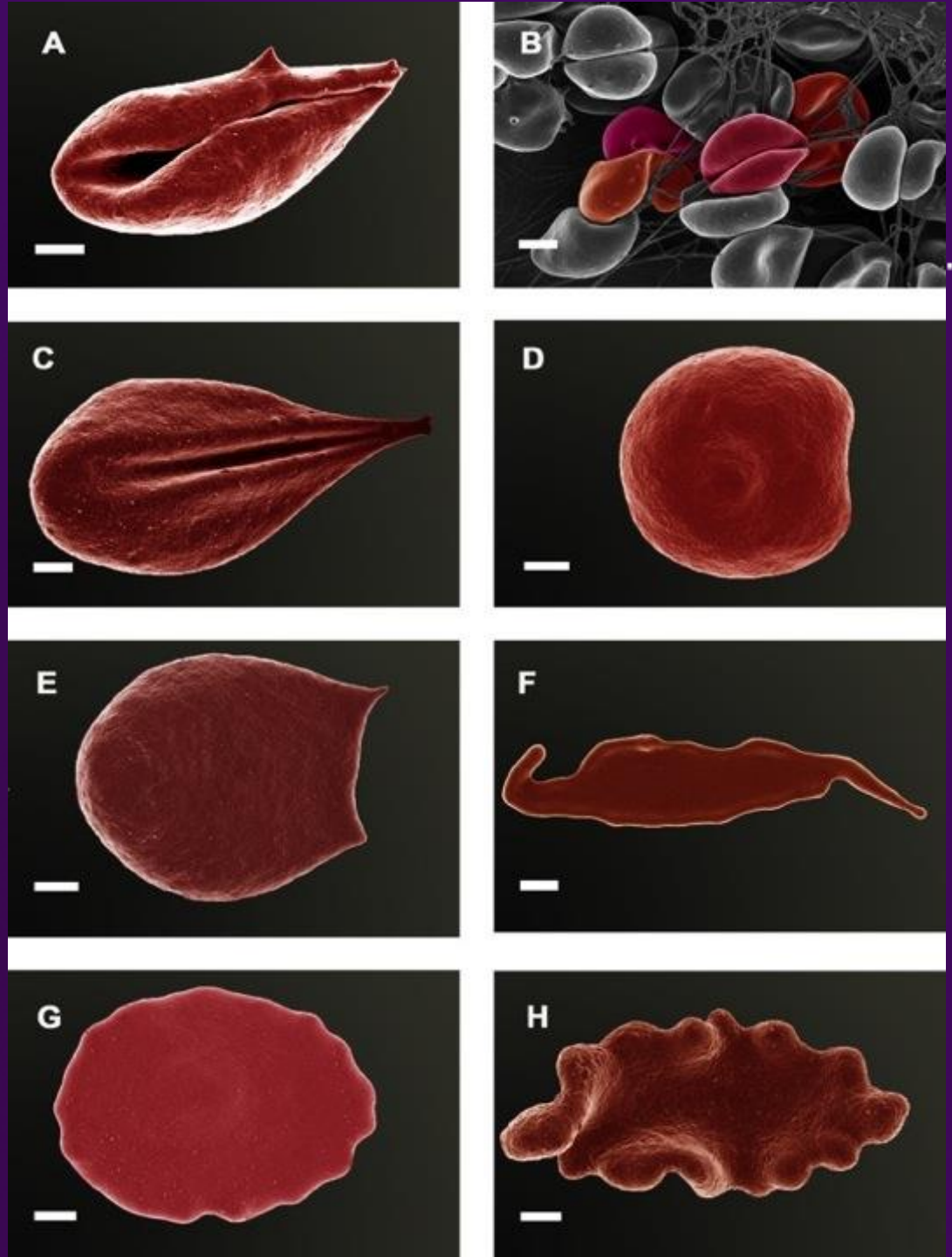




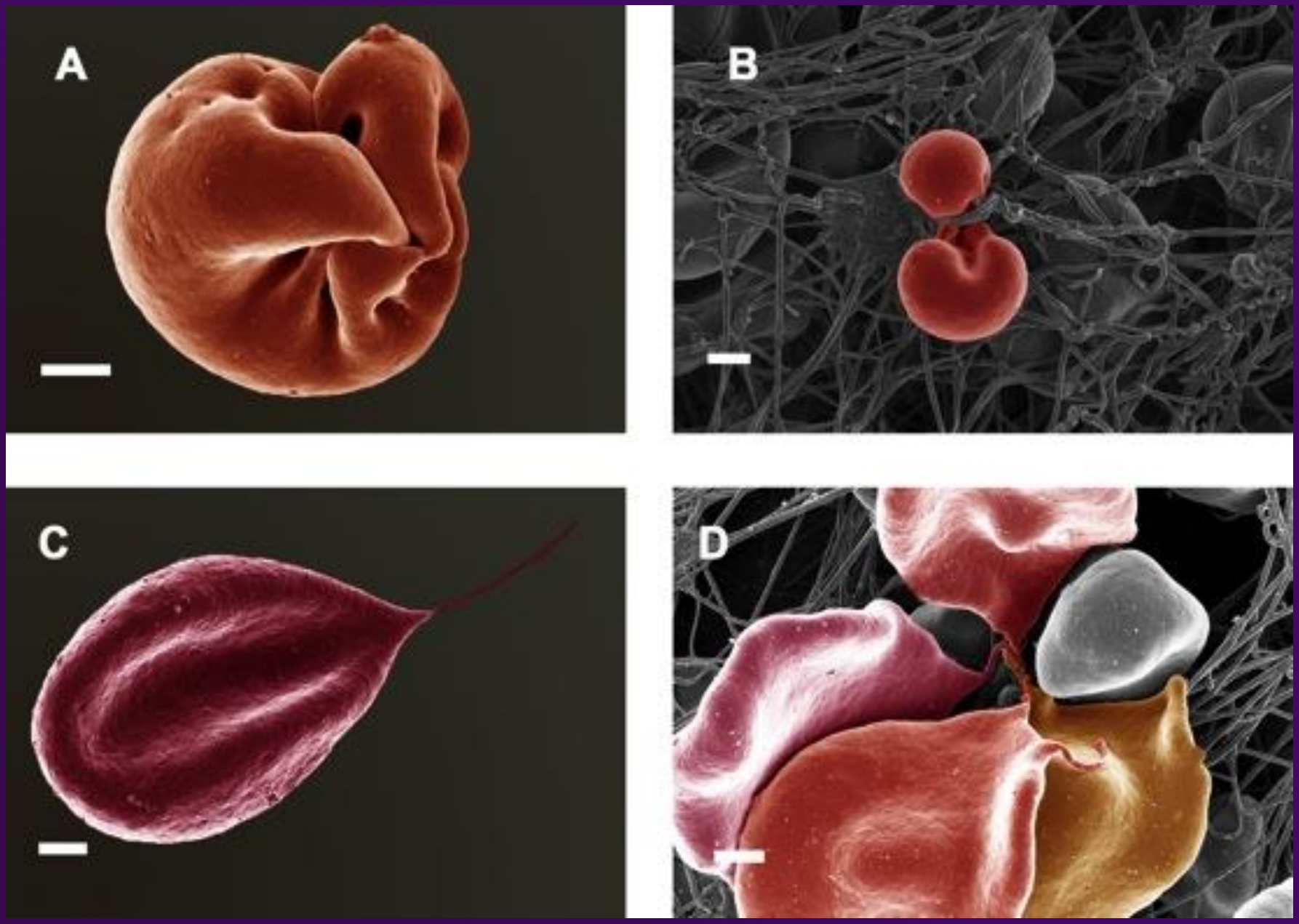

A


B

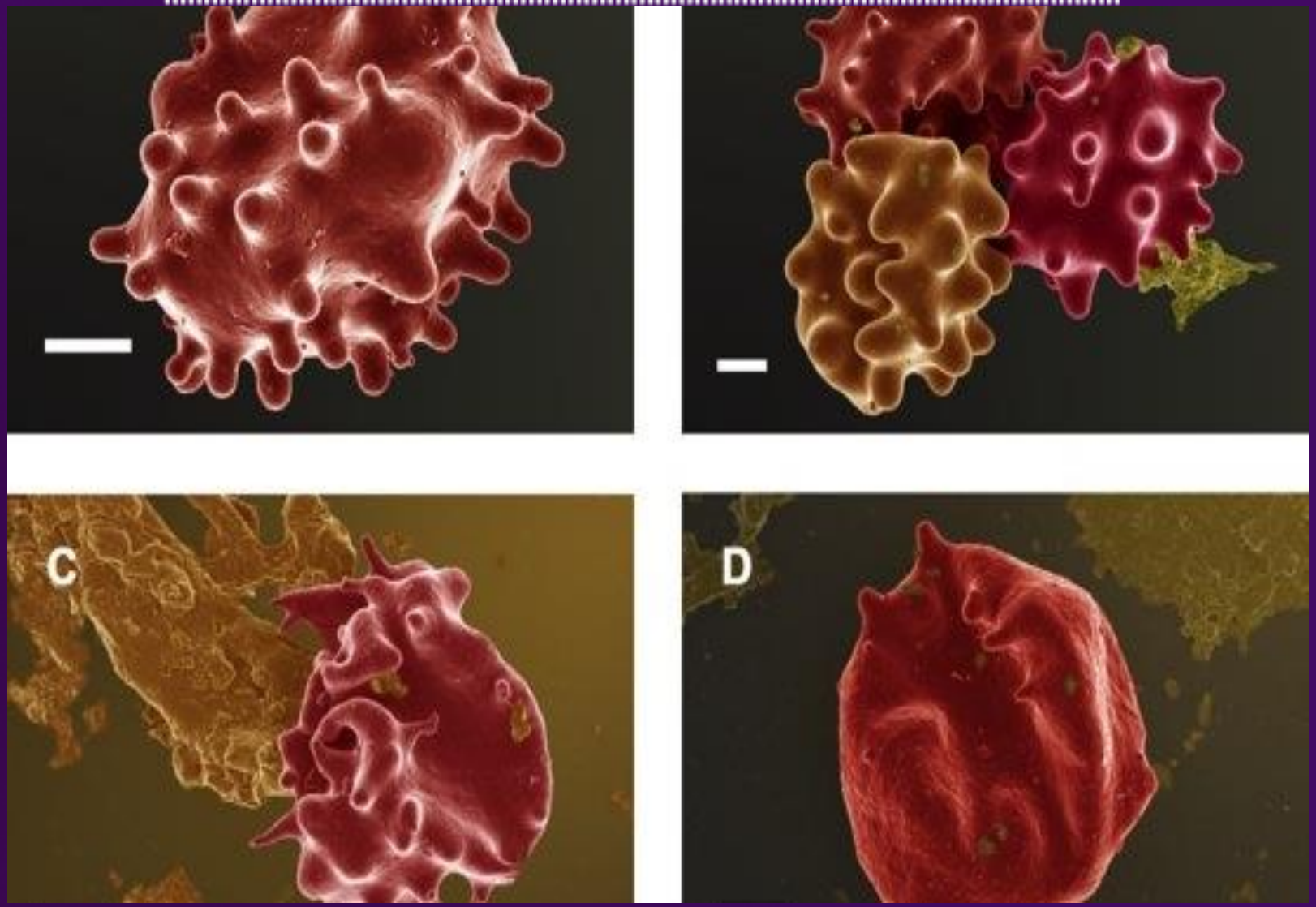

C
WBC



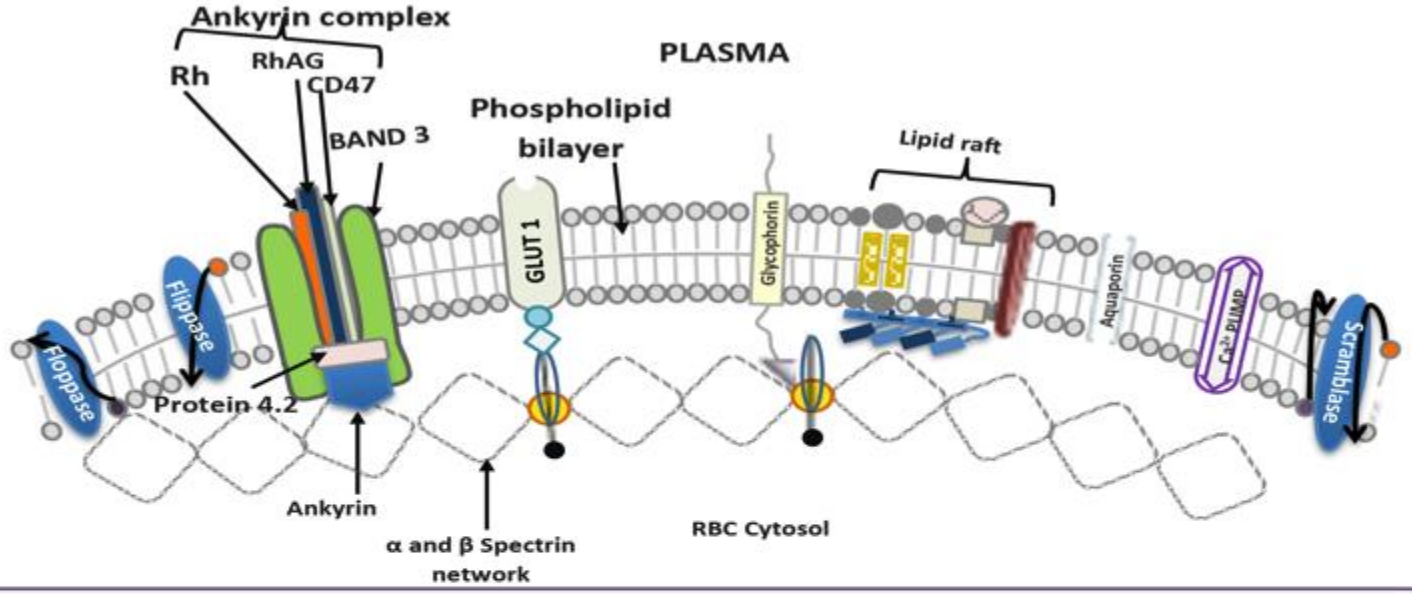
Poikilocytosis abnormally shaped RBCs

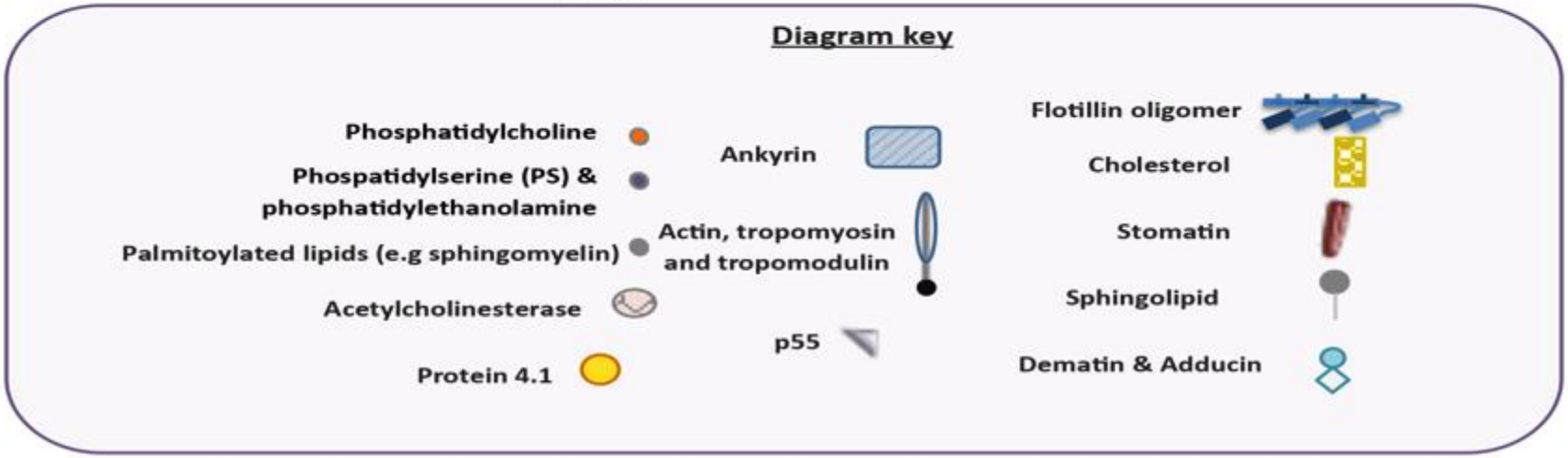

A
B
C
D

Eryptosis



The biochemical reasons for shape change





Abnormal fibrin formation and platelets = hypercoagulation:

HALLMARK OF INFLAMMATION

Hypercoagulability and hypofibrinolysis

Integrative Biology

ROYAL SOCIETY OF CHEMISTRY

PERSPECTIVE

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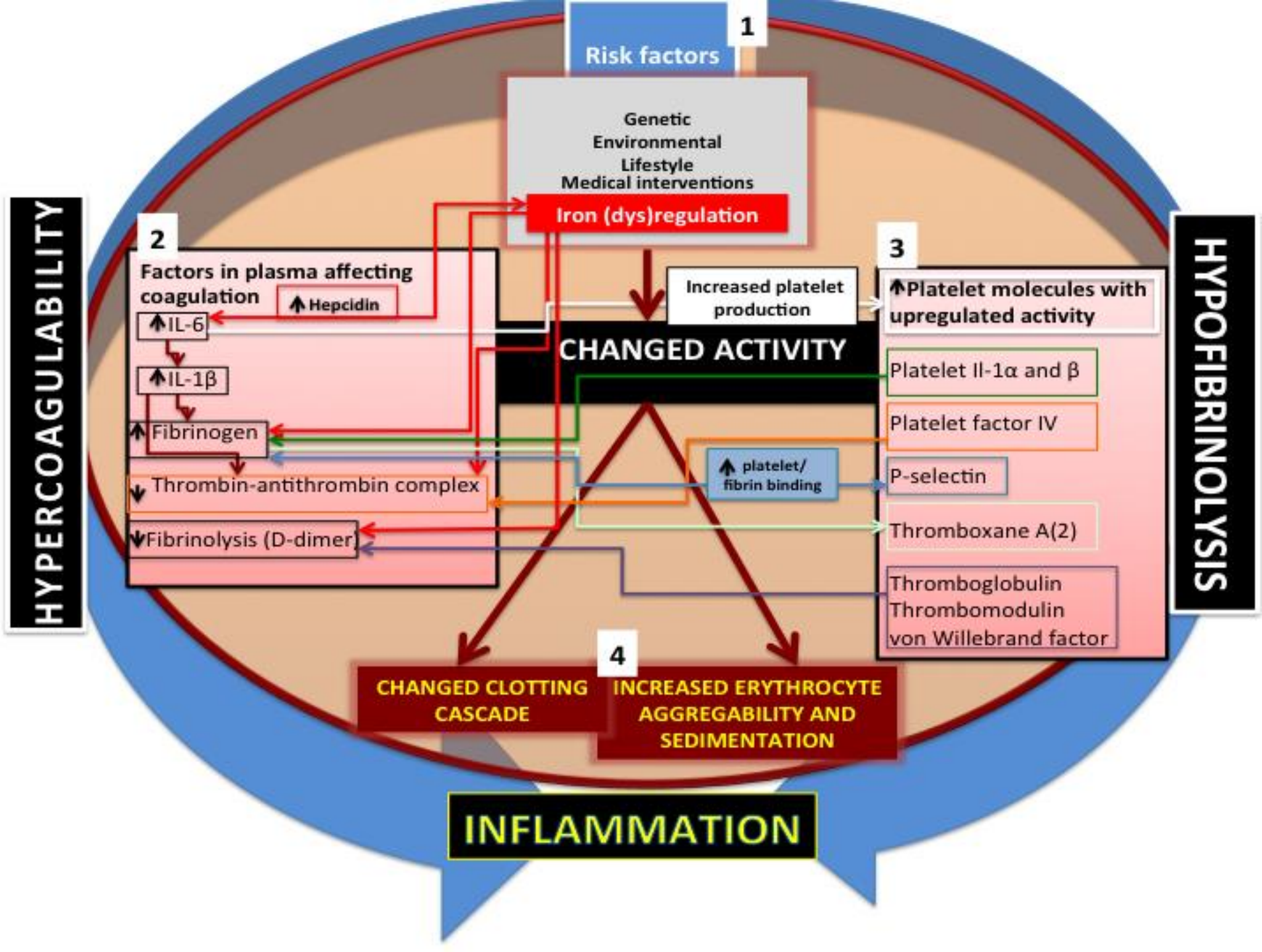
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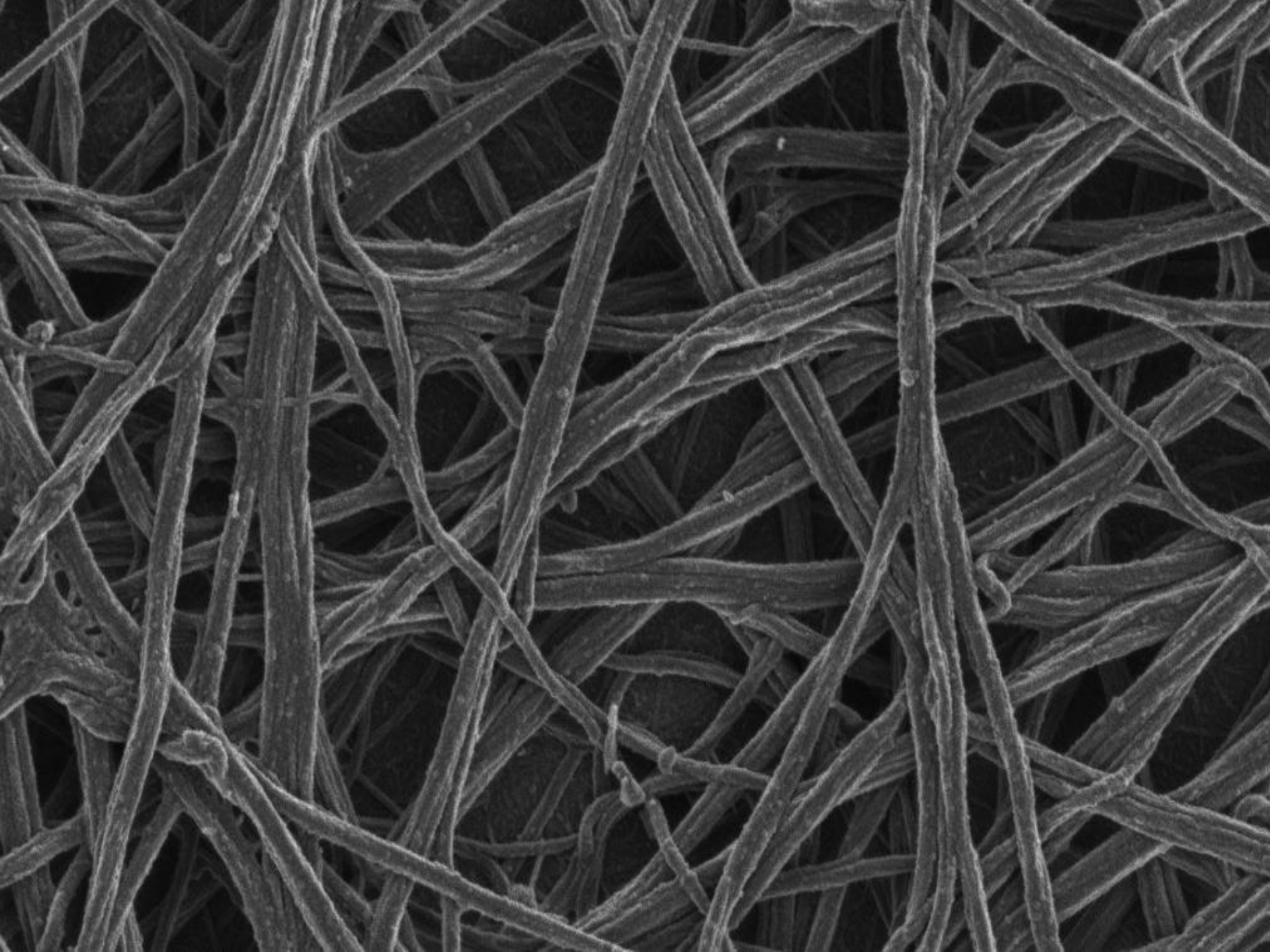
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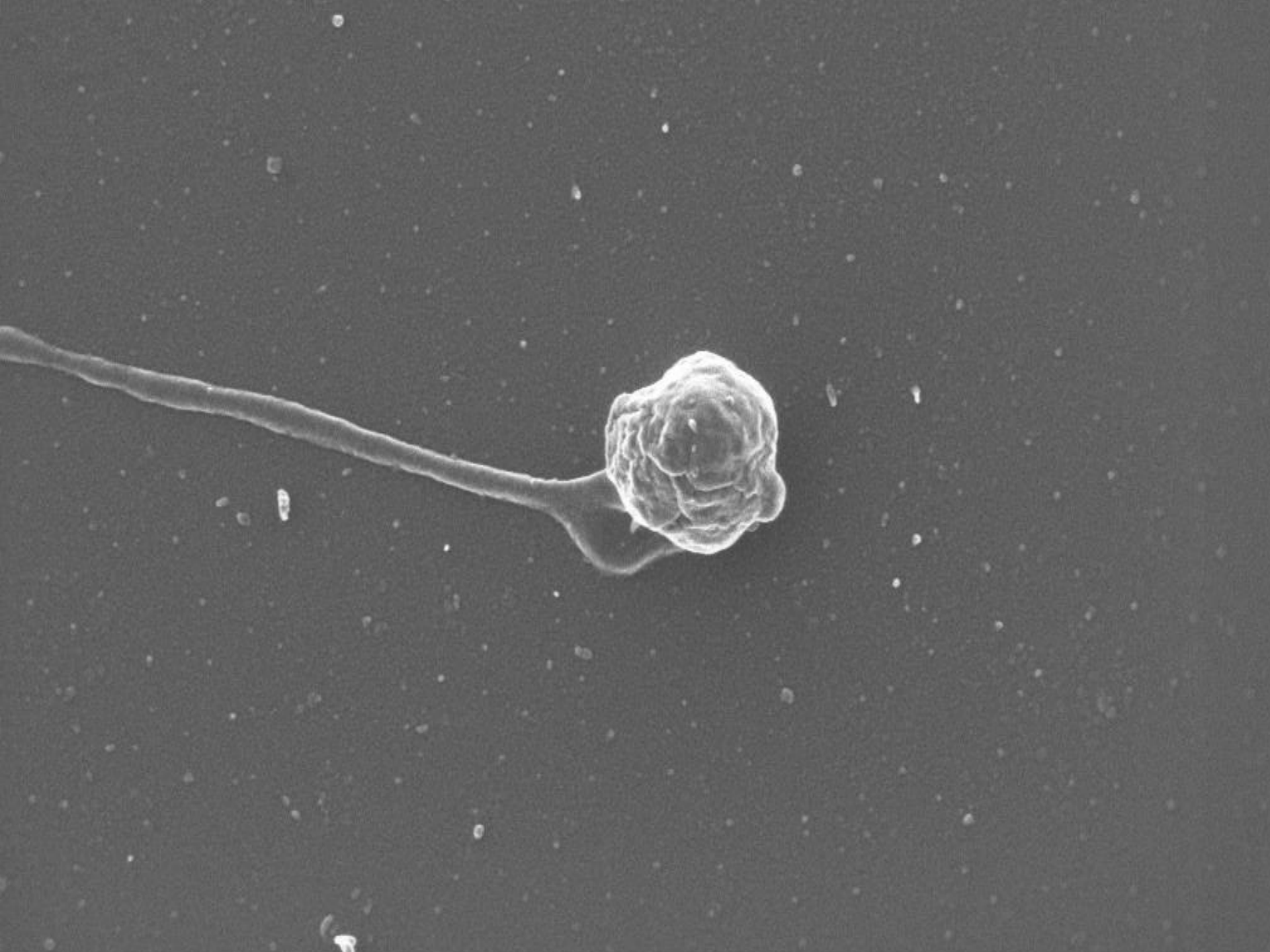
The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen)

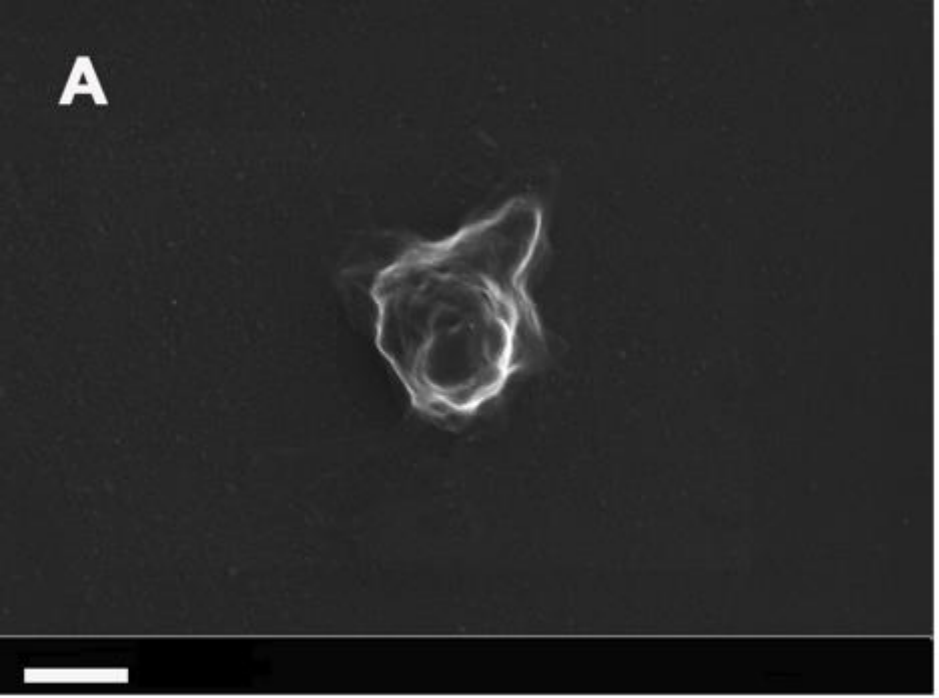
Douglas B. Kell*[a] and Etheresia Pretorius*[b]

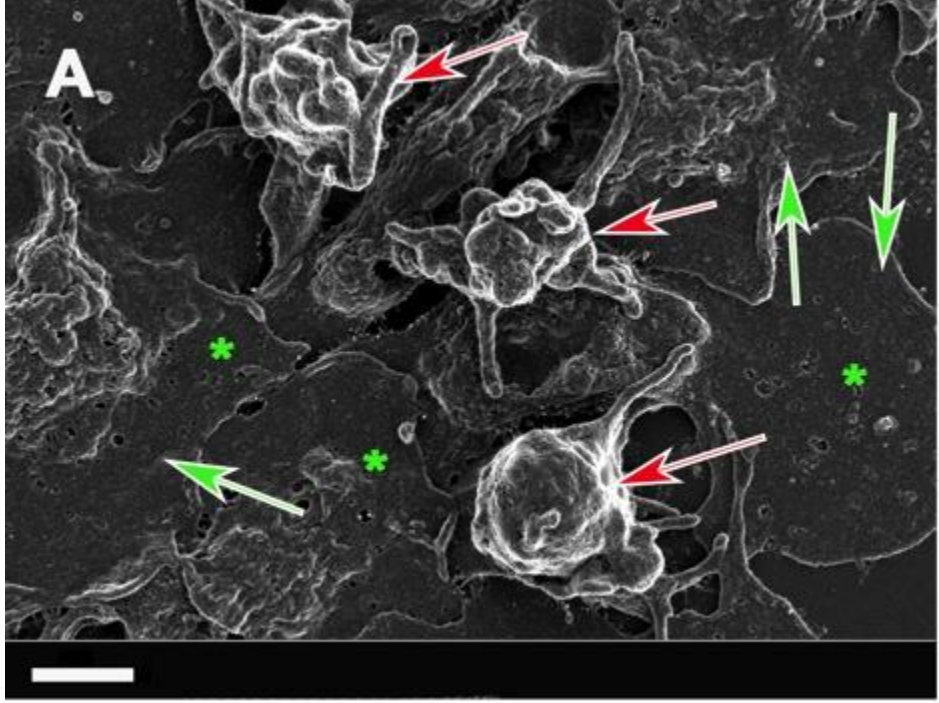
Integrative Biology (2015) 7: 24-52


HYPERCOAGULABILITY
HYPOFIBRINOLYSIS
1
Risk factors
Genetic
Environmental
Lifestyle
Medical interventions
Iron (dys)regulation
2
Factors in plasma affecting coagulation
Hepcidin
IL-6
IL-1β
Fibrinogen
Thrombin-antithrombin complex
Fibrinolysis (D-dimer)
3
Increased platelet production
CHANGED ACTIVITY
Platelet molecules with upregulated activity
Platelet Il-1α and β
Platelet factor IV
platelet/ fibrin binding
P-selectin
Thromboxane A(2)
Thromboglobulin
Thrombomodulin
von Willebrand factor
4
CHANGED CLOTTING CASCADE
INCREASED ERYTHROCYTE AGGREGABILITY AND SEDIMENTATION
INFLAMMATION



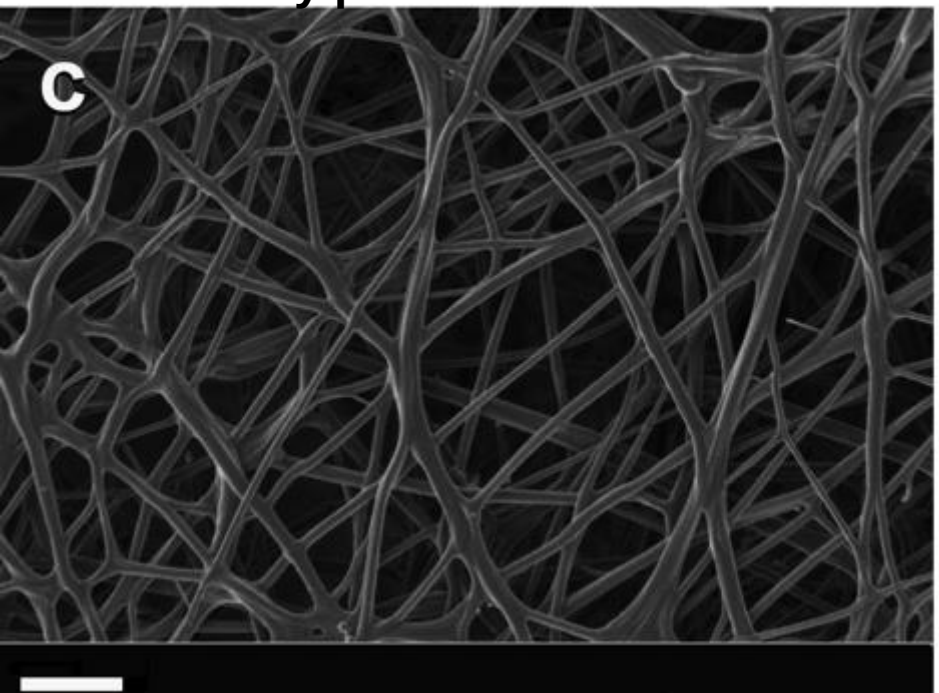


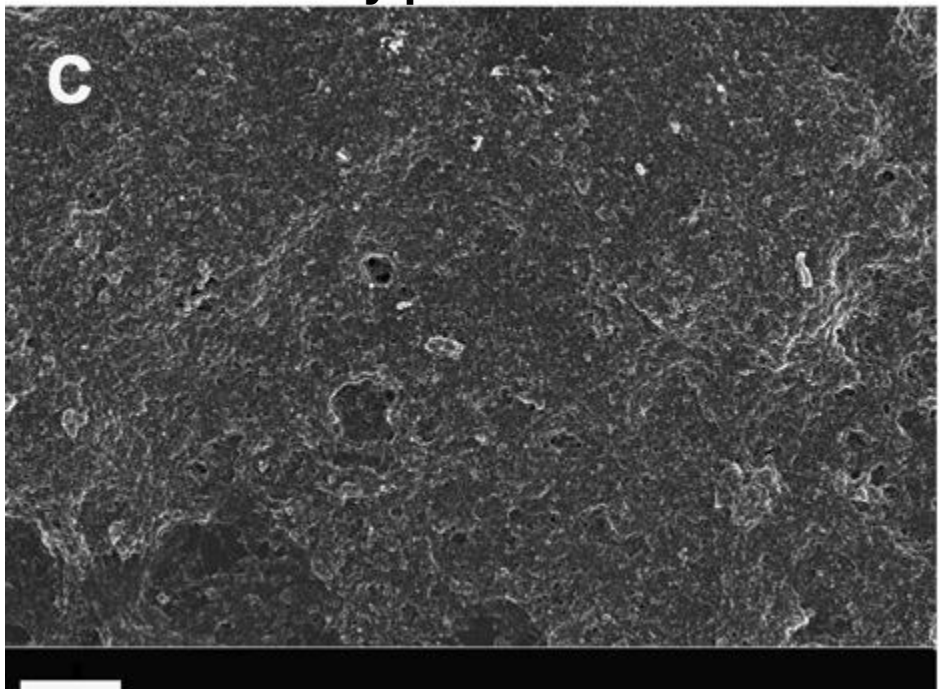




Healthy platelet and fibrin

Inflammatory platelets and fibrin





How can we measure coagulation function?

Thromboelastography

THROMBOELASTIC PARAMETERS		
R value: reaction time	Minutes	Time of latency from start of test to initial fibrin formation (amplitude of 2mm); i.e. initiation time
K: kinetics	Minutes	Time taken to achieve a certain level of clot strength (amplitude of 20mm); i.e. amplification
A (Alpha): Angle (slope between the traces represented by R and K) (	Angle in degrees	The angle measures the speed at which fibrin build up and cross linking takes place, hence assesses the rate of clot formation; i.e. thrombin burst
MA: Maximal Amplitude	mm	Maximum strength/stiffness of clot. Represents the ultimate strength of the fibrin clot, i.e. overall stability of the clot
Maximum rate of thrombus generation (MRTG)	$Dyn.cm^{-2}.s^{-1}$	The maximum velocity of clot growth observed
Time to maximum rate of thrombus generation (TMRTG)	Min	The time interval observed before the maximum speed of the clot growth
Total thrombus generation (TTG)	$Dyn.cm^{-2}$	The clot strength: the amount of total resistance (to movement of the cup and pin) generated during clot formation. This is the total area under the velocity curve during clot growth representing the amount of clot strength generated during clot growth

The hematological system in diabetes

- High blood glucose and context of insulin resistance, and relative insulin deficiency, disturb the architecture and functions of RBCs, platelets and fibrin
- ALSO glycation of haemoglobin, reduced deformability and reduced lifespan of RBCs
- Membrane cholesterol alter fluidity and bending stiffness of RBCs
- Altered RBC rheology
- Hypercoagulation due to systemic oxidative stress

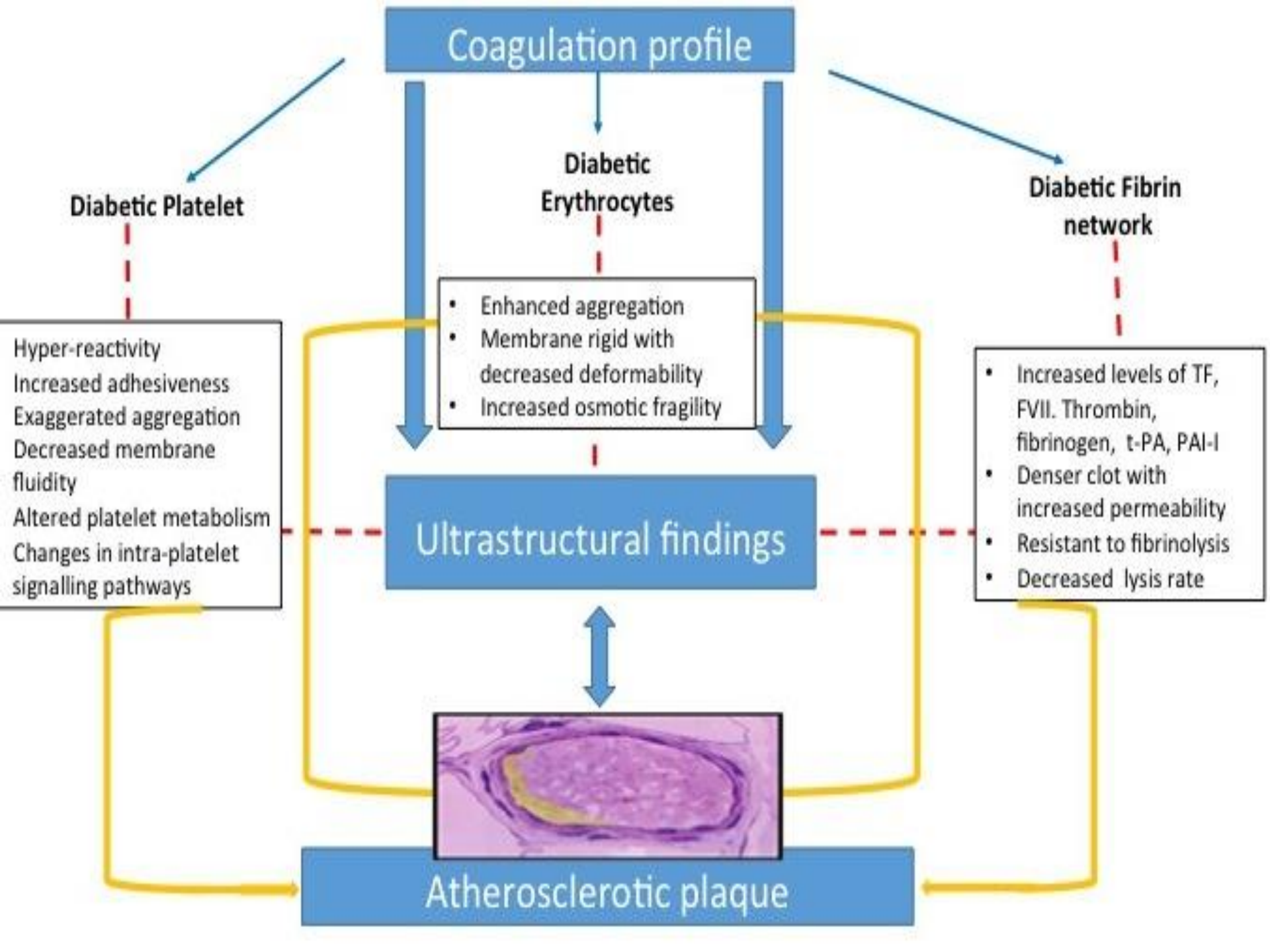
Increased HbA1c

DESPITE

Medication for:

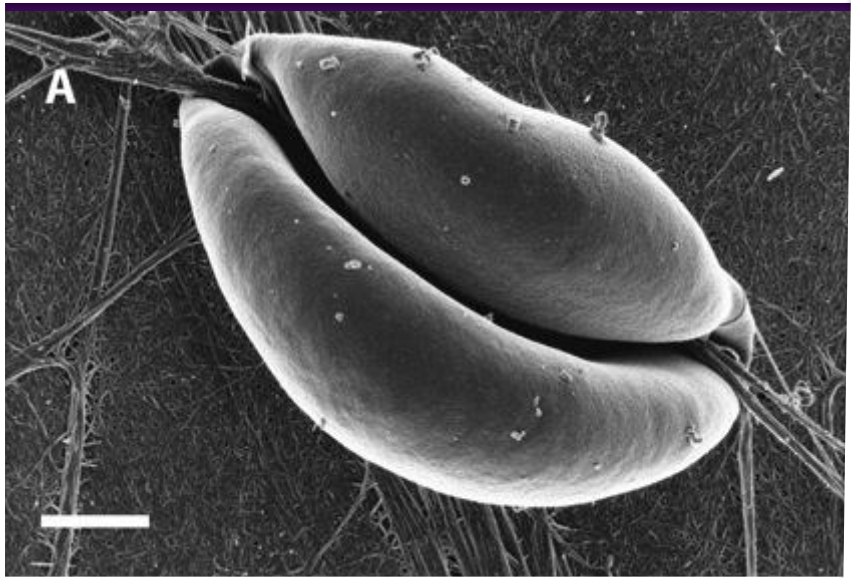
- Dyslipidemia
- blood sugar
- Hypertension
- Anti-clotting

ender	Age	Iron ($\mu mol.L^{-1}$) 11.6-31.3	Transferrin ($g.L^{-1}$) 2.2-3.7	% saturation 20 -50%	Serum ferritin ($ng.mL^{-1}$) M=20-250 F=10-120	HbA1c (%) < 7%	Dyslipidemia: Simvastatin Atorvastatin	Met-formin / Oral	Actrapid Humulin Protoph-ane Actra-phane	HT: Coversyl Amlodopine Carvedilol Adalat	Aspirin/ Disprin
	68	25.5	3.6	28	67		x	x		x	x
	56	49	3	65	86	11.9		x		x	
	71	28.1	2.2	51	210	8.3		x		x	
	80	15.5	2.6	24	96	7	x	x		x	x
	37	18.5	2.8	26	191	7.6		x			
	56	10.6	2.7	16	39	7.7		x		x	
	71	19.9	2.3	35	20	8	x	x		x	x
	48	15.1	3.3	18	46	6.2	x	x		x	x
	70	5.2	3.9	5	7	7.5		x		x	
	82	33.6	3	45	101	8.9	x	x		x	x
	62	25.6	2.3	45	202	5.9		x		x	
	71	17.8	2.4	30	243	10.5	x	x		x	x
	70	19.7	2.2	36	75	10.4		x		x	
	58	36.5	2.9	50	164	6.0		x			
	61	18.6	2.8	27	29	8.2		x		x	
	56	32.1	2.9	44	152	8.6	x	x		x	x
	42	40.7	2.3	71	217	13.6		x			
	62	16.2	4.1	16	24	10.6	x	x		x	x
	72	19.8	2.1	38	332	6.7		x	x	x	x
	59	18.5	2.8	26	191	?	x	x			
	75	14	1.7	33	218	6.6		x	x	x	x
	41	28.2	3	38	235	8.3	x	x	x		x
	81	23.8	2.3	41	62	9	x		x	x	x
	41	33.4	2.7	49	101	5.5	x	x	x		x
	80	39.5	2.7	59	142	6.8	x	x		x	
	64	13.2	2.3	23	50	?		x	x		
	63	22.9	2.4	38	265	10.2	x	x	x	x	x
	49	7.1	3.4	8	12	6.8	x	x	x	x	
	72	25.3	2.4	42	386	6.7		x	x	x	x
	46	21	2.4	35	233	11.6		x			
	40	14.5	2.4	24	96	8.7			x	x	
	55	10.3	2.2	19	88	5.8		x	x	x	
	62	BLOOD CLOTTED				8.2	x			x	
	52	25.9	3	35	155			x		x	
	59	18.5	2.5	30	125	11.6	x		x	x	
	58	16.5	2.5	26	79	6.0	x	x	x	x	x
	62	14.1	2.9	19	64	10.2	x	x	x	x	
	60	6.1	2.6	9	93	7.5	x	x	x	x	x
	57	24	2.7	36	60	8.0	x	x	x	x	x
	63	31.4	2.5	50	77	7.6	x	x	x	x	
	59	15.4	2.8	22	151	8.5	x		x	x	x
	73	29.5	2.9	51	154	8.0	x		x	x	
	58	20.3	2	41	81	10.6	x	x	x	x	
	66	28.6	2.6	44	359	10.0	x	x	x	x	
	60	18	2.4	30	192	12.2	x	x	x	x	
	69	9.6	2.3	17	74	7.4	x	x	x	x	
	62	21.1	3.1	27	28	9.2	x	x		x	
	58	28.2	2.8	40	605	11.9	x	x	x	x	x


Coagulation profile
Diabetic Platelet
Diabetic Erythrocytes
Diabetic Fibrin network
Hyper-reactivity
Increased adhesiveness
Exaggerated aggregation
Decreased membrane fluidity
Altered platelet metabolism
Changes in intra-platelet signalling pathways
Enhanced aggregation
Membrane rigid with decreased deformability
Increased osmotic fragility
Increased levels of TF, FVII. Thrombin, fibrinogen, t-PA, PAI-I
Denser clot with increased permeability
Resistant to fibrinolysis
Decreased lysis rate
Ultrastructural findings
Atherosclerotic plaque

Changes in red blood cell membrane structure in type 2 diabetes: a scanning electron and atomic force microscopy study

Antoinette V Buys[1], Mia-Jean Van Rooy[2], Prashilla Soma[2], Dirk Van Papendorp[2], Boguslaw Lipinski[3] and Etheresia Pretorius[2*]



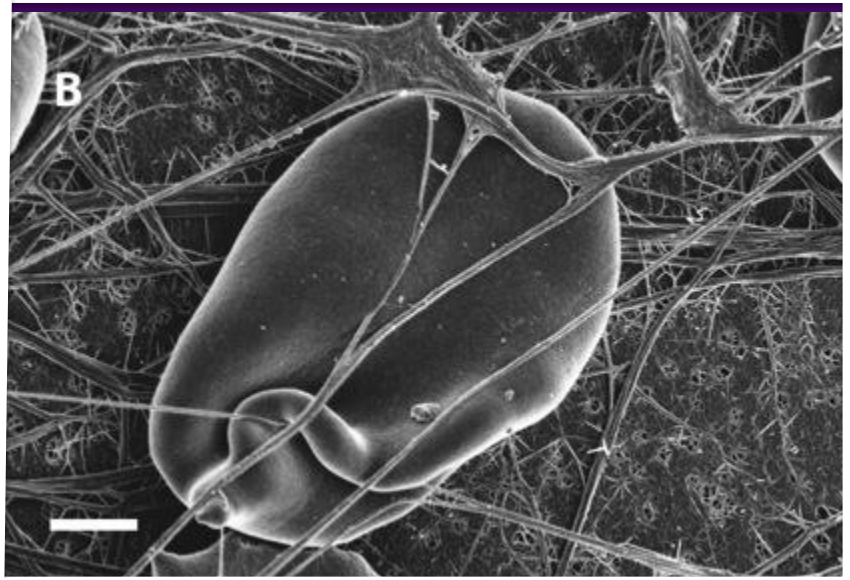


Table 1 Macroparameter measurement results from healthy and diabetic erythrocytes

	Control	Diabetic	p-value
Diameter	7.22 μm ± 0.16 μm	6.80 μm ± 0.08 μm	0.028584*
Height	2.68 μm ± 0.04 μm	1.48 μm ± 0.11 μm	2.44057 E-07*
Concave Depth	358.2 nm ± 117.6 nm	153.9 nm ± 58.6 nm	0.00296363*

*All p-values indicated significant difference between two groups.

Membranes have decreased roughness more deformable

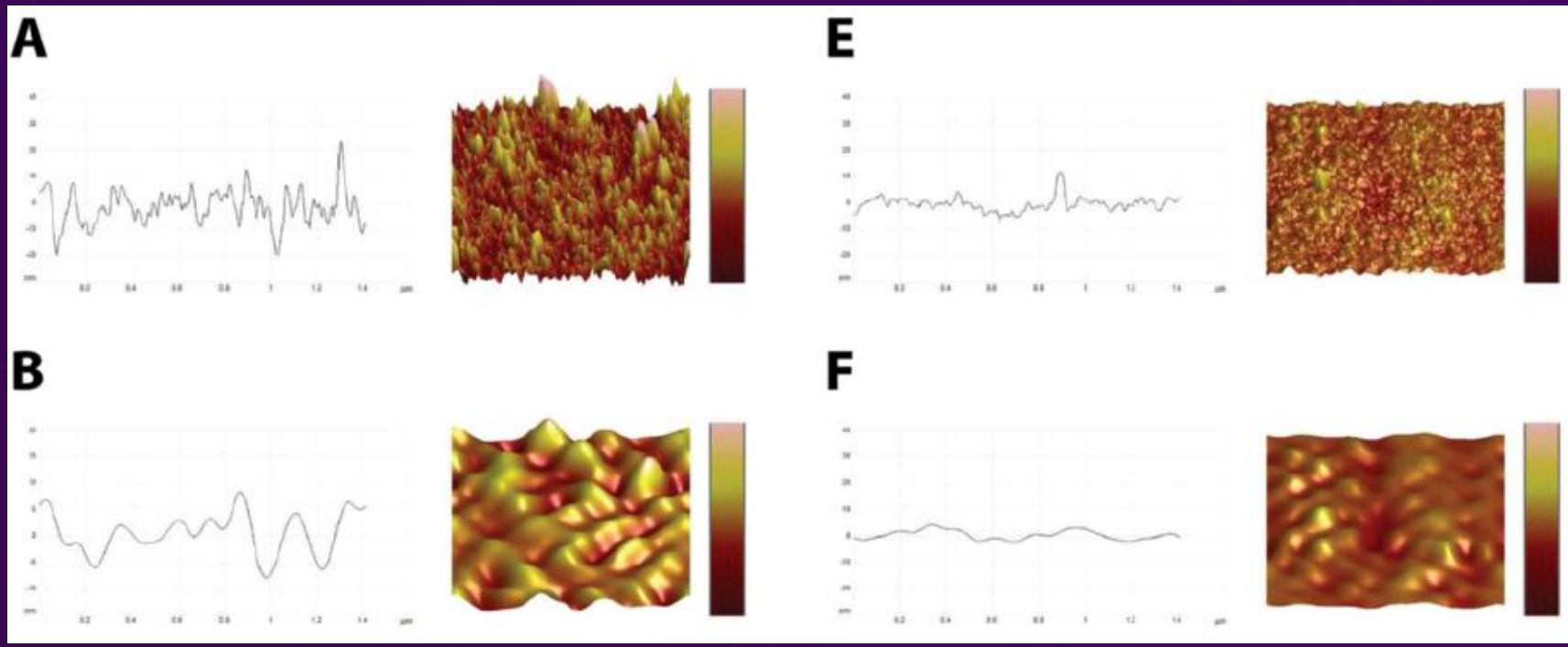
Changes in red blood cell membrane structure in type 2 diabetes: a scanning electron and atomic force microscopy study

(2013) Cardiovasc Diabetol 12, 25

Antoinette V Buys[1], Mia-Jean Van Rooy[2], Prashilla Soma[2], Dirk Van Papendorp[2], Boguslaw Lipinski[3] and Etheresia Pretorius[2*]

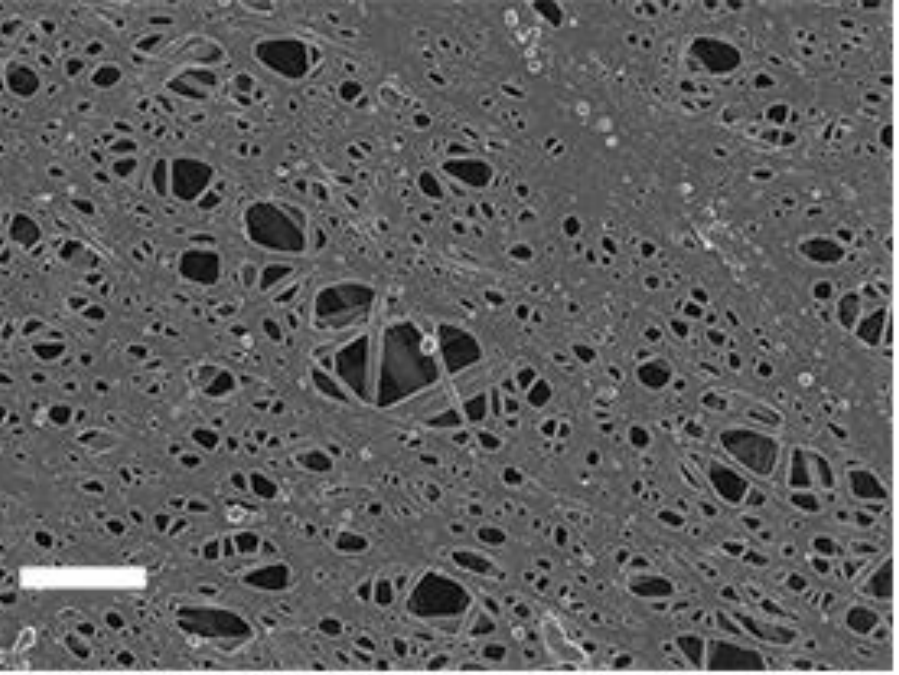
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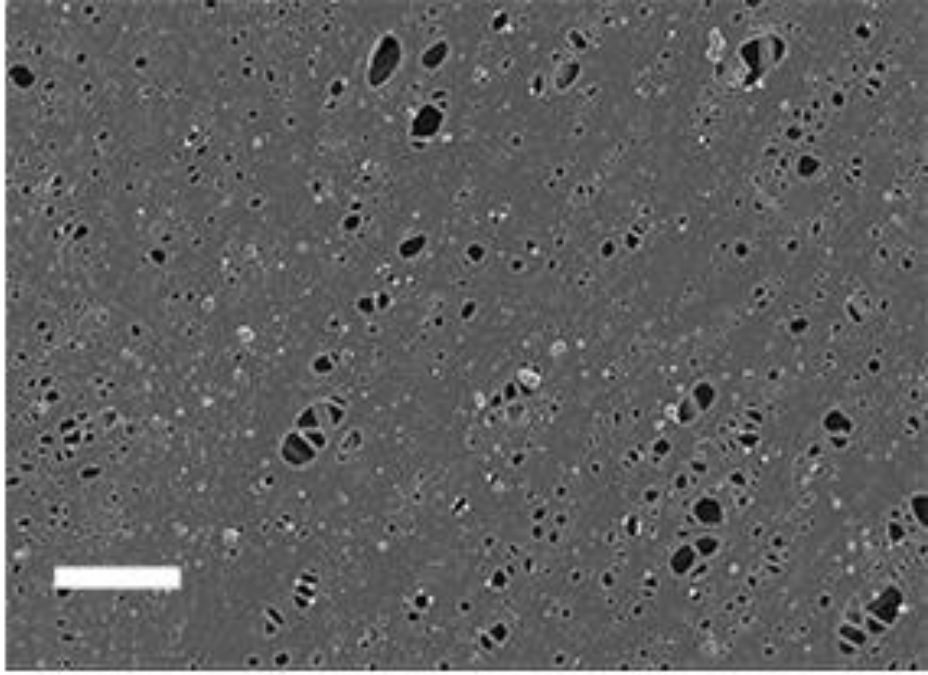
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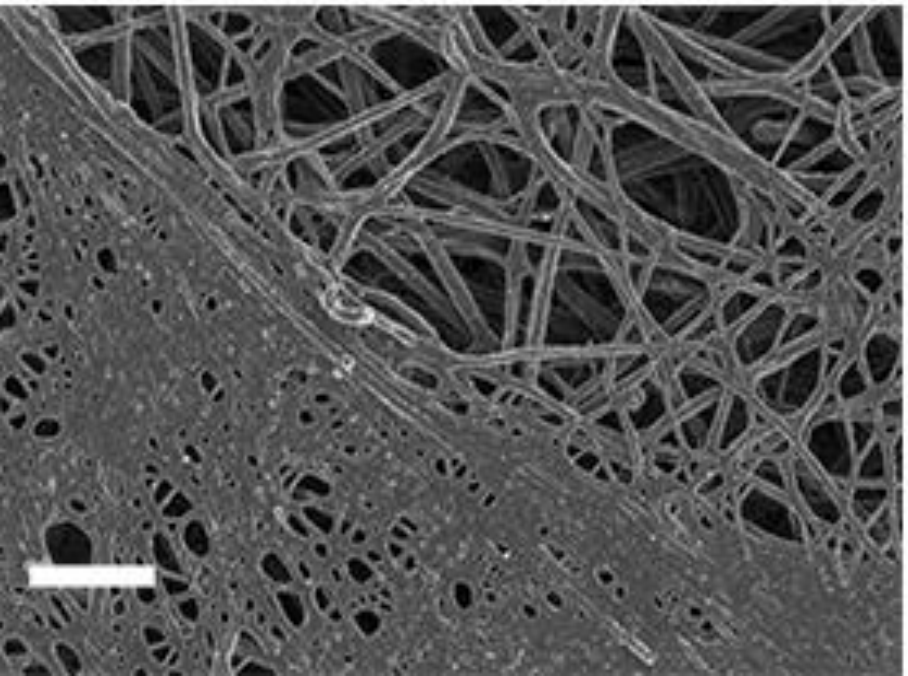


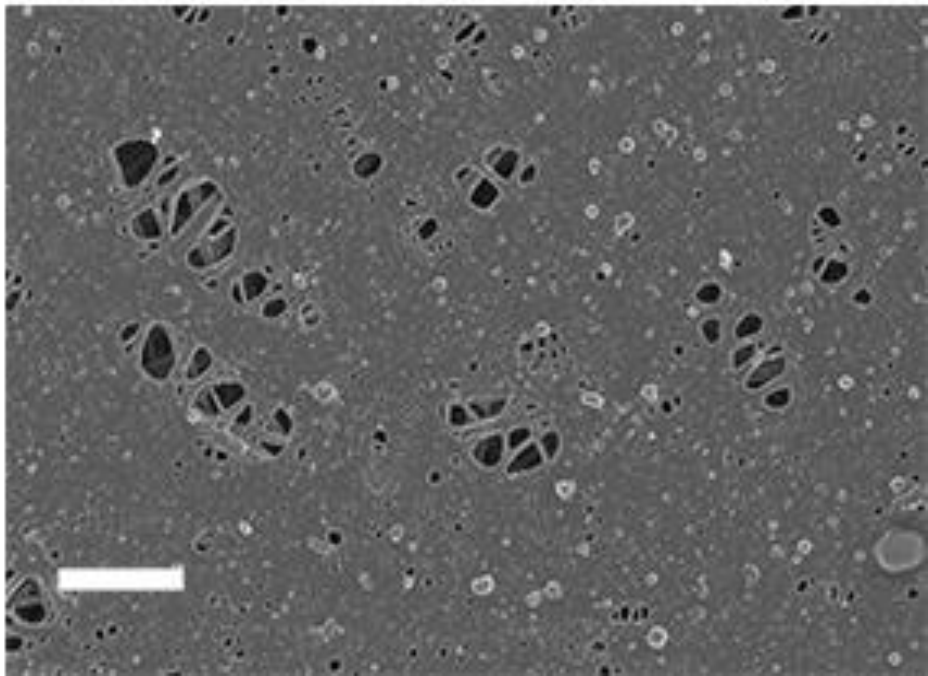
Control

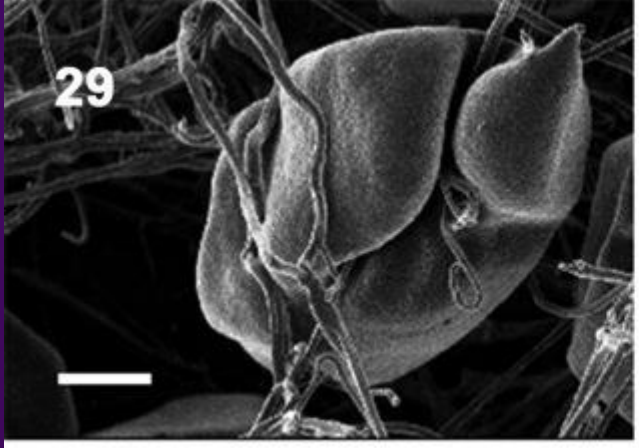
Diabetic

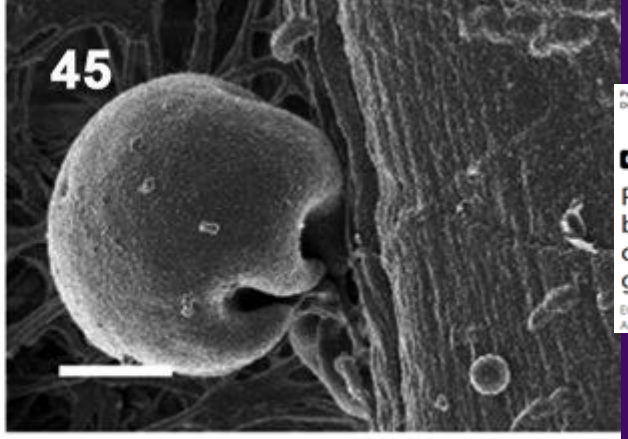


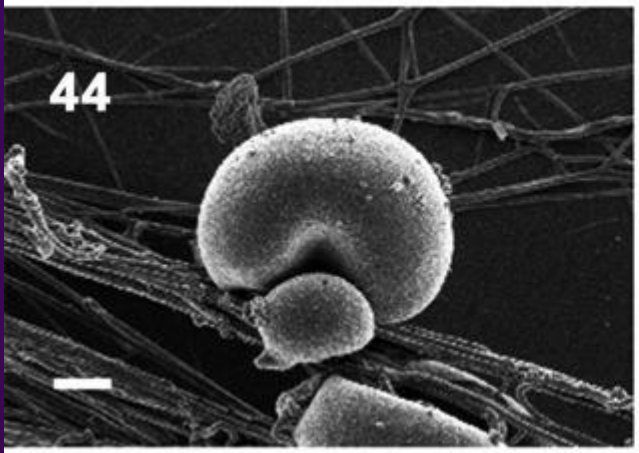


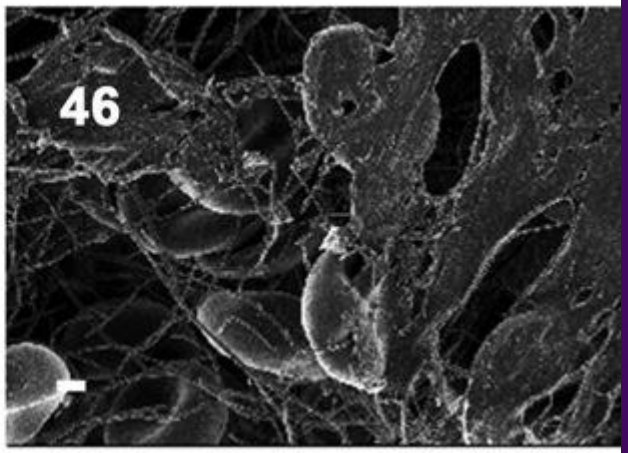


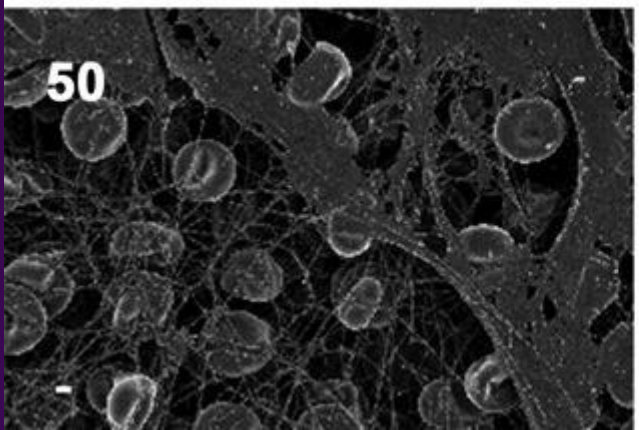


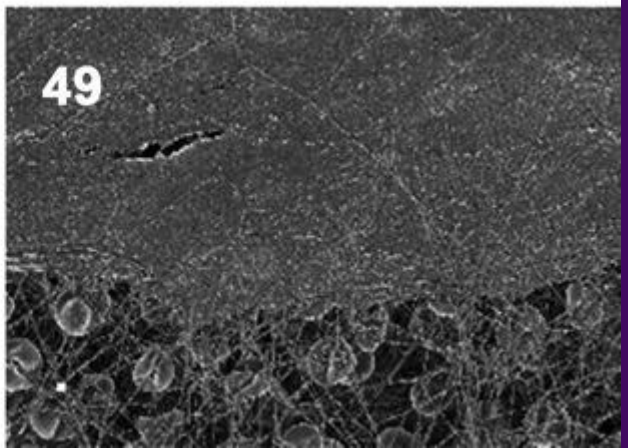












Pretorius et al. Cardiovascular Diabetology (2015) 14:30
DOI 10.1186/s12933-015-0192-5

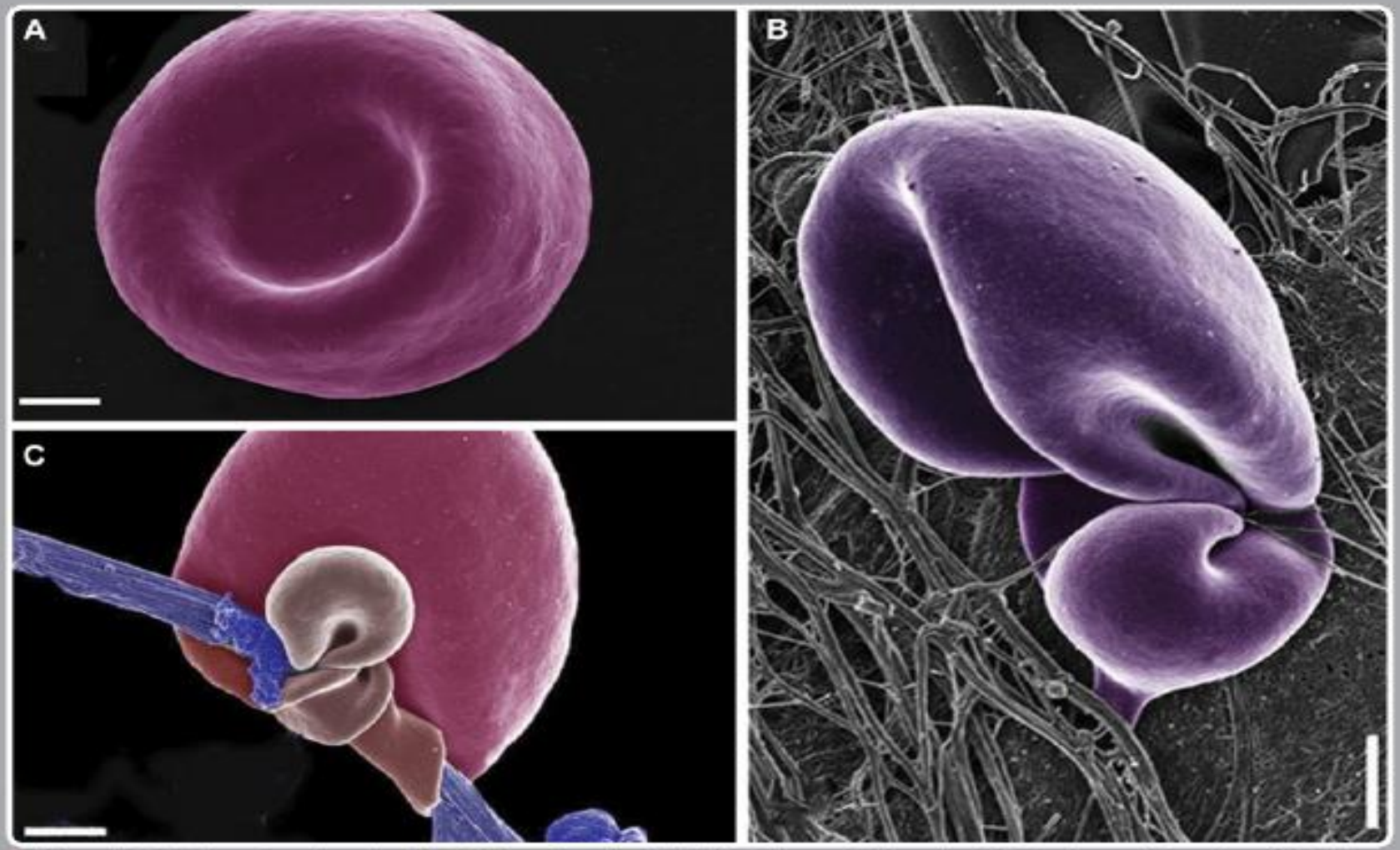
CARDIO VASCULAR DIABETOLOGY

ORIGINAL INVESTIGATION Open Access

Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics

Etheresia Pretorius[1*], Janette Bester[1], Natasha Vermeulen[1], Sajee Alummoottil[1], Prashilla Soma[1], Antoinette V Buys[2] and Douglas B Kell[3*]

Iron alters red blood cell morphology



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A Normal, B diabetic with raised iron, C added Fe^{3+}

Many potential uses in diagnostics

Integrative Biology

REVIEW ARTICLE

Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases

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In line with the NIH goals for an individualized patient-centered precision medicine approach: Collins FS and Varmus H: A new initiative on precision medicine. N Engl J Med, 2015; 372:793-795

Individualized patient care precision medicine?

- Because type II diabetes is such an extensive health issue,
- We suggest that the answer to adequate treatment is based on an *individualized patient-centered approach*, in combination with the latest NIH precision medicine approach
- COLLINS, F. S. & VARMUS, H. 2015. A new initiative on precision medicine. *N Engl J Med,* 372, 793-5.
- A combination of viscoelastic and ultrastructural methodologies may be used in a personalized patient-centered regime

Why?

The biological insight of this line of though is that biophysical and biochemical changes to RBCs are a significant accompaniment to type II diabetes.

The technological innovation is the use of advanced microscopies and viscoelastic measurements together with other more run-of-the-mill tests used by practitioners.
Together, quick biophysical and biochemical readouts done individually the effect of medication can be seen quickly

The benefit of this integration are that these readouts are at a much higher physiological or phenotypic level than typical biomarkers and that RBC shape changes may form an essential role in the envisaged individualized, precision medicine approach.

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