ORIGINAL ARTICLE

The stabilizing effect of an oligomeric proanthocyanidin on red blood cell membrane structure of poorly controlled Type II diabetes

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Type II diabetes (T2D) is a pandemic characterized by pathological circulating inflammatory markers, high-glucose levels and oxidative stress. The hematological system is especially vulnerable to these aberrant circulating molecules, and erythrocytes (RBCs) show aberrant rheology properties, owing to the direct contact with these molecules. Pathological levels of circulating inflammatory markers in T2D therefore have a direct effect on the molecular and cellular structure of RBCs. Previous research has suggested that antioxidants may reduce oxidative stress that results from the pathological inflammatory markers. Particularly, polyphenol antioxidants like oligomeric proanthocyanidins (OPCs) may act as a hydroxyl mopping agent, and may have a positive effect on the deformability and membrane protein structure of RBCs from T2D. In this paper, we look at the effect of one such agent, *Pinus massoniana* bark extract (standardized to 95% oligomeric proanthicyanidins), on the RBC membrane structures and RBC shape changes of T2D, after laboratory exposure at physiological levels. Our methods of choice were atomic force microscopy and scanning electron microscopy to study RBC elasticity and ultrastructure. Results showed that in our hands, this OPC could change both the eryptotic nature of the RBCs, as viewed with scanning electron microscopy, as well as the elasticity. We found a significant difference in variation between the elasticity measurement values between the RBCs before and after OPC exposure (P-value < 0.0001). In conclusion, the data from both these techniques therefore suggest that OPC usage might contribute to the improvement of RBC functioning.

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INTRODUCTION

Type II diabetes (T2D) is commonly associated with comorbidities including dyslipidaemia[1–4] and hypertension.[4,5] The main cause of morbidity and mortality in individuals with T2D is cardiovascular disease (CVD), with up to 80% of diabetes subjects dying as a result of cardiovascular complications.[1,3,6] The risk of athero-thrombotic events is also comparable to individuals without diabetes but with a history of ischemic heart disease.[1] Furthermore, the prognosis in these individuals following an event remains poor, despite major advances in treatment[1] such as antiplatelet therapies, and the control of the modifiable risk factors like hypertension, obesity, smoking and dyslipidaemia.[7]

Changed inflammatory marker levels, oxidative stress and chronic (systemic) inflammation, all lead to a pathophysiological coagulation system that is characterized by hypercoagulability. Systemic inflammation has a fundamental role in many chronic conditions, including T2D and CVD.[8–12] CVD and vascular dysfunction are fundamental parts of the etiology of T2D and this condition is also known to have a changed coagulation system. Therefore, all these factors serve as early indicators of vascular dysfunction. Thus, there are important links between oxidative stress, a changed inflammatory marker profile and inflammation, the progression of T2D and ultimately, vascular dysfunction.[4]

Dysregulated inflammatory markers, either upregulated or downregulated, are associated with diabetes.[4] These inflammatory markers are mainly C-reactive protein,[13,14] coagulation factors,[14–18] nitric oxide,[19–23] tumor necrosis factor-α, nuclear factor kappa-B, cyclooxygenase-2,[24–28] prostaglandin-2,[29,30] Interleukin-6[16,17,29,31,32] and thromboxane A2.[33,34]

A fundamental part of a pathological coagulation system and a systemic hypercoagulability, is an altered erythrocyte (RBC) function. All of the above-mentioned circulating inflammatory markers have a noticeable effect on RBC functioning, specifically on their rheology properties. Hypercoagulability affects fibrin (ogen) and this in turn also directly impacts on RBC rheology. It is also thought that abnormalities of high blood glucose as is the case in insulin resistance, and relative insulin deficiency, may have a negative effect on the structure and function of RBCs at molecular scale.[4]

Pathological levels of circulating inflammatory markers, high-glucose levels and the associated oxidative stress in T2D therefore have a direct effect on the molecular and cellular structure of RBCs. Their membranes consist of the typical lipid bilayer, the actin–spectrin cortex and the integral and peripheral membrane proteins,[35,36] of which ~20 are major proteins and at least 850 minor ones.[35] In particular, flippases, floppases and scramblases (integral proteins) facilitate the transmembrane passage, as well as the structural arrangement of lipids RBC membranes.

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Structural alterations, owing to the systemic inflammatory profile in T2D, are known to affect the RBC membrane lipids, as well as band 3 and spectrin, which in turn cause RBC structural shape changes.[36,37] For an in-depth review of the RBC membrane structure, see reference Pretorius *et al*.[38]

AS oxidative stress is known to have a role in the progression of T2D[4,23,39,40] and biophysical shape changes of RBCs,[36,41] the question that now arose is whether there is a polyphenol antioxidant that may act as a hydroxyl mopping agent, and may have a positive effect on the deformability and membrane protein structure of RBCs from T2D that can be added to the diet. A group of (polyphenolic) antioxidants found in pine bark extract and grape seed extract that may have an effect, is the oligomeric proanthocyanidins (OPCs). Various preliminary clinical trials have been conducted investigating the effect of OPCs in several indications and clinical contexts including asthma, CVD and diabetes.[42–47]

It has been shown that OPCs increase erythrocyte membrane fluidity *in vitro*, potentially due to inducing modification of the lipid bilayer and lipid–protein interactions as shown by Sivonova and co-workers in 2004.[42] In an *in vitro* study, OPCs (0.07 mg ml^{-1}) resulted in 50% inhibition of the formation of erythrocytes with elevated density (increased adherence to neutrophils, platelets and vascular endothelial cells) from patients with sickle cell anemia.[48] OPCs have also been shown to have anti-inflammatory effects. It has been demonstrated to inhibited cyclooxygenase-1 and -2 activity[49] and reducing the production of interleukin-1beta and its associated mRNA *in vitro*, as well as the expression of IL-2.[50] Activation of two major transcription factors heavily involved in interleukin-1beta gene expression, nuclear factor kappa-B and activator protein-1, have also been shown to be reduced.

In the current study, we therefore investigated the effect of the addition of physiological levels of OPCs (*Pinus massoniana* bark extract standardized to 95% oligomeric proanthicyanidins), on the RBC membrane structures and RBC shape changes of T2D. Shape changes or patho-morphological changes are closely associated with RBC properties including area deformability, such as membrane elasticity changes due to membrane biochemical changes as discussed previously, and directly translate as biophysical indicators. In this study, atomic force microscopy (AFM) was used to evaluate membrane elasticity, whereas scanning electron microscopy (SEM) was used to evaluate physical changes. Although a randomized control trial is obviously the most useful way to establish the effect of any molecule (dietary or otherwise) it is of great importance to first establish if the molecule has any effect *in vitro*.

MATERIALS AND METHODS
This study was approved by the Ethical Committee of the University of Pretoria (South Africa). A written form of informed consent was obtained from all healthy donors (available on request). The methods were carried out in accordance with the approved guidelines. Blood was collected and methods were carried out in accordance with the relevant guidelines of the ethics committee (ethics number: 506/2014 and 298/2016: E Pretorius and J Bester: principal investigators for use of control blood; ethics number: 151/2006 for T2D study). We adhered strictly to the Declaration of Helsinki.

Samples used
We recruited 20 healthy individuals with no systemic inflammation, and who did not use any chronic medication, as our control group. T2D individuals were voluntarily recruited, from the Diabetic Clinic at the Steve Biko academic hospital, Pretoria, South Africa. Demographic data including age, gender, glucose level at time of sample collection, hemoglobin A1c (HbA1c) measures and medication used for typical comorbidities of T2D were recorded. Inclusion criteria consisted of both male and female participants, aged between 19 and 70, with a diagnosis of T2D for >3 months prior to screening and without any signs of infection. Smoking was an exclusion criteria and our samples consisted of individuals (control

and T2D) who did not smoke. Diabetic individuals were diagnosed according to the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMSDA) guidelines. These guidelines follow the American Diabetes Association (ADA) criteria to define T2D.

An important consideration might be that these OPCs should not interact negatively with the currently used medications that typical poorly controlled diabetes patients may be taking. Therefore, a literature search was also done to investigate possible interactions of OPCs with medication typically prescribed for comorbidities associated with T2D.

Dosage of OPCs
The standard daily dosage of pine bark extract standardized to 95% OPCs for people with diabetes is 50–200 mg per day.[43,47,51,52] The extract that was used is a 4:1 concentrated extract. Therefore, in order to reach a daily dosage of 200 mg, 50 mg was used. Based on an average individual of 70 kg, the final concentration in the blood is expected to be 9 µg ml^{-1}. Therefore, the exposure concentration for both the AFM and SEM experiments was 9 µg ml^{-1}.

Sample preparation for AFM
Whole blood samples were centrifuged at 145 g for 30 s. The supernatant consisting of plasma, platelets and leukocytes, was discarded. The remaining RBCs were prepared for AFM by fixing in 4% formaldehyde made up in phosphate-buffered saline for 30 min, at room temperature (22 °C) followed by dehydration. RBCs were suspended in undiluted (161.39 g mol^{-1}) hexamethyldisilazane onto a glass cover slip by placing a drop and spreading the fluid by tilting the cover slip sideways, ensuring an even distribution of cells. The cover slips were then dried and stored until AFM analysis.

AFM imaging and measurement
The cells were characterized with a commercial AFM system (Dimension Icon with ScanAsyst, Bruker, Santa Barbara, CA, USA) using the PeakForce QNM (Quantitative Nanomechanical Property Mapping) imaging mode. This method is similar to the standard tapping mode of scanning probe microscopy, where the probe and the sample are brought together intermittently. See Bester and co-workers 2013,[53] for a comprehensive discussion of the AFM methods used in this paper. This mode operates by controlling the maximum force applied by the probe to the sample[54] and allows that at every pixel a rapid force–distance curve is generated. The cantilever's deflection sensitivity and spring constant is calibrated before measurements, therefore the curve can be analyzed quantitatively to obtain a series of specific property maps of the sample. The retract curve can then be used to calculate the slope of the curve (which is called the modulus) and the minimum of the curve (otherwise known as adhesion images). The deformation can therefore be calculated and this is the variation between the zero and maximum force. Following this, the area between the approach and retract curve can then be used to calculate energy dissipation.[53,55,56] The stiffness of an elastic material can be measured using Young's modulus.[36,57,58] Young's modulus is the stress divided by the corresponding strain, with greater values indicating increased stiffness or decreased deformability. As each force curve's data, can also be stored individually, and quantitative measurements of the Young's modulus can be generated by fitting the slope of any force–distance curve of the image to an appropriate model (in this instance; the Derjaguin–Muller–Toporov Model.[59]

Silicon Nitride probes (TAP525 – MPP 13120 – 10, Bruker, USA) were employed in all AFM measurements and these probes were specified to have a nominal force constant of 200 N.m-1, a resonant frequency between 430 and 516 kHz (measured by the manufacturer), and a nominal tip radius of 15 nm. Ten cells per participant were analyzed by selecting a 1µm by 1µm scan area on the periphery of the RBC and performing 128 by 128 data points of individual force curve measurements with a peak force of 6 µN. As there might be differences in concavity of RBCs, the periphery of the cells was chosen to ensure that an area that is not affected by the concavity of the specific RBC is measured. The scans were performed at 0.6 Hz, which is a tip velocity of 1.2 µm s^{-1} and 50 force curves were chosen at random within the stated area. The offline software (NanoScope Analysis version R3, Bruker, USA) was used to process the force curves. A goodness-of-fit model was used to obtain the coefficient of determination (R^2) between the modulus model and the data given by the acquired curve, determined by calculating the ratio of explained variation to total variation

in the data set. We only used force curves with a coefficient of determination of 0.85 and above for modulus measurements.

Atomic force microscopy

To have statistical power of 95% to detect a statistically significant treatment effect at a 1% significance level, a sample of 35 T2D patients is required, whereas 47 patients are needed for statistical power of 99% (see Rosner[60]) for details on sample size calculations in before–after studies). A sample of 60 T2D patients was recruited and AFM data (RBC membrane elasticity measurements) were available for 56 of these individuals. The elasticity measurement distributions were analyzed for each patient. Owing to the asymmetry of these distributions, it was decided to calculate for each patient the median elasticity measurement value from the corresponding nave T2D blood sample. The same was done for each patient for the T2D blood sample exposed to OPCs. Thus, we obtained 56 matched (paired) median values to be utilized as reference values in the various graphical and statistical analyses done. These analyses included the drawing of histograms and box and whisker diagrams, testing for normality, testing for a difference in the medians between the nave T2D blood samples and T2D blood samples exposed to OPCs and testing for a difference in the variation (spread) between these two groups of blood samples.

Statistical analyses were done using SAS/STAT and SAS/IML software, Version 9.4 of the SAS System for Windows (SAS Institute, Cary, NC, USA). The histograms and box and whisker diagrams were drawn with Wolfram Mathematica, Version 10.4 (Wolfram Research, Champaign, IL, USA).

Scanning electron microscopy

The SEM experiment was conducted by comparing the naive T2D blood samples with T2D blood samples exposed to OPCs. Whole blood samples from the 20 healthy individuals were also collected, scanned and analyzed as a control group. After the blood was collected, 10 µl of whole blood with 5 µl thrombin (to create an extensive fibrin network) were placed directly on a glass cover slip, fixed, dehydrated, dried, mounted and coated with carbon according to previously described methods.[4,61] A Zeiss ULTRA Plus FEG-SEM with InLens capabilities was used and micrographs were taken at 1 kV.

RESULTS

The demographic data for the group of 60 patients with T2D is summarized in Table 1. The information collected included glucose measured at the time of sample collection, HbA1c values as well as chronic medication typically administered to T2D patients. Table 1 shows that, although almost all T2D participants (97%) are taking hypoglycaemic medication, the samples group still represents a poorly controlled diabetic population. Besides medication for glucose control, the T2D group also includes individuals on medication for dyslipidaemia and hypertension, whereas some are taking anticoagulants. The control group of 20 healthy individuals was selected to be age and gender matched to the T2D group.

An important consideration for this study was the possible interactions of OPCs with the currently used mediations that typical poorly controlled diabetes patients may be taking (see

Table 1. Summary of patient demographic data and medication

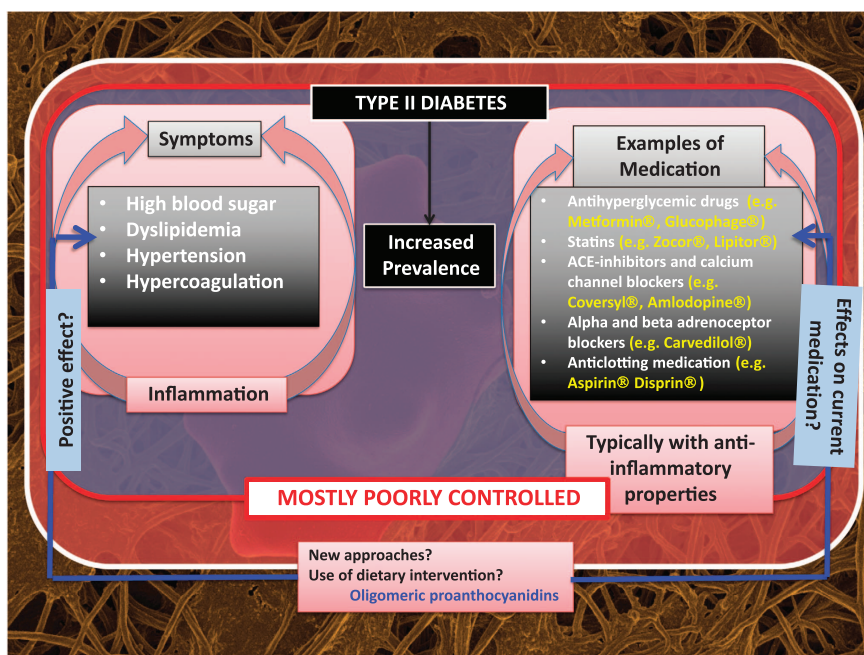| *TYPE II diabetes demographic data and medication summary*	
% Distributions	*Individuals with diabetes*
Number of female patients (%)	34 (57%)
Number of male patients (%)	26 (43%)
Total no of patients	60
Average age ± s.d.	56.7 ± 9.9
Average HbA1c (%) ± s.d.	9.0 ± 2.2
Average glucose level (mmol l^{-1}) ± s.d.	10.1 ± 5.1
Number of patients taking anticholesterol medication (%)	40 (67%)
Number of patients taking hypoglycaemic medication (%)	58 (97%)
Number of patients taking anticoagulants medication (%)	31 (52%)
Number of patients taking antihypertensive medication (%)	44 (73%)

Figure 1. Type II diabetes, symptoms suggestive of systemic inflammation, typical medication for the comorbidities and solution for disease tracking based on an individualized, precision medicine approach.

Table 2. Medication typically administered to diabetes type II patients and possible interactions with OPCs

Medication	Selected references
Glucose control	
Effect on blood glucose levels in general	No evidence of interaction
Antihyperglycemic drug dimethylbiguanide (metformin/glucophage)	No evidence of interaction
Actraphane (mixture of fast-acting insulin and long-acting insulin)	No evidence of interaction
Actrapid (human soluble insulin)	No evidence of interaction
Humulin (70% human insulin isophane suspension and 30% human insulin injection (rDNA origin))	No evidence of interaction
Protophane (intermediate-acting insulin)	No evidence of interaction
Hypertension control	
Effect on blood pressure in general	Had no effect on blood pressure or heart rate.[46,55] In patients with mild blood pressure, blood pressure was normalized and thromboxane levels lowered.[47,56]
Coversyl (active ingredient is perindopril arginine which is a angiotensin-converting enzyme inhibitor)	No evidence of interaction
Amlodopine (calcium channel blockers)	Reduced the dosage of the calcium channel blocker nifedipine significantly; plasma levels of endothelin-1 were reduced and concentrations of prostacyclin were elevated[47,57]
Carvedilol (beta and alpha adrenoceptor blocker with antioxidant activity)	No evidence of interaction
Adalat (nifedipine) calcium channel blocker)	No evidence of interaction
Anti-clotting medication	
Aspirin (acetylsalicylic acid)	An inhibitory effect on platelet aggregation similar to aspirin.[44,47,55,56,58] Does not affect INR (bleeding tendency) in patients taking aspirin.[46]
Cholesterol medication	
Effect on blood lipid levels in general	Decreases LDL-cholesterol, increases HDL-cholesterol levels, no significant change in total cholesterol or triglycerides.[46,59] No statistically significant improvement in total cholesterol or LDL levels compared with placebo.[44,60]
Simvastatin	No evidence of interaction
Lipitor	No evidence of interaction

Table 3. Descriptive statistics and normality tests for the group of participants with type II diabetes (naive T2D) and for the same group of participants treated with OPCs (T2D+OPCs)

	Naive T2D	T2D group treated with OPCS
Descriptive statistics		
Sample size	56	56
Mean	30197.76	32730.20
s.d.	17693.60	21559.00
Median	26622.00	29326.25
75 percentile	36202.50	46812.00
25 percentile	16952.00	15545.80
Interquartile range	19250.50	31266.20
Maximum	106986.50	103698.00
Minimum	8093.00	4499.00
Range	98893.50	99199.00
P-values for normality tests		
Anderson–Darling	0.0001	0.0270
Shapiro–Wilk	< 0.0001	0.0047

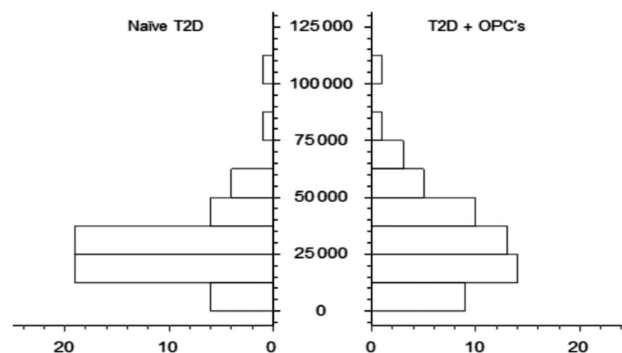Abbreviations: OPCs; oligomeric proanthocyanidins; T2D, type II diabetes.

Figure 2. Paired histograms comparing the group of participants with type II diabetes (naive T2D) and the same group of participants treated with OPCs (T2D+OPCs).

Figure 1). Table 2 shows possible interactions of OPCs with typically prescribed chronic medication.

Descriptive statistics for the naive T2D group and the T2D group treated with OPCs are summarized in Table 3, whereas Figures 2 and 3 illustrate paired histograms and box and whisker diagrams for the two diabetes groups. It is clear from these diagrams that both groups are positively skewed and hence non-normal. The non-normality was confirmed with various tests for normality,

including the Anderson–Darling[62] and Shapiro–Wilk tests[63] (see Table 3). Figure 4 shows a box and whisker diagram for the control group of 20 healthy individuals.

Owing to the non-normality of the data, non-parametric hypothesis tests were utilized to compare the elasticity measurement values between the two groups of blood samples. First, with the Wilcoxon matched pairs test[64] no statistically significant difference in the median elasticity measurement values between the naive T2D blood samples and T2D blood samples exposed to OPCs was found (P-value = 0.3083). This is also evident from the box and whisker diagrams in Figure 3. However, these box and whisker diagrams do suggest that the variation in elasticity
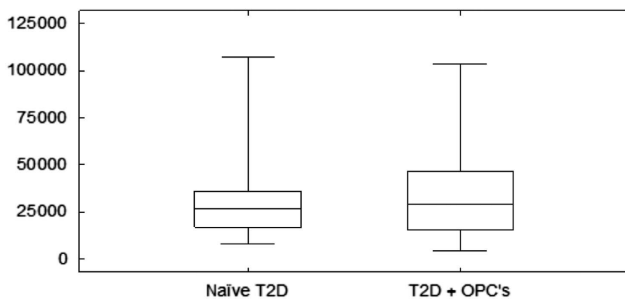
Figure 3. Box and whisker diagrams for the group of participants with type II diabetes (naive T2D) and the same group of participants treated with OPCs (T2D+OPCs).
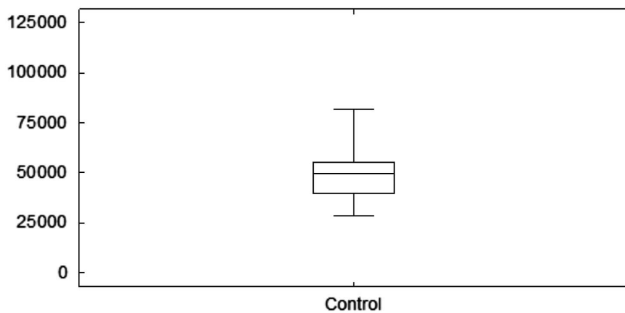

Figure 4. Box and whisker diagram for the control group of 20 healthy individuals.

measurement values for the T2D group treated with OPCs is larger than the variation in elasticity measurement values for the naive T2D group. Using the modification of Wilcox[65] of the Morgan–Pitman test,[66,67] a significant difference in variation between the elasticity measurement values of the two groups of blood samples was found (P-value < 0.0001).

Scanning electron microscopy
SEM followed the same trends as the AFM results, and it seems as if the OPC changes the RBC membrane to be comparable to that of a typical healthy RBC membrane (see Figure 5). A healthy RBC membrane shows a smooth undulating structure, where RBCs from T2D are known to be eryptotic, with a membrane that is much rougher.[4,61]

Eryptosis is programmed RBC cell death, similar to that of apoptosis, and is found in (all) inflammatory conditions. For comprehensive reviews on this process, see Pretorius et al.[38] With the addition of the OPC, the eryptotic RBC structure is reversed, and the membrane roughness are slightly improved, compared with that of a typical healthy RBC.

DISCUSSION

In a healthy RBC, the neutral phosphatidylcholine and sphingomyelin are mostly found on the outside, and the charged phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine, are found mostly on the inner membrane leaflet.[36,41] In most inflammatory conditions, RBCs have been noted to show eryptosis (similar to apoptosis, but called eryptosis owing to theabsence of mitochondria and nuclei); for extensive reviews on eryptosis see various publications.[38,68–73] During eryptosis, a membrane phosphatidylserine-flip occurs, and phosphatidylserine is then present on the outside leaflet of the RBC membranes.[74–79] Ultimately, eryptosis results in an inflammatory RBC presence in
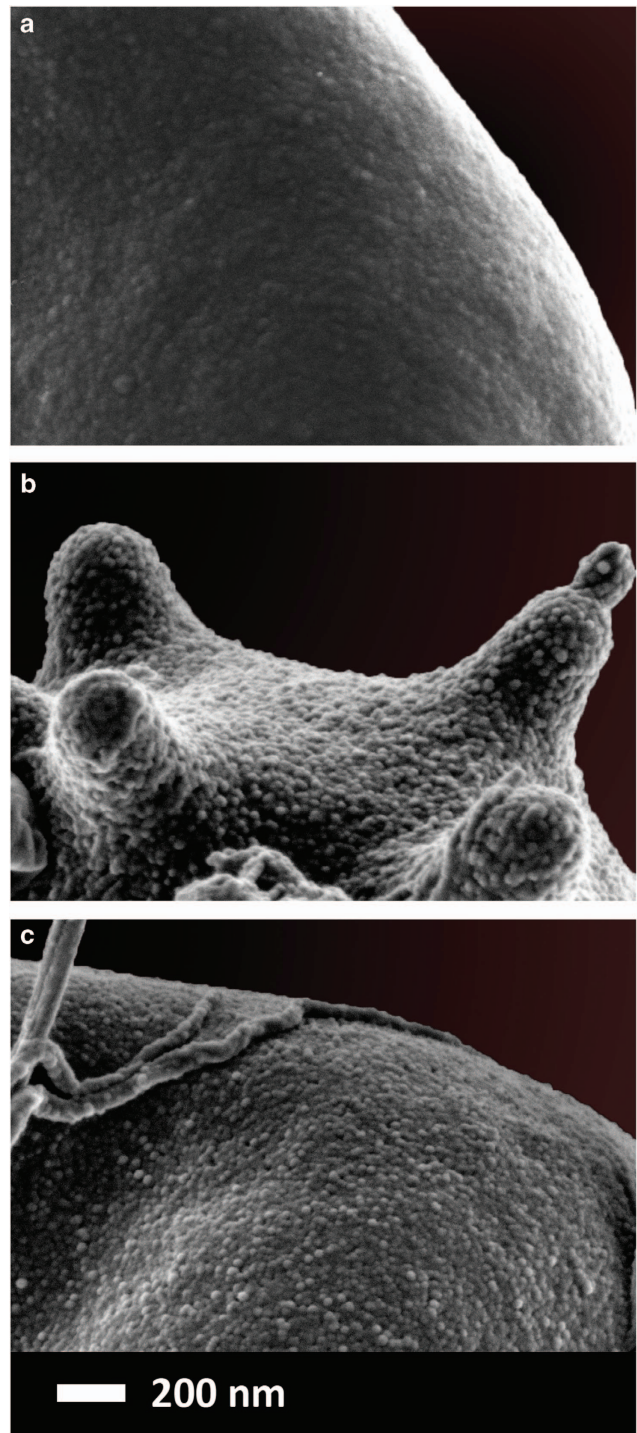

Figure 5. (**a**) A typical RBC membrane from a healthy individual. (**b**) A typical RBC membrane from a type II diabetes individual. (**c**) A typical RBC membrane from a type II diabetes individual, treated with an OPC.

the circulation. Eryptotic RBCs are known to themselves produce membrane microparticles and these microparticles in itself are inflammatory.[80] In all inflammatory conditions, RBCs demonstrate enhanced eryptosis and a pathological membrane structure with a changed elasticity. This was previously also demonstrated in T2D.[61,81] The pathological nature T2D RBCs, together with the general hypercoagulability seen in the condition,[82] fundamentally influences the inflammatory profiles of the patients. Eryptosis is

6

also seen in conditions like anemia.[83] Previously, it was also shown that pathological RBC structure, with an accompanying changed viscoelasticity and hypercoagulability of whole blood and plasma, are found in conditions like Alzheimer's and Parkinson's disease,[84–86] hyperferritinemia and haemochromatosis.[87]

Procyanidins and proanthocyanidins have been shown to have antioxidative and anti-inflammatory properties.[88,89] Preliminary clinical trials have been conducted in a number of indications and clinical contexts, including asthma, CVD and diabetes.[42–47,51,90,91] It has been shown that OPCs increase RBC membrane fluidity *in vitro*, potentially owing to inducing modification of the lipid bilayer and lipid–protein interactions as shown by Sivonová et al.[42] Sivonová et al. studied the *in vitro* effect of polyphenol rich plant extract, trademarked as Pycnogenol, on RBC membrane fluidity. They found that the extract significantly increased the membrane fluidity especially at the membrane surface as compared with untreated RBCs. They also found that the extract had protective effect against lipid peroxidation, generation of thiobarbituric acid reactive products and oxidative hemolysis induced by H_2O_2. They stated that, even though the exact mechanisms are unknown, it is thought that pycnogenol can reduce the lipid peroxidation and oxidative hemolysis either by reducing free radicals or by chelating metal ions, or by both. They concluded that the extract possibly modifies membrane-dependent processes not only by its chemical action, but also by interacting directly with cell membranes and/or penetrating the membrane and therefore inducing modification of the lipid bilayer and lipid–protein interactions.[42]

In the current study, the protective antioxidant effect of OPCs on the local mechanical properties of the erythrocyte membrane was evaluated, and therefore the membrane elasticity of erythrocytes of patients with T2D, using AFM techniques.

The Wilcoxon matched pairs test[64] showed no statistically significant difference in the median elasticity measurement values between the T2D sample group and the same group exposed to OPCs (*P*-value = 0.3083). From this data it can be interpreted that the OPCs had no effect, or at least not a measurable effect, on the local mechanical properties of the erythrocyte membrane by means of AFM, and therefore the membrane elasticity of erythrocytes.

Using the modification of Wilcox[65] of the Morgan–Pitman test,[66,67] a significant difference in variation between the elasticity measurement values of the two groups was found (*P*-value < 0.0001). Oxidative stress has been associated with premature erythrocyte aging.[92] It can therefore be assumed that in T2D more erythrocytes would be expected to measure as aged, lesser deformable erythrocytes[92] as seen in this study and to have smaller Young's modulus values and a smaller variance in membrane elasticity as seen by the AFM data (refer to Figure 3). The results showed that at least some of the samples were positively affected in the treated T2D group. It can therefore be presumed that the protective antioxidant effect of OPCs on the local mechanical properties of the erythrocyte membrane, and therefore the membrane elasticity of patients with T2D as measured by AFM, has an improving effect in comparison with the untreated T2D group.

These results are comparable to the findings of Pretorius (2015) where the authors investigated the effect of iron chelators deferoxamine or deferasirox on T2D erythrocytes by means of SEM and AFM. They found that treatment with either deferasirox or deferoxamine improved the Young's modulus values towards normal values. This indicates a possible improvement in the elasticity of the cells.[4] However, when they compared the elasticity measurements individually after treatment, a more complex picture emerges owing to the substantial variation in the two groups.[4] Similar variation was found in this study. In this study we found that a detailed analysis showed that the effect of the iron chelators on membrane elasticity was patient-specific.[4]

This is further supported after closer inspection, at ×150 000 magnification (refer to Figure 5), the erythrocyte membranes are visible. The T2D membrane ultrastructure appears much more rough and textured than that of healthy erythrocytes, possibly due to membrane phospholipid scrambling, associated with oxidative stress.[86] The OPCs-treated sample shows a visibly smoother surface of the erythrocyte membranes, as compared with the untreated T2D erythrocytes.

It is also important to note that AFM is generally considered to be an informative technique that is frequently used in erythrocyte structural studies using Young's modulus to measure membrane stiffness and elasticity.[36,57,58] However, in this study relatively complex statistical methodology was needed to statistically compare treated and untreated T2D groups. The technique was also found to be a timeous technique when a larger samples group is being studied. In addition, it is considered to be a complicated method to implement as a patient-orientated approach.[36]

CONCLUSION

In conclusion, the protective antioxidant properties of OPCs improved membrane elasticity as shown by a significant difference (*P*-value < 0.0001) in variation between the elasticity measurement values of the two groups T2D treated and untreated) when using the modification of Wilcox[65] of the Morgan–Pitman test.[66,67] Using SEM analysis these findings are further supported by the visibly smoother membrane surface viewed at ×150 000 magnification. The data from AFM showed that this is possibly due to an improvement towards a more normal variance of elasticity, and possibly contributing to the improvement of biophysical shape changes as seen in SEM analyses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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