Proceedings

Blood compatibility of originally synthesized amphiphilic dendrons — perspective drug nanocarriers †

Simon Suty [1], Veronika Oravczova[1], Zuzana Garaiova [1], Veronika Subjakova [1], Maksim Ionov [2], Dzmitry Shcharbin [3], Zuzana Šimoníková [4], Peter Bartek[4], Jean-Pierre Majoral [5], Maria Bryszewska [2], Tibor Hianik [1] and Iveta Waczulíková [1,*]

[1] Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
[2] Faculty of Biology and Environmental protection, Department of General Biophysics, University of Lodz, Poland
[3] Institute of Biophysics and Cell Engineering of NASB, Minsk, Belarus
[4] Department of Hematology and Transfusiology, St. Elizabeth Cancer Institute, Bratislava, Slovak Republic
[5] Laboratoire de Chimie de Coordination Du CNRS, Toulouse, France
* Correspondence: simon.suty@fmph.uniba.sk
† Presented at the 1st International Electronic Conference on Biomedicine, 01–26 Juner 2021; Available online: https://ecb2021.sciforum.net/.

Published: 1 June 2021

Abstract: Dendrons are branched synthetic polymers suitable for the fabrication of nanosized drug delivery systems. Compounds intended for use in humans must be completely compatible with blood. Here we studied the effect of originally synthesized amphiphilic phosphorous dendrons of various generations on blood components and model membranes to assess the presence and nature of interactions leading to a potential safety concern. The changes in hematological and coagulation parameters upon the addition of dendrons in the concentration range of 2–10 μM were monitored. We found that only the combination of higher concentration and higher generation of dendron altered some clinically relevant parameters: it significantly decreased platelet count, plateletcrit, shortened thrombin time, and increased activated partial thromboplastin time. At the same time small-size platelet clumps in blood films under the light microscope were observed. We further investigated aggregation propensity of the positively charged dendrons in model conditions using zwitterionic and negatively charged liposomes. The observed changes in size and zeta potential indicated the electrostatic nature of the interaction. Overall, we proved the low-generation amphiphilic dendrons compatible with blood within the studied concentration range. However, interactions of high-generation dendrons at higher concentrations with platelets and clotting factors cannot be excluded.

Keywords: nanoparticles; amphiphilic dendrons; blood compatibility, liposomes

1. Introduction

Dendrons are synthetic molecules, a constituting subunit of larger, nanosized supramolecular structures – dendrimers. Dendrimers are widely accepted as a fourth class of polymers [1]. The size of dendrons and dendrimers is dependent on the number of repetition cycles, called generations (G). Based on their unique structure and composition, the dendritic nanoparticles (NP) are widely studied in various areas, including medical applications, e.g. as drug carriers. The drug molecules can be entrapped within their inner cavities or chemically conjugated on their surface [2-5]. A new concept of forming dendron corona at virus surfaces was reported for attaching the cell

targeting groups/drug molecules to improve the biodistribution and capacity in virus-assisted gene therapy [6].

In targeted drug delivery, the first tissue that NP carriers encounter is usually blood tissue. Therefore, compatibility with blood and its elements is a necessary requirement for the application of NP *in vivo* and especially their interactions with platelets and the enzymes of coagulation cascade since these play a central role in hemostasis and its regulation [7,8]. The potential incompatibility of nanomaterials with platelets remains to be completely understood. In this sense, hemocompatibility of NP is a crucial factor in hindering undesired activation of elements of coagulation cascade and the following blood coagulation and thrombosis, which could even lead to emboli [9]. Due to the substantial role of platelets in thrombosis, the study of interactions between NP and platelets (activation or inhibition) might define their thrombogenicity and biocompatibility [10].

Several studies have reported that the surface chemistry of NP is one of the most underlying factors through which NP can activate platelets [11]. Studies performed by Dobrovolskaia et al. using 12 different formulations of PAMAM dendrimers with diverse sizes and surface charges (G3, G4, G5 and G6 functionalized with succinamic acid (anionic), amine (cationic) and amidoethanol groups (neutral)) on human platelets showed that only large (G4-G6) cationic dendrimers, but not the small (G3) cationic or anionic or neutral dendrimers, exhibited collagen-induced platelet aggregation [12]. Between different nanoplatforms cationic dendrimers noticeably showed their effect on thrombocyte activation through inducing aggregation of platelets *in vitro* and *in vivo*. Cationic PAMAM dendrimers (with amine end-group) G7 may cause coagulopathies through aggregation of negatively charged blood proteins such as fibrinogen and albumin [13]. Marrink et al. used computational strategies and proposed that PAMAM dendrimers induce platelet aggregation by altering the cell membrane, affecting its integrity [14]. The activation/inhibition of platelets through electrostatic interaction with NP is a controversial phenomenon which should be studied in each case separately because these effects are also dependent on different physico-chemical properties such as size, shape and chemical composition of the NP [15,16].

In vitro assays using human blood along with experiments using cell models appear to be a suitable technique to study mechanisms of dendron-based nanomedicines interference with hemostasis and to optimize their hemocompatibility. Therefore, in this work we have focused on the interactions of originally synthesized amphiphilic dendrons with model lipid membrane – liposomes and with whole human blood to ascertain how these dendrons affect the blood elements as well as the coagulation pathway.

2. Materials, Subjects and Methods

2.1. Chemicals and dendrons

We used 10 mM phosphate buffer, pH 7.4 was adjusted using 1M HCl or 1M NAOH at the temperature 25°C. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-Dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerolu) sodium salt (DMPG) and cholesterol (Chol). Lipids were purchased from Sigma Aldrich. In this work, amphiphilic phosphorus dendrons of the first (D1) and second (D2) generation were studied. The chemical structures and molar weights were: $C_{124}H_{198}Cl_{10}N_{34}O_8P_8S_5$, Mw = 3055.77 g/mol, and $C_{264}H_{418}Cl_{20}N_{74}O_{18}P_{18}S_{15}$, Mw = 6664.18 g/mol, respectively (Figure A1).

2.2. Preparation of liposomes

We used liposomes as a simple model of cell membranes. For the composition of liposomes, we used a mixture of DMPC and DMPG in the respective ratios. DMPG represented an amount of 10 mol % from the DMPC, and Chol was added in an amount of 30 mol % of the mixture of DMPC and DMPG. The dry lipid film was hydrated with 10 mM Na-phosphate buffer, pH 7.4, resulting

in 10 mg/ml of the lipids in the solution. The liposomes were extruded through a polycarbonate membrane with a 400 nm pore size to obtain unilamelar liposomes.

2.3. Size and Zeta potential measurements

Size of prepared liposomes upon the interactions with D1 and D2 dendrons was measured by dynamic light scattering technique (DLS) and Zeta potential was measured by phase analysis light scattering technique based on laser Doppler velocimetry using a spectrophotometer Zetasizer-Nano ZS90 (Malvern Instrument, UK). Concentration of lipid vesicles was 1 mg/ml. Dendrons were titrated into the liposomal solution by adding small aliquots to get desired dendron concentrations (0 μM, 0.1 μM, 1 μM, 3 μM, 4 μM, 5 μM, 7.5 μM, 10 μM, 12.5 μM, 15 μM) by both all measurements at 25°C. Z-average size – the hydrodynamic size parameter (also known as the cumulants mean) and width parameter of that intensity weighted size distribution known as polydispersity index - PDI were analyzed using Malvern software. Samples for zeta potential measurements were measured in zeta potential folded capillary cell (DTS1070).

2.4. Blood compatibility study

Fasting blood specimens were collected from healthy volunteers in the morning. To control for age, sex, and other potential confounders we used a fully within-subject study design including balanced numbers of male (n = 7) and female (n = 7) participants aged between 20 and 28 years. The study protocol was approved by the Institutional Ethics Committee at the St. Elizabeth Cancer Institute in Bratislava - No. of the approval: 03-2020/EK OÚSA, signed on the 4th of March 2020.

For the investigation of hematological parameters, we used the automatic hematology analyzer COULTER DxH 800 (Beckman Coulter, U.S.A) and for coagulation parameters we used the automatic hemostasis analyzer ACL Top 500 CTS (Instrumentation Laboratory, U.S.A). The blood was taken by blood vacutainers containing sodium citrate for investigating coagulation parameters and vacutainers containing K_2EDTA for investigating blood elements. Both generations, D1 and D2, were tested *in vitro* at each of selected final dendron concentrations, 2 μmol/l (C1) a 10 μmol/l (C2). Hematological investigation was completed with examination of fixed and stained blood smears (films) of each sample. All samples were viewed and described by an experienced hematologist.

2.5. Statistical analysis

Analysis of variance (ANOVA) for two-factor fully within-subject design was performed to assess effects of both factors (concentration and generation) as well as their interaction (concentration × generation). The restricted maximum likelihood approach for estimating components of variance was used in order to control for "nuisance" variables, e.g. sex or fitness.

Statistical analyses were performed using StatsDirect 3.3.5 software (Stats Direct Ltd., Cheshire, UK) and GraphPad Prism 7.0 (GraphPad Software, Inc., US). All P-values were considered significant at a two-tailed P-value of < 0.05.

3. Results and Discussion

3.1. Dendrons' blood compatibility assessment

No apparent hemolysis or discolouration occurred upon addition or after incubation with D1 and D2 dendrons. Analysis of the red component of blood did not reveal any clinically relevant red blood cells (RBC) aggregation at the selected concentrations C1 and C2, as evaluated by RBC count, mean cell volume, distribution width, mean corpuscular hemoglobin concentration and by hematocrit values under all combinations of experimental conditions (Table A1). No abnormalities in size, shape and colour of RBC were identified in the examined smears under the light microscope (Figure 1). Similarly, no abnormal results were obtained for the white component of blood except for an insignificant increase in the apparent count of leukocytes (WBC) upon the incubation at D2C2 experimental condition. The morphological analysis of platelets included the estimation of the number of platelets (PLT), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution

width (PDW) and several other parameters (Table A1). Upon incubation with D2C2 we have found spuriously low platelet counts and lower PCT (Figure 2) - likely due to formation of platelet microaggregates that could be misclassified by the hematology analyser as leucocytes. This notion is supported by the above mentioned finding of slightly increased WBC counts and by no significant change in PDW. Pseudothrombocytopenia due to time/anticoagulant-induced platelet aggregation was excluded by comparing the treated samples with the respective control samples – evaluated before and after incubation period.

The microaggregates were confirmed by viewing blood smears presenting small platelet clumps of 5-20 cells (Figure 1). Some samples showed signs of hypogranulation or degranulation, which points to that, upon incubation with D2 at C2, some platelets might have been activated and caused changes in coagulation indices TT5, PT and APTT (Figure 4, Table A1).

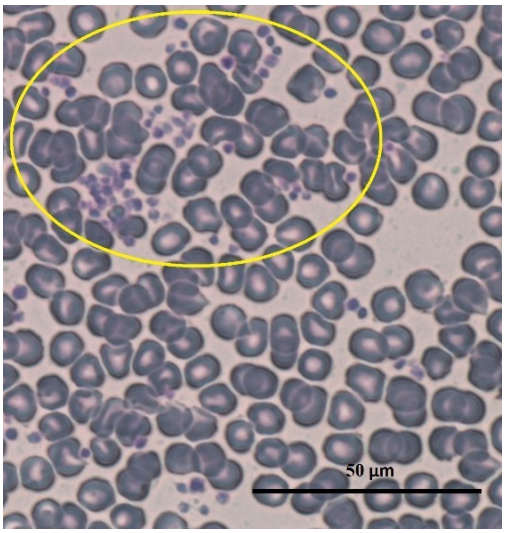

Figure 1. Images from light microscopy at 100× magnification after adding and incubation of the second generation dendron D2 with the concentration of 10 µM (C2). Small purple particles represent platelets, which under these experimental conditions created clumps (yellow ellipse).

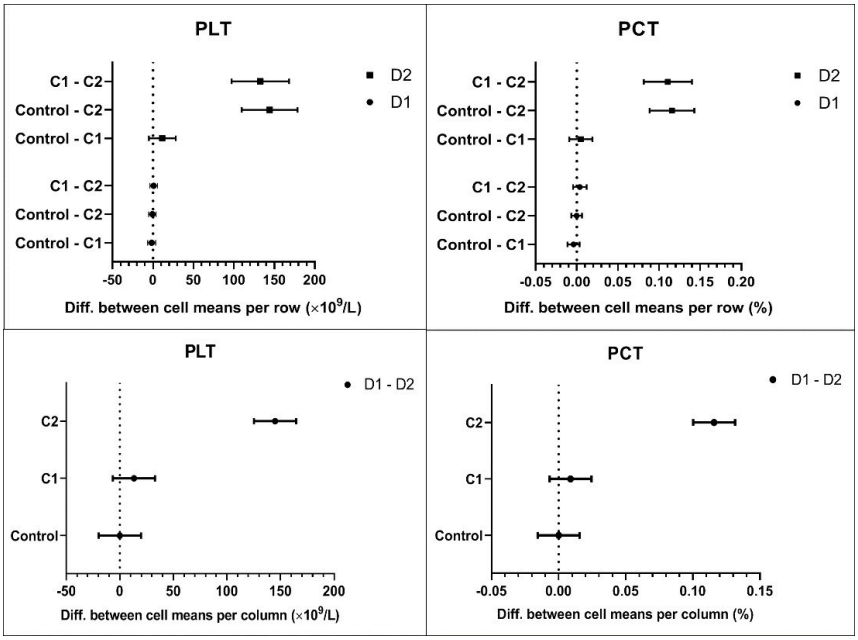

Figure 2. Changes in platelet's parameters expressed as mean within-subject differences in one factor within the levels of the other factor. The upper plots show simple main effects of concentration and

the lower plots the effect of generation. The symbols denote mean responses with 95% confidence intervals. Abbreviations: PLT - absolute number of platelets; PCT – plateletcrit.

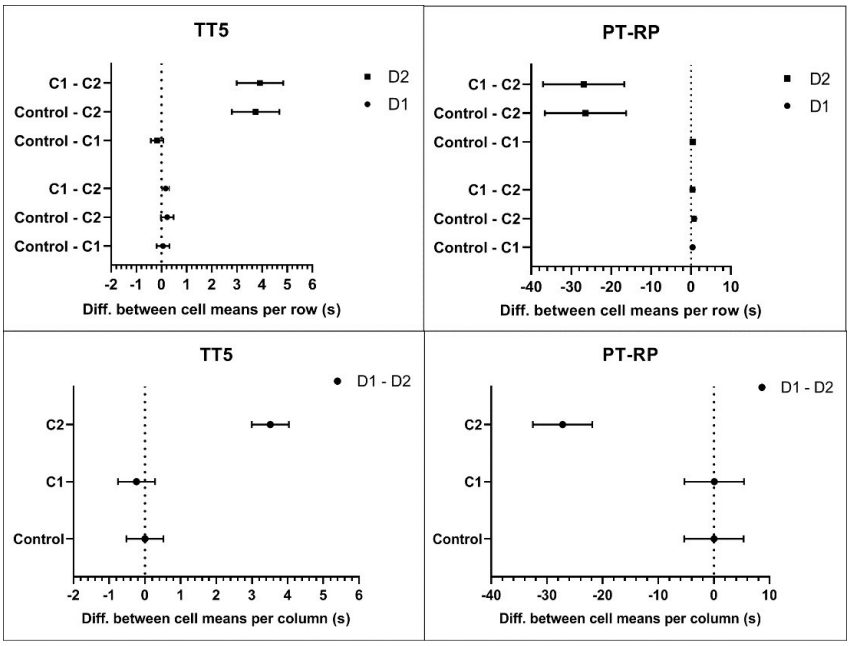

Figure 3. Changes in coagulation parameters expressed as differences in one factor within the levels of the other factor. The upper plots show simple main effects of concentration and the lower plots the effect of generation. The symbols denote mean responses with 95% confidence intervals. Abbreviations: TT5 – thrombin time; PT-RP - prothrombin time (RP - recombiplastine). A note: changes in APTT-SS – activated partial thromboplastine time (reagent: SynthA Sil) followed the same pattern as those for PT-RP.

Statistical analysis yielded a significant interaction between both experimental factors (dendron concentration × dendron generation). There was no effect of generation at C1, but there was a large effect of generation at C2: the bigger dendron (D2) applied at higher concentration (C2) caused changes in the platelet indices, whereas the smaller dendron (D1) did not. Figure 2 (PLT and PCT) and Figure 3 (TT5 and PT-RP) visually confirm a clear non-additive effect of the combination of higher concentration and higher generation of the amphiphilic dendrons. The differences obtained for the rest of hematology and coagulation indices did not differ from their respective reference values.

3.2. Interaction of dendrons with model lipid membranes- liposomes

To further investigate the mechanism of interaction, experiments on the model lipid membranes were performed. In the first series of experiments, we studied the interaction of D1 and D2 with DMPC-DMPG-Chol liposomes. Results were obtained by titration of the dendrons with concentrations from 0.1 μM up to 15 μM. Our measurements were carried out at 25°C. After data evaluation, we observed a significant increase in (Z-average size) from 250 nm up to more than 1 μm with higher dendron concentrations at 25°C (see Figure 4). Similarly, we observed an increase in PDI from 0.2 - a uniform distribution in particle size up to 1 - highly polydispersed sample. In addition, measurements at 37°C had later onset of aggregates for both dendrons (not shown). Zeta potential results showed an increasing pattern in correspondence to dendron concentration applied. The negative zeta potential of liposomes changed from –45.72 ± 7.82 mV up to as high as –12.9 mV.

To confirm electrostatic nature of dendron interaction with lipid membrane, we prepared neutral DMPC liposomes. As presumed, dendrons did not interact with the neutral DMPC liposomes

in terms of hydrodynamic diameter. Following addition of any dendron concentration, Z-average size was in the range of 182.21 ± 8.31 nm. Zeta potential values did not fluctuate as much as in the case of negatively charged DMPC-DMPG-Chol liposomes.

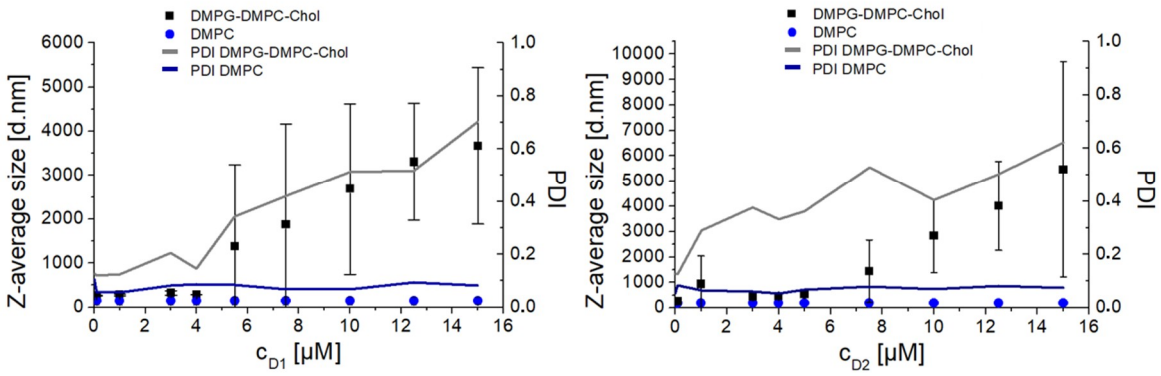

Figure 4. The hydrodynamic diameter (Z-average; [d.nm+SD]) and polydispersity index (PDI) of zwitterionic DMPC and negatively charged DMPG-DMPC-Chol liposomes upon the increasing concentration of D1 (left) and D2 (right) dendrons in Na-phosphate buffer (10 mM, pH 7.4).

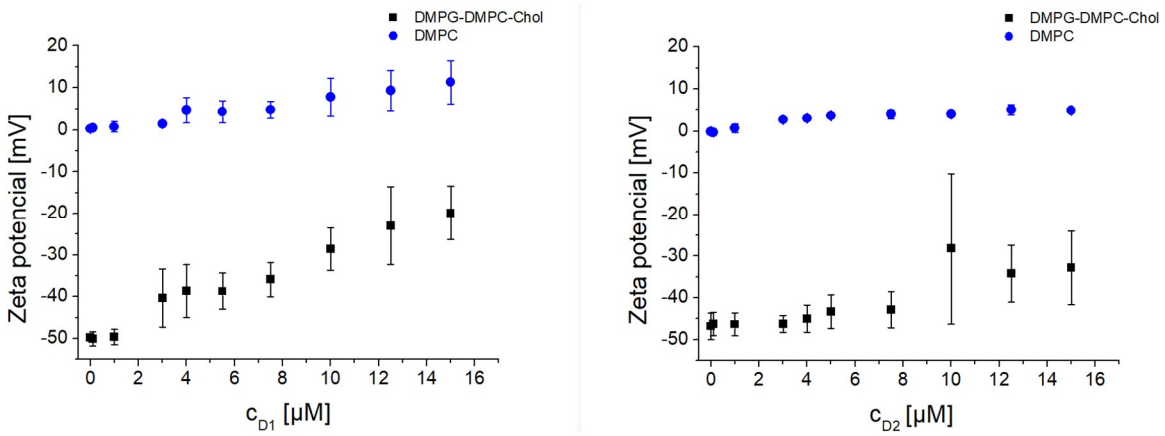

Figure 5. Zeta potential of zwitterionic DMPC liposomes and negatively charged DMPG-DMPC-Chol liposomes upon the increasing concentration of D1 (left) and D2 (right) dendrons in Na-phosphate buffer (10 mM, pH 7.4).

4. Discussion

In vitro methods are critical components in the biological safety assessment of NP intended for medical use in humans, as they provide valuable information on cell and tissue compatibility prior to preclinical and clinical studies. While such approaches utilize simplified systems compared to the complex *in vivo* milieu, they are important because they provide insight into potential *in vivo* tissue and cellular responses. Findings of such studies can be compared to those obtained with experimental models.

To study *in vitro* effect of generation and concentration on the blood components we have chosen a within-subject study design that can help to reduce errors associated with individual differences. The blood samples from the same participants were subjected to both experimental and control conditions, which enabled us to adjust the observed effects to account for inter-subject variability as part of the statistical analysis. Our results on *in vitro* assessed blood compatibility were largely satisfying – most hematology indices did not deviate from their baseline values. We did not observe any unusual RBC aggregation or adhesiveness to other cells upon adding dendrons. No morphological alterations occurred in RBC and WBC as a result of their exposure to NP. The RBC are negatively charged, which prevents cells from coming into contact [17]. Thus, we can assume that the

balance between attractive and repulsive electrostatic forces between red blood cells was not disturbed by the amphiphilic dendrons.

Our results showed that the best blood compatibility was achieved with the combination of lower generation and lower concentration (D1C1 experimental condition), while a lower but still satisfying compatibility was detected for D1C2 and D2C1 conditions. Only the 2nd generation dendron at a final concentration of 10 μM exerted effects with potential clinical relevance. That means the main effects of the factors generation and concentration were not additive, which might be attributed to the nonlinear increase in the number of functional end groups increasing positive charge on the nanoparticle surface. The observed platelet abnormalities could be described as signs of pseudo-thrombocytopenia (decreased PLT and PTC) and a consumption coagulopathy (shortened TT5 and prolonged PT-RP and APTT-SS). The former condition is linked to platelet clumping - as a result reported platelet counts were lower than their actual counts in the blood because automated counters cannot differentiate platelet clumps from individual cells. The latter could be linked to a deficiency of the plasma factors prothrombin, fibrinogen, factor V, or factor X [17,18]. Assuming that the reference range of the APTT is 30-40 seconds and the reference range of the PTT is 60-70 seconds [19], the observed out-of-range prolongation in APTT-SS was linked to the increasing number of accessible positively charged groups in D2C2 experimental condition. This is in line with the higher toxicity observed for cationic amino-modified NP [12]. The impact of the surface charge of commercial PAMAM G4 dendrimers on their interactions with human serum albumin, and with alpha-1-microglobulin, were studied by Serchenya et al. [20]. Both proteins can bind and transfer various ligands in blood and have immunoreactivity properties, which can be affected by the interaction with the NP. The authors have found out that the weakest interaction was observed for anionic dendrimers and that the effect of neutral and cationic dendrimers was comparable. They confirmed that the binding of cationic dendrimers to proteins can be explained by electrostatic forces acting between positively charged dendrimers and negatively charged regions of proteins and that the effect of cationic dendrimers on proteins is generation-dependent [20,21]. Their most important finding was that dendrimer-induced reduction in immunoreactivity of the proteins was only partial even if the protein was fully bound by the dendrimers. Thus, that the application of dendrimers *in vivo* might not eliminate the immunochemical properties of these proteins [21].

Based on acquired results, we assume that these interactions were most likely of electrostatic nature. As seen in Figure 5, the dendrons did not interact with neutral DMPC liposomes in terms of changes in their hydrodynamic diameter. In contrast, a significant interaction was observed with negatively charged DMPG-DMPC-Chol liposomes, where large aggregates were observed. PDI corresponds with our assumptions. In the case of neutral DMPC liposomes, PDI values are less than 0.2 for any dendron concentration applied. On the other hand, with negatively charged liposomes, PDI reached values as high as 1. Zeta potential values increased in both experimental conditions with increasing concentrations of D1 and D2, which is consistent with the positive surface charge of the dendrons.

5. Conclusion

In conclusion, our results suggest very good blood compatibility for lower-generation dendrons reaching low concentration levels at the site of action or accidental release. However, treatment with higher generation dendron at higher concentration affected several hematological and coagulation parameters in a clear concentration and generation dependence. We showed that increase in positive surface charge reduced interparticle repulsion, which may lead to their interaction with plasma coagulation factor and/or with platelets and eventually lead to platelet activation. Obtained data indicated that thrombogenic propensity might not be the only issue in nanomedicine safety. Coagulation abnormalities may also relate to prolonged clotting time.

Thus, some precautions must be taken while using higher generation amphiphilic dendrons at higher concentrations, since interactions of the naked or prematurely released nanoparticles with platelets and clotting factors cannot be excluded.

Acknowledgments:

Supported by the APVV: SK-BY-RD-19-0019 and SK-PL-18-0080, by the KEGA: 041UK-4/2020, VEGA 1/0756/20 and by the NAWA International Academic Partnership Programme EUROPARTNER: Strengthening and spreading international partnership activities of the Faculty of Biology and Environmental Protection for Interdisciplinary Research and Innovation of the University of Lodz.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Tomalia, D. A. Birth of a new macromolecular architecture: dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry, *Prog. Polym. Sci.* **2005**, 30, 294–324.
2. Prashant, K.; Keerti, J.; Narendra, J.K. Dendrimer as nanocarrier for drug delivery. Prog. Polym. Sci. **2014**, 39. 268–307. doi: 10.1016/j.progpolymsci.2013.07.005.
3. Svenson S. Dendrimers as versatile platform in drug delivery applications, *Eur. J. Pharm. Biopharm.* **2009**, 71, 445–462.
4. De La Harpe, K.M.; Kondiah; P.P.D.; Choonara, Y.E.; Marimuthu, T.; Du Toit, L.C.; Pillary, V. The Hemocompatibility of Nanoparticles: A Review of Cell-Nanoparticle Interactions and Hemostasis, *Cells* **2019**, 8, 1209; doi:10.3390/cells8101209
5. Garaiova, Z.; Melikishvili, S.; Michlewska, S.; Ionov, M.; Pedziwiatr-Werbicka, E., Waczulikova, I.; Hianik, T.; Gomez-Ramirez, R.; Javier De La Mata, F.; Bryszewska, M. Dendronized Gold Nanoparticles as Carriers for gp160 (HIV-1) Peptides: Biophysical Insight into Complex Formation, *Langmuir* **2021,** *37* (4), 1542-1550, doi: 10.1021/acs.langmuir.0c03159
6. Wagner, J.; Li, L.; Simon, J.; Krutzke, L.; Landfester, K.; Mailänder, V.; Müllen, K.; Ng, D.Y.W.;Wu, Y.; Weil, T. Amphiphilic Polyphenylene Dendron Conjungates for Surface Remodeling of Adenovirus 5, *Angew Chem Int Ed Engl.* **2020**, 59(14), 5712-5720
7. Kargl, R., Kleinschek, K. S. How can we understand the influence of nanoparticles on the coagulation of blood? *Nanomedicine (Lond.)* **2020**, 15 (20),1923-1926, DOI: 10.2217/nnm-2020-0177
8. Sanfinsg, E.; Augustsson, C.; Dahlbäck, B.; Linse, S.; Cedervall, T. Size-dependent Effects of Nanoparticles on Enzymes in the Blood Coagulation Cascade. *Nano Lett.* **2014**, 14, 8, 4736-4744
9. Urbán, P.; Liptrott, N.J.; Bremer, S. Overview of the blood compatibility of nanomedicines: A trend analysis of in vitro and in vivo studies, *WIREs Nanomed Nanobiotechnol.* **2019,** 11, e1546
10. Ziemba, B.; Matuszko, G.; Bryszewska, M., Klajnert, B. Influence of dendrimers on red blood cells; *Cell Mol Biol Lett* **2012**, Volume 17, 21-35
11. Svenson S.: The dendrimer paradox – high medical expectations but poor clinical translation; *Chem. Soc. Rev.* **2015**, 44, 4131
12. Dobrovolskaia, M.A.; Patri, A.K.; Simak, J.; Hall, J.B.; Semberova, J.; De Paoli Lacerda, S.H.; McNeil, S.E., Nanoparticle size and surface charge determine effects of PAMAM dendrimers on human platelets in vitro, *Mol. Pharm.* **2011**, 9 (3), 382–393
13. Jones, C.F. et al.: Cationic PAMAM dendrimers aggressively initiate blood clot formation, *ACS Nano* **2012**, 6, 9900–9910, doi: 10.1021/nn303472r
14. Matus, M.F.; Vilos, C.; Cisterna, B.A.; Fuentes, E.; Palomo, I. Nanotechnology and primary hemostasis: Differential effects of nanoparticles on platelet responses; *Vascular Pharmacology* **2018**, 101, 1-8, DOI: 10.1016/j.vhp.2017.11.004

15. Ilinskaya, A.N., Dobrovolskaia, M.A. Nanoparticles and blood coagulation system. Part II: Safety concerns, *Nanomedicine (Lond.)*, 2013, 8 (6), 969-981
16. Pagana, K.D.; Pagana, T.J., Pagana, T.N.; *Mosby's Diagnostic & Laboratory Test Reference*. 14th ed. St. Louis, Mo: Elsevier; **2019**
17. De La Cruz, G.G.; Rodríguez-Fragoso, P.; Reyes-Esparza, J.; Rodríguez-López, A.; Gómez-Cansino, R.; Rodriguez-Fragoso, L. Interaction of Nanoparticles with Blood Components and Associated Pathophysiological Effects, IntechOpen **2018**, chapter 3, doi: 10.5772/intechopen.69386
18. Ilinskaya, A.N., Dobrovolskaia, M.A. Nanoparticles and blood coagulation system. Part II: Safety concerns, *Nanomedicine (Lond.)*, **2013**, 8 (6), 969-981
19. Pagana, K.D.; Pagana, T.J., Pagana, T.N.; *Mosby's Diagnostic & Laboratory Test Reference*. 14th ed. St. Louis, Mosby: Elsevier; **2019,** ISBN: 9780323609692
20. Serchenya, T.; Shcharbin, D.; Shyrochyna, I.; Sviridov, O.; Terekhova, M.; Dzmitruk, V.; Abashkin, V.; Apartsin, E.; Mignani, S.; Majoral, J.P.; Ionov, M.; Bryszewska, M. Immunoreactivity changes of human serum albumin and alpha-1-microglobulin induced by their interaction with dendrimers, *Colloids Surf B Biointerfaces* **2019**, 179, 226-232, doi: 10.1016/j.colsurfb.2019.03.065
21. Janaszewska, A.; Lazniewska J.; Trzepiński, P.; Marcinkowska, M.; Klajnert-Maculewicz, B. Cytotoxicity of Dendrimers, Biomolecules **2019**, 9, 330; doi:10.3390/biom9080330

Appendix A

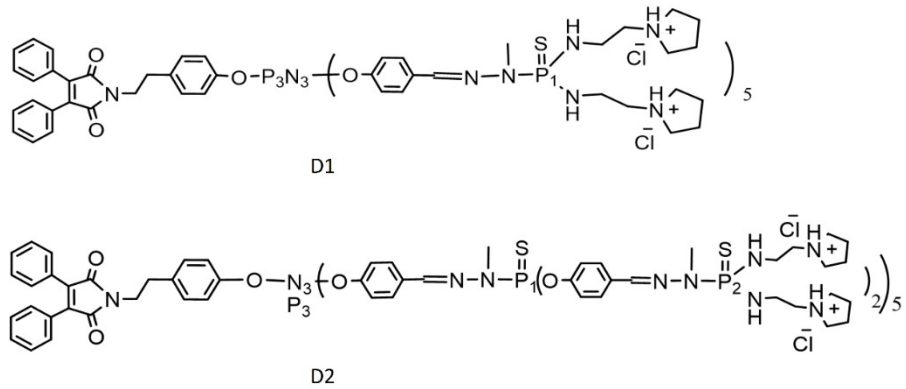

Figure A1. Chemical structure of the studied amphiphilic dendrons of the first (D1) and second (D2) generation.

Table A1. Descriptive statistics and differences in the selected hematology and coagulation parameters across experimental conditions.

	D1: jq108				D2: jq111			
	Mean 1	Mean 2	Diff	Adjusted P	Mean 1	Mean 2	Diff	Adjusted P
RBC (×10^{12}/L)								
Control vs. C1	4.80	4.89	-0.088	0.0001	4.80	4.84	-0.043	0.0242
Control vs. C2	4.80	4.84	-0.039	0.0567	4.80	4.82	-0.024	0.1979
C1 vs. C2	4.89	4.84	0.049	0.0383	4.84	4.82	0.019	0.3048
HBG (g/L)								
Control vs. C1	141.1	144.4	-3.357	<0.0001	141.1	142.2	-1.143	0.0487
Control vs. C2	141.1	143.6	-2.500	<0.0001	141.1	141.9	-0.786	0.1713
C1 vs. C2	144.4	143.6	0.857	0.1388	142.2	141.9	0.357	0.4067
PLT (×10^{9}/L)								
Control vs. C1	225.7	227.4	-1.643	0.6465	225.7	214.2	11.50	0.1794
Control vs. C2	225.7	226.4	-0.714	0.9057	225.7	81.50	144.2	<0.0001
C1 vs. C2	227.4	226.4	0.929	0.8488	214.2	81.50	132.7	<0.0001
MPV (fL)								
Control vs. C1	9.31	9.41	-0.100	0.2754	9.31	9.61	-0.300	0.0275
Control vs. C2	9.31	9.34	-0.021	0.9333	9.31	11.44	-2.129	<0.0001
C1 vs. C2	9.41	9.34	0.079	0.4836	9.61	11.44	-1.829	<0.0001
PCT (%)								
Control vs. C1	0.209	0.213	-0.0039	0.3554	0.209	0.204	0.0050	0.6057
Control vs. C2	0.209	0.209	-0.0001	0.9995	0.209	0.094	0.1158	<0.0001
C1 vs. C2	0.213	0.209	0.0038	0.4495	0.204	0.094	0.1108	<0.0001
PDW (%)								
Control vs. C1	16.88	16.91	-0.029	0.879	16.88	17.11	-0.229	0.0631
Control vs. C2	16.88	16.84	0.036	0.8377	16.88	17.19	-0.307	0.1616
C1 vs. C2	16.91	16.84	0.064	0.3207	17.11	17.19	-0.079	0.8976
TT5 (s)								
Control vs. C1	14.37	14.31	0.064	0.7748	14.37	14.54	-0.171	0.1792
Control vs. C2	14.37	14.14	0.229	0.0650	14.37	10.63	3.743	<0.0001
C1 vs. C2	14.31	14.14	0.164	0.0216	14.54	10.63	3.914	<0.0001
PT-RP (s)								
Control vs. C1	11.73	11.34	0.386	0.0003	11.73	11.29	0.443	0.0082
Control vs. C2	11.73	10.96	0.764	0.0010	11.73	38.16	-26.43	<0.0001
C1 vs. C2	11.34	10.96	0.379	0.1227	11.29	38.16	-26.87	<0.0001
aPTT-SS (s)								
Control vs. C1	29.77	32.80	-3.029	<0.0001	29.77	50.81	-21.04	0.0006
Control vs. C2	29.77	34.18	-4.407	0.0007	29.77	152.4	-122.6	<0.0001
C1 vs. C2	32.80	34.18	-1.379	0.1690	50.81	152.4	-101.6	<0.0001
FBG (g/L)								
Control vs. C1	2.760	2.804	-0.044	0.6181	2.760	2.786	-0.026	0.8923
Control vs. C2	2.760	2.701	0.059	0.3756	2.760	2.829	-0.069	0.2544
C1 vs. C2	2.804	2.701	0.104	0.0375	2.786	2.829	-0.043	0.6422