

PAPER • OPEN ACCESS

Carbon Coating on Surface of PVC Blood Bags

To cite this article: V S Chudinov and I N Shardakov 2021 *J. Phys.: Conf. Ser.* **1945** 012068

View the [article online](#) for updates and enhancements.

You may also like

- [Scaling deterministic lateral displacement arrays for high throughput and dilution-free enrichment of leukocytes](#)
David W Inglis, Megan Lord and Robert E Nordon
- [A computer system for the leukocytes classification in medical images](#)
E M Chernykh and V M Mikhelev
- [Profile of Leukocytes in Pig \(*Sus scrofa domestica*\) Before and After Heart Infarct Surgery](#)
Gunanti, Sus Derthi Widhyari, Dwi Utari Rahmiati et al.



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

Carbon Coating on Surface of PVC Blood Bags

V S Chudinov ^a, I N Shardakov

ICMM UB RAS, Academician Korolev Street, 1, Perm, Russian Federation

E-mail: ^a chudinovsl@mail.ru

Abstract. In this work, we studied the effect of a carbon coating created on the surface of a polyvinyl chloride blood storage bags on protein adsorption, cell culture proliferation, and on whole blood leukocytes. Based on the results obtained, it can be assumed that the preservation of leukocytes is more effective in the blood storage bags treated by the ion-plasma method, in comparison with the untreated one. The adhesion of blood cells occurs on the carbon coating of the plasma-treated surface of the blood storage bags. This can be eliminated by preliminary application of proteins that prevent cell adhesion on the walls of the blood storage bags.

1. Introduction

According to the WHO, about 118.5 million blood donations are collected annually in the world. Blood and its components are widely used for blood transfusion and diagnostics in medicine, in biotechnological processes of the pharmaceutical industry and in scientific research. Rapid destruction of blood cells *ex vivo* limits logistics and storage. In blood transfusion medicine, whole blood is usually processed into various components for specialized storage within 24 hours. Aggregation of blood cells, collateral damage due to the activation of leukocytes, lysis of cells with the release of chemokines significantly affect healthy and living cells [1, 2, 3]. The material of the wall of the polymeric blood storage bag can also be a factor in the destruction of blood cells outside the body [4, 5]. This factor is not sufficiently described in the scientific literature, and may also be significant. The surface of the polymer container can have a destructive effect on cells, cause a reaction of leukocytes, creating an aggressive environment for the cell mass. Earlier in our work [6] it was shown that the carbon surface obtained by the ion-plasma method on polyurethane promotes improved proliferation of human cell culture in comparison with the surface of an untreated polymer. This work is a pilot to create a carbon coating on the inner surface of a polyvinyl chloride blood storage bag and to assess the possibility of better preservation of blood cells in it.

2. Results and Discussion

We used 6 Ravimed (Poland) polyvinyl chloride (PVC) blood storage bag with a volume of 450 ml. One of the containers was cut into pieces to create a carbon coating using the standard technology of ion-plasma processing of flat samples, as well as for use as a control untreated flat sample. The method of plasma-immersion ion implantation (PIII) was used. The samples were treated with 20 keV nitrogen ions for 80, 400, and 800 s, which corresponded to fluences of 10^{15} , 5×10^{15} , and 10^{16} ions/cm². 4 blood storage bags were processed internally to maintain their shape, and one untreated was designated as a control.

The method of processing the inner surface of the blood storage bag was used, in which a plasma flow was created in its cavity between the high-voltage electrode and the wall of the vacuum chamber using a pulsed processing mode. A metal mesh electrode was placed around the blood storage bag, which made it possible to observe the plasma discharge inside the blood storage bag. High voltage rectangular pulses of several kV were applied to the electrode. Nitrogen gas was supplied through a supply tube inserted into the cavity of the blood storage bag through a small incision. In this case, the pressure P_{in} in the supplying polymer tube was 2.2-2.3 Torr, and the pressure in the chamber P_{ch} was no higher than $2.6-2.7 \times 10^{-4}$ Torr (Figure 1). Such a difference, according to Paschen's law [7], made it possible to



create a plasma discharge only in the cavity of the blood storage bag and the supply tube, which was observed visually through the window of the vacuum chamber (Figure 2). Only the inner surface of the blood storage bag was treated with high energy ions. We used 3 modes for 4 blood storage bags, the data on them are given in Table 1.

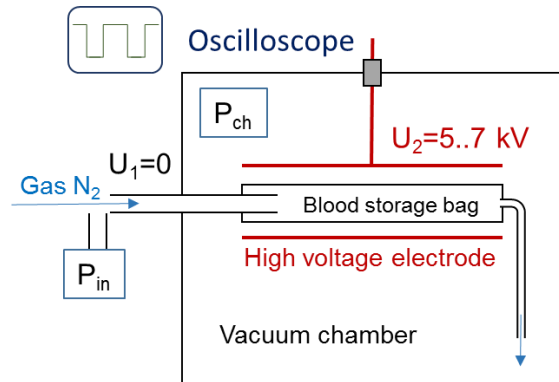


Figure 1. Scheme of treatment for inner surface of blood storage bag.

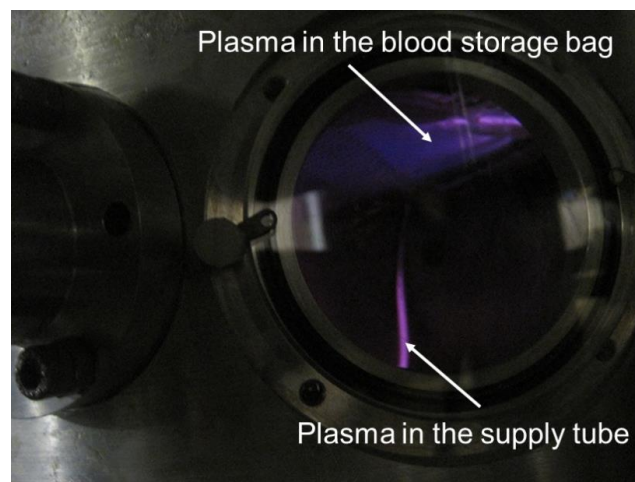


Figure 2. Photo of treatment for inner surface of blood storage bag.

Table 1. Regimes of plasma treatment on inner surface of the blood storage bag.

Number of bags	Voltage, kV	Pulse frequency, Hz	Pulse width, uSec	Treatment time, Sec
1	5.3	400	20	1800
2*	5	400	20	800
1	6.6	50	20	1020

To study changes in the molecular structure of the surface layer, Fourier Transform Infrared Spectroscopy in Attenuated Total Reflection mode (FTIR ATR) was used. The spectra were recorded on a Digilab spectrometer (Australia) with a spectral resolution of 4 cm^{-1} and a number of scans of 500. ATR attachment (Harrick, USA) with a $1 \times 5\text{ cm}^2$ germanium crystal with a beam incidence angle of 45 degrees was used. In this case, the spectra were recorded both from flat samples, untreated, treated with plasma, and from a blood storage bag, processed using the ion-plasma method inside (the processing mode is marked * in the first column in Table 1). The blood storage bag with the treated inner surface was cut into several pieces, and the spectra were recorded at different sites to determine the uniformity

of the ion treatment. Due to the difference in the penetration depth of the infrared beam into the polymer (from 400 nm to 800 nm, depending on different wavenumbers) and the thickness of the carbon layer up to 70-80 nm, spectra were subtracted. The spectrum of the initial PVC was subtracted from the spectrum of PVC after plasma treatment, which made it possible to evaluate changes in the surface layer.

In the subtracted spectra of all plasma treated PVC samples, a vibration line of hydroxyl and amine groups with a maximum at 3440 cm^{-1} is observed. A complex shape of the C=O and C=N vibration lines is observed with maxima at 1700 and 1630 cm^{-1} . A broad absorption contour of C-O vibrations with a maximum at 1170 cm^{-1} is observed. The standard deviation of the intensity in the spectra taken at different parts of the hemacontainer, treated inside, for the C = C groups is 20%, for the hydroxyl groups it is 10%, which is acceptable. Due to the difference by several times in the energies of ions implanted into flat samples and into the surface of the blood storage bag treated internally, it can only be assumed that the fluence during internal processing was more than $2.5 \times 10^{14}\text{ ions / cm}^2$.

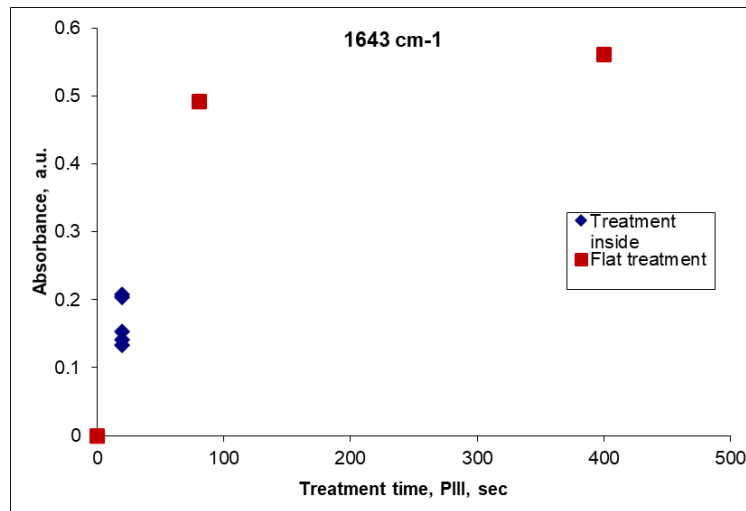


Figure 3. Absorbance at wavenumber of 1643 cm^{-1} (group C=C) for flat samples processed by the standard ion-plasma method with a planar electrode geometry (red squares) and for samples cut from different places in the blood storage bag treated with high-energy ions inside (blue rhombuses).

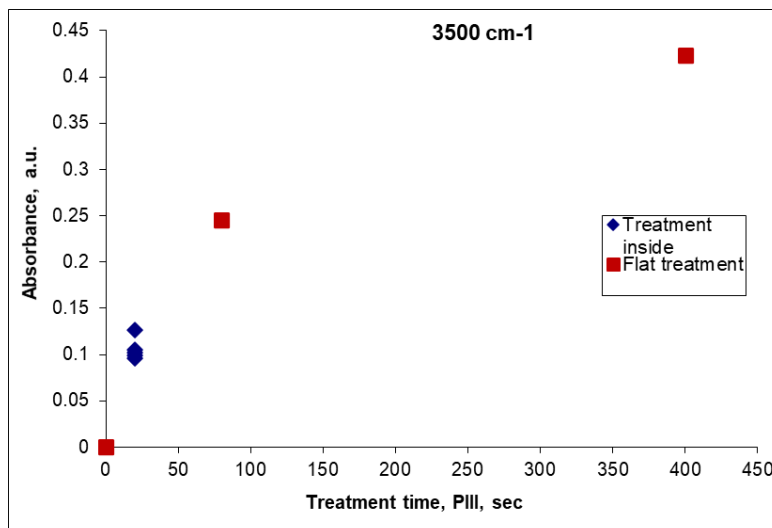


Figure 4. Absorbance at wavenumber of 3500 cm^{-1} (hydroxyl group) for flat samples processed by the standard ion-plasma method with a planar electrode geometry (red squares) and for samples cut from different places of the blood storage bag treated with high energy ions inside (blue rhombuses).

On flat samples of polyvinyl chloride, the attachment of serum bovine albumin was investigated. For this, the untreated and treated with ions with an energy of 20 keV and a fluence of 10^{16} ion/cm² PVC samples were immersed in buffer solution with the protein with a concentration of 20 µg/ml. Then the samples were washed in two different ways: the first is 5 times washing in a buffer solution, the second is a washing in 2% solution of sodium dodecyl sulphate (SDS) at 80°C for an hour, followed by 5 times washing in a buffer solution. After additional washing in deionized water and drying the samples in air for 24 hours, the surface of the samples was analyzed using FTIR ATR spectroscopy. To analyze the spectra, we used the subtraction of the surface spectra of the same samples that were not subjected to the protein attachment procedure. As a result of the analysis, similar patterns were revealed, as in our works with polyurethane [6]. First, the amount of adsorbed protein on PVC samples with a carbon layer is significantly higher than on untreated ones. Secondly, after washing in a detergent, the protein is washed off the untreated PVC and remains firmly bound to the carbon surface of the treated one. This allows us to conclude that the albumin protein is covalently adsorbed on the PVC surface of the blood storage bag after ion-plasma treatment, as a result of the formation of a carbon layer containing free radicals stabilized in the π -electron cloud of polycondensed aromatic structures [8, 9].

On PVC samples treated by ions with an energy of 20 keV and a fluence of 10^{16} ion/cm² with a planar geometry of the electrode, the proliferation of a culture of human aorta cells was investigated. All manipulations were performed under sterile conditions in a laminar flow hood with preliminary sterilization of samples in ultraviolet radiation with a wavelength of 254 nm for 10 minutes on both sides of the sample. The concentration of cells applied was 2×10^4 cells per millilitre. After applying the cells to the samples, the plate was placed in a sterile incubator maintained at 37°C in an atmosphere of 5% CO₂. 2 days after sowing the cells on a TCP culture plastic, untreated and treated PVC samples, the number of cells and the uniformity of the cell spreading on the PVC surface were assessed. To stain the actin structures of the cytoplasm, we used a labelled fluorescent dye phalloidin (Phalloidin-TRITC). For visualization of nuclei, cells were stained with DAPI fluorescent dye. According to the results of the analysis, it was revealed that on the surface of the treated PVC, the cells spread and grew in about the same way as on specialized culture plastic and slightly better than on untreated PVC. After confirmation by a cell culture experiment of the non-toxicity of plasma-treated PVC, the whole blood study was continued to assess the safety of blood cells in high-energy ion-treated PVC blood storage bag.

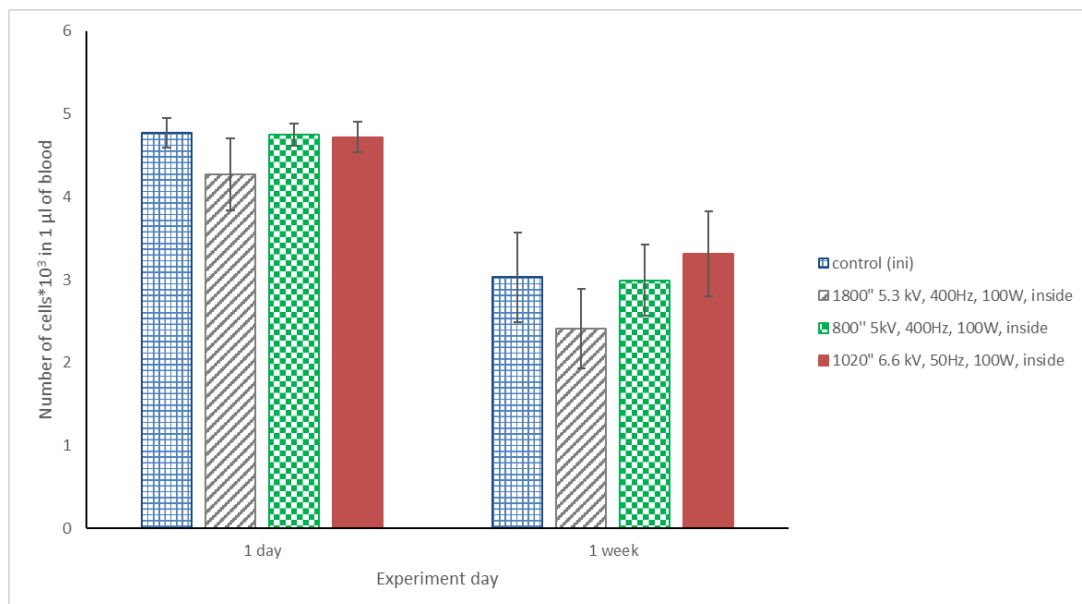


Figure 5. Dependence on the storage time of the number of unbroken leukocytes $\times 10^3$ in 1 µl of blood in blood storage bags, untreated and internally processed by different modes of the ion-plasma method.

In the final experiment, whole blood of a healthy donor was used with the addition of anticoagulant sodium citrate in a ratio of 5:1. Each blood storage bag was filled with 100 ml of blood. The study was carried out in 5 blood samples from a blood storage bag. Leukocytes were counted according to the standard method in the Goryaev chamber [10]. The choice of leukocytes for counting is due to a number of reasons: the simplicity of the method due to the lack of the need for staining, a relatively small specific number of cells compared to platelets and erythrocytes, the participation of leukocytes in the immune response to a foreign agent - the wall of the polymer material. The experiment was carried out for a week. At the end of the exposure, the blood in the blood storage bag was replaced with a buffer solution in the same amount - 100 ml. The buffer solution was stirred by shaking the blood storage bag in order to wash the cells from its walls. Then the counting was carried out by the same method in the Goryaev chamber. Figure 5 shows leukocyte count versus storage time diagrams for untreated and plasma-treated blood storage bags. No statistically significant difference was found between the amount in the treated and untreated blood storage bags.

However, after changing the blood to a washing buffer solution, it was noted that blood cells adhered to the surface of the treated blood storage bag, and no blood cells were found in the control blood storage bags according to the method of counting in the Goryaev chamber (Figure 6). It can be assumed that previously adhered cells are not included in the count of leukocytes in volume when taking blood doses from the blood storage bags. The detection of cells after washing allows us to consider the number of leukocytes in the volume of plasma-treated blood storage bags underestimated (Figure 5). These facts allow us to formulate the conclusion that there is some difference in the number of leukocytes in blood storage bags, untreated and treated with high energy ions, in favor of the latter. To determine the absolute values of this difference, additional experiments are needed to solve the problem of cell adhesion on the carbon layer. This solution can be performed by preliminary application of a protein to the surface, or a group of proteins, for example, from blood serum, where blood coagulation complexes have been removed. It is also possible that it was the adhesion of blood cells to the walls of the treated PVC that contributed to their preservation. And this also requires additional experimental work.

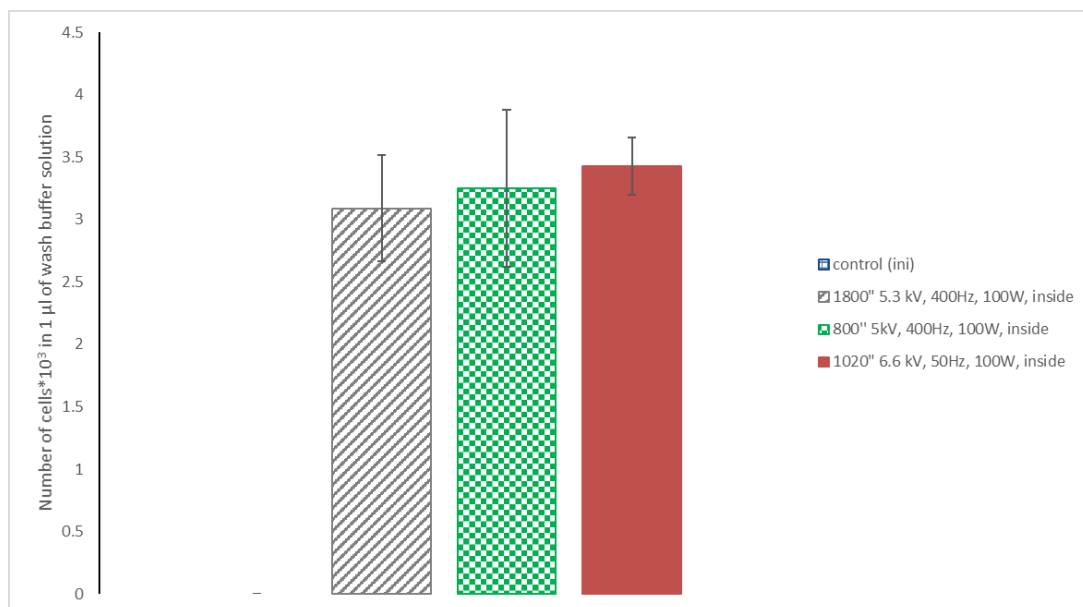


Figure 6. The number of unbroken leukocytes $\times 10^3$ in 1 μ l of wash buffer solution in blood storage bags, internally treated with different modes of the ion-plasma method

3. Conclusion

On the inner surface of the polyvinyl chloride blood storage bag, a carbon coating was created using the described method of ion-plasma treatment for hollow polymer. The applied method makes it possible to achieve a satisfactory uniformity of processing of the entire inner surface of the blood storage bag. The

carbon layer on PVC contains free radicals stabilized in the π -electron cloud of polycondensed aromatic structures. Their existence in the carbon layer ensures the covalent adsorption of proteins to the surface of polyvinyl chloride, creating a favourable environment for the adhesion and proliferation of cell cultures. The method of ion-plasma treatment of the surface of blood storage bag can contribute to the preservation of whole blood cells for hematransfusion and diagnostic medicine, production and scientific research. This assumption is based on the first pilot experiment and requires a wider range of studies.

Acknowledgments

One of the authors (V.S. Chudinov) thanks Prof. M. Bilek, Prof. D. McKenzie and A. Kondyurin for their permission to work in their laboratories.

References

- [1] Wong K, Sandlin R, Carey T, Miller K, Shank A, Oklu R, Maheswaran S, Haber D, Irimia D, Stott S and Toner M 2016 The Role of Physical Stabilization in Whole Blood Preservation *Sci Rep* **6** 21023 Online: <https://doi.org/10.1038/srep21023>
- [2] Barshtein G, Arbell D, Livshits L and Gural A 2018 Is It Possible to Reverse the Storage-Induced Lesion of Red Blood Cells *Front. Physiol.* **9** 914 Online: doi: 10.3389/fphys.2018.00914
- [3] Tsvetkova N, Torok Z, Satpathy G, Banerjee M, Bali R, Dwyre D, Kheirrolomoom A, Little E, Crowe J and Tablin F 2004 New Technology for Red Blood Cell Preservation *Blood* **104** 2710 Online: <https://doi.org/10.1182/blood.V104.11.2710.2710>
- [4] Gulliksson H, Meinke S, Ravizza A, Larsson L and Höglund P 2017 Storage of red blood cells in a novel polyolefin blood container: a pilot in vitro study *Vox Sang* **112** 33-39 Online: <https://doi.org/10.1111/vox.12472>
- [5] Volkova LV and Grishina TA 2019 Effect a haemo container different manufacturers on the productive capacity of mammalian cells *Russian Journal of Biopharmaceuticals* **11** 11-15
- [6] Chudinov VS, Kondyurina IV, Shardakov IN, Svistkov AL, Osorgina IV and Kondyurin AV 2018 Polyurethane modified by plasma ion implantation for medical application *Biophysics* **63** 330–9
- [7] Paschen F 1889 Ueber die zum Funkenübergang in Luft, Wasserstoff und Kohlensäure bei verschiedenen Drucken erforderliche Potentialdifferenz (german) *Annalen der Physik und Chemie* **273** 69–96
- [8] Kondyurin A, Nosworthy NJ and Bilek M 2011 Effect of low molecular weight additives on immobilization strength, activity, and conformation of protein immobilized on PVC and UHMWPE, *Langmuir* **27** 6138–48
- [9] Kondyurin A and Bilek M 2008 *Ion Beam Treatment of Polymers. Application aspects from medicine to space* (Oxford: Elsevier)
- [10] Polyanskaya GG, Efremova TN, Koltsova AM, Musorina AS, Sharlaimova NS and Yakovleva TK 2019 *A training manual for working with cell cultures of animals and humans* (St.Petersburg: Politech-Press)