

## **Plasma Health Care: promises and difficulties for haemostasis**

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The effectiveness of plasmas in the sterilization process and surface treatment, is now well established. The emergence of the production of a wide range of plasma at low temperature and atmospheric pressure allows various uses in medicine, since it is not possible to place patients in partial vacuum and because that mammalian cells do not tolerate temperature above 50-55°C even for a short period. The future contribution of plasma technology in the food industry is currently probably underestimated. It's a way to increase food resources for the global population, knowing that at present one third of food produced is lost, whether in developing countries or developed countries. For cold plasmas health care, the following fields of application are well identified: 1) dermatology: therapy of skin infections, tissue regeneration, wound healing, sterilisation of infected ulcer. 2) blood coagulation. 3) treatment of cancer cells, pending other applications that we have not yet imagined. In Toulouse, we have initiated studies on the effects of plasma in three areas: cell signaling, treatment of skin infections due to parasites, haemostasis in collaboration with the GREMI (Orleans). We will discuss only the latter subject.

This is Alexander Fridman who first demonstrated the possibility of accelerating blood clotting by using a Floating Electrode Dielectric Barrier Discharge. Other teams have since confirmed its work. The major interest is in the treatment of external bleeding in multiple trauma or in patients with congenital diseases of haemostasis (haemophilia, von Willebrand disease, platelet disorder, etc.). For our part, like other teams, we obtained a coagulation layer surrounding the samples of blood or blood plasma, treated with DBD plasma jets or afterglows, but no clotting in the heart of the sample. But the originality is to obtain here a reversible layer. It is difficult to demonstrate the mechanism: the dosage of coagulation factors including fibrinogen, show no difference in rates of factors, before and after treatment of the sample by DBD plasma. We assume that this is an incomplete polymerization of fibrin monomers linked by weak electrostatic forces, without the intervention of factor XIII, which normally creates covalent bonds. In collaboration with the Gremi, the "plasma gun" causes a statistically significant decrease in bleeding time performed on rat tail. This reflects a stimulation of primary haemostasis, a phenomenon dependent on blood platelets. The metrological study of coagulation has required a methodological adaptation. Indeed, the plasma treatment of blood samples in the measuring wells produces a surface layer. The lower part of the sample remains liquid. So, the analyzers used are unable to measure the clotting time. We therefore used an indirect technique according to a description made in microbiology by Kamgang-Youbi in 2009. We treated calcium chloride by a DBD direct plasma for 30 seconds. Use of this plasma activated calcium chloride resulted in a statistically significant decrease in clotting times measured. Despite these results, we fail to understand the physiological mechanisms behind these effects. Depending on the variation of parameters (plasma source, exposure distance, type of gas mixture, gas flow, etc), we observe contradictory phenomena, sometimes even with longer clotting times. The study shows that blood platelets can be activated or inhibited, as can be seen after exposure to NO.

In conclusion, although these contradictory effects are not really surprising given the existence of many loops of activation or inhibition in haemostasis as in many other biological systems, this situation proves that there is still much work to provide for understanding the physiological mechanisms induced by plasmas, in the haemostatic system. It remains to design experiments in view to isolate the role of each component (ROS, RNS, heat, UV, etc.) and measure a possible threshold effect and then be able to create the most suitable plasma depending on the wanted application.