

## **Mechanism of Blood Coagulation by Non-Thermal Atmospheric Pressure Dielectric Barrier Discharge Plasma.**

M. Balasubramanian MD<sup>1</sup>, G. Nagaraj MD<sup>2</sup>, S. Kalghatgi<sup>3</sup>, A.S. Wu MD<sup>4</sup>, A.D. Brooks MD<sup>5</sup>, G. Fridman<sup>6</sup>, M. Cooper<sup>7</sup>, V. Vasilets PhD<sup>8</sup>, A. Gutsol PhD<sup>9</sup>, A. Fridman PhD<sup>10</sup>, G. Friedman PhD<sup>11</sup>

<sup>1</sup>Dept. of Pathology, Drexel University College of Medicine (DUCOM), <sup>2</sup>Dept. Internal Medicine (DUCOM), <sup>4,5</sup>Dept. of Surgery (DUCOM), <sup>3,11</sup>Dept of Electrical and Computer Engineering, Drexel University (DU), <sup>6</sup>School of Biomedical Engineering (DU), <sup>7,8,9,10</sup>Dept. of Mechanical Engineering (DU)

*Introduction:* Non-thermal atmospheric pressure dielectric barrier discharge plasma (cold plasma due to its non-thermal nature) has emerged as a promising new tool in medicine due to its ability to coagulate blood rapidly, sterilize tissue without thermal damage and induce apoptosis in malignant tissue. The potential clinical applicability of non-thermal plasma lies in its use in controlling intra-operative microvascular bleeding in organs and in endoscopy. Non-thermal plasma can also be used to treat superficial wounds in trauma through hemostasis while simultaneously inducing surface sterilization. We have previously demonstrated that non-thermal plasma hastens blood coagulation on cut tissue surfaces and accelerates clot formation in whole blood five times faster than natural coagulation. A series of experiments were undertaken to investigate the mechanism of coagulation by non-thermal plasma.

*Methods & Results:* We initially postulated that changes in pH and/or extracellular Ca<sup>2+</sup> as a possible mechanism for non-thermal plasma mediated coagulation. Our studies however showed no significant changes in pH or Ca<sup>2+</sup> in treated blood. Thermal energy triggered coagulation as seen in conventional electrocautery as well as electric field effects were eliminated as other possible mechanisms. The role of reactive oxygen species (ROS) in coagulation was studied, as non-thermal plasma is known to produce ROS in water. ROS production in blood was blocked with sodium pyruvate, an ROS scavenger, and the results showed no effect on non-thermal plasma induced coagulation.

Specific effects of non-thermal plasma on citrated blood samples revealed extremely rapid coagulation with surface gel formation, while clotting studies (PT, aPTT) performed on the plasma beneath the gel revealed consumption of coagulation factors. Examination of the clot formed by non-thermal plasma using Scanning Electron Microscopy (SEM) showed platelet activation with pseudopodia formation, aggregation, and fibrin formation. The effects of non-thermal plasma on fibrinogen solution treated at physiologic pH showed a change in opacity suggesting clot formation. Dynamic Light Scattering (DLS) was used to measure particle size distributions of treated and untreated fibrinogen solutions. Treated fibrinogen exhibited a multi-modal distribution of sizes with the largest size corresponding to the size of fibrin-like structures. This suggests that non-thermal plasma may coagulate blood by the conversion of fibrinogen to fibrin. Evaluation of albumin, our control protein given its non-involvement in coagulation, showed no changes upon exposure to non-thermal plasma.

*Conclusion:* Non-thermal plasma likely promotes coagulation by enhancing the physiologic coagulation process through direct activation of fibrinogen as well as platelet activation and aggregation. Future research will further evaluate the mechanisms of non-thermal plasma induced platelet activation and effects on other proteins in the coagulation cascade. This will lead to newer insights into the physiological aspects of coagulation and clinical utility of non-thermal plasma in medicine.