Low-temperature plasma treatment is a new tissue processing technology - Plasma-induced blood coagulation limits the excessive host responses-

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Abstract: One of the benefits to using plasma treatment for bleeding control is that it can suppress the cellular responses such as migration of immune cells and platelet activation in wounds, resulting in the minimization of unfavorable tissue remodelings such as fibrosis and atrophy parenchyma. Indeed, the effect by plasma treatment so-called coagulation, remind us to use it to engineer the biomaterial and the regenerated tissues. In my talk, I will figure out our concept for LTP effect and the feasible to use in engineering for biomaterials.

Keywords: protein aggregation, protein aggregation, repulsion force, glycosylation

1. The classical concept for blood coagulation by plasma treatment

Developments in low-temperature plasma sources have occurred in the fields of surface reformation, sterilization, tissue dissection, dermatological therapy, cancer therapy, and agricultural productivity as means to provide a mild electron supply using a wireless circuit with no contact resistance [1]. In 1990, low-temperature plasma, which can accelerate blood clot formation, attracted plasma scientists' attention, and several groups worldwide began studying this mechanism of blood coagulation[1]. Blood clot formation (also described as blood coagulation) by low-temperature plasma (LTP) treatment was pointed out in earlier reports as shortening whole-blood clotting time [1-4]. The underlined mechanism merely is linked with the natural clot formation process due to the finding of acceleration of blood coagulation. Indeed, it was pointed out that LTP treatment activated platelets and coagulation factors, but for any changes on the erythrocytes in the clot and serum proteins such as albumin and immune globulins to discuss [2-4].

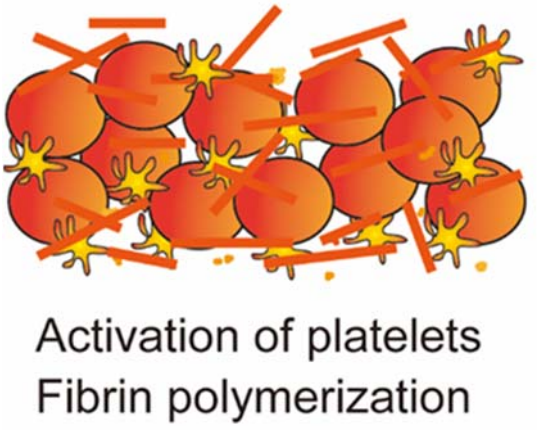

Fig. 1. The classical concept for Plasma Induce Coagulation. Referred from Miyamoto K et al. Archives of Biochemistry and Biophysics, 605 2013 (Ref. 7)

2. An effect for serum proteins by plasma treatment

Contrast to the general understandings in earlier studies, and we have focused on the plasma effects on the secreted serum proteins that were not involved in blood coagulation and the fibrogenesis system because albumin and immunoglobulins are a much higher concentration than the fibrinogen and the other molecules to form clot [5-7]. In our previous paper, we reported the success that plasma treatment generated single protein scaffolds from serum proteins such as albumin, immunoglobulin, and fetuin without the presence of platelets, coagulation factors, and any chemical linkers.

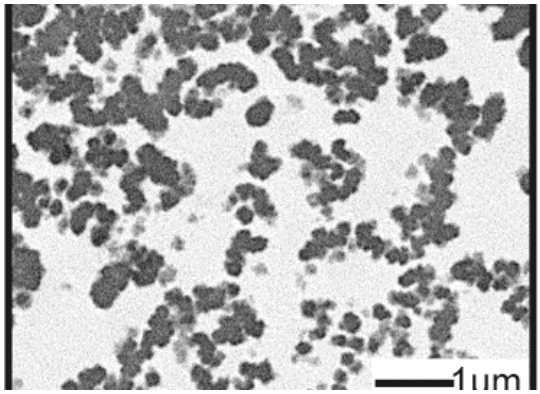

Fig. 2. Trans electron microscopic image for plasma induced protein micelle in albumin solution. Referred from Ikehara S. et al.Plasma Process Polym, 12 2015 (Ref. 6)

3. Effect on erythrocytes (red blood cells) by plasma treatment

We focused on the plasma effects on erythrocytes because the volume occupies much higher than the platelets. To increase knowledge for the correlation between hemolysis and plasma treatment, we constructed an experimental system to measure the electric current originating from low-temperature plasma and hemolysis efficacy [7, 8]. Using this system, we succeeded to show that there is a strong correlation between the measured electric current value and the hemoglobin concentration[8].

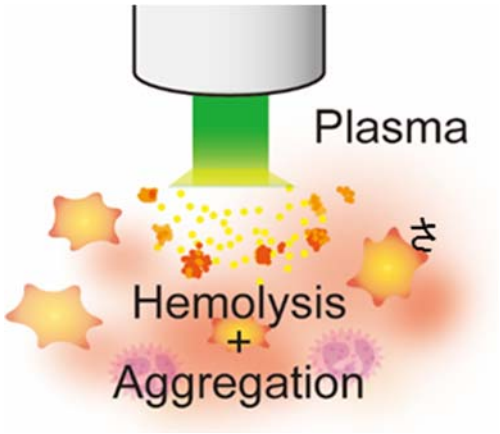

Fig. 3. Shame for plasma induced clot formation through the hemolysis. Referred from Miyamoto K et al. Archives of Biochemistry and Biophysics, 605 2013 (Ref. 7)

4. Plasma instruments

We developed our tool to produce plasma using a dielectric barrier discharge (66 kHz, sinusoidal peak-to-peak voltage of 6.0 kV applied to an electrode [5, 9]. In in vivo experiments, the plasma treatment succeeded to form clots solidly more than the naturally formed clot by the continuous contact with the plasma flare [5, 6, 10]. This result and protein aggregation were reproduced in the experiment using the instrument from Prof. Hori group.

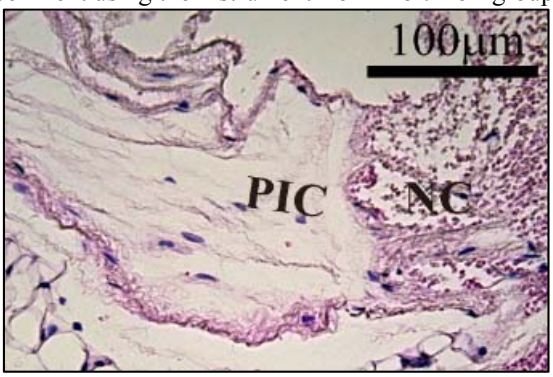

Fig. 4. Plasma Induced Coagulation(PIC) and Natural Clot (NC). Referred from Ikehara Y. Et al. J, Photopolym. Sci. Technol., 26(4) 2013 (Ref. 4)

5. Immunological Significance of plasma induced blood coagulation

Recently, we reported that myosin light chain (Myl) 9 and Myl12 are functional ligands for CD69 protein, suggesting that Myl9/Myl12 released from platelets might act as a mediator for inflammatory responses by interacting with CD69 on the leukocyte surface [11]. The interaction between Myl9/Myl12 and CD69 is a new therapeutic target on unfavorable tissue repair such as fibrosis in asthma due to the inhibitory antibodies to block the interaction that insulate the distribution of Myl9 nets. It is noteworthy that platelets release Myl9 and Myl12 from the cytoplasm to form intravascular net-like structures that retain inflammatory cells [11], and that Myl9 nets in pulmonary stroma promote to expression Heparan sulfate that act as a coreceptor for the activation signaling by both amphiregulin and osteopontin due to the highly negative charge. [5-7], whereas LTP treatment shut down the immunological responses started from Myl9/Myl12 release.

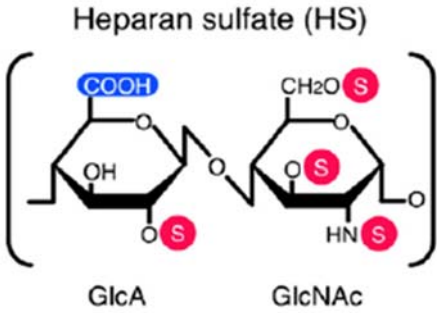

Fig. 5. Chemical structure of Heparan sulfate (HS). HS is a sugar chain consists of sulfated polysaccharides made of repeating disaccharides Glucuronic acid(GlcA) and *N*-acetylglucosamine(GlcNAc).

6. Acknowledgments

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7. References

[1] Shimizu T *et al.* Journal of Physics D: Applied Physics **50 (50)**, 503001 (2017).

[2] Fridman G *et al.* Plasma Chemistry and Plasma Processing **26**, 425-442 (2006).

[3] Kalghatgi SU *et al.* IEEE Transactions on Plasma Science **35**, 1559-1566 (2007).

[4] Kim J *et al.* Plasma Medicine **5**, 99-108 (2015).

[5] Ikehara Y *et al.* J Photopolym Sci Tehnol **26(4)**, 555-557 (2013).

[6] Ikehara S *et al.* Plasma Process Polym **12**, 1348-1353 (2015).

[7] Miyamoto K *et al.* Arch Biochem Biophys **605**, 95-101 (2016).

[8] Miyamoto K *et al.* Plasma Process Polym **In press**, (2019).

[9] Sakakita H *et al.* WO2012/005132, (2012).

[10] Miyamoto K *et al.* Arch Biochem Biophys **605**, 95-101 (2016).

[11] Hayashizaki K *et al.* Science Immunology **1**, 9154 (2016).