Surface Protein Destruction Using Cold Atmospheric Plasmas

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It is well known that surgical instruments and medical devices are contaminated by both bacteria and infectious proteins. However most bio-decontamination studies reported using cold atmospheric plasmas are limited to bacterial inactivation and their ability to destroy infectious protein residual left on surgical instruments has so far received relatively little attention. In this paper, we present a systematic study of surface protein removal and destruction using a helium-oxygen atmospheric plasma source. It is shown that such cold atmospheric plasmas are effective in destroying surface protein.

1. General:

Biological lethality of cold atmospheric gas discharges has been extensively studied for more than 15 years. A vast majority of their bio-decontamination studies has been focused on inactivation of microorganisms, and their results have been essential in establishing the capability of cold atmospheric plasmas against a wide range of bacteria and virus. Clearly, this is very significant for hospital sterilization and food hygiene control. It is however important to recognize that surgical instruments, medical devices and hospital wards are contaminated by both bacteria and infectious proteins. Similarly, work surfaces used in food manufacturing are often subject to both bacterial and proteinaceous contamination. For cold atmospheric plasmas to be developed into a widespread sterilization technology for healthcare facilities, their ability to destroy surface protein left on surgical instruments following surgery must be studied. In this paper, we present a systematic study of surface protein removal and destruction using a helium-oxygen atmospheric plasma source.

2. Surface protein destruction

2.1. Plasma source

The plasma source used was an atmospheric plasma plume generated in a flowing helium oxygen gas stream with an excitation voltage of 9 kV and 30 kHz. The plasma was truck inside a dielectric tube and it was carried by the flowing gas to the ambient air to form a 2 cm long plume with a small diameter of 3 mm. Therefore the plasma was a dielectric-barrier discharge. Nanosecond images were taken, from which no streamers were observed. Optical emission spectroscopy indicated abundant fluxes of atomic oxygen, OH radicals and nitride oxide were found. UV emission was however weak.

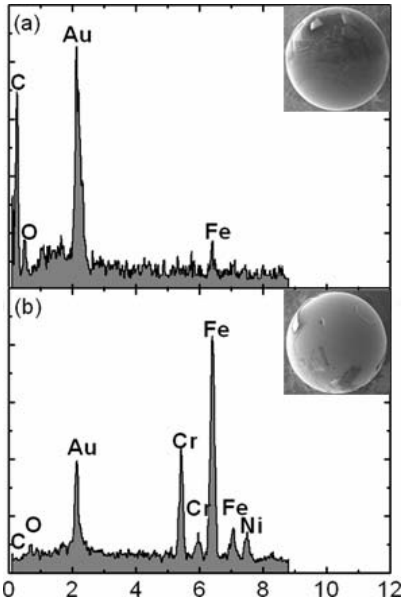

Figure 1: SEM and EDX of BSA-coated stainless steel balls – (a) untreated; and (b) treated with plasma.

2.2. Protein samples

Protein of the greatest clinical significance is prion, the misshaped protein of Creutzfeldt-Jakob diseases. However, since prion protein is extremely infectious, we employed a purified protein, bovine serum albumin (BSA), as a model. To model surgical instruments, stainless steel balls and stainless steel disks, both of 6 mm diameter were made and used as the sample substrate. Quantities of BSA, up to 20 μl per droplet, were diluted in distilled water and then their droplets were deposited on stainless steel surfaces to dry at 50^{o}C before plasma treatment. The diameter of the BSA deposit on the stainless steel disk was typically 5 mm. All data of plasma protein inactivation experiments were in triplicate from three independent experiments.

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2.3. Scanning-electron microscope results

BSA coated stainless steel balls were treated by the plasma plume for 3 minutes, and then subject to analysis using scanning electron microscopy (SEM) and electron energy dispersion X-ray (EDX) analysis. Figure 1 shows both the untreated and treated samples. From the SEM images, it is clear that large amount of BSA coating was removed thus demonstrating the ability of the cold atmospheric plasma jet to remove proteinaceous matters from stainless steel surface (and hence from surgical instruments). As an alternative evaluation of the plasma treatment, EDX was used to measure the reduction in elements, particularly oxygen and carbon. These elements were used here as signature of proteins. It is clear that the reduction of carbon and oxygen was very significant.

2.4 Laser-induced fluorescence results

To provide a quantitative evaluation of the plasma treatment, a laser induced fluorescence method was used in which the surface protein was tagged through protein binding and then irradiated by a laser beam. The irradiated protein emitted fluorescence at a longer wavelength than the laser wavelength. It was found experimentally that the florescence intensity is proportional to the amount of surface protein, from which a calibrated surface protein detection technique was developed. Figure 2 shows the reduction of surface protein as a function of plasma treatment time. It is clear that significant protein reduction has been achieved for more than three orders of magnitude. This highlights the clear ability of cold atmospheric plasmas in reducing surface protein.

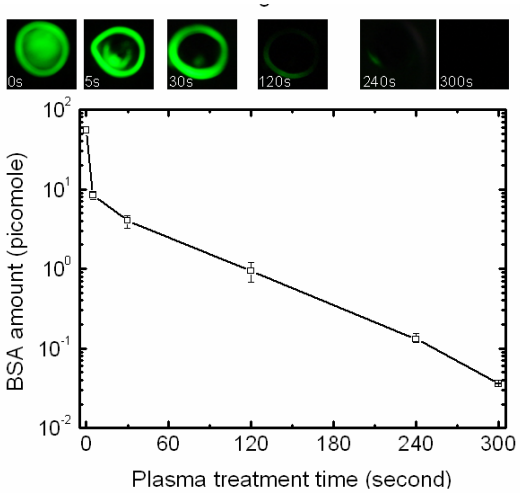

Figure 2: Protein reduction as a function of plasma treatment time with images showing the fluoresced protein samples at different points of plasma treatment.

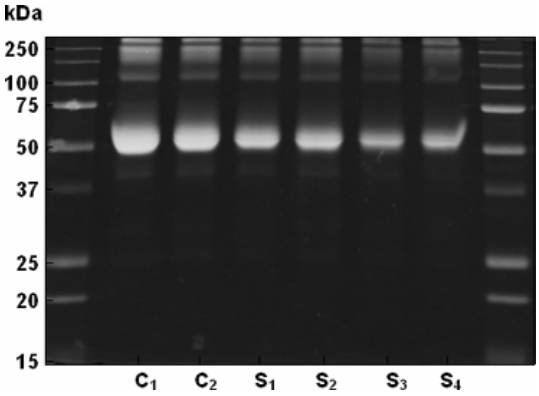

Figure 3: Electrophoresis images of six BSA samples. C_1 and C_2 are two control samples that were left in open-air and treated with an unionized He-O_2 flow, both for 300 seconds. S_1, S_2, S_3 and S_4 are samples that were treated by plasma for 4, 60, 180, and 300 seconds respectively.

2.5 Protein degradation and destruction:

To After plasma treatment, the removed BSA protein may have been fragmented into basic chemical elements such as oxygen, nitrogen, carbon, and hydrogen. The un-removed proteins that remained on the stainless steel surface were likely to be less damaged than the removed proteins. Although direct measurement data of possible BSA fragmentation are at present unavailable, insight can be gained by using electrophoresis to analyze the un-removed BSA proteins. To this end, buffer-diluted BSA droplets were first deposited on stainless steel disks and then carefully washed off the disks so that they can be collected in equal amounts of buffer solution for subsequent electrophoresis experiments. Triplicate experiments were performed to ensure the consistency of electrophoresis results. Fig. 3 shows images of two controls - C_1 samples were left in open air for 300 s and C_2 samples were treated with a He-O_2 flow also for 300 s. Their electrophoresis images do not show any significant difference from one another. When plasma treatment was applied in four different durations (e.g. 4 s, 60 s, 180 s, and 300 s), the 50 kDa band became progressively thinner and weaker as shown in figure 3. This suggests that the plasma-treated but un-removed proteins underwent considerable degradation. Two useful conclusions may be drawn from this. Firstly, proteins removed from the stainless steel were likely to have sustained much greater damages, destructed potentially into basic chemical elements, than the un-removed proteins. Secondly for protein residues remained on the sample surface, their integrity was compromised. Therefore even when plasma treatment cannot completely remove all

proteinaceous matters from stainless steel surfaces, the plasma-treated but un-removed proteins are likely to have a reduced infectivity, if any, and pose less risk than untreated proteins.

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3. Conclusions

It has been shown that cold atmospheric plasmas can effectively remove protein matters from the surface of stainless steel. Its removal was found to be through destruction and degradation into to either elements or damaged fragments. When combined with their bactericidal capability that has already been established, the protein-destruction ability of cold atmospheric plasmas is significant. It means that cold atmospheric plasmas could be used as a generic technology platform capable of both bacterial and protein decontamination.