Research Article

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Achieving Antimicrobial Activity of Highly Stretchable Hydrogel Upon DBD Plasma Treatment

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Abstract
Recently, we developed a highly stretchable and moldable solid hydrogel based on polyacrylamide (PAAm) and gelatin. The hydrogel may serve as a material for wound healing and/or wound treatment due to its stretchability and low young's module. For wound healing or wound treatment applications, the hydrogel should be antimicrobial. We demonstrate in this work that upon a treatment using dielectric barrier discharge plasma (DBD), the hydrogel possesses the required antimicrobial efficacy.

Keywords: nonthermal plasma; DBD; hydrogel; antimicrobial; wound healing; wound treatment.

Introduction
Efficient wound management is essential to tackle the ongoing clinical problems with acute and chronic wound healing and wound treatment. Current methods for acute and chronic wound management are susceptible to infection and permanent scarring after initial treatment [1]. Therefore, there is a need to develop techniques to properly clean the wound, protect it from further infection, and promote wound healing. A solution to this problem may be to use hydrogel as wound dressings due to their high absorption capacity, easy removal from the wound, acceleration of healing and reduction of pain and inflammation [2].

A hydrogel that is high stretchable with low tensile strength would be ideal due to its maximum comfort for patients. Recently, we developed a high stretchable gel that is made of the combination of polyacrylamide and gelatin.[3] This gelatin/polyacrylamide hydrogel is capable of withstanding strains of 5000%, i.e. 50 times of its initial length, before failure and a weak tensile strength of 0.30MPa at failure.[3] The gels were stable over thousands of continuous stretching cycles, demonstrating their good reliability for would healing applications.

For the wound healing applications, methods to obtain the antimicrobial activity of hydrogels are required [4]. In this study, we report our preliminary study on the antimicrobial efficacy of the gelatin/polyacrylamide hydrogel after a treatment using dielectric barrier discharge plasma (DBD). DBD plasma refers a non-thermal plasma under ambient conditions that is generated between two electrodes when either one or both electrodes are insulated. [5–7]. A microsecond or nanosecond pulsed power supply is used to prevent the buildup of charges on the electrodes. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl radicals, nitric oxide, and nitrogen dioxide [8] are generated in DBD, [9] which can deactivate bacteria. [10]

Materials and Methods
Chemicals and Hydrogel Preparation
Gelatin (isolated from bovine skin, molecular weight 38-43 KDa), N,N,N',N'-tetramethylethane-1,2-diamine (TEMED), ammonium persulfate (APS) were purchased from Sigma-Aldrich (USA), Acrylamide, N,N′-methylenebisacrylamide (MBAA) were purchased from TCI America. All chemicals are used as purchased without further purification.

The hydrogel was fabricated according to our published work [3]: Acrylamide (0.4 g) and gelatin (0.05 g) were dissolved in distilled water in a vial. MBAA (the crosslinker, 0.06 wt% of acrylamide), were added into the vial with ammonium persulfate (the initiator, 0.50 wt% of acrylamide). After degassing the solution for 5 mins, TEMED (crosslinking accelerator for polyacrylamide, 0.25 wt% of acrylamide) was added into the vial. After degassing for another 3 mins, the vial was transferred into the UV reactor (Raytheon, twelve 8W UV lamps at 254 nm wavelength) and the hydrogel was cured in 1 hour. When the photoreaction was done, the vial was transferred to a box at humidity 50% for one day to stabilize the reaction. Once stabilized, the hydrogel was taken out of the vial and N2 was used to remove the surface water. The hydrogel was then ready for testing.

Plasma Setup and Hydrogel Treatment
Plasma was generated using a nanosecond-pulsed power supply with alternating polarity (FPG 20-N, FID Technology, (Burbach,

Germany) and a floating electrode-dielectric barrier discharge setup (Figure 1 left) identical to that used by Li et al. [11]. This pulse generator provided a 1–10 ns pulse width with a rise time of 5 kV/ns between the dielectric barrier electrodes [12]. The power supply provides an adjustable frequency of 500 Hz to 1.5 kHz with a maximum amplitude of 20 kV. A 1-mm thick quartz glass acts as the insulating layer for the high-voltage copper electrode. Plasma was applied to the hydrogel sample on a glass side (Figure 1 right) at a fixed gap of 1 mm between the top electrode and the hydrogel. Samples were treated under atmospheric conditions with no gas flow.

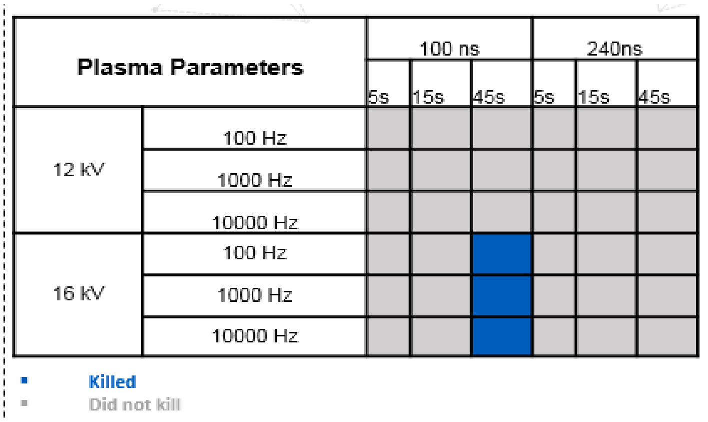
Figure 1: Left: DBD schematics for plasma treatment of the gelatin/polyacrylamide hydrogel. Right: A piece of gelatin/polyacrylamide hydrogel on a glass slide.

Results and Discussion

Treatment of the gelatin/polyacrylamide hydrogel with DBD plasma was done with different parameters, including pulse width, treatment time, frequency, and voltage. After the treatment, the hydrogel was placed inside a petri dish harboring E. Coli bacteria. After incubating for at least one day, the transparency of the hydrogel was then observed to verify its antimicrobial efficacy, which is based on principle that the clearer the hydrogel, the higher the possibilities of it being antimicrobial. Figure 2 shows the plasma treated hydrogel demonstrated the antimicrobial activity, while the hydrogel without treatment has no antimicrobial activity.

Figure 2: Left: Hydrogel before plasma treatment. Right: Hydrogel after plasma treatment. The pulse, voltage, treat time, and the frequency of the plasmas were 100 ns, 16 kV, and 45 s, and 1,000 Hz, respectively.

Table 1 shows that high voltage (16 kV), shorter pulse time (100 ns), and longer treatments (>45 s) provided better results in achieving the antimicrobial efficacy of the hydrogel. The frequency, from 100Hz to 10,000 Hz, does not seem to significantly affect the antimicrobial activities of the treated hydrogel (Figure 3).



Plasma Parameters		100 ns			240ns		
		5s	15s	45s	5s	15s	45s
12 kV	100 Hz	⬜	⬜	⬜	⬜	⬜	⬜
	1000 Hz	⬜	⬜	⬜	⬜	⬜	⬜
	10000 Hz	⬜	⬜	⬜	⬜	⬜	⬜
16 kV	100 Hz	⬜	⬜	🟦	⬜	⬜	⬜
	1000 Hz	⬜	⬜	🟦	⬜	⬜	⬜
	10000 Hz	⬜	⬜	🟦	⬜	⬜	⬜

- 🟦 Killed
- ⬜ Did not kill

Table 1: The antimicrobial activity of hydrogels when treated with DBD plasma with various pulse width, treatment time, frequency, and voltage.

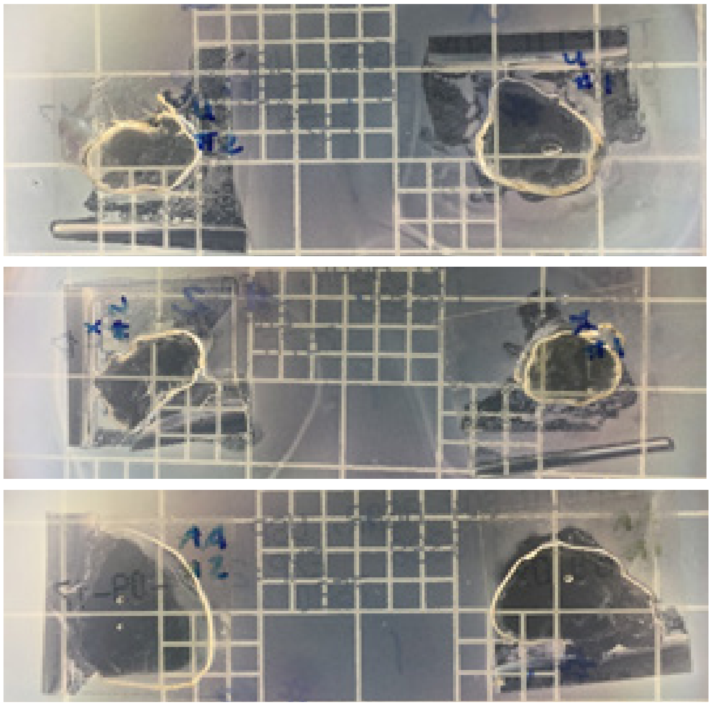


Figure 3: The antimicrobial activity of the plasma treated hydrogel when the pulse, the voltage, and treat time of the plasmas were 100 ns, 16 kV, and 45 s, respectively. The frequencies were 100 Hz (top), 1,000 Hz (middle), 10,000 Hz (bottom).

Conclusions

In conclusion, our results show that the highly stretchable gelatin-polyacrylamide hydrogel demonstrated antimicrobial activity after a treatment of DBD plasma at 16 kV and 45 s. The data is promising since we expect the hydrogel maybe be coated on textile and used as a bandage tape. The hydrogel may be treated with a handheld DBD plasma device right before applying on wound for wound treatment and healing. Research is ongoing in our group to develop a light weight and easy to deploy system that can be used by medical and non-medical personnels for treating wound at or near the point of injury under prolonged field care.

Conflicts of Interest: The authors declare no conflict of interest.

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