Uptake and diffusion of plasma-generated reactive nitrogen species through keratinized membrane

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Abstract: Plasma medicine has attracted strong interest due to its potential applications in life science, but still suffers from the absence of basic knowledge of plasma interactions with biomaterials. As an effort to address this challenging issue, surface kinetics and transport of reactive nitrogen species (RNS) through a keratinized membrane are modelled for an experimental system consisting of a surface microdischarge (SMD) in air, designed for the purpose of theoretical modelling with minimal assumptions. Modelling results yield spatial and temporal concentration profiles of RNS inside the bovine hoof, leading to the detailed understanding for the uptake and diffusion of plasma generated RNS through the keratinized biomembrane.

Keywords: heterogeneous reaction, diffusion, bovine hoof, plasma medicine

1. Introduction

Atmospheric-pressure air plasmas have been researched for wide applications in plasma biotechnology due to their promising merits for convenient generation of reactive species at ambient conditions. Furthermore, it has been demonstrated that atmospheric-pressure, ambient temperature gas plasmas can be considered as promising platforms for a wide range of environment and biomedical applications such as antibacterial action and disinfection of wounds [1].

Emerging plasma technologies for therapeutic usage of reactive oxygen and nitrogen species (RONS) have faced many challenges because of their extreme complexities for plasma interactions with biomaterials in spite of apparently superior potential [2-3]. To address these issues, the basic understanding of plasma interactions with biomaterials is a significant initial step to take, in order to enable applications of plasma medicine.

This work focuses on reactive nitrogen species diffusion and heterogeneous reactions inside biomaterial. For this work, a well-characterized SMD device was used for the generation of RNS [2]. Bovine (cow) hoof in the form of disks were taken as a simplified research platform to understand the delivery behavior into biomaterials for plasma-generated species. This work also has relevance to treating fungal infections in human nails, since the bovine hoof is an accepted model for human nail. The hoof material can be thought of as a keratinized membrane. Based on the predesigned experimental setup, the delivery of RNS inside the model bio-membrane was analyzed using a transient reaction-diffusion model. The penetration of RNS through the biomaterial was monitored by conventional solution assays. By performing comparison studies with the reaction-diffusion modeling, the basic transport and uptake of RNS inside the keratin membrane is discussed in detail in this work.

2. Experimental and theoretical details

As shown in Fig. 1, a SMD device was used to generate RNS under the nitrogen oxides-dominated mode, which has been described in detail in a previous report [4]. The concentrations of RNS inside this test chamber were monitored by UV/VIS spectrometry.

![Fig. 1. Schematic diagram of the experiment. Species generated in the plasma diffuse into, and react within, the hoof disk. RNS that penetrate the disk are detected using Greiss reagent in water.](image)

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Hoof disks with different thicknesses were exposed to RNS generated from the SMD device for a fixed time. Water with Griess reagent was used for absorption of the penetrated RNS via the hoof disks, as shown in Fig. 1. For comparison with modelling results, the concentration of NO2− ions with Griess reagent was also measured by UV/VIS spectrometry. In addition, the effective diffusivity in the hoof disk, in the absence of reaction, was measured directly using an experimental setup.
similar to the one depicted in Fig. 1, employing CH$_4$ and CO$_2$.

A transient, one-dimensional reaction-diffusion model was formulated for RNS within the keratinized material and then absorbing into water. In the absence of published data for the heterogeneous reactions of RNS with keratin material such as hoof disk, we developed a model based on gas kinetic theory and an Eley-Rideal mechanism. In part, the model is based on data from the atmospheric chemistry literature [5]. Finally, the one-dimensional transient reaction-diffusion equations were solved numerically using Mathematica$^\text{®}$.

3. Results

The RNS concentrations measured by UV/VIS spectrometry were applied as boundary conditions to the one-dimensional reaction-diffusion model in the bovine hoof. To verify the model, the predicted concentrations of RNS as a function of hoof thickness were compared with experimental data. The modelling results as a function of hoof thickness showed good agreement with experimental data, indicating that the RNS transport with heterogeneous chemical reactions inside the bovine hoof can be explained reasonably.

Fig. 2 represents a typical model result for RNS concentration and fractional coverage of the hoof disk as a function of the exposure time with our SMD device. The propagation of RNS species inside the hoof is characterized by relatively slow diffusion through the pores of the membrane as well as by the uptake of RNS by water known to coat the keratin [6]. After a specific exposure time (typically a few tens of minutes), the RNS concentration spatial profile becomes linear, as the uptake of RNS inside the hoof becomes saturated. Based on this result, we conclude that the delivery of RNS inside a biomaterial such as this keratinized membrane can be understood by a coupled reaction-diffusion model with uptake of RNS in the porous biomaterial.

4. Conclusion

The delivery and interactions of RNS generated by atmospheric pressure plasma inside the cow hoof model of human nail were investigated. The experimental results were modelled with a transient reaction-diffusion model accounting for the uptake of RNS in the porous membrane. The theoretical approach was verified by experimental measurements, and showed the detailed transport and kinetic behaviour for the delivery of RNS into the biomaterial. We believe that this work provides basic insights to better understand plasma-induced chemical and physical effects for more complex biomaterials such as cells and living tissue. This is important for many applications of plasma biomedicine.

5. References