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ORIGINAL PAPER

A Study on the Effect of Non-thermal Plasma on Macrophage Phenotype Modulation

Takia Sharfuddin[1] · Diego Jacho[1] · Dalton Mitchey[1] · Eda Yildirim-Ayan[1] · Halim Ayan[1]

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Abstract
Non-thermal plasma has gained significant popularity in the field of biology due to its ability to generate reactive oxygen and nitrogen species, which can alter gene expression within cells. Plasma treatment has been used to address various medical issues, such as cancer treatment, blood clotting, and bacterial sterilization. In our study, we investigated the effect of non-thermal plasma treatment on monocyte-derived macrophages (U937) in vitro. To create an in vivo-like environment, the cells were embedded in a collagen matrix and treated with a di-electric barrier non-thermal plasma system using a combination of air and helium flow. Our results showed that plasma in air might modulate macrophages through changes in gene expression. Further experiments revealed the presence of reactive species responsible for these changes. By evaluating cell viability and gene expression, we determined that 120 s of plasma treatment in air is optimal for Fibroblast cells within collagen. Our study suggests that non-thermal plasma in air can modulate monocyte-derived macrophages embedded in collagen.

Keywords Non-thermal plasma · Polarization of macrophages · 3D collagen matrix · Reactive species

Introduction

Plasma is the fourth state of matter and consists of a combination of negatively charged electrons, positively charged protons, reactive species, radiation, free radicals, and an electromagnetic field [1–3]. There are two types of plasma found in nature: thermal and non-thermal, which are distinguished based on the equilibrium state between negative and positive particles. Thermal plasma has a much higher temperature for positive particles than electrons and can reach temperatures up to 3000 °C, making it unsuitable for use on living cells. In contrast, non-thermal plasma, which is in an equilibrium state, is suitable for use on living organisms without causing harm to tissues or cells [4, 5]. Non-thermal plasma is widely used in the medical field, including cancer treatment [6], sterilization [7], tooth whitening, blood coagulation, and wound healing.

✉ Halim Ayan
halim.ayan@utoledo.edu

[1] Department of Bioengineering, University of Toledo, Toledo, OH, USA

Plasma treatment generates reactive species that alter gene expression within cells, making it a promising tool in the medical field. In the 1990s, plasma treatment was first used for sterilization. Further research and experimentation revealed that the changes were caused by generating reactive species during plasma treatment [8]. In the 2000s, non-thermal plasma promoted wound healing [9].

Monocytes are white blood cells originating in the bone marrow and traveling through the blood to reach peripheral tissues. When homeostasis or inflammation occurs, monocytes leave the blood and migrate into tissues, transforming into dendritic or macrophage cells depending on growth factors or cytokines [10]. External stimuli, such as cytokines, can alter the expression of macrophage genes, resulting in their polarization into either M1 or M2 phenotypes. M1 macrophages are known for their ability to kill, such as killing tumor cells or viruses, while M2 macrophages are known for their role in healing, such as wound healing [11]. Therefore, many researchers have explored effective ways to polarize macrophages. Polarization of macrophages has been observed using specific miRNAs, such as let-7b [12]. MiR-127 has been found to be responsible for the expansion of pro-inflammatory cytokines, leading to the polarization of macrophages into the M1 phenotype [13]. MiR-146a has been found to modulate macrophages by inhibiting the Notch1 pathway [14].

Non-thermal plasma has been applied to monocyte THP-1 cells, A375 melanoma cells, and monocytes derived from human blood using a KINPen argon plasma jet. Changes in gene expression were observed in M2 markers. Further investigation revealed the presence of reactive species after plasma treatment, which may be responsible for modulating THP-1 monocytes [15]. Non-thermal plasma was applied to both monocytes and monocyte-derived macrophages. It was observed that monocytes were susceptible to plasma treatment after 30 s, while macrophages gradually polarized to the M2 type with plasma treatment [16]. Macrophage polarization through plasma treatment on macrophage cells derived from THP-1 cells has been shown to induce cancer apoptosis [17]. The Raw 264.7 and B16F10 cell lines were treated with microwave plasma with nitrogen and oxygen gas, and plasma-generated nitrogen oxide was found to differentiate macrophages to the M1 type, which plays a valuable role in anti-cancer activity [18]. While there have been studies on the plasma treatment of macrophage cells, none have been conducted on macrophage cells embedded within collagen scaffolds. Most research studies use 2D cell cultures. However, due to limitations and inconsistent results with in vivo studies, many researchers have moved to 3D cultures, which respond more similarly to in vivo [19, 20].

To this end, the objective of this study is two-fold: (a) understanding the differentiation of monocyte-derived naïve human macrophages within the 3D collagen matrix upon air- and helium DBD plasma treatment, and (b) investigating the effect of plasma treatment time on pro- and anti-inflammatory differentiation of macrophages.

Materials and Methods

Cell Culture

Human-derived monocytes (U-937; ATCC) were thawed and cultured in RPMI-40 media with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in a T-75 flask, according to ATCC protocols. Upon confluency, monocytes were differentiated into macrophages via phorbol 12-myristate 13-acetate (PMA). U-937 monocytes were treated with 100 ng/ml

of PMA in 10 mm Petri dishes for 24 h. Following PMA treatment, cells were embedded within collagen scaffolds. Briefly, M0 cells were encapsulated at a 2×10^6 cells/ml seeding density within rat tail collagen type-I mixed with PBS, and NaOH to achieve a neutral pH of 7.5. Then, 200 uL of encapsulated collagen scaffolds were plated in 12-well plates and incubated for four hours at 37 °C with a complete RPMI-40 medium.

Additionally, Human dermal fibroblasts (ATCC, HHF1;USA) were cultured in a complete media of Dulbecco's modified Eagle's medium (DMEM) (ATCC, USA) supplemented with 15% FBS, and 1% penicillin–streptomycin. Fibroblast (HFF1) cells were used to assess viability after plasma treatment.

Dielectric Barrier Discharge Plasma System and Characterization

A well-established custom built dielectric barrier discharge (DBD) plasma system was used in this study [6, 21–24]. A copper electrode surrounded by an insulator was inserted into a 6 mm test tube. The test tube end functioned as a dielectric barrier when a high voltage was applied. The optimum distance between the electrode tip and the collagen surface was 2 mm for plasma generation. A lid for the wells of 12-well plates was also designed and 3D printed in our lab to create an enclosed system for plasma treatment. It had inlet and outlet features for gas flow into and out of the well plate. The inlet was connected to an air or helium gas tank. The plasma system was then connected to an oscilloscope for measuring voltage and current, as shown in Fig. 1.

A closed view of the plasma treatment system is shown in Fig. 2. Figure 2a shows a zoomed picture of the experiment consisting of a 12-well plate, a 3D lid with an inlet, outlet, and electrode holder while Fig. 2b shows a picture of non-thermal plasma generated during the experiment.

To characterize the generated plasma, the voltage-current wave form collected from oscilloscope was used. Briefly, a high voltage probe with a ratio of 1000:1 was used to measure voltage, and a high current probe was used to measure current. The results were displayed on a digital oscilloscope (TDS 2016, Tektronix, USA). The voltage probe was

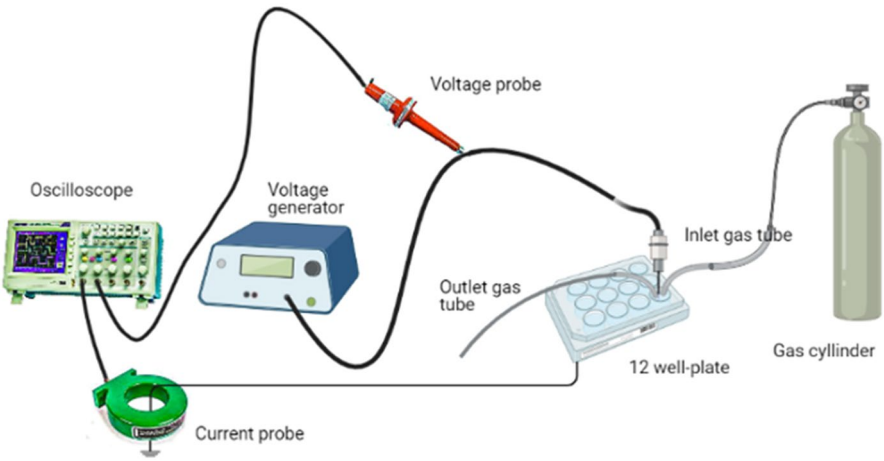


Fig. 1 An overview of the plasma treatment setup for electrical plasma characterization. The electrode is connected to a high-voltage source, and the high-voltage and current probe display signals on the oscilloscope. The inlet of the lid is connected to a tank to ensure gas flow

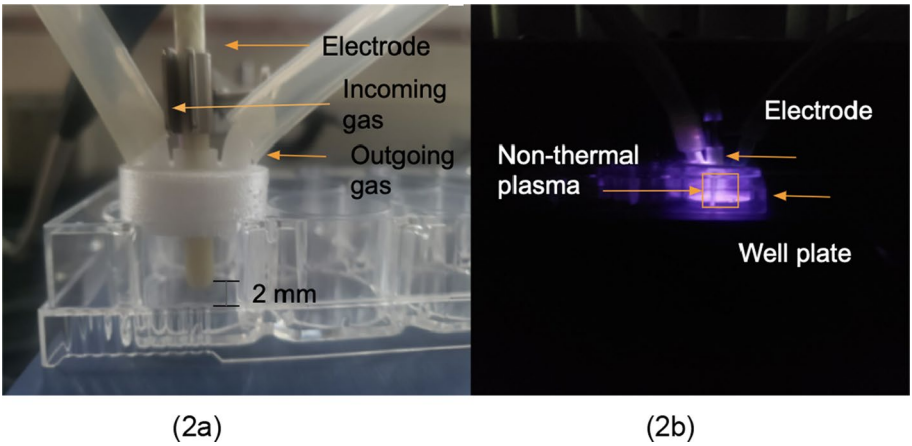


Fig. 2 Experimental set-up for enclosed DBD plasma system

connected in parallel with the electrode, and the cable was passed through the current probe.

To identify optimal plasma exposure time, cell viability and gene expression were performed in fibroblast cells. Plasma in the air was applied to fibroblast cells for 0, 15, 30, 60 and 120 s. After selecting the optimized time, experiments proceeded without 120 s plasma treatment. Plasma in the air was applied on M0 cells embedded within collagen scaffolds for 0, 15, 30 and 60 s, and gene expression was performed.

Assessing Optimal Plasma Treatment Duration Using Cell Viability

Prior running extensive biological studies, the optimal plasma treatment time was assessed though cell viability data. For this study, cells were directly exposed to the plasma treatment rather than encapsulated within a 3D matrix to test the durability of the cells to the plasma treatment without cushion around them. For viability study, the cells were seeded on 12-well plate with 300,000 cells/well seeding density and incubated in the incubation (37C,5% CO2) for two hours. Then, plasma treatment was applied for 0, 15, 30, 60, and 120 s with air as a working gas. Following the treatment, the cells were incubated with Calcein AM (Thermo Fisher, USA) for 30 min. The Calcein AM is a green fluorescent marker tagging the live cells through penetrating inside the live cells through the cell membrane. After incubation, the cells were washed twice with PBS and observed under a fluorescent microscope.

Assessing the Naïve Macrophage Differentiation upon Plasma Treatment

The naïve macrophages were embedded within collagen type I (3 mg/ml) (Corning, USA) with a seeding density of 1×10^6 cells/ml and then incubated in incubator (37C, 5% CO2) for 4 h. After the incubation period, plasma was applied to 3D cell-embedded construct for 0 and 60 s using an air or helium flow of 500 mL/min. After plasma treatment, gene expression was analyzed to observe molecular changes. Cells treated with plasma in air and plasma in helium were evaluated immediately after the experiment.

For gene expression, there were four groups (control for plasma in air, control for plasma in Helium, 60 s plasma treatment in air, 60 s plasma treatment in Helium) for plasma treatment on monocytes derived macrophages within collagen. Gene expression was analyzed using the RT-qPCR method, where RNA was extracted using an RNA extraction kit, and complementary DNA (cDNA) was synthesized from the RNA through reverse transcription. The COX2, MMP13, TNF-α, IL-10, CD163, and CCL18 genes were analyzed, with GAPDH as a housekeeping gene. The forward and reverse primer sequences for these genes are listed in Table 1 and were selected based on published articles [25]. The Q BIO-RAD PCR system was used to analyze the PCR data, which were further analyzed using the manufacturer's comparative threshold (CT) method. All C_T values were normalized to the GAPDH value, and the ΔC_T value was obtained. The $\Delta\Delta C_T$ value was calculated using the equation $\Delta\Delta C_T = \Delta C_{T\_Sample} — C_{T\_Control}$. The fold changes were calculated using the value $2^{-\Delta\Delta C_T}$.

Evaluation of Generation of Reactive Species After Plasma Treatment

Reactive oxygen and nitrogen species are generated after plasma treatment due to the oxidative stress that is produced in cells. These reactive species have been shown to be responsible for addressing various health issues [35, 36]. In this study, 400,000 macrophage cells were plated in 12 well plates and embedded in collagen. Plasma was applied to the macrophages, and the H2DCFDA (2',7'-dichlorodihydrofluorescein diacetate) dye was added to the cells and incubated. H2DCFDA (Life Technology) is a dye that binds with reactive oxygen species, emitting bright green light. After incubating for 30 min, the dyes were removed, and the cells were washed with PBS twice. Then PBS was added to the cells and incubated for 10 min. 50 µL of the supernatant was collected and transferred to 96 well plates, and fluorescence was measured using a fluorescent microplate reader, with excitation/emission at 485/540 nm.

Similarly, to detect reactive nitrogen species, the DAF-2DA (di-aminofluorescein) (Caymen Chemicals) dye was added to cells embedded in collagen and incubated for 30 min in an incubator. The cells were washed with PBS twice after the dyes were removed. Then the cells were incubated with PBS for 10 min in an incubator. 10 µL of the supernatant was added to 96-well plates and observed at 490/525 nm wavelengths for excitation/emission using a microplate reader.

Table 1 Forward and reverse primer sequences for genes

Genes	Forward Primer 5'-3'	Reverse Primer 5'-3'	Ref
IL 10	CCTGTGAAAACAAGAGCAAGGC	TCACTCATGGCTTTGTAGATGCC	[26]
CD 163	TCTGTTGGCCATTTTCGTCG	TGGTGGACTAAGTTCTCTCCTCTTGA	[27]
CCL 18	AAGAGCTCTGCTGCCTCGTCTA	CCCTCAGGCATTCAGCTTAC	[28]
COX-2	CGGTGTTGAGCAGTTTTCTCC	AAGTGCGATTGTACCCGGAC	[29]
MMP-13	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT	[30]
TNF-α	AGAGGGAAGAGTTCCCCAGGGAC	TGAGTCGGTCACCCTTCTCCAG	[31]
IL-12	CGGTCATCTGCCGCAA	AACCTAACTGCAGGGCACAG	[32]
CD 206	CTTTAACGTGGCACCAGGCG	GCCAACCGCTGTTGAAGCTC	[33]
GAPDH	CCACCCATGGCAAATTCCATGGCA	TCTAGACGGCAGGTCAGGTCCACC	[34]

Statistical Analysis

All groups in the experiments contained a minimum of three replicates. The results from the experiments were analyzed statistically using a t-test in R studio. A *p*-value less than 0.05 was considered to indicate a significant difference and was marked with an asterisk (*). For experiments with more than two groups, one-way ANOVA with Tukey's test was performed in R studio. In the time optimization experiment for plasma treatment in air, a statistical difference of $p < 0.05$ between the control group and one of the plasma groups (15 s, 30 s, and 60 s) was represented by an asterisk (*). A statistical difference of $p < 0.05$ between the 15 s and 30 s or between the 15 s and 60 s groups was represented by an ampersand (&). A statistical difference of $p < 0.05$ between the 30 s and 60 s groups was represented by a dollar sign ($).

Results

Electrical Characterization of Plasma

Voltage and Current waveform concerning time are displayed in Fig. 2. 13.5 kV was applied with a 500 Hz frequency (Fig. 3).

Plasma Treatment with Air-Modulated the Macrophages

Macrophage cells embedded within collagen were treated for 0 and 60 s with both plasma in air and plasma in helium. After plasma treatment, gene expression was analyzed for COX-2, MMP-13, TNF-α, CCL-18, CD 163, and IL 10. A t-test was performed between the groups, and a p-value less than 0.05 was considered significant. The expression of

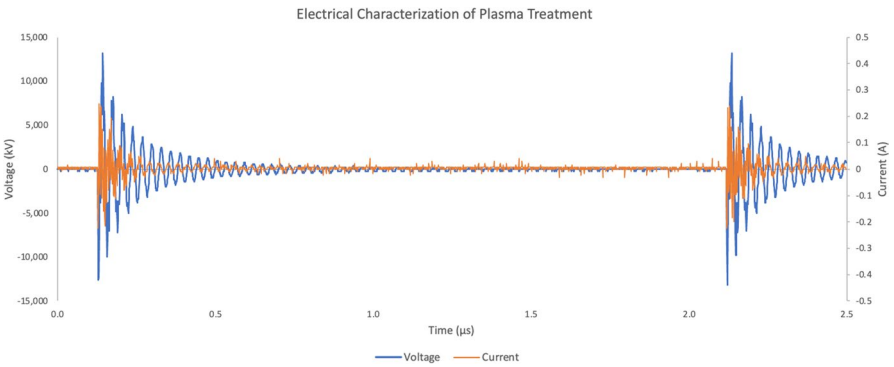
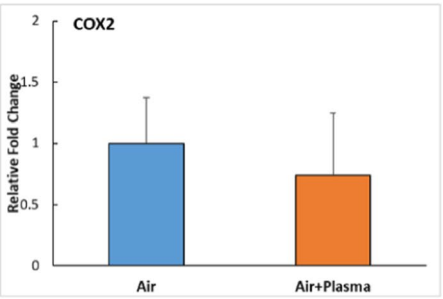
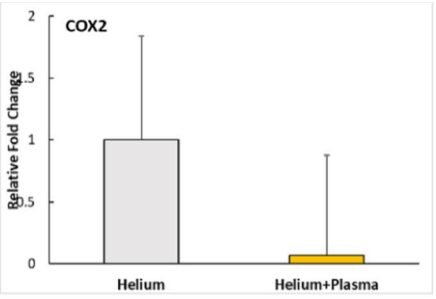


Fig. 3 Voltage and current waveform with data collected from an oscilloscope. Voltage is represented by a blue waveform and current is represented by an orange waveform

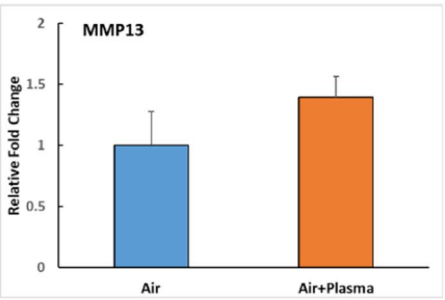
COX-2, an M1-related gene, was found to be decreased after both plasma in air and plasma in helium treatment (Fig. 4a). MMP-13 gene expression was upregulated after plasma in air treatment but significantly downregulated after plasma in helium treatment (Fig. 4b). TNF-α, an M1-related gene, was observed to be significantly downregulated after both plasma in air and plasma in helium treatment (Fig. 4c).

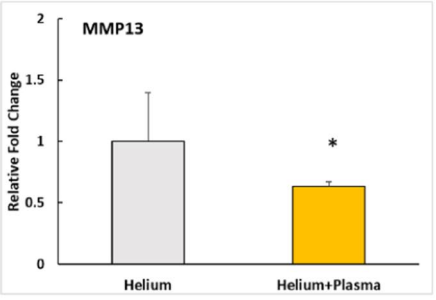
Contrary to the decrease in M1-related genes after plasma treatment, M2-related genes increased after plasma treatment in air. M2-related genes IL-10 (Fig. 4d) and CD163 (Fig. 4e) were significantly upregulated after plasma in air treatment but



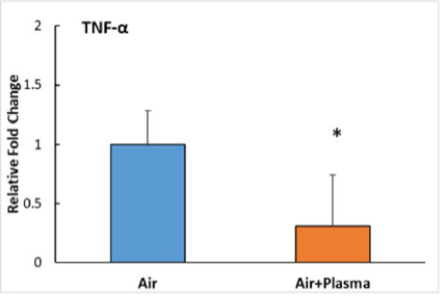


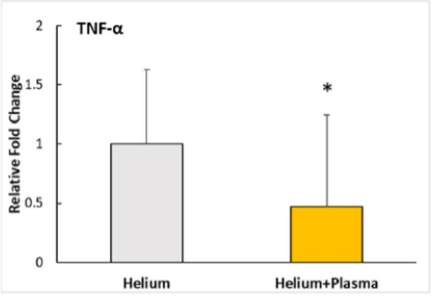
(a) Expression of M-1 related gene Cox-2 after plasma treatment in air and plasma treatment in Helium for 0 and 60s.





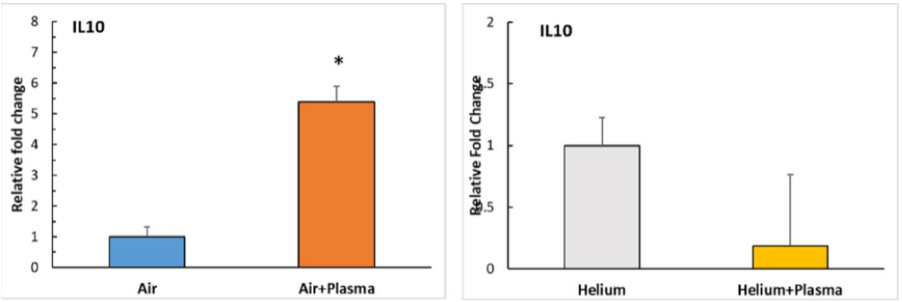
(b) Expression of M-1 related gene MMP-13 after plasma treatment in air and plasma treatment in Helium for 0 and 60s



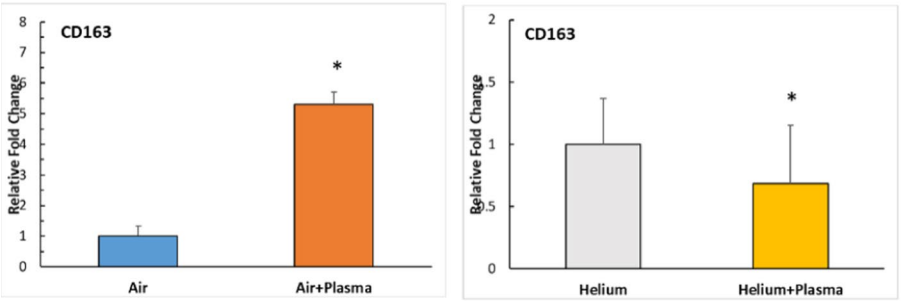


(c) Expression of M-1 related gene TNF-α after plasma treatment in air and plasma treatment in Helium for 0 and 60s.

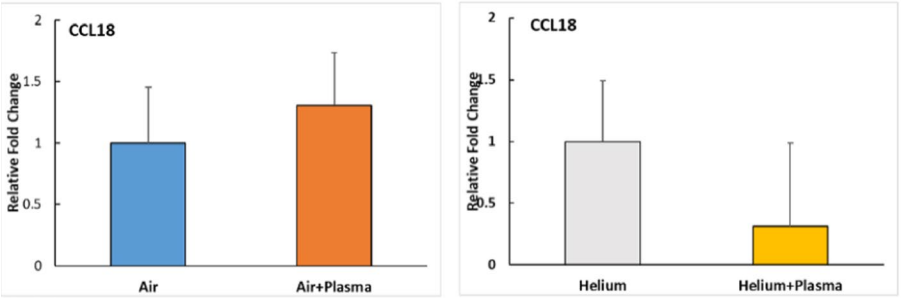
Fig. 4 Gene expression after plasma treatment in air and Helium for 0 and 60 s.T-test was performed for statistical analysis and * denotes a *p*-value less than 0.05. Each expression for each sample was evaluated based on data obtained from three biological replicates



(d) Expression of M-2 related gene IL-10 after plasma treatment in air and plasma treatment in Helium for 0 and 60s.



(e) Expression of M-2 related gene CD163 after plasma treatment in air and plasma treatment in Helium for 0 and 60s.



(f) Expression of M-2 related gene CCL18 after plasma treatment in air and plasma treatment in Helium for 0 and 60s.

Fig. 4 (continued)

downregulated after plasma in helium treatment. CCL18 was upregulated in air but significantly downregulated in helium (Fig. 4f).

Plasma in Air Might have Generated Reactive Species Within Cells

Since changes in gene expression were observed after plasma treatment, further experiments were conducted to determine the cause of these changes. Reactive oxygen and nitrogen species were evaluated, as these species are known to produce changes within cells.

Plasma was applied for 60 s in air, and fluorescence was measured in a fluorescent microplate reader with the addition of a fluorescence dye. The difference in fluorescence between the control group and 60 s of plasma in air was minimal when evaluating reactive oxygen species (Fig. 5a). However, after 60 s of plasma treatment, a higher level of fluorescence was observed, indicating the presence of more reactive nitrogen species (Fig. 5b). These results suggest that nitrogen species were detected after 60 s of plasma in air.

Cells were Susceptible to Damage or Death when Exposed to Plasma for 120 s

Plasma in air was applied to fibroblast cells for 0, 15, 30, 60, and 120 s to evaluate its toxicity. The viability of the HFF1 cells was assessed using a fluorescent microscope after staining the cells with Calcein AM. The number of fluorescent cells can identify live cells as the dye binds to living cells. The control group is shown in Fig. 6a. There was little difference in fluorescence between the control and the 15 s plasma treatment groups (Fig. 6b), indicating that 15 s of plasma treatment was not toxic to the Fibroblast cells.

Similarly, 30 s of plasma treatment did not affect cell viability of Fibroblast cells (Fig. 6c). Fluorescence was slightly reduced after 60 s of plasma treatment (Fig. 6d) but was significantly reduced after 120 s of plasma treatment in air (Fig. 6e). This suggests that 120 s of plasma treatment was toxic to the fibroblast cells, reducing their viability. As a result, 120 s of plasma treatment was eliminated from further experiments.

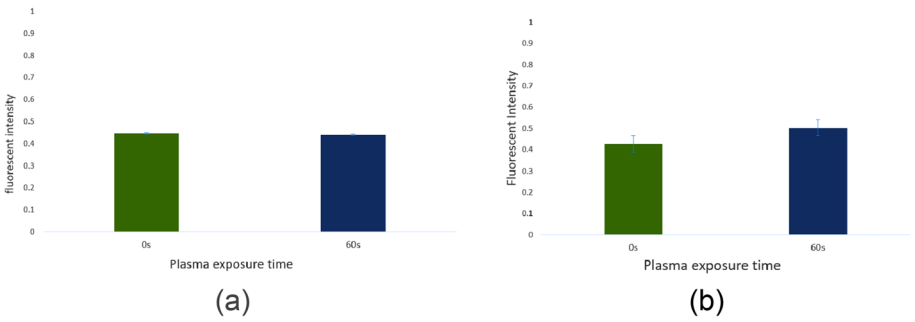


Fig. 5 Detection of **a** Oxygen reactive species and **b** Nitrogen reactive species after plasma treatment. Plasma was applied to Macrophages for 0 and 60 s. After incubating with dyes and washing with PBS, supernatants of cells were placed in 96 microplates and observed under a fluorescent microplate reader. Each plasma treatment sample was evaluated based on data obtained from four biological replicates

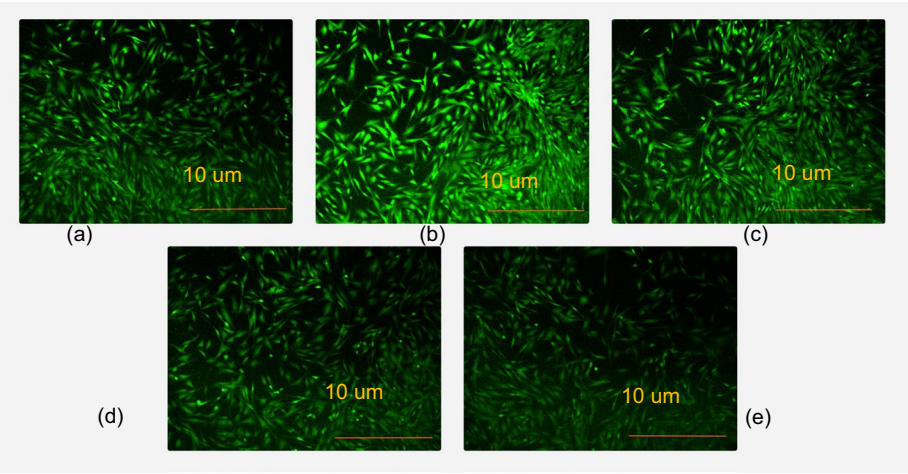


Fig. 6 Fibroblast cell viability observed under fluorescent microscopy after **a** 0 s **b** 15 s **c** 30 s **d** 60 s and **e** 120 s of plasma treatment. All images are in 10X magnification. All scale bars represent 10 µm. Green staining represents live cells

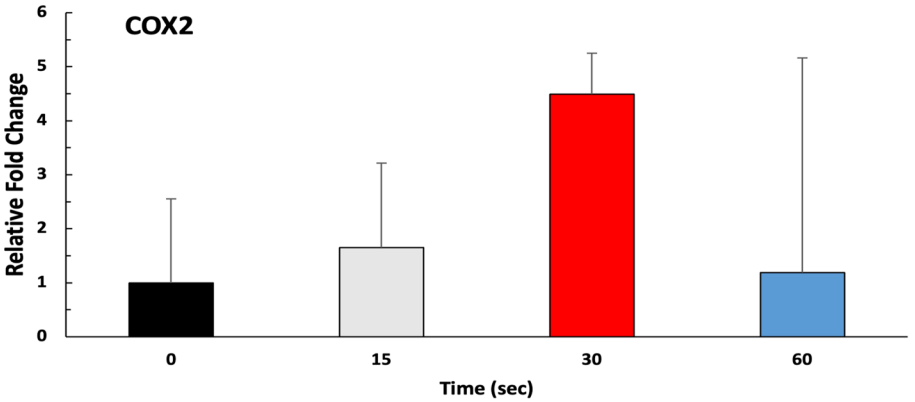
Plasma Treatment in Air Might have Modulated Macrophages with Increased Treatment Time

After selecting the appropriate working gas for plasma treatment, the optimal time for plasma exposure to cells was determined using a live/dead assay. Plasma in air was applied to macrophages embedded within collagen for 0, 15, 30, and 60 s and incubated for 24 h. The expression of COX-2, MMP-13, IL-12, CCL-18, IL-10, and CD206 genes was evaluated (Fig. 7).

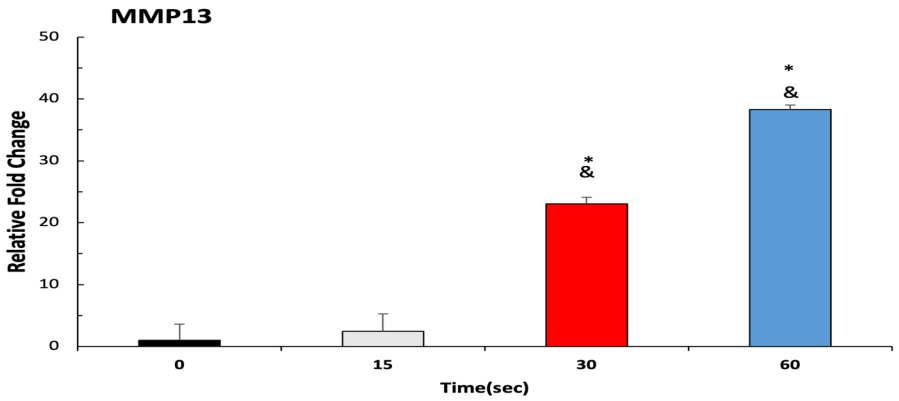
Based on the gene expression analysis results, plasma treatment in air has a significant effect on M1 and M2-related genes. COX-2, an M1-related gene, was initially upregulated with increased plasma exposure time but was eventually downregulated after 60 s of treatment. MMP-13, also an M1-related gene, was gradually upregulated with increased treatment time, with significant upregulation observed after 30 and 60 s of treatment. In contrast, M2-related genes such as IL-10, CCL-18, and CD206 were significantly upregulated after 60 s of treatment. IL-12, another M1-related gene, was also upregulated with increased treatment time, with significant upregulation observed after 30 and 60 s of treatment. These results suggest that plasma treatment in air may have modulated the macrophages embedded within collagen, potentially impacting wound healing.

Discussion

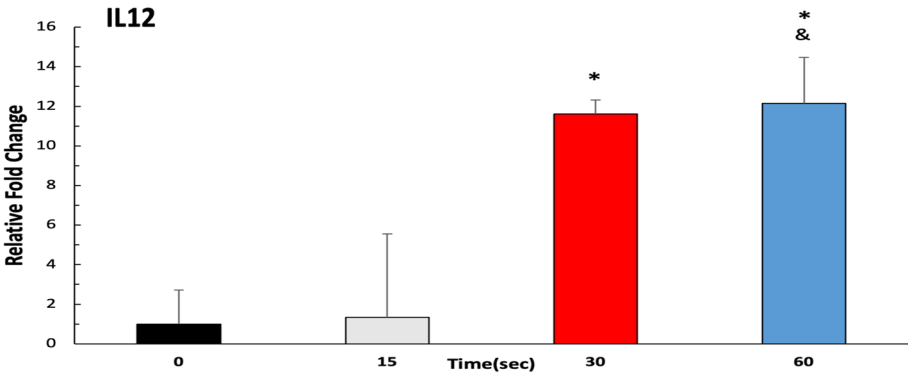
Non-thermal plasma has been a popular topic for research in the medical field, such as for cancer treatment [6], disinfection from bacteria [7], and surface treatment [37]. Non-thermal plasma treatment is also known for regulating macrophage polarization [16]. The polarization of macrophages is of utmost importance, as polarized macrophages are known to influence healing from diseases. For example, M1-type macrophages are activated and



(a) Gene expression of COX-2 after plasma treatment in air for 0,15,30 and 60s.

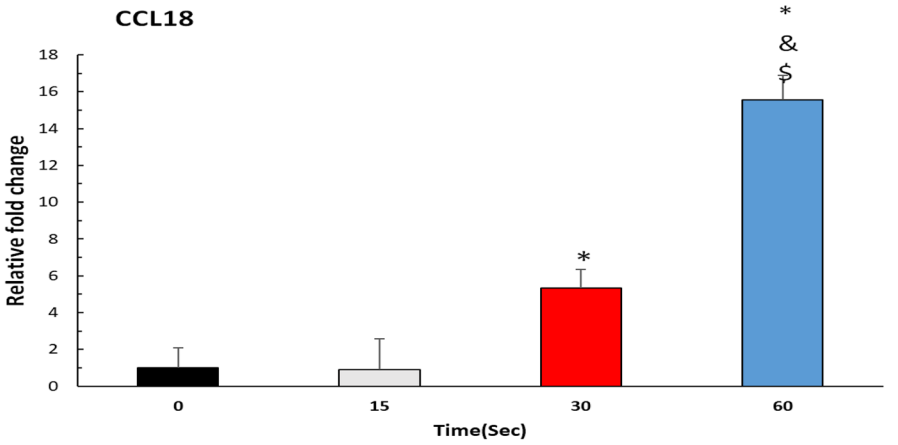


(b) Gene expression of MMP-13 after plasma treatment in air for 0,15,30 and 60s.

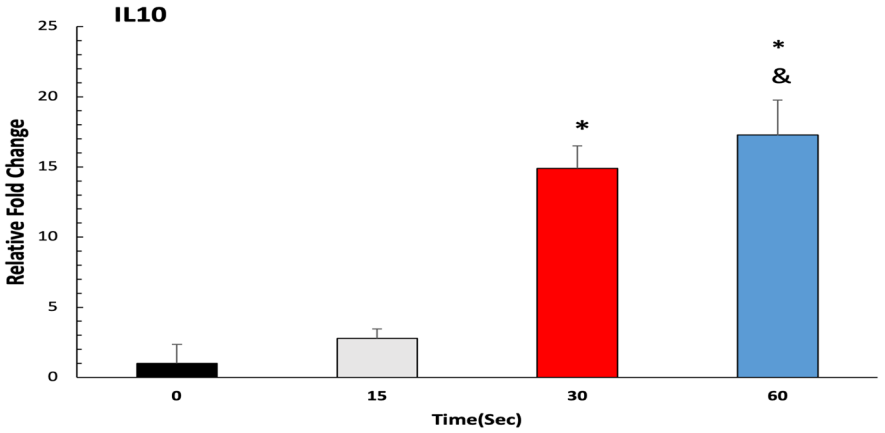


(c) Gene expression of IL-12 after plasma treatment in air for 0,15,30 and 60s.

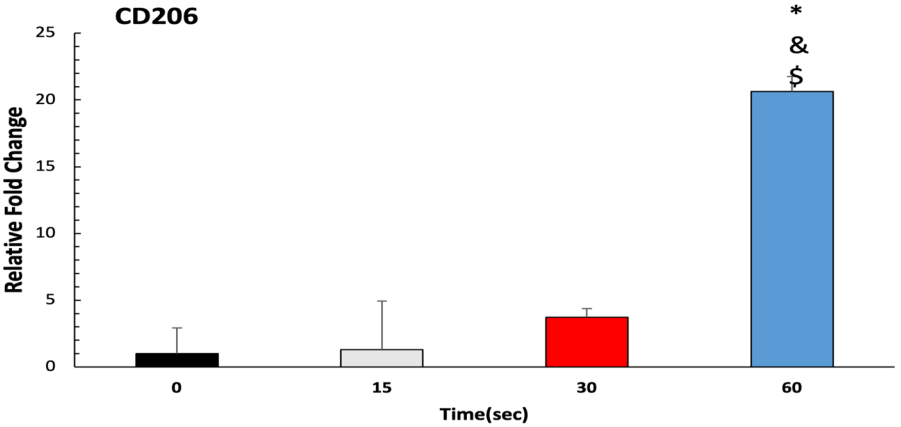
Fig. 7 Gene expression after plasma treatment in air for 0, 15, 30 and 60 s.After plasma treatment cells were incubated for 24 h, PCR was performed. Anova was performed for statistical analysis since there were more than two groups for this experiment. *P* value less than .05 between control and one among plasma groups (15 s, 30 s, 60 s) is represented by *. *P* value less than .05 between 15 and 30 s or between 15 and 60 s groups is represented by &. *P* value less than .05 between the 30 s and 60 s groups is represented by $. Each expression for a different amount of plasma treatment was evaluated based on data obtained from six biological replicates



(d) Gene expression of CCL-18 after plasma treatment in air for 0,15,30 and 60s.



(e) Gene expression of IL-10 after plasma treatment in air for 0,15,30 and 60s.



(f) Gene expression of CD206 after plasma treatment in air for 0,15,30 and 60s.

Fig. 7 (continued)

help solve inflammatory diseases, while M2-type macrophages are known to activate in chronic or parasite diseases [38]. Therefore, methods for polarizing macrophages have been a popular research topic [39]. Non-thermal plasma has been known to influence the polarization of macrophages [15].

In our research study, plasma in air and plasma in helium was applied to monocyte-derived macrophages embedded within collagen. In the existing literature, only plasma in air has been used to treat monocyte-derived macrophages [16]. In this study, non-thermal plasma in environmental air was applied on monocyte-derived macrophages in a monolayer. In contrast, in our study, the monocyte-derived macrophages were embedded in collagen and the non-thermal plasma was applied using the flow of air instead of ambient air. Previously, the effect of helium jet plasma has been studied on monocyte-derived macrophages in a monolayer [40]. Still, the study of non-thermal plasma treatment on monocyte-derived macrophages embedded in collagen in an enclosed DBD plasma system with a helium gas flow has been done for the first time in this study.

Our first objective was to select a working gas for our experiment. To do this, we used an enclosed DBD plasma setup with both air and helium flow individually. Macrophages were derived from monocytes and embedded in collagen, and plasma was applied for 0 and 60 s. The expression of the COX-2, MMP13, TNF-α, IL10, CD163, and CCL18 genes was analyzed. After plasma in helium treatment, the genes were downregulated for all genes except COX2. After plasma treatment in air, the M1-related genes COX2 and TNF-α were downregulated, but MMP13 was upregulated. The M2-related genes IL10, CD163, and CCL18 increased after plasma treatment in air for 60 s. This is similar to the results obtained in a study [16] where DBD plasma was applied to monocyte-derived macrophages in a monolayer, and the macrophage was modulated after plasma treatment. We concluded that plasma in air modulated the macrophages within collagen, and in future experiments, we only performed plasma treatment in air. Since plasma treatment in air modulated the macrophages, we performed further experiments to identify the reason for this change. We measured reactive oxygen and nitrogen species and detected reactive nitrogen species after 60 s of plasma treatment. Reactive oxygen and nitrogen species produced by non-thermal plasma treatment are known to cause changes within cells. As shown in a previous study [15], reactive oxygen and nitrogen species were detected after plasma treatment, which was responsible for modulating monocytes.

After selecting the working gas, we varied the plasma exposure time to determine the optimum plasma exposure time. Plasma in air was applied to fibroblast cells for 0, 15, 30, 60, and 120 s. Using a live/dead assay, we found that cell viability was not reduced until 60 s of plasma treatment. However, after 120 s of plasma treatment, the live/dead ratio was reduced. Therefore, 120 s of plasma exposure was considered toxic for our current experimental setup and was eliminated from further experiments. We then applied plasma for 0, 15, 30, and 60 s to macrophages within collagen and analyzed the expression of the COX2, MMP13, IL12, CCL18, IL10, and CD206 genes. All genes except COX2 gradually increased with increased plasma treatment time. All genes except COX2 and CD206 were significantly upregulated after 30 and 60 s of plasma exposure compared to the control. The M1-related gene MMP13 was significantly upregulated after 30 and 60 s of exposure compared to the control. The M1-related gene IL12 was also observed to increase after 60 s of plasma exposure. The M2-related genes CCL18 and CD206 were significantly upregulated after 60 s of exposure compared to all other groups (0 s, 15 s, 30 s). The M2-related gene IL10 was upregulated compared to the control and 15 s group after 60 s of plasma treatment. The expression of IL10, a widely known cytokine during macrophage differentiation, demonstrates the inflammatory effect of plasma treatment.

Overall, the gene expression results suggested that within 60-s plasma treatment compared to helium generated plasma, the air generated plasma affected the gene expression profile of native macrophages within the 3D collagen matrix. Predominantly, the air generated plasma upregulated the hallmark M2 related genes (IL-10 and CD163, Fig. 4) statistically significantly ($p < 0.05$) compared to helium generated plasma. While IL-10 and CD163 expressions increased sixfold in air plasma group, there was no statically significant changes in IL-10 and slight decrease in CD163 expressions in helium plasma treated macrophages. Thus, it can be concluded that to push naïve macrophage into M2 lineage, as a working gas, air is a better alterative than helium. Nevertheless, it is essential to exercise caution when claiming that air plasma treatment induces the differentiation of naive macrophages into the M2 (anti-inflammatory) phenotype. Such a claim would necessitate substantiation through protein-level analysis employing surface markers and/or M2-associated antibodies. Considering all the changes after plasma treatment in air, it can be reasonably concluded that plasma treatment in air modulated the monocyte-derived macrophages within the 3D collagen matrix.

Conclusion

In this research study, non-thermal DBD plasma in the air was applied to monocyte-derived macrophages embedded within collagen. The results reasonably suggest that non-thermal plasma in air modulated the macrophages within collagen. Plasma treatment was also performed on macrophages within collagen with helium. With our plasma setup, plasma in helium did not modulate the macrophages within collagen, but the setup could be varied to obtain a different result. The presence of reactive species was the reason for the changes in the macrophages. The time was optimized for plasma treatment in air, and 120 s of plasma was toxic to cells within collagen in our plasma treatment setup. With non-thermal plasma treatment, macrophages within collagen can be modulated which can subsequently impacting the tissue healing.

Authors' Contribution TS conducted the experiment and composed the manuscript. HA and EY conceived the original idea and provided project supervision. DJ contributed to the execution of the gene expression analysis, while DM conducted the live/dead assay.

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Declarations

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