Biomedical Applications of Low Temperature Atmospheric Pressure Plasmas to Cancerous Cell Treatment and Tooth Bleaching

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Abstract: The biomedical applications of low temperature atmospheric pressure plasmas to cancerous cell treatment and tooth bleaching were investigated. Gold nanoparticles conjugated with cancer-specific antibodies have been introduced to cancerous cells to enhance selective killing of cells, and the mechanism of cell apoptosis induced by plasma has been investigated. Tooth exposed to plasma jet with hydrogen peroxide has become brighter and the productions of hydroxyl radicals from hydrogen peroxide have been enhanced by plasma exposure.

Keywords: atmospheric pressure plasma, cancerous cell, tooth bleaching

1. Introduction

Low temperature atmospheric pressure plasmas (APPs) have been developed for the past decade and attracting great interests due to their interesting characteristics and wide ranges of applicability.¹² The APPs have been finding new applications in the medical field thanks to their nonthermal property to allow them to interact with the living tissues, cells, synthetic polymeric biomaterials, and inorganic materials in the human body.³ It has been investigated the biomedical applications of APPs such as blood coagulation, wound healing, decontamination, and cancer cell treatment.⁴⁻⁸

A novel approach to treat cancerous cells using APPs has been suggested to achieve the selective cell death.⁹,¹⁰ Gold nanoparticles conjugated with a cancer-specific antibody for the protein over-expressed in cancerous cells were bound to cells and the enhanced death of the cells with the antibody-conjugated gold nanoparticles was demonstrated while exposing the cells to APPs, suggesting the selective cancer cell treatment.

Another interesting biomedical application of APPs is to use them for tooth bleaching that is a popular esthetic therapy in dentistry.¹¹,¹² Conventional tooth bleaching therapy normally exposes tooth deposited bleaching gel including hydrogen peroxide to light sources so that the decomposition of hydrogen peroxide into ‘OH is enhanced by heating the bleaching gel. It was demonstrated that tooth exposed to APP jet with hydrogen peroxide gets brighter compared to the case treated by hydrogen peroxide alone.

In this paper, we show the cancerous cell treatment of APPs with gold nanoparticles conjugated with cancer-specific antibody, the mechanism of apoptosis induced by APPs, and the effect of APP jet on the external and internal tooth bleaching.
Figure 1. Comparison of the cell death rate which is significantly increased in the case of anti-FAK antibody-conjugated gold nanoparticles.9)

2. Biomedical Applications of APPs

2.1 Cancerous cells treatments

The conventional therapies for cancerous diseases are based on surgical extirpation, chemotherapy, radiation. However, these therapies have not yet made a conquest in the war with cancers and they have a limitation in use due to their significant side-effects. It has been reported that non-thermal APPs were effective in killing cancer cells.8,13,14) The cancer treatment by APPs has to achieve selectivity between normal and cancerous cells so that it is used as a reliable cancer therapy.

Gold nanoparticles (gNPs) are widely used as diagnostic and drug delivery tools recently thanks to the advances in nanotechnology. It was suggested that gNPs can be conjugated with cancer-specific antibody and the conjugated gNPs are able to be selectively bound to the targeted cancerous cells and the cancerous cells bound to the gNPs can be dramatically affected by the exposure to APPs compared to normal cells.13)

Three cell groups were prepared to examine the enhancement and the selectivity of plasma treatment with FAK-gNPs: (1) cells cultured in only media, (2) cells cultured in media containing gNPs, and (3) cells cultured in media containing FAK-gNPs. When the three groups of cells were exposed to the dose-controlled air plasma, the cell death rates were 14%, 36%, and 74%, respectively, as shown in Fig. 1.

Since the epidermal growth receptor (EGFR) and transferring receptor (TFR) have been known to be over-expressed in many oral cancer cells, these receptors were chosen to be targets for plasma cancer therapy.

In order to examine the selective effect of plasma exposure with anti-EGFR antibody-conjugated gNPs (FAK-gNPs) or anti-TFR antibody-conjugated gNPs (TFR-gNPs), SCC25 oral cancer cells were cultured in four different media: (a) pure media; (b) media containing gNPs; (c) media containing TFR-gNPs; and (d) media containing EGFR-gNPs. When these four groups of cells were exposed to plasma for 30 s, the cell death rate were 5%, 21%, 66%, 92%, respectively, as shown in Fig. 2.

It was reported that APPs can induce apoptosis in mammalian cells, which is a programmed cell death.8) However, the mechanism of apoptosis induced by plasma treatment has been unclear yet.

The mechanism of apoptosis was investigated via Western blot analysis, as shown in Fig. 3(a). It was demonstrated from the detection of the increase of gamma-H2A.X at 3 h after treatment that DNA damage such as DNA double strand breaks (DSBs) was induced by plasma treatment. The cleaved caspase-3 was clearly observed at 3 h following plasma exposure indicating caspase-associated apoptosis. The air plasma treatment also induced the accumulation of the p53. Figure 3(b) shows the analysis of the cellular location of cytochrome C via the immunocytochemistry. For the control, the
cytochrome C was localized in the mitochondria represented by green spots. Cytochrome C was released from the mitochondria to the cytosol at 1h (smeared green fluorescence) and gradually released with time. It is likely that the cellular organelles such as mitochondria were damaged by plasma exposure. It could be suggested that the effects of plasma on cells involve genetic signaling cascade from DNA damage and nongenetic physical damage in cellular membrane system.

2.2 Tooth Bleaching

In dental clinic, the bleaching material including high concentration of hydrogen peroxide is deposited on tooth and it is exposed to light sources to enhance the reactivity of the bleaching material. The substantial role of the light sources, however, has been in a controversy.

It was suggested to use APP jet for tooth bleaching on behalf of light sources while using hydrogen peroxide. The extracted human teeth were used to demonstrate the effect of APP jet on tooth bleaching while dropping hydrogen peroxide (28%, 20 μl every 30 seconds). The teeth used were cut in half longitudinally, and the control group was treated by using hydrogen peroxide alone. Figure 4 shows the changes of tooth surface brightness after treatment of hydrogen peroxide with and without plasma exposure. The tooth treated by plasma with hydrogen peroxide became brighter than the control group (Fig. 4(c)). It is likely that the enhanced efficacy of tooth bleaching is caused by the increase of -OH generation from hydrogen peroxide by plasma treatment.

It was demonstrated the effect of nonthermal APPs on intracoronal tooth bleaching in blood stained human teeth. The extracted single-root human teeth were used and standard access cavities inside teeth were created with a diamond-coated bur with a high-speed engine. Then they were artificially stained by the hemoglobin-rich hemolysed blood. The control groups were treated with 30% hydrogen peroxide (20 μl every 5 min.) alone in the pulp chamber for 30 min. and the experiment groups were treated with 30% hydrogen peroxide (20 μl every 5 min.) with the APP jet in the pulp chamber for 30 min., as shown in Figs. 5(a) and 5(b).

It was shown that the tooth color of the experiment group and the control group after 30 min. treatment. It was observed a clear high efficacy for the APP jet and hydrogen peroxide bleaching compared to the control group. The average (n=20) ΔE value after treatment for 30 min. was 9.24 in the experiment group and 4.47 in the control group. It suggests that the nonthermal APP jet could bleach teeth within a very short time and it has potential use in the intracoronal bleaching of intrinsic discolored teeth.
3. Summary

In this paper, we discussed about cancerous cell treatment and tooth bleaching of biomedical applications using low temperature APPs. The novel method to treat cancerous cells used gold nanoparticles conjugated with cancer-specific antibody for the protein over-expressed in cancerous cells and the high selectivity for the targeted cell death was achieved by exposing to APPs. Low temperature APP jet was introduced to tooth bleaching therapy and it was demonstrated that the use of APP jet with hydrogen peroxide induced the significant color change of tooth stained by extrinsic and intrinsic causes.

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References