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The effects of non-thermal atmospheric pressure plasma jet on attachment of osteoblast

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ABSTRACT

Despite the high success rate of dental implant surgery, the failures are still being reported and investigation have been undergone to improve attachment of osteoblast on the surface of implant material. With increasing interest in non-thermal atmospheric pressure plasma jet (NTAPPJ), the effects of it on the cellular mechanisms have been previously reported. Hence in this experiment, effects of NTAPPJ on osteoblast for improved attachment and possible application in dental implant surgery were investigated.

Mouse osteoblast cells of MC3T3-E1 were first directly treated with NTAPPJ with air for various durations. Also to investigate the effects by culture media, culture media were separately treated with NTAPPJ for the same durations. Cell attachments were then assessed following 4 and 24 h of cell culture using Water Soluble Tetrazolium salt (WST) assay and confirmed by automated cell counter and examining under confocal laser microscope.

The results showed that there was significantly improved osteoblast attachment with relatively short duration of NTAPPJ treatment. Also results indicated that NTAPPJ possibly improved osteoblast attachment through interactions with proteins in culture media that in turn interacted with cells.

Hence the application of NTAPPJ on osteoblast improves cellular attachment and would be useful tool for dental implant surgery.

1. Introduction

Since the development of dental implant surgery, titanium has been used as the main material due to its advantages such as high resistance to corrosions and low modulus of elasticity [1]. However, despite reporting relatively high success rate of over 90% during past 10 years [2,3], reports on dental implant failure following surgeries are still being made [3,4].

The success of dental implant surgery is depended on the formation of bone tissue surrounding the dental implant, where the osteoblast attachment to the material is the initial and the most essential event for such process to take place [5,6]. Hence, studies to improve the osteoblast attachment rate through change in topographies of titanium have been carried out where numbers of different methods such as acid-etching [7] or nanotubes formation [8] have been investigated. Although some of these methods seem to significantly improved the osteoblast attachment through *in vitro* and *in vivo* studies, the results have been sometimes difficult to reproduce in real clinical situations due to the nature of titanium implant that significantly loses the biological properties of it as the time progresses, which the term has been defined as the biological aging of titanium [9,10].

The term plasma refers to partially ionized state of gas in physics, which previously have been widely used for various industrial applications [11,12]. Along with recent development of non-thermal atmospheric pressure plasma, the technology has been applied extensively on the field of biomedical studies from modification of biomaterial surfaces [13–16] to sterilization of microorganisms [17–19].

Due to the recent advances of non-thermal atmospheric pressure plasma, design of its application can be carried out as the jet form (non-thermal atmospheric pressure plasma jet, NTAPPJ) which allows precise treatment of tissue or cells without thermal damages [20,21]. Using such NTAPPJ, high numbers of studies have been undergone to investigate effects of plasma on cells, especially in relation to apoptosis of cancer and cell proliferation with regard to regenerations/wound healing [11,21–24]. However, there has been yet a study that considered the effects of NTAPPJ on osteoblast or any related cells.

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Therefore in this study, effects of NTAPPJ on osteoblast cells were investigated as the possible application in dental implant surgery. Consideration of applying NTAPPJ directly on the site where titanium dental implant would be placed was investigated to aid the direct activation of osteoblast for improved attachment, while avoiding effects from biological aging of materials.

2. Materials and methods

2.1. Non-thermal atmospheric pressure plasma jet (NTAPPJ)

Non-thermal atmospheric pressure plasma jet (NTAPPJ) was manufactured and provided by Kwangwoon University (Plasma Bioscience Research Center, Kwangwoon University, Korea) which has been used and described in previous studies [19]. The plasma was formed by passing 0.5 L/min of nitrogen gas through NTAPPJ while applying maximum voltage of 15 kV. Each of test samples were treated under NTAPPJ by setting the distance between tip of flume to media at 3 mm and exposed for 10, 30, 60, 120, and 240 s where control samples were not exposed to the plasma.

2.2. Cell culture and culture media

The mouse osteoblast cell line of MC3T3-E1 (Sub-clone 4, American Type Culture Collection, ATCC, USA) with passage between 5 and 9 were used in this experiment. 1×10^5 cells/100 μl was prepared from the confluent cells for the both 'direct' and 'media' treated experiment which is further explained below. All of culture media used in this experiment was Alpha Modified Eagles Medium (α-MEM, Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 1% antibiotics (penicillin/streptomycin, Gibco, USA).

2.3. Direct treatment of NTAPPJ

In order to mimic the real clinical application of NTAPPJ, it was applied directly to the media with osteoblast cells. The method was adapted from previous studies [20], where cells were in the state of free suspension by placing the cell suspension in 1 ml of culture media at standard 12-wells plate (SPL, Korea) immediately before the NTAPPJ treatment. Number of cells attached on the plate was determined by removing unattached cells at 4 and 24 h, and counting number of attached cells through Water Soluble Tetrazolium salt (WST, Daeil-lab, Korea) assay. The method of using WST assay is similar to other WST or MTT-based methods [8,18] where cell counting is based on the WSTs that change into yellow/orange color due to the reduction by viable cells. The reading of optical absorbance at 450 nm using the reader (Epoch, BioTek, USA) would give the indication of number of cells attached and the results were expressed as the percentage of cells attached compare to the control. Additionally, number of cells attached on each test and control samples following 24 h of culture were assessed by automated cell counter (Luna™ automated cell counter, Logos Biosystems, Korea) to confirm the results.

2.4. Media treatment of NTAPPJ

In order to determine whether the effects above are due to the direct interactions between plasma and cells or due to the interactions between plasma and culture media, culture media were pre-treated with NTAPPJ without the presence of cells as with previous studies [11]. Briefly, 1 ml of culture media without cells were treated with NTAPPJ and 100 μl suspensions with cells were immediately placed in each of culture media. As with the direct treatment, numbers of cells attached were determined after 4 and 24 h of cell culture using the WST assay and number of cells attached after 24 h were counted using the automated cell counter.

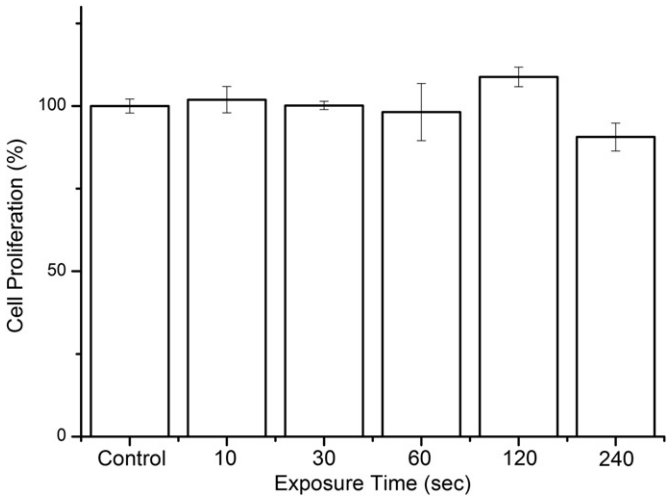

Fig. 1. Osteoblast attachment after 4 h of cell culture following different duration of direct plasma exposure to cell and culture media, assessed by Water Soluble Tetrazolium salt assay (*: Statistically significant at $p < 0.05$).

2.5. Observation under confocal laser microscope

Following 4 h of NTAPPJ treatment, osteoblast of each direct and media treated group was observed under confocal laser microscope (LSM700, Carl-Zeiss, USA) following calcein and ethidium homodimer-1 (Molecular Probes, USA) staining for general observation of attached cells as well as the actin (Rhodamine Phalloidin, Invitrogen, USA) and nucleus (DAPI, Invitrogen, USA) staining for morphological analysis.

2.6. Statistical analysis

The statistical analyses of results were carried out using one-way ANOVA test by IBM SPSS statistics 20 program (IBM, USA). The statistical significance was declared at $p < 0.05$.

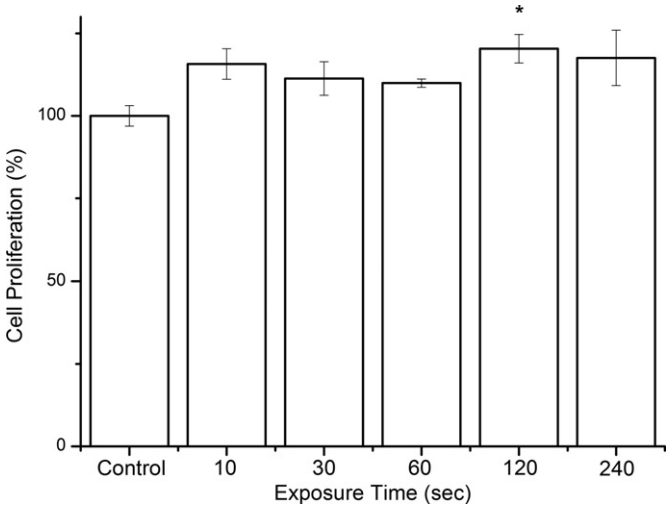

Fig. 2. Osteoblast attachment after 24 h of cell culture following different duration of direct plasma exposure to cell and culture media, assessed by Water Soluble Tetrazolium salt assay (*: Statistically significant at $p < 0.05$).