Non-thermal atmospheric plasma induces osteogenic activity in osteoblasts through the WNT signaling Pathway

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INTRODUCTION

Non-thermal plasma (NTP: an ionized gas) is being employed for the biological and medical treatment of living tissues. Plasma comprises free charges (electrons, ions) and free radicals, generating a transient electric field. Reactive oxygen and nitrogen species (ROS and RNS) produced by NTP are thought to be the primary factors that interact with the proteins to exert their biological effects. NTP has been reported as a potent stimulator for various biological activities, like anticancer, sterilization, promoting wound healing, etc. Recent studies have shown a positive effect of NTP on osteogenic induction in stem cells and bone regeneration. However, a clear insight into the mechanism of action of NTP on the osteoblasts is still warranted.

METHODS

Two types of (dielectric barrier discharge (DBD) or Jet) NTPs were treated to serum-free cell culture media with various time intervals (5, 10, 20 min) to generate various plasma–media interfaces for ion exchange (JNTPM). The cell viability and cytotoxicity of the MC3T3-E1 cell line (Mice osteoblast cell line) treated with various concentrations of JNTPM were confirmed through MTT and LDH assays. To confirm the osteogenic effect on osteoblasts, MC3T3-E1 cells were treated with various concentrations of JNTPM for 48h, and ALP activity was assessed. Also, mRNA and protein expression levels of osteogenic markers like Osterix, Runx2, Collagen1α, Bone sialoprotein, and Osteopontin were measured by real-time RT-PCR. Mineralization and collagen synthesis were observed through Alizarin red S and Sirius red S staining in JNTPM-treated MC3T3-E1 cells for 7 days. Further, the possibility of the role of JNTPM involving the WNT/β-catenin signaling pathway was examined by Axin-2 reporter luciferase assay and protein expression of β-catenin.

RESULTS

Ionized media showed no mitogenic or cytotoxic effects on murine preosteoblast cells, MC3T3 E-1. Among various NTP ionized mediums, Jet NTP medium (JNTPM, 10 min) showed induction of ALP activity of preosteoblasts after 48 h of treatment. A dose-dependent (% of JNTPM to complete medium) treatment of JNTPM to MC3T3 E-1 cells demonstrated an increased ALP activity from 25% to 100% of media treatment. Since 25% of JNTPM was enough to induce maximum ALP activity in preosteoblasts, 25% of JNTPM was used for further experiments. Apart from ALP activity, JNTPM enhanced the mRNA expressions of osteogenic markers of osteoblasts like Osterix, Runx-2, bone sialoprotein, Collagen 1, and osteopontin. Moreover, the 7 d JNTPM-treated MC3T3 E-1 cells showed increased collagen synthesis and mineralization. Treatment of JNTPM to MC3T3 E-1 cells transfected with Axin-2 reporter construct showed time-dependent increased reporter activity, implying activation of the Wnt signaling pathway. The increased stabilization of β-catenin and phosphorylation of GSK3-β further corroborated the activation of Wnt signaling.

DISCUSSION

The treatment of N-acetyl-l-cysteine (NAC) treatment suppressed the Axin-2 reporter activity and stabilization of β-catenin in JNTPM-treated MC3T3 E-1 cells, suggesting a role of ROS in the induction of Wnt signaling and thus osteogenic activity in preosteoblasts. Thus, JNTPM induces osteogenic activity in osteoblasts via the Wnt signaling pathway and is utilized for stimulating bone formation. However, further studies are required to delineate the cross-talk between ROS and Wnt signaling to understand the osteogenic stimulatory properties of JNTP.

SIGNIFICANCE/CLINICAL RELEVANCE

New biologic agents in clinical trials include anti-sclerostin and anti-Dickkopf antibodies that stimulate the WNT/β-catenin signaling pathway in osteoblasts, leading to new bone formation. Alternatively, this objective can also be achieved by Jet Non-thermal plasma. Non-thermal atmospheric plasma at a particular strength has the osteogenic stimulatory property on osteoblasts and can thus induce bone formation at localized sites like in fracture healing by stimulating the WNT signaling pathway.

REFERENCES

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ACKNOWLEDGEMENTS

This research was funded by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2020R1C1C1008694 & NRF-2020R111A3074575).

Keywords
Non-thermal plasma, osteoblasts, osteogenic activity, WNT signaling pathway

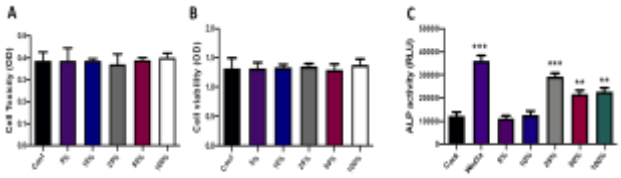

Figure 1. Effect of various concentration of Jet Non thermal Plasma ionized mediums. (A) MTT Assay. (B) LDH Assay. (C) ALP Activity.

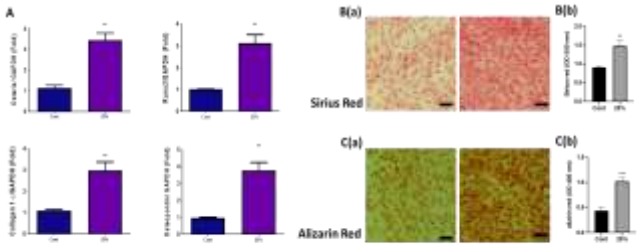

Figure 2. Osteogenic stimulatory abilities of Non thermal Plasma ionized mediums. (A) Real-Time RT-PCR. (B) Sirius Red S staining. (C) Alizarin Red staining

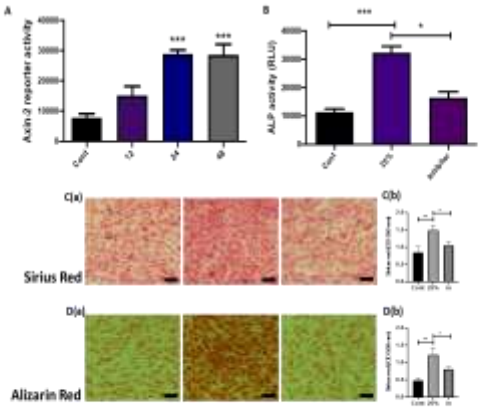

Figure 3. Activation of WNT/β-catenin signaling pathway by Non thermal Plasma ionized mediums. (A) Axin-2 reporter assay. N-acetyl-l-cysteine (NAC) Treatment suppressed the (B) ALP activity (C) Collagen synthesis and (D) Mineralization in JNTPM treated Osteoblasts.