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A Novel Study Elucidating the Effects of Glucose and Static Magnetic Field on Osteoblast Differentiation Markers

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Abstract

In this study, we explored the expression dynamics of osteoblast differentiation marker genes under the influence of static magnetic fields (SMF) in high glucose conditions. High glucose can significantly impair osteoblast function and differentiation- particularly in diabetic patients with persistent hyperglycemia. In the in-vivo diabetic-like environment that is under glucose level, SMF's role in bone formation was evaluated using Human osteoblastic cells that were treated with SMF for up to 7 days. The expression of key osteogenic markers such as alkaline phosphatase (ALP), osteocalcin (OCN), and collagen type I (COL1A1) was analysed using qRT-PCR and ELISA. The findings demonstrated that high glucose led to pronounced inhibition of osteoblast differentiation markers. Conversely, being exposed to an SMF dramatically increases the expression of markers for osteoblast differentiation. Additionally, the inhibitory effects of glucose on osteoblast development appear to be lessened upon SMF exposure. These results emphasize the significance of taking into account the positive effects of SMF, which may have therapeutic potential in bone repair.

Keywords: *Bone Regeneration, Bone Health, Diabetes, Health Care, Runx2, Static Magnetic Field.*

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by elevated blood sugar levels (hyperglycemia) resulting from either deficient insulin secretion or insulin resistance. Chronic hyperglycemia, as highlighted by the American Diabetes Association, can lead to dysfunction in various organs, such as the eyes, kidneys, nerves, heart, and blood vessels [1]. Notably, DM also has adverse effects on human bones and is associated with an increased risk of developing osteoporosis. Diabetic animals experience compromised bone integrity due to various factors that negatively affect cell processes responsible for bone formation and remodelling [2-4]. As a result, abnormal bone microstructure develops, and there is an accumulation of advanced glycation end products (AGEs) in the bone matrix. Osteoporosis is characterised by loss of bone mass linked to the risk of fractures that lead to significant morbidity and mortality, notably in developed nations. In children with type 1 diabetes (T1D), bone mass may reduce between 5-21%, and diabetic patients face a high risk of fractures in the hip, spine and tibia because of low mineral density [5]. Furthermore, static magnetic fields (SMFs) offer benefits in alleviating diabetic complications. For instance, high glucose levels not only suppress the proliferation of MC3T3-E1 cells and reduce the expression of P2X7 while also decreasing calcification and lower expression of ALP and osteocalcin (OCN) [6]. Magnetotherapy utilises static, pulsed and alternating electromagnetic fields to offer a secure and non-invasive method

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to treat injuries directly, reduce pain and inflammation and manage a range of medical issues. PEMFs have been widely used in clinical treatments for treating non-united bone fractures for many years [7]. Additionally, it's important to acknowledge that PEMFs are generated through the use of electrical devices. SMFs, a type of magnetic field utilized in clinical applications, are particularly prevalent in the dental field [8]. The advantage of SMFs lies in their independence from power devices, unlike PEMFs. They are also convenient for long-term use and pose no heat or electric hazards to surrounding tissues. Rare-earth magnets, which generate SMF, have found application in various dental procedures, such as magnetic retention appliances for implant- or tooth-retained overdentures, maxillofacial prostheses after trauma and cancer surgery, and orthodontic treatments like space closure, molar distalization, intrusion, the traction of impacted teeth, and palatal expansion [9-11]. Studies have indicated that SMFs can enhance implant stability and reduce bone loss during the early weeks of healing.

Glucose serves as the primary energy source for the body, and its levels are regulated by various hormones, particularly insulin and glucagon secreted by the pancreas' beta and alpha cells, respectively. Maintaining overall homeostasis involves controlling the influx and outflow of glucose into glycogen reserves, balancing glycolysis and gluconeogenesis, and promoting protein catabolism when needed [12]. Recent evidence suggests a strong connection between glucose metabolism and bone health [13-15]. Osteoporosis has been identified as one of the diabetic complications, known as DM-induced bone fragility. Osteoblasts play a vital role in enhancing bone mass through collagen synthesis and matrix mineralization, making them a crucial target for pharmacological interventions. Osteoblasts primarily utilize aerobic glycolysis to utilize glucose for their activity.

Recently, in cellular experiments, MC3T3-E1 cells or primary osteoblasts and RAW264.7 cells were cultured in a diabetic marrow microenvironment stimulated with 25 mM high glucose [16]. During differentiation induction, these cells were exposed to a static magnetic field (SMF). The SMF exposure resulted in the promotion of osteogenesis, as evidenced by increased levels of ALP (alkaline phosphatase) and greater mineralization deposition in osteoblasts. Understanding the landscape of osteoblast differentiation under high glucose and its influence on SMF in this scenario will help us design therapies involving SMF for bone regeneration in DM patients. SMFs have been found to modulate intracellular signalling pathways involved in osteoblast differentiation. They can activate various signalling molecules, including calcium ions and cyclic adenosine monophosphate (cAMP), which play crucial roles in osteoblast differentiation and function.

We speculate that SMF could serve as a promising therapeutic approach to enhance bone health in individuals with type 1 diabetes. To study this, we have exposed human osteoblastic cells to high glucose, SMF separately and a combined exposure for a period of 7d and assessed the expression pattern of osteoblastic differentiation marker genes such as Runx2, Col-l, ALP and OCN.

Methodology

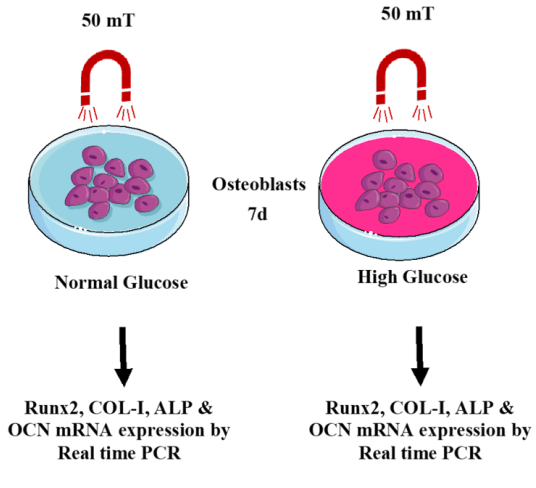
Cell Culture

Rat bone marrow-derived mesenchymal stem cells were isolated by a previous protocol, and cells were maintained under standard culture conditions (10% FBS-DMEM, 37 °C, 5% CO_2). Human MG-63 cells were purchased from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were subcultured and used in the cell investigations that were described.

SMF Exposure System

A neodymium (Nd2Fe14B) disc magnet was used in conjunction with 4-well plastic culture

plates to create a static magnetic field (SMF) exposure system. The magnet was positioned horizontally beneath the wells, with its north (N) side facing the well. Different desired field intensities (50 mT) were achieved by adjusting the distance between the magnet and the culture plates, the SMF intensity was measured accurately using a Gauss meter. To avoid interference from adjacent SMFs, each culture plate had only one magnet disc attached, and sufficient space was provided between adjacent culture plates.



High Glucose And SMF Exposure

The cells were grown under normal and high glucose conditions, where the high glucose conditions were simulated by adding 24.5 mmol/l D-glucose powder to the medium. The cells were then maintained under standard culture conditions for seven days with medium changes once in three days. The cells were exposed to a static magnetic field of 50 mT using the neodymium magnet for seven days. The overall methodology is represented in Figure 1.

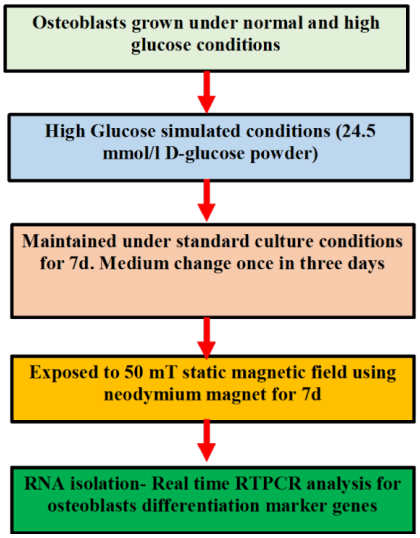


Figure 1. Overall Schematic Representation of the Methodology Involved in the Current Study. Osteoblasts were Exposed to a Static Magnetic Field (SMF) Under Normal and High Glucose Conditions for a Period of 7d and Osteoblast Differentiation Markers were Assessed by qPCR Analysis.

Realtime PCR Analysis

MG-63 cells following a treatment period of 7d were subjected to total RNA extraction using the TRIzol method followed by centrifugation at 4°C and 16,000 × g for 10 minutes. The total RNA was then dissolved in ddH2O, and its concentration was determined at a wavelength of 260 nm using a spectrophotometer. Subsequently, the total RNA underwent reverse transcription with the High-Capacity cDNA reverse transcription kit at 42°C for 15 minutes, followed by 95°C for 3 minutes. RT-qPCR was performed on the ABI 7500 Real-time PCR system using SYBR green. Appropriate primers for the target genes were selected and used during PCR analysis. The relative mRNA expression levels were analyzed using the $2^{-\Delta\Delta Cq}$ method and expressed in fold change.

Statistical Analysis

The experiments were performed in triplicate and data were reported as the mean ± standard deviation (SD). A paired t-test was used in SPSS to assess statistical significance, with a p-value of 0.05 or less considered significant.

Results and Discussion

High glucose levels have been shown to have an impact on osteoblast differentiation. Specifically, elevated glucose concentrations can hinder or impair the normal process of osteoblast differentiation, leading to reduced

bone formation and mineralization. This disruption in osteoblast differentiation is one of the mechanisms contributing to the negative effects of diabetes on bone health and the increased risk of developing conditions like osteoporosis in diabetic individuals.

Stimulation of high glucose conditions with 25mM/l glucose significantly reduced the expression of Runx2, ALP (Figure 2), Col-I and OCN genes (Figure 3) in osteoblastic cells. We observed a significant increase in the marker genes' expression upon exposure to 50 mT SMF. Another interesting finding is that, when cells are grown under HG and exposed to SMF, there was a significant increase in the expression levels of Runx2, ALP, and OCN when compared to the HG group alone. However, there was no change in the COL-I expression even after exposure to SMF under HG conditions. Overall, the process of osteoblast differentiation is revived back to normal following SMF exposure.

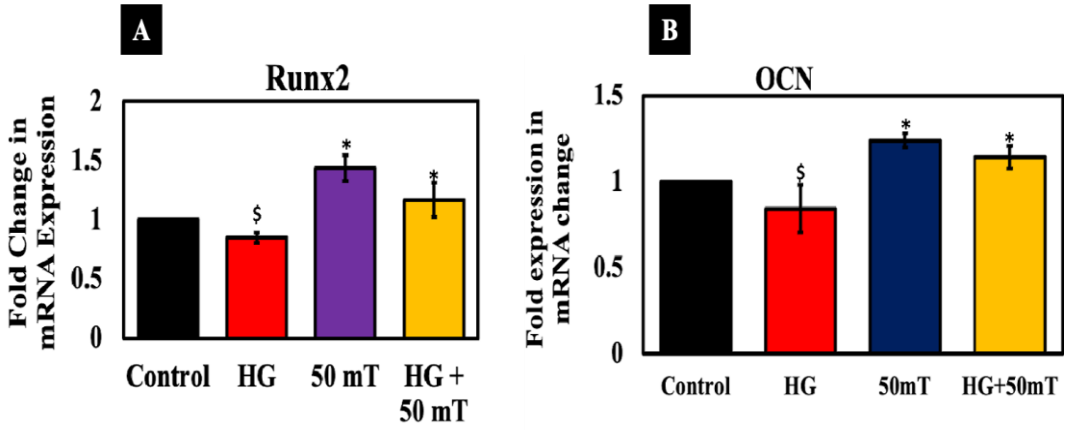


Figure 2. qPCR Analysis of Runx2 and OCN Genes in MG-63 Cells Following 7d Treatment Period. HG Downregulates the Expression of These Genes, While Exposure to SMF Upregulates the Expression. SMF is Found to be Osteoprotective in Nature at 50 mT. *- Indicates Significant Increase Compared to Control; $- Indicates Significant Decrease Compared to Control. ($p \leq 0.05$; n=3)

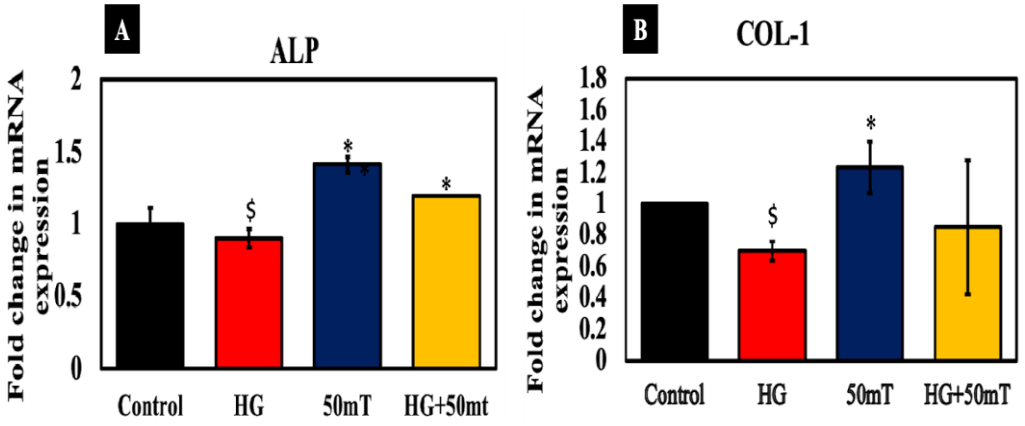


Figure 3. qPCR Analysis of ALP and COL-I Genes in MG-63 Cells Following 7d Treatment Period. HG Downregulates the Expression of These Genes, while Exposure to SMF Upregulates the Expression. SMF is Found to be Osteoprotective in Nature at 50 mT. *- Indicates Significant Increase Compared to Control; $- Indicates Significant Decrease Compared to Control. ($p \leq 0.05$; n=3)

Runx2 is a key transcription factor that orchestrates osteoblast differentiation and plays a critical role in bone formation and maintenance. Its proper regulation is essential for maintaining bone health and preventing bone-related disorders. Runx2, also known as

Runt-related transcription factor 2, is a crucial transcription factor that plays a significant role in osteoblast differentiation [17]. It is considered a master regulator of osteogenesis and is essential for the commitment and maturation of osteoblasts, which are the bone-forming cells. Runx2 regulates the expression of various genes involved in bone formation, including collagen type I, alkaline phosphatase (ALP), and osteocalcin (OCN), among others [18]. The upregulation and coordinated expression of ALP and COL-I are crucial for the successful differentiation and functioning of osteoblasts. ALP plays a vital role in bone mineralization by dephosphorylating organic phosphate compounds, releasing inorganic phosphate, and providing a suitable environment for calcium and phosphate deposition. The increased ALP activity is often used as an indicator of osteoblast activity and bone formation during osteoblast differentiation [19]. Collagen is the most abundant protein in the bone matrix, and type I collagen is the predominant collagen type found in bone tissue. During osteoblast differentiation, the expression of COL-I is induced. Collagen provides the structural framework and tensile strength to the bone, forming a network that mineralizes with calcium and phosphate to create the bone's organic matrix [20-23]. These are early differentiation markers, while OCN (Osteocalcin) and OPN (Osteopontin) are non-collagenous proteins that are synthesized by osteoblasts and are present in the extracellular matrix. These proteins serve as late markers of osteoblast differentiation.

Our study provides evidence for the correlation between glucose content and osteoblast differentiation. High glucose levels are inhibitory to the differentiation of osteoblasts. This observation is consistent with previous studies that have reported the detrimental effects of hyperglycemia on bone formation and maintenance. Hyperglycemia has been shown to impair osteoblast function and decrease bone formation, leading to an increased risk of osteoporosis. The positive effect of magnetic fields on osteoblast differentiation markers is also in line with previous studies. Magnetic fields have been reported to affect the behaviour of various cell types, including osteoblasts. However, the mechanism by which magnetic fields affect osteoblast differentiation is not yet fully understood. Some studies have suggested that magnetic fields can enhance the activity of growth factors and signalling molecules involved in osteoblast. Differentiation, while others have suggested that magnetic fields can affect intracellular calcium signalling, which is essential for osteoblast differentiation.

SMF has the potential to stimulate osteoblast differentiation through the activation of several signalling pathways. These pathways include Wnt, p38, JNK/MAPK, and NF-κB signalling pathways [24]. The stimulation in the expression of differentiation markers in osteoblasts in our study could be by either of these signaling pathways stimulation. Very recently, it has been shown that SMF has a potential therapeutic approach for enhancing bone health in individuals with type 1 diabetes. This effect is achieved by regulating iron homeostasis metabolism and redox status, which play crucial roles in maintaining bone health. It is reported in the literature that biocomposite scaffolds can promote regeneration [25, 26]. SMF can also be used to treat critical-sized bone defects along with other biocomposite scaffolds.

One limitation of this study is that we have used only one dose of SMF and the next is that we have limited it to only under osteogenic conditions. Therefore, future studies on different doses of SMF and utilizing a normal medium are envisaged to decipher the exact dose of SMF needed to promote osteoblast differentiation under HG conditions. Additional in vivo studies are also required to concretely assess the role of SMF in

ameliorating the detrimental effects of high glucose.

Conclusions

In conclusion, we found that high glucose significantly affected osteoblastic differentiation by downregulating all the vital marker genes and exposure to SMF reversed the effect thereby promoting osteoblastic differentiation. Therefore, SMF could be a potential alternative and additional therapy for bone regeneration in diabetic individuals.

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Conflict of Interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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