Chitooligosaccharide enhances Osteogenic differentiation: An In Vitro Assessment

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Abstract:

This article presents a comprehensive investigation into the influence of chitooligosaccharide exposure on osteoblastic cells over a carefully monitored 7-day timeframe. The study involved the extraction of total RNA from these cells, followed by a meticulous analysis of the expression profiles of pivotal osteoblast marker genes, notably Runx2, ALP (Alkaline Phosphatase), and type I collagen (Col I). The findings of this research are striking, as they unveil a remarkable phenomenon: chitooligosaccharide exposure induced a significant and notable upregulation of these key genes. This discovery highlights the potential of chitooligosaccharide as a potent modulator of osteoblastic activity. It carries significant implications for advancing our understanding of the intricate regulatory mechanisms governing osteoblast function and bone matrix formation(1). Furthermore, it opens promising avenues for innovative therapeutic strategies for bone health and regenerative purposes I.e particularly in addressing conditions marked by compromised bone density and quality. Further exploration of the underlying molecular pathways is imperative to fully harness the therapeutic potential of chitooligosaccharide in promoting bone health and regeneration.

Keywords: Chitooligosaccharide; Runx2; ALP; type I collagen; bone

Introduction

Chitooligosaccharide (COS) degraded chitin oligomers and its N-deacetylated precursor, chitosan, are in high demand in pharmacological and clinical applications due to their water solubility, biocompatibility, and improved functionality. Previous studies have investigated the potential of COS in bone diseases like osteoporosis and osteopenia. Osteoblast plays a crucial role in maintaining bone homeostasis and regulating the bone remodelling process. Assessing the impact of COS on osteoblast markers is vital to understand its potential as a therapeutic agent for bone-related disorders.

Osteoinductive biomaterials like demineralized bone matrix (DBM) are often used in tissue engineering. Supporting and inducing new bone growth in vivo [1] will be possible if DBM is combined with osteoprogenitors and implanted in animals. In addition, in vitro and in vivo studies have shown that DBM can stimulate osteoinductivity. Bone morphogenetic proteins (BMPs) and basic fibroblast growth factor (bFGF) (2)is two examples of noncollagenous proteins that may contribute to the DBM's osteoinductivity [2]. DBM was shown to have much better biocompatibility, biodegradability, osteoconductivity, and osteoinductivity than the scaffold when tested alongside chitosan and polymethylmethacrylate in a rat model of radial bone defect [3]. The bone repair around the autologous cortical bone transplant was also greatly aided by DBM.

Hydrolysis of collagen yields gelatin, a natural polymer that is extremely it is biological (low-dose immunogenic), biodegradable, non-cytotoxic and non-antigenic[4]. Cell adhesion, migration, and proliferation are all aided by gelatin's arginine-glycine-aspartic acid (RGD) pattern [4]. Since gelatin's physicochemical characteristics are malleable, it has been shown in prior research [5] that it may be utilized to create three-dimensional scaffolds for bone tissue regeneration. Because of this, gelatin is used as the foundation for creating the collagen-based scaffolds used in bone tissue engineering.

Because of its structural resemblance to natural GAG, chitosan is classified as a glycosaminoglycan (GAG) analog [3]. Deacetylated chitin polysaccharide chitosan is a chitin-derived amino polysaccharide with alternating units of -(1, 4)-2-acetamido-2-deoxy-D-glucose and -(1, 4)-2-amino-2-deoxy-D-glucose. Antimicrobial activity, anticancer effects, and immunomodulatory biological assets are only some of its appealing features [6]. In addition, chitosan has been shown in studies [7] to hasten neovascularization and stimulate bone growth. The molecular weight of chitosan varies greatly depending on its origin and the methods used to prepare it. Recent research has shown that low-molecular-weight chitosan is better than high molecular weight chitosan in promoting the growth of mouse fibroblasts[8].

The physical technique, chemical hydrolysis, and enzymatic degradation are all viable routes to producing chitooligosaccharide (COS), a water-soluble chitosan with a low molecular weight [9]. Tissue engineers have shown a growing interest in chitosan and related polysaccharides have been extensively studied in recent years[9]. Gelatin-based scaffolds have been used in vitro to enhance the attachment, growth, and osteogenetic differentiation of rat MSC derived from bone marrow, as reported by Ratanavaraporn et al. [10]. The osteogenic differentiation capacity of osteoblastic cells was significantly enhanced by COS [10, 11]. In particular, bone cells showed improved osteogenic differentiation when cultured with COS [11].

Materials and methods

1.1. Cell Culture and Maintenance

In order to get MG63 cells, we had to go to NCCS which is cultivated in DMEM (Dulbecco's Modified Eagle's Medium) in Pune, India, supplemented with 24 mM Sodium Bicarbonate; 25 mM Hydroxyethyl ester (HEPES); 100 Units/L Penicillin; 100 Units/L Streptomycin; 30 Grams/L Gentamicin; 2.5 Grams/L Amphotericin B(Invitrogen, USA),10% fetal bovine serum (Invitrogen, USA). Trypsin (0.05%) and EDTA (0.025 M) were used to sub-culture MSCs when they reached confluence (70-80%). In one group, the cells were left untreated whereas in another, COS (0.005%) was added to their environment.

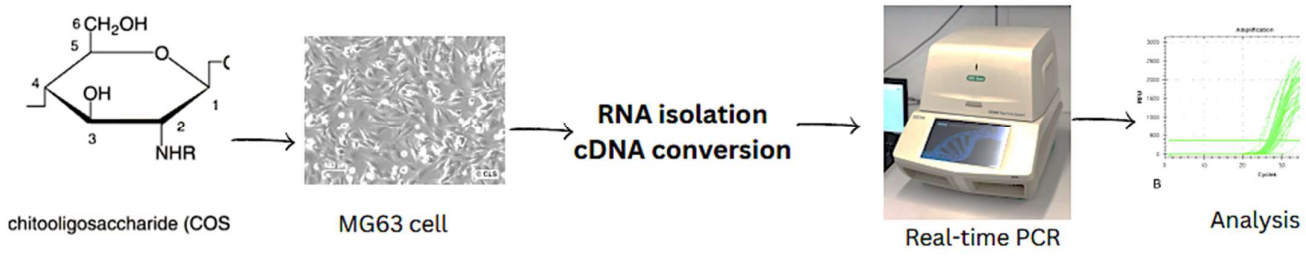

Figure 1. Cohesive Methodology Framework Employed for Investigating the Impact of Chitooligosaccharide Exposure on Osteoblastic Cells and the Modulation of Osteoblast Marker Gene Expression: A Comprehensive Overview"

1.2.RT-PCR Study

Mechanistic of reverse transcriptase chain reaction (RT-PCR) was used to evaluate the expression in runx2, Type I collagen, alkaline phosphatase, osteocalcin, and osteonectin in mesenchymal stem cells. After COS-induced osteoblastic differentiation, the cells' sum total RNA was derived and being analyzed by the validation of the genes Runx2, alkaline phosphatase (ALP), and type I collagen. After validating the quality of the extracted RNA using Nanodrop (Thermo Fisher Scientific, USA), the

amount of RNA was normalized. We then used a KAPA SYBR FAST qPCR Kit was analyzed by amplification. Table 1 in the appendices contains the complete list of primers. Using the Ct technique, we determined the relative mRNA fold change [12].

Table 1. Represents the Primer sequences used for real time RT-PCR analysis.

1.3 Statistical analysis

Gene		5′⚜3′ Sequence
Runx2	Forward	CAGTTCCCAAGCATTTCATC
	Reverse	TCAATATGGTCGCCAAACAG
ALP	Forward	AGGCAGGATTGACCACGG
	Reverse	TGTAGTTCTGCTCATGGA
Type-1 collagen	Forward	TAACCCCCTCCCCAGCCACAAA
	Reverse	TTCCTCTTGGCCGTGCGTCA
GAPDH	Forward	TTGATGTCATCATACTTGGCAGGT
	Reverse	CAG TCAAGGCTGAGAATGGGA

The Factual Bundle for the Sociologies (SPSS) rendition 17.0 (SPSS Inc.) was utilized for all measurable examinations.At least three independent replications of each experiment were performed. The information were introduced as a mean SD. Understudy t-test was utilized to look at the control and exploratory gatherings. P-values < 0.05 were considered to be significant statistically.

Result

The effect of chitooligosaccharide exposure on osteoblastic cells was assessed over a 7-day period. Total RNA was isolated from these cells, and the expression levels of key osteoblast marker genes, including Runx2 (Figure 2), ALP (Figure 3), and type I collagen (Col I) (Figure 4), were analyzed. Remarkably, chitooligosaccharide significantly increased the expression of these genes, demonstrating its potential as a modulator of osteoblastic activity.

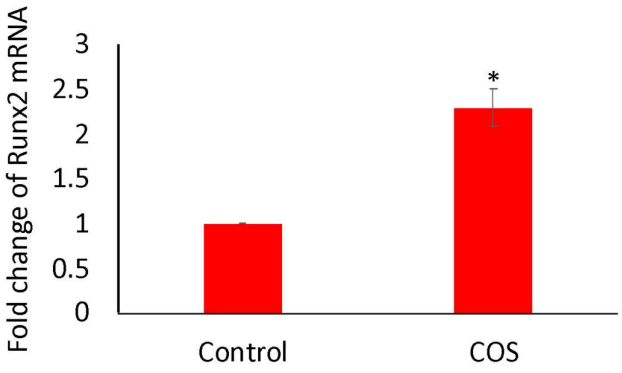

Figure 2. Runx2 expression(Runt related transcription factor). MG63 cells were exposed to chitooligosaccharide (0.005%) for 7 days and total RNA was separated and Constant RT-PCR examination was performed.The fold change increased in 1.5 percent from the control group to the COS significantly stating that the osteoblast profoundly increased in the expression of Runx2 enzyme.

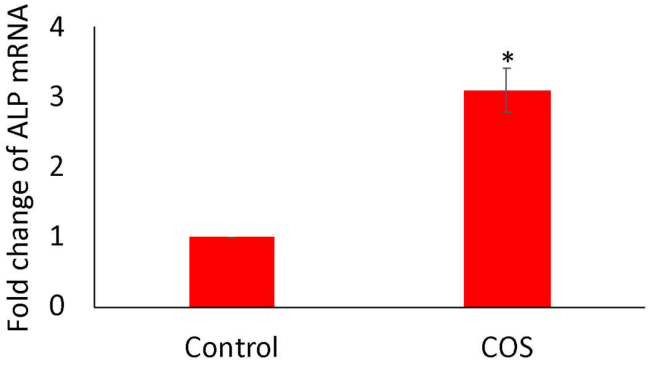

Figure 3. ALP expression(Alkaline phosphatase). MG63 cells were presented to chitooligosaccharide (0.005%) for 7 days and absolute RNA was secluded and Ongoing RT-PCR investigation was performed.Results explained that there is 2 percent increase in fold change from the control to the chitooligosaccharide sample explaining ALP enhance the osteogenic potential.

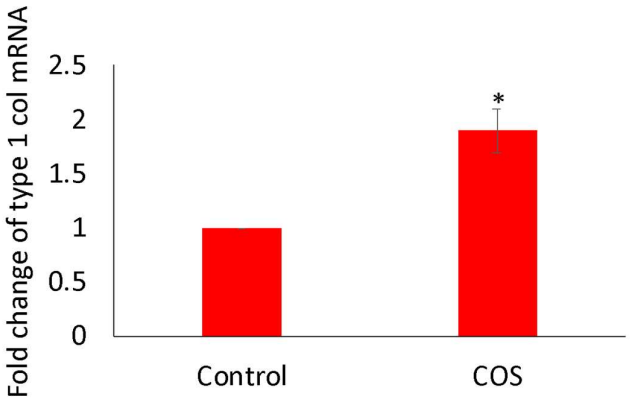

Figure 4. Col 1(Type-1:Collagen). MG63 cells were exposed to chitooligosaccharide (0.005%) for 7 days and complete RNA was detached and Ongoing RT-PCR investigation was performed.This explains increase of 1 percent fold change from the control group to the COS enhancing the joint cartilage of the bone with the effect of this enzyme.

Discussion

Osteoblasts play a pivotal role in bone formation and maintenance, making them a crucial focus in understanding bone health and regenerative strategies. The current review examined the effect of chitooligosaccharide openness on osteoblastic cells and observed noteworthy changes in the expression of key osteoblast marker genes. Runx2, a master regulator of osteogenesis, is essential for osteoblast differentiation and bone development [13]. Our findings revealed a significant increase in Runx2 gene expression following chitooligosaccharide treatment. This upregulation suggests that chitooligosaccharide promotes osteoblast differentiation, a pivotal move toward bone development (14). Alkaline phosphatase(ALP) is an early marker of osteoblast separation and is essential for bone mineralization(3). The substantial increase in ALP gene expression observed in our study further supports the notion that chitooligosaccharide enhances osteoblast activity. Elevated ALP levels suggest an enhanced capacity for bone matrix mineralization, a hallmark of healthy bone formation [13-16]. Type I collagen (Col I) is the most plentiful protein in the bone network,and its synthesis is a fundamental process in bone formation. Our results demonstrate a significant increase in Col I gene expression in response to chitooligosaccharide treatment. This suggests that chitooligosaccharide not only promotes osteoblast differentiation but also enhances the production of critical extracellular

matrix components, contributing to improved bone quality.COS positively influence osteoblast activity and bone formation(4). It enhances osteoblast
proliferation, differentiation and mineralisation in various mode

The mechanism underlying these observed effects may involve different flagging pathways, including Wnt/β-catenin and BMP/Smad pathways, both known to be involved in osteoblast differentiation and bone formation. Further examinations are expected to clarify the particular sub-atomic components through the chitooligosaccharide that exerts its osteogenic effects [13-16]. These findings have significant implications for bone health and regenerative medicine. Chitooligosaccharide, with its ability to enhance osteoblast activity and bone matrix formation, holds promise as a potential therapeutic agent for conditions characterized by compromised bone density and quality, such as osteoporosis and bone fractures.

Conclusion

In conclusion, our study demonstrates that chitooligosaccharide exposure significantly upregulates the declaration of basic osteoblast marker qualities, including Runx2, ALP and Col I. These findings suggest that chitooligosaccharide has osteogenic potential and warrants further investigation as a potential therapeutic agent for bone-related disorders and regenerative medicine applications. Understanding the underlying molecular mechanisms will be critical in harnessing the full therapeutic potential of chitooligosaccharide in bone health and regeneration.

Scope of future research

In this field, we can add to the advancement of novel helpful stratergies for promoting bone formation and addressing skeletal disorders.

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Conflict of interest

There is no conflict of interest.

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