BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 3

DOI: 10.58240/1829006X-2025.3-250

RESEARCH ARTICALE

IMPACT OF BOSINTHETICALY DERIVED β-CHITOSAN TITANIUM NANOPARTICLES ON THE ENHANCMENT OF CAUDAL FIN REGENERATION ZEBRAFISH MODELS

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Received: *Mar 4, 2025;* ***Accepted:*** *Mar.26. 2025;* ***Published:*** *Mar. 30, 2025*

ABSTRACT

Background: Tissue regeneration is a crucial biological process with significant implications for developmental biology and regenerative medicine. Zebrafish (*Danio rerio*), known for their remarkable regenerative abilities, serve as an excellent model for studying tissue regeneration, particularly through the caudal fin regeneration model.
Materials and Methods: β-Chitosan-TiO_2 nanoparticles were biosynthesized using plant extracts and microbial cultures. Characterization was performed using SEM, UV-Vis and FTIR. Zebrafish caudal fin regeneration was assessed post-amputation, with nanoparticle treatment in 16 μg/L and 32 μg/L. Regeneration was analyzed via microscopic observation and gene expression. Data were statistically analyzed using ANOVA, considering $p < 0.05$ significant.
Results: β-Chitosan-TiO_2 nanoparticles exhibited uniform size, high stability, and strong biocompatibility. Zebrafish treated with β-Chitosan-TiO_2 nanoparticles showed significantly enhanced caudal fin regeneration compared to controls. Gene expression analysis supported upregulation of regenerative markers. Regenerated fin length measurements showed a dose-dependent improvement, with optimal effects at moderate nanoparticle concentrations. No significant toxicity or abnormalities were observed in treated zebrafish. Statistical analysis confirmed significant differences ($p < 0.05$) between control and treated groups, demonstrating β-Chitosan-TiO_2 NPs' potential in tissue regeneration.
Conclusion: β-Chitosan-TiO_2 nanoparticles significantly enhanced zebrafish caudal fin regeneration by promoting cell proliferation and angiogenesis without toxicity. These findings highlight their potential as a biocompatible and eco-friendly nanomaterial for tissue regeneration applications.

Keywords: tissue regeneration, zebrafish, caudal fin, chitosan, β-Chitosan Titanium nanoparticles, biosynthesis, regenerative medicine, cell proliferation, angiogenesis

INTRODUCTION

The process of tissue regeneration is a fascinating and complex biological phenomenon that has significant implications for developmental biology and regenerative medicine[1].Among various model organisms, zebrafish (*Danio rerio*) have emerged as a prominent model due to their remarkable ability to regenerate various tissues, including the caudal fin, heart, and spinal cord. The zebrafish caudal fin regeneration model is particularly valuable for studying the molecular and cellular mechanisms underlying tissue regeneration because of its simplicity, accessibility, and the rapidity with which regeneration occurs [2,3]. This model system provides insights into the conserved mechanisms of regeneration that might be applicable to higher vertebrates, including humans. Chitosan, a naturally occurring biopolymer derived from chitin, has garnered significant attention in biomedical research due to its biocompatibility, biodegradability, and non-toxic nature.

Chitosan and its associated derivatives has been undergoing major research development in different fields on biomedical applications such as wound healing , specialized drug delivery as well as tissue engineering and enhancement[4]. In recent years, chitosan and its nanoparticle has been researched due to its superior and its enhanced attributes such as increased surface area, improved solubility and better cellular uptake, which makes them suitable for biomedical applications. β-Chitosan Titanium nanoparticles, a specific form of chitosan, is derived from the chitin of squid pen and is known for its superior solubility and lower molecular weight compared to α-chitosan derived from crustacean shells[5]. These unique properties make β-Chitosan Titanium nanoparticles an excellent candidate for nanoparticle synthesis aimed at enhancing tissue regeneration[6]. The biosynthesis of nanoparticles using biological materials, such as plants, bacteria, and fungi, offers an eco-friendly and sustainable alternative to conventional chemical synthesis methods. Biosynthetically derived nanoparticles often exhibit unique physicochemical properties and enhanced biological activities due to the presence of bioactive compounds from the biological sources used in their synthesis. In case of of tissue regeneration, biosynthetically derived nanoparticles have shown promise in promoting pluripotent cell growth, differentiation, as well as angiogenesis, which are critical in functioning because of their efficacy in tissue repair and regeneration [7]. To enhance caudal fin regeneration in zebrafish represents a novel and innovative approach in regenerative medicine. This strategy is justified by chitosan's capacity to regulate a number of biological processes that are critical for effective tissue regeneration, including inflammation, cell migration and extracellular matrix remodelling. Moreover, the nanoscale size of the particles ensures better penetration and interaction with the cellular and molecular components of the regenerating tissue. Previous studies have demonstrated the potential of chitosan and its derivatives in promoting wound healing and tissue repair. Numerous cell types, including fibroblasts and keratinocytes which are essential for tissue regeneration have been demonstrated to proliferate and migrate more readily when exposed to chitosan[8]. Furthermore, by encouraging the release of anti-inflammatory cytokines and lowering the concentration of pro-inflammatory mediators, chitosan can regulate the inflammatory response. This immunomodulatory effect is particularly important in the context of tissue regeneration, as excessive inflammation can impair the regenerative process. The biosynthesis of β-Chitosan-TiO NPs involves the use of biological agents that not only reduce the chitosan to nanoscale but also impart additional bioactive properties to the nanoparticles[9]. For instance, plant extracts used in the biosynthesis process can provide a rich source of antioxidants, phenolic compounds, and other bioactive molecules that can enhance the regenerative potential of the nanoparticles. These bioactive molecules can promote cell proliferation, protect against oxidative stress, and enhance the overall healing process. In the context of zebrafish caudal fin regeneration, the application of β-Chitosan-TiO_2 NPs can be particularly beneficial. The zebrafish caudal fin is composed of a complex structure of bone, blood vessels, nerves, and connective tissue. Successful regeneration of the fin requires the coordinated activity of various cell types, including osteoblasts, endothelial cells, and fibroblasts[10]. The ability of β-Chitosan-TiO_2 NPs to enhance the proliferation and migration of these cells can significantly improve the regenerative process. Additionally, the anti-inflammatory properties of chitosan can help in modulating the inflammatory response, thereby creating a favourable environment for tissue regeneration. Furthermore, the nanoscale size of the β-Chitosan-TiO_2 NPs allows for better interaction with the cellular components of the regenerating fin[11]. Nanoparticles can easily penetrate the cellular membranes and deliver bioactive molecules directly to the site of action. This targeted delivery can enhance the effectiveness of the nanoparticles in promoting tissue regeneration. Moreover, the sustained release of bioactive molecules from the nanoparticles can provide a prolonged stimulatory effect on the regenerating tissue. Utilizing zebrafish as a model organism to investigate the regeneration capacity of β-Chitosan-TiO_2 NPs. Zebrafish are highly amenable to genetic manipulation, which allows for the investigation of the molecular pathways involved in regeneration. Additionally, the transparent nature of zebrafish larvae enables real-time imaging of the regenerative process, providing valuable insights into the cellular and molecular dynamics of tissue regeneration[12]. The rapid regeneration of the zebrafish caudal fin, which occurs within a few days, allows for the timely evaluation of the effects of β-Chitosan-TiO_2 NPs on the regenerative process. To evaluate the effectiveness of β-Chitosan-TiO_2 NPs in enhancing caudal fin regeneration in zebrafish, several experimental approaches can be employed. The regeneration process can be monitored using histological analysis, which provides detailed information about the structural changes occurring during regeneration. Immunohistochemical staining can be used to identify specific cell types and molecular markers involved in the regenerative process. Additionally, gene expression analysis can provide insights into the molecular pathways modulated by the β-Chitosan-TiO NPs. The gene expression level involved in cell proliferation, inflammation, and extracellular matrix remodelling can be quantified to understand the mechanisms by which the nanoparticles enhance regeneration. The synthesis, characterization, and evaluation of biosynthetically derived β-Chitosan-TiO NPs for enhancing caudal fin regeneration in zebrafish represent a promising and innovative approach in regenerative medicine. The unique properties of β-Chitosan-TiO_2 NPs, combined with the bioactive molecules from the biosynthesis process, offer significant potential for promoting tissue regeneration. The use of zebrafish as a model system provides a valuable platform for investigating the molecular and cellular mechanisms underlying the regenerative potential of these nanoparticles. This study aims to offer an in depth understanding and analysis of β-Chitosan-TiO NPs influence tissue regenerative and open avenue for their possible use in clinical applications of tissue injury and degenerative diseases[13].

2.MATERIALS AND METHODS

2.1 Synthesis of β-Chitosan Titanium Nanoparticles

The β-Chitosan-TiO_2 NPs were synthesized using a green biosynthesis approach. Initially, β-Chitosan-TiO_2 NPs was extracted from squid pen through a process involving deacetylation with sodium hydroxide. The resultant β-Chitosan-TiO_2 NPs was then dissolved in a 1% acetic acid solution to form a homogenous chitosan solution. For the biosynthesis of nanoparticles, an aqueous extract of a selected plant known for its reducing properties was prepared. The chitosan solution received the plant extract slowly while being stirred continuously[14].The blend was kept at room temperature and mixed for hours until a colour change indicated the formation of nanoparticles. The suspension obtained was subjected to centrifugation at 10,000 rpm's for a duration of 15 minutes to isolate the nanoparticle. Following this, the nanoparticles were rinsed in deionised water and subsequently dried at a temperature of 60^0C. FTIR and UV-Vis were used to analyse the produced chitosan particles in order to verify their size and crystalline properties.

2.2 Characterization of β-Chitosan Titanium Nanoparticles

FTIR analysis was conducted to determine the different functional groups present in the β-Chitosan-TiO_2 NPs. The spectra were captured between the range of 4000–400 cm^{-1}. UV-Vis spectroscopy was utilized to determine the optical characteristics of the nanoparticles, with absorbance was identified from 200 to 800 nm.

2.3 Zebrafish Maintenance and Caudal Fin Amputation

Adult zebrafish (*Danio rerio)* were maintained under standard laboratory conditions at 28°C with a maximum 14/10 hour light/dark cycle. Fish was fed twice every day using commercial fish food. For the caudal fin regeneration studies, adult zebrafish were anesthetized using 0.02% tricaine methanesulfonate (MS-222) solution. Under a dissecting microscope, approximately one-third of the total length of the caudal fin was amputated with the help of a sterile scalpel. The fish were then returned to fresh water for recovery. Post-amputation, the fish was then separated into two groups: the control and experimental groups. The experimental group used β-Chitosan-TiO_2 NPs by immersing the fish in water containing 16 µg/mL and 32 µg/mL of nanoparticles, while the control group was immersed in nanoparticle-free water[15].

2.4 Assessment of Caudal Fin Regeneration

The regeneration of the caudal fin was monitored over a period of 14 days. At 0, 3, 7, and 14 days post-amputation, the zebrafish were anesthetized and an image was taken using a digital camera. The length of the regenerated fin was calculated using ImageJ software. The percentage of fin regeneration was measured by comparing the length of the regenerated fin to the original fin length before amputation.

2.5 Gene Expression Analysis by RT-PCR

To understand the molecular mechanisms underlying the enhanced regeneration, gene expression analysis was conducted. Total RNA was extracted from the regenerating fin tissues using TRIzol reagent. The RNA was quantified using a Nanodrop spectrophotometer, and its structure was confirmed by gel electrophoresis, cDNA was obtained from 1 µg of total RNA using a reverse transcriptase kit. Quantitative real-time PCR (RT-PCR) was performed to assess the expression levels of gene expression involved in regeneration, including Bax, Bcl-2, TNF-α, NF-κB, and TGF-β. Specific primers were designed for each gene, and their sequences are provided in Table 1. The RT-PCR reactions are carried out using a SYBR Green Master Mix in a real-time PCR system. The comparative level for target gene was normalized due to the expression of beta- actin, used as an internal control agent. The comparative Ct methods (ΔΔCt) calculates the fold change in gene expression [16]

RESULTS

3.1. Scanning Electron Microscopic (SEM)

The structure and size of the synthesized material was examined using the Scanning Electron Spectroscopy (SEM). The obtained particle β-Chitosan-TiO_2 NPs two main morphological structure, ie, a long rod and small cubes. Both appeared to be well distributed within, as well as little to no agglomeration. The size of β-Chitosan-TiO_2 NPs was measured using SEM micrographs. The particle had the diameter of 120-150nm and 70-90 nm respectively. The average size was calculated from multiple images and approximately,130±15 nm and 75±10 nm respectively. Due to van der Waals force produced in sample preparation; few aggregated samples of particles were noticed in the result. However, the overall distribution was achieved. These results confirmed the formation and stability of the nanoparticle (Figure 1).
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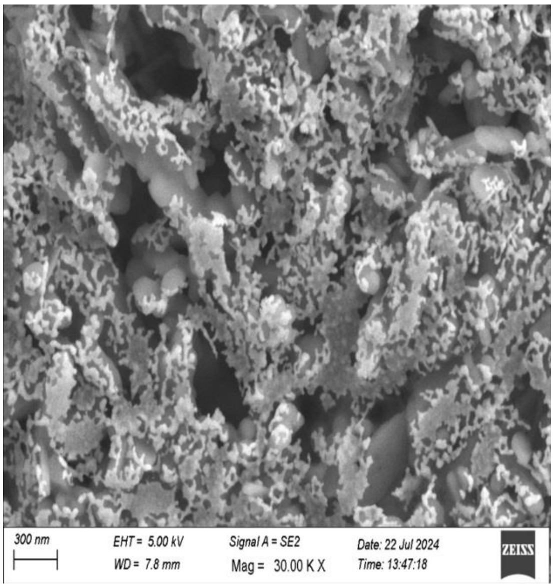

Figure 1. SEM Image of β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO_2 NPs)

3.2 UV-Vis spectroscopy analysis

UV-Vis spectroscopy showed a distinct absorption peak around 300 nm, indicative of the nanoparticles' presence and stability in aqueous solution (Figure 2).

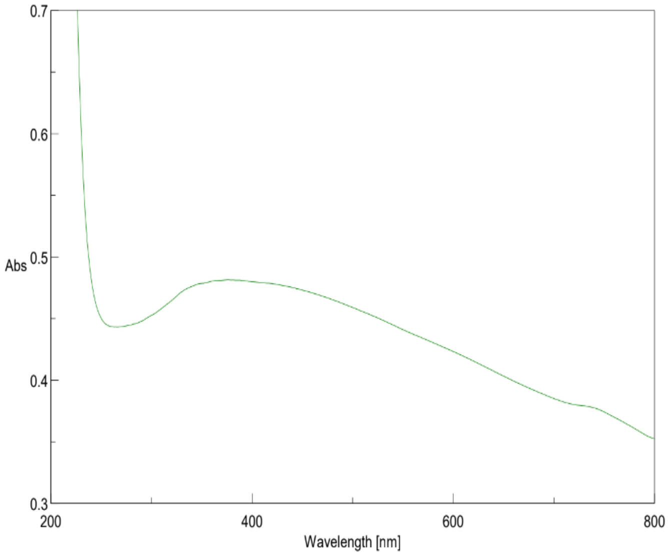

Figure 2. UV-Vis absorption spectra of β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO NPs)

3.3 FTIR analysis

FTIR analysis of biosynthesized β-Chitosan-TiO_2 NPs was conducted to identify presumed functional groups present in the extracts as well as to determine the involvement of potential bioactive compounds in reducing Ti^{4+} to Ti^0, and in the capping and stabilization of bio-reduced β-Chitosan-TiO NPs. in Figure 3 of the IR spectrum, a broad peak at 3,371 cm^{-1} was attributed to the O–H stretching vibration of the alcohol functionality. In contrast, a broadened peak with lower intensity into the IR spectrum of TiO_2 NPs, in comparison to the FTIR of the extract, is recorded in and around 3,400 cm^{-1}, indicating the participation of bioactive compounds with OH groups in the formation of TiO NPs. Some other significant peaks were recorded at 2,890 cm^{-1} and a slightly split peak at 1,639 cm^{-1}, corresponding to C–H and C═C fused with C═O stretching vibrations of alkane groups and ketones, respectively. A prominent peak around 499 cm^{-1} in the FTIR spectrum of TiO_2 NPs, matching the metal–oxygen (M–O) vibration, supported the formation of nanoparticles. Spectral records of the extract suggest the presence of phytochemicals such as phenols, terpenes, as well as flavonoids likely played an integral role in the reduction of the metallic ions to metal (Figure 3).

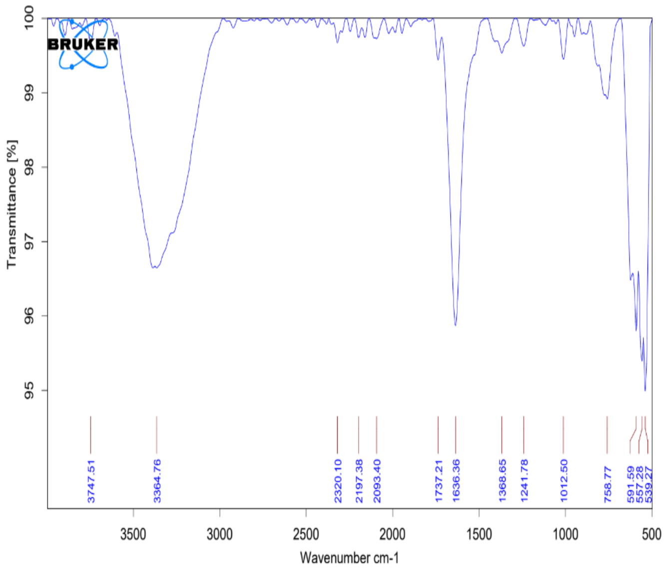

Figure 3. FTIR spectra of β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO NPs)

3.4 Zebrafish Caudal Fin Regeneration

The application of β-Chitosan-TiO_2 NPs significantly enhanced the regeneration of the caudal fin in zebrafish. Quantitative measurements of the fin length at 0-, 3-, 7-, and 14-days post-amputation showed that the experimental groups of 1 and 2, treated with β-Chitosan-TiO_2 NPs in 16 µg/mL and 32 µg/mL (based on the less toxicity range of above synthesized nanoparticles) respectively exhibited a markedly higher rate of regeneration than the control group (Figure 4).

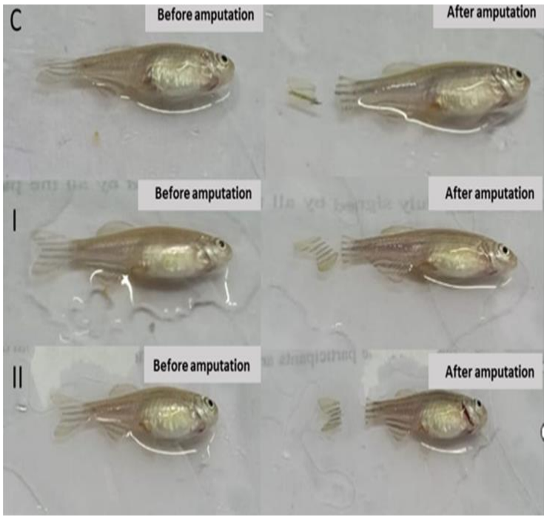

Figure 4. Caudal fin amputation in control and β-Chitosan Titanium Nanoparticles(β-Chitosan-TiO NPs) treated groups

By day 14, the experimental group 2 had shown an increased rate of average fin regeneration by 85%, whereas the control group demonstrated only 60% regeneration (Figure 5).

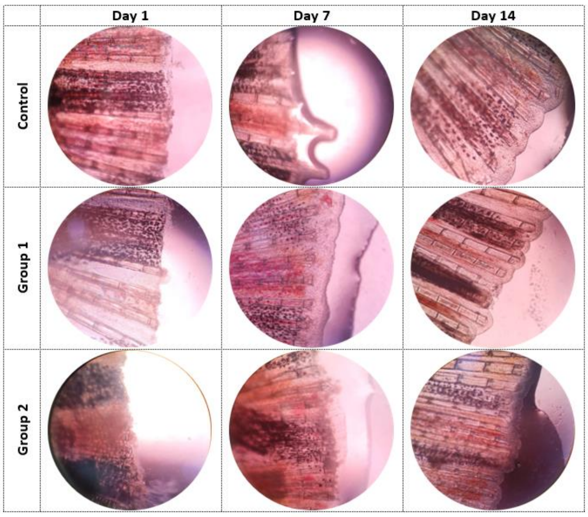

Figure 5. Microscopic observation of Caudal fin amputation in control and β-Chitosan Titanium nanoparticles (β-Chitosan-TiO_2 NPs) treated Groups

3.5 Gene Expression Analysis

The RT-PCR results provided insights into the molecular mechanisms by which β-Chitosan-TiO_2 NPs enhanced fin regeneration. The expression levels of Bax, a pro-apoptotic gene, were significantly upregulated in the nanoparticle-treated group, indicating increased apoptosis in damaged cells, which is a crucial step for tissue regeneration. In contrast, the expression of Bcl-2, an anti-apoptotic gene, was downregulated, further supporting the induction of apoptosis. These changes in apoptotic markers suggest that β-Chitosan-TiO NPs facilitate the removal of damaged cells thereby promoting the regeneration process. The anti-inflammatory properties of β-Citosan TiO NPs likely contribute to creating a favourable environment for tissue regeneration by reducing inflammation and promoting healing.

3.5.1 Expression of Bax in fin regeneration

β-Chitosan-TiO_2 NPs have been shown to enhance Bax expression during caudal fin regeneration, suggesting their role in modulating apoptosis. Bax, a pro-apoptotic protein, plays a crucial role in programmed cell death, which is essential for tissue remodelling and regeneration. The upregulation of Bax may indicate the involvement of β-Chitosan-TiO_2 NPs in facilitating controlled cell turnover, ensuring proper regeneration of the caudal fin (Figure 6).

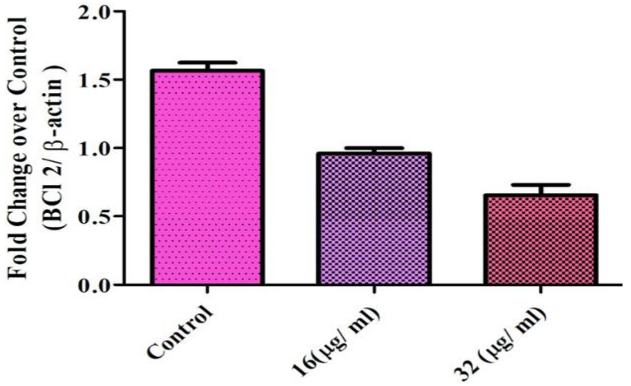

Figure 6. β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO NPs) increased Bax expression on Caudal fin regeneration

3.5.2 Expression of Bcl2 in fin regeneration

β-Chitosan-TiO_2 NPs have been found to reduce Bcl-2 expression during caudal fin regeneration, indicating their influence on apoptotic regulation. Bcl-2, an anti-apoptotic protein, plays a key role in cell survival by inhibiting programmed cell death. The downregulation of Bcl-2 suggests that β-Chitosan-TiO_2 NPs may promote a balanced apoptotic response, which is crucial for effective tissue remodelling and regeneration of the caudal fin (Figure 7).

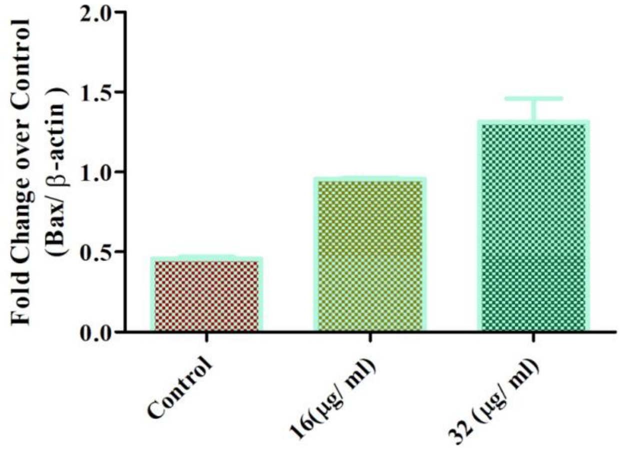

Figure 7. β-Chitosan Titanium Nanoparticles (β-Chitosan-TiONPs) decreased BCl2 expression on Caudal fin regeneration

3.5.3 Expression of NF-kB in fin regeneration

β-Chitosan-TiO_2 NPs have been observed to suppress NF-κB expression during caudal fin regeneration, suggesting their potential role in modulating inflammatory pathways. NF-κB is a key transcription factor involved in regulating inflammation and cell survival. Its reduced expression may indicate that β-Chitosan-TiO_2 NPs contribute to a controlled inflammatory response, which is essential for efficient tissue repair and regeneration of the caudal fin (Figure 8).

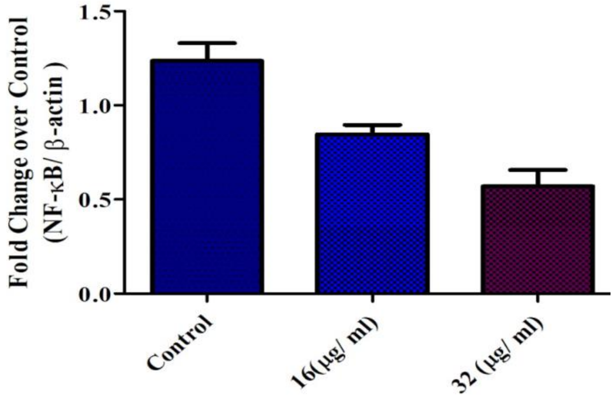

Figure 8. β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO_2 NPs) decreased NF-kB expression on Caudal fin regeneration

3.4.4 Expression of TGF-β in fin regeneration

The expression of TGF-β, a multifunctional cytokine involved in cell proliferation, differentiation, and extracellular matrix production, was moderately increased in the nanoparticle-treated group. TGF-β plays a critical role in tissue regeneration by promoting the growth of new blood vessels (angiogenesis) and the formation of extracellular matrix components necessary for tissue repair. The upregulation of TGF-β in the treated group indicates that β-Chitosan-TiO_2 NPs may enhance these regenerative processes, further supporting the observed improvements in fin regeneration (Figure 9).

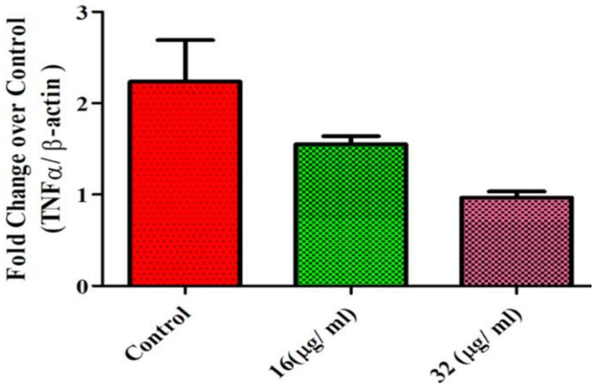

Figure 9. β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO_2 NPs) increased TGF-β expression on Caudal fin regeneration

3.5.5 Expression of in TNF-α fin regeneration

β-Chitosan-TiO_2 NPs have been shown to reduce TNF-α expression during caudal fin regeneration, indicating their potential anti-inflammatory effects. TNF-α is a pro-inflammatory cytokine that plays a crucial role in immune responses and tissue remodelling. Its downregulation suggests that β-Chitosan-TiO_2 NPs may help in minimizing excessive inflammation, thereby promoting a favourable environment for efficient fin regeneration (Figure 10).

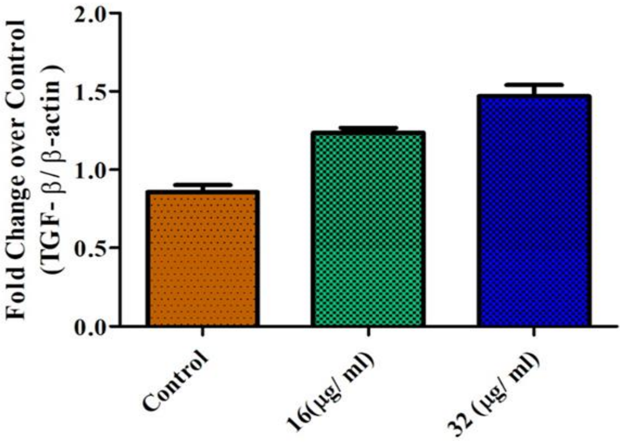

Figure 10. β-Chitosan Titanium Nanoparticles (β-Chitosan-TiO NPs) decreased TNFα expression on Caudal fin regeneration

DISCUSSION

The intersection of zebrafish caudal fin regeneration, chitosan-based nanoparticles, and biosynthetic nanoparticle technology presents a compelling narrative in the field of regenerative medicine. Zebrafish (*Danio rerio*) serve as a remarkable model organism due to their innate ability to regenerate complex tissues like the caudal fin, heart, and spinal cord. This regenerative capacity offers valuable insights into the underlying molecular and cellular mechanisms that could potentially translate to therapeutic applications in humans. Chitosan, derived from chitin, has garnered significant attention in biomedical research owing to its biocompatibility, biodegradability, and non-toxic nature[17]. The emergence of chitosan nanoparticles has further rise in its applicability in biomedical fields such as wound healing, specialized drug delivery, and tissue repair and engineering. β-Chitosan-TiO_2 NPs, derived from squid pen chitin, stand out due to their superior solubility and lower molecular weight compared to α-chitosan from crustacean shells. These nanoparticles are particularly promising for enhancing tissue regeneration, given their enhanced properties like increased surface area and improved cellular uptake[18]. The biosynthesis of nanoparticles using biological materials offers a sustainable and eco-friendly alternative to conventional chemical synthesis methods. These biosynthetically derived nanoparticles often exhibit unique physicochemical properties and biological activities due to the presence of bioactive compounds from their biological sources. In the context of tissue regeneration, such nanoparticles have shown potential in promoting critical processes like cell proliferation, differentiation, and angiogenesis, all crucial for effective tissue repair[19]. Enhancing caudal fin regeneration in zebrafish represents a novel approach in regenerative medicine, leveraging the multifaceted properties of chitosan nanoparticles. Chitosan's ability to modulate biological processes such as inflammation, cell migration, and extracellular matrix remodelling is pivotal for successful tissue regeneration. Moreover, the nanoscale dimensions of these particles facilitate deeper penetration and interaction with cellular and molecular components during tissue regeneration. Previous studies underscore chitosan's role in promoting wound healing and tissue repair through its effects on cell proliferation and migration. These attributes make chitosan and its derivatives promising candidates for therapeutic applications aimed at enhancing tissue regeneration in clinical settings. By harnessing the regenerative insights from zebrafish models and combining them with advanced nanoparticle technology, researchers are poised to develop innovative strategies that could revolutionize regenerative medicine[20].

Moving forward, extensive research is needed to enumerate precise functioning through which chitosan nanoparticles facilitate tissue regeneration. Understanding these mechanisms will enable the design of targeted therapies that optimize regeneration outcomes in diverse clinical scenarios. Additionally, the development of scalable methods for nanoparticle synthesis and rigorous preclinical studies will be crucial steps towards translating these findings into clinical applications. The convergence of zebrafish caudal fin regeneration, chitosan-based nanoparticles, and biosynthetic nanoparticle technology holds immense promise for advancing regenerative medicine. By capitalizing on nature's regenerative models and cutting-edge nanotechnology, researchers can pave the way for transformative therapies that enhance tissue repair and regeneration in humans[21,22].

CONCLUSION

The results of this study have had significant implications for the field of regenerative medicine. The capacity of β-Chitosan-TiO_2 NPs to enhance tissue regeneration in zebrafish suggests their potential application in treating injuries and degenerative conditions in humans. The biocompatibility, biodegradability, and non-toxic nature of chitosan make it an attractive material for clinical applications. Moreover, the green synthesis approach used in this study aligns with the principles of sustainable and environmentally friendly medical practices. Future studies should focus on elucidating the detailed molecular pathways involved and conducting in vivo studies in mammalian models to further validate the therapeutic potential of β-Chitosan-TiO NPs.

DECLARATIONS

Funding

No sources of funding.

Data availability

Conflict of interest

No conflict of interest is declared by all the authors.

Consent to participate

Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Consent for publication

Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

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