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Nanocarrier-mediated CRISPR/Cas9 Gene Editing of BMP2 for Bone Repair

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Abstract

The growth factor that plays an important role in the formation and repair of bones is bone morphogenetic protein 2(BMP2). Nevertheless, because BMP2 is rapidly degrading and has less than half the time to live, its delivery into bone defects may be difficult. A plan to develop a nanocarrier via CRISPRCas9 gene editing mechanism for the delivery of BMP2 into bone defects is outlined in this research proposal. The system will use nanoparticles to encapsulate the CRISPR/Cas9 components and BMP2 gene and will target the delivery of the system to bone-forming cells. BMP2 clinical use is impeded by fast degradation of in vivo, which limits efficacy to a shorter period of time. In order to address these problems a new nanocarrier mediated, CRISPRCas9 gene editing system targeting BMP2 delivery for bone healing and repair is proposed in this research.

Keywords: BMP2 gene editing, CRISPR/Cas9 technology, Nanocarriers, Bone regeneration, Osteoblasts

INTRODUCTION

The annual impact of bone fractures on millions of people in the world is significant [1]. Fractures of the bone are a common problem and are significantly increasing. as per studies suggested. They can have substantial effects on a person's quality of life. The current treatments for fractures are surgery and the use of bone graft. However, these treatments may be invasive and may not be successful. For the treatment of bone fractures, gene therapy may be more efficient and less invasive. The bone morphogenetic protein 2 (BMP-2) gene, sometimes referred to as the BMP2 gene, is a gene that codes for the protein [2]. BMP-2 belongs to the TGF-β superfamily of proteins, which is responsible for transforming growth factor-beta [3]. The growth factor BMP2 has a fundamental role in bone formation and repair. BMP2 contributes to the proliferation and differentiation of osteoblast cells which are responsible for bone formation. In fact, BMP2 treatment with bone defects may face difficulties due to its rapid degradation and limited duration of use. Consequently, the use of BMP2 in clinical applications has been limited.

A powerful gene editing technology called CRISPR/Cas9, allows scientists to edit DNA in precise ways. CRISPR/Cas9 can be used to deliver BMP2 to bone defects by targeting the delivery of a BMP2 gene to bone-forming cells. This would permit BMP2 to be released continuously and with a controlled release, which could lead to improved bone healing.

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Why is BMP2 gene editing important for bone repair [4]?

Due to BMP2's critical role as a growth factor in all phases of bone healing, BMP2 gene editing is necessary for bone repair [5]. The cells that create new bones, called osteoblasts, are stimulated to proliferate and differentiate from mesenchymal stem cells. Collagen and other extracellular matrix proteins that are necessary for the development of bones are also produced in greater amounts when

BMP2 is present [6]. By using nanocarrier-mediated CRISPR/Cas9 gene editing of BMP2, we will demonstrate how to speed up bone regeneration in a mouse model of critical-sized calvarial lesions [7]. In addition, the scientists demonstrate that the altered BMP2 gene expresses itself for a considerable amount of time at the site of the bone defect, which could potentially be linked to improved bone healing.

Overall, the research shows that CRISPR/Cas9 gene editing of BMP2 via nanocarriers is a novel and promising method for bone healing.

BMP2 gene editing can be used to enhance bone regeneration for the following applications [8]:

- to cure bone abnormalities or non-healing fractures resulting from surgery, illness, or trauma.
- to raise the proportion of successful bone-repair surgeries and other bone grafting.
- to create better scaffolds for bone regeneration and other medical equipment for bone restoration.
- to research and develop novel gene treatments for bone conditions like osteoporosis and osteoarthritis.

Though BMP2 gene editing is still in its infancy, it has the potential to completely change how diseases and abnormalities of the bones are treated [9].

What are nanocarriers?

Drugs, genes, and other therapeutic agents can be delivered to cells and tissues via nanocarriers, which are microscopic particles. Biodegradable materials like metals, polymers, and lipids are commonly used to make them. It is possible to engineer nanocarriers with certain characteristics, like size, shape, surface chemistry, and release kinetics [10].

The CRISPR/Cas9 gene editing tools were delivered to bone cells using lipid nanoparticles [1]. Phospholipid bilayers are the building blocks of lipid nanoparticles, which are tiny, round particles. The CRISPR/Cas9 gene editing tools may be effectively delivered to cells and encapsulated to prevent degradation [10].

To introduce CRISPR/Cas9 gene editing tools to bone cells, various kinds of nanocarriers have been working, such as:

- Nanoparticles of polymers [11]
- Metal nanoparticles [4]
- Viral vectors and exosomes [6, 7]

Implications of CRISPR/Cas9 gene editing of BMP2 using nanocarriers for bone regeneration. Using nanocarriers to alter the BMP2 gene using CRISPR/Cas9 for bone repair has plenty of perks:

- CRISPR/Cas9 gene editing instruments can be effectively delivered to bone cells and shielded from deterioration by nanocarriers [10].
- Osteoblasts and mesenchymal stem cells are two examples of the particular cell types found in the bone that can be targeted via nanocarrier engineering [10].
- It may be possible to optimize the effectiveness and reduce negative effects of CRISPR/Cas9 gene editing tools by designing nanocarriers that release the enzymes at a regulated rate [1].

All things considered, delivering CRISPR/Cas9 gene editing tools to bone cells for bone healing by nanocarriers is a promising new strategy.

Every variety of nanocarrier has benefits and drawbacks of its own. For instance, whereas lipid nanoparticles are easily fabricated and biodegradable, their effectiveness in delivering CRISPR/Cas9 gene editing tools to specific cell types may be compromised. A promising new technique for giving bone cells access to CRISPR/Cas9 gene editing tools is the use of nanocarriers. The effectiveness and safety of CRISPR/Cas9 gene editing for bone healing may be enhanced by these.

How can nanocarriers be used to deliver CRISPR/Cas9 gene editing tools to bone cells?

There are several approaches to use nanocarriers to introduce CRISPR/Cas9 gene editing tools into bone cells. One popular method is to incorporate the CRISPR/Cas9 gene editing instruments inside the nanocarrier. Next, the nanocarrier can be designed to specifically target osteoblasts or mesenchymal stem cells, as there are two types of cells found in bones [6]. Upon reaching the intended cell, the nanocarrier can discharge the CRISPR/Cas9 gene editing instruments into the cell's cytoplasm.

Adding the CRISPR/Cas9 gene editing instruments to the nanocarrier's surface is an additional strategy. An assortment of chemical linkers can be used for this. Afterwards, the nanocarrier can be designed to specifically target different bone cell types.

Lipid nanoparticles will be employed to introduce CRISPR/Cas9 gene editing instruments into bone cells. Phospholipid bilayers are the building blocks of lipid nanoparticles, which are tiny, round particles. The CRISPR/Cas9 gene editing tools may be effectively delivered to cells and encapsulated to prevent degradation [7].

Getting nanocarriers to target particular cell types in the bone can be a challenge as well. The last possibility is that nanocarriers might cause the body to mount an immunological reaction [14].

Notwithstanding these obstacles, CRISPR/Cas9 gene editing instruments for bone mending can be delivered to bone cells using nanocarriers, which is a fascinating novel strategy [8]. To overcome these obstacles and create enhanced CRISPR/Cas9 gene editing systems mediated by nanocarriers for bone regeneration, researchers are shifting in this direction.

CRISPR/Cas9 gene editing tools can also be delivered to bone cells using other kinds of nanocarriers, such as:

- Narrow particles of polymers
- Nanoparticles of metal
- Exosomes Vectors of viruses

Every variety of nanocarrier has benefits and drawbacks of its own. For instance, whereas lipid nanoparticles are easily fabricated and biodegradable, their effectiveness in delivering CRISPR/Cas9 gene editing tools to specific cell types may be compromised.

What are the advantages of using nanocarriers for CRISPR/Cas9 gene editing of BMP2 for bone repair [4]?

Several benefits of BMP2 gene editing using CRISPR/Cas9 for bone repair:

1. *Improved delivery effectiveness:* Compared to alternative distribution techniques like naked DNA or viral vectors, nanocarriers can shield CRISPR/Cas9 gene editing instruments from deterioration and deliver them to bone cells more effectively [9].
2. *Deliveries that are specifically targeted:* Mesenchymal stem cells and osteoblasts are two examples of the cell types that can be specifically targeted by nanocarriers. This can optimise the effectiveness of gene editing and reduce the possibility of adverse effects [12].
3. *Controlled release:* CRISPR/Cas9 gene editing instruments can be released at a predetermined pace via nanocarrier design. This may contribute to the achievement of long-lasting therapeutic benefits and an extension of the gene editing process [13].

LITERATURE REVIEW

Nanotechnology in Bone Regeneration

Nanotechnologies have become a valuable tool for increasing the bioavailability of therapeutic agents, e.g. BMP2, to particular sites of action. Nanoparticles, in order to improve their therapeutic efficiency, can protect BMP2 from degradation and prolonging its release while facilitating targeted delivery of the bone forming cells [8, 14]. In animal models, studies have shown that in comparison to free BMP2 administration nanoparticles containing BMP2 significantly enhance bone healing and repair [9, 14].

CRISPR/Cas9 Technology in Bone Regeneration:

In various areas of medicine, including bone regeneration, CRISPR Cas9, a revolutionary gene editing technology, has gained widespread acceptance. CRISPRCas9 enables a precise modification of genes that allows BMP2 to be targeted for bone formation cells [8]. The prospect of promoting bone regeneration and repair is offered by this approach. In animal models of broken bones, studies have shown that overexpression of BMP2 by CRISPRCas9 is associated with increased bone regeneration [2]. Therefore, a potential therapeutic strategy for bone fractures could be delivery of CRISPRCas9 via BMP2 mediated pathways.

Nanocarrier-mediated CRISPR/Cas9 Gene Editing of BMP2 for Bone Repair

Combining the benefits of nanotechnology and CRISPR/Cas9 technology, nanocarrier-mediated CRISPR/Cas9 gene editing of BMP2 offers a promising approach to enhance bone healing and repair. In order to facilitate their targeted delivery into bone defects, nanoparticles may contain CRISPR and Cas9 components and BMP2 gene [6, 10]. The targeted delivery can increase the local BMP2 concentration leading to increased differentiation of osteoblasts, proliferation and matrix synthesis thus promoting bone regeneration.

Proposed Objectives

The research proposal focuses on investigating the potential of nanocarrier mediated CRISPRCas9 gene editing to improve bone regeneration, in particular for Bone Morphogenetic Protein 2 (BMP 2) Using CRISPR andCas9 technologies via nanocarriers may offer targeted, efficient modification of BMP2, which has a major role in bone formation and regeneration.

i. To obtain an extensive understanding of BMP2 as a bone repair agent, the present challenges and recent advances in CRISPRCas9 gene editing techniques, it is necessary to carry out a thorough literature review.
ii. To assess whether certain nanocarriers may be able to deliver the CRISPR Cas9 components in bone cells, such as polymers, metals and viral vectors. The advantages and disadvantages of each type of nanocarrier shall be assessed.
iii. Design CRISPR/Cas9 to specifically target BMP2 genes. Optimise the gene editing system for efficient and precise modification, while minimising unwanted effects.
iv. Utilise cell culture models to assess the effectiveness of nanocarrier-mediated CRISPR/Cas9 in modifying BMP2 genes within bone cells. Assess the effects on BMP2 expression and subsequent osteogenic differentiation.
v. To study the effectiveness and safety of a new nanocarrier mediated CRISPRCas9 system, using animal models in vivo. Assess bone regeneration and in the host tissue integration of edited cells.
vi. To ensure that the selected nanocarriers and CRISPR Cas9 system are safe in bone cells and surrounding tissues, perform a thorough biocompatibility and toxicity study.
vii. Determine the functional efficacy of BMP2 gene editing to repair bone tissues, including biomechanical characteristics, bone density and histological analyses.
viii. Ensure that the research is conducted in accordance with ethical standards and guidelines. In vitro and in vivo gene editing experiments need to obtain necessary ethical approvals.
ix. Use appropriate statistics to analyse the results obtained. Assess the outcomes to arrive at important conclusions concerning the effectiveness and safety of nanocarrier mediated CRISPRCas9 gene editing for BMP2 in bone repair.

In order to achieve these objectives, this research is aimed at contributing valuable insights on the potential of nanocarriers and CRISPRCas9 gene editing as a new strategy for increasing bone repair with specific focus on BMP2.

METHODOLOGY

Nanoparticle Synthesis and Characterization

1. Design and synthesis nanoparticles capable of encapsulating the CRISPR/Cas9 components and BMP2 gene, ensuring their stability and protection during delivery. Employ biodegradable materials, such as poly(lactic-co-glycolic acid) (PLGA), for nanoparticle fabrication.
2. Characterize the physical and chemical properties of the nanoparticles using various techniques, including:
    a. *Size and Zeta Potential Analysis:* Determine the size and surface charge of the nanoparticles using dynamic light scattering (DLS) and zeta potential measurements.
    b. *Morphological Analysis:* Evaluate the morphology and structure of the nanoparticles using transmission electron microscopy (TEM).
    c. *Encapsulation Efficiency:* Assess the encapsulation efficiency of the CRISPR/Cas9 components and BMP2 gene within the nanoparticles using appropriate analytical methods, such as HPLC or qPCR.
3. *Functionalization with Targeting Ligands:* Conjugate targeting ligands, such as antibodies or peptides, to the surface of the nanoparticles to specifically deliver the gene editing machinery and BMP2 to bone-forming cells in bone defects. Validate targeting efficiency using in vitro cell culture experiments.

Vector Construction

1. Construct a lentiviral vector containing the CRISPR/Cas9 components and BMP2 gene under the control of a bone-specific promoter, such as osteocalcin promoter.
2. Verify the correct sequence and integrity of the vector using restriction enzyme digestion and Sanger sequencing.
3. Amplify the vector using high-fidelity PCR to generate sufficient DNA for transfection.
4. To achieve targeted delivery of CRISPR/Cas9 components and BMP2 gene to bone-forming cells, a lentiviral vector will be engineered with the following features:
    i. *Bone-Specific Promoter:* To ensure targeted expression of BMP2 gene in bone-forming cells, a bone-specific promoter, such as osteocalcin promoter, will be incorporated upstream of the BMP2 gene. This will restrict BMP2 expression to osteoblasts and mesenchymal stem cells (MSCs), the primary bone-forming cells, minimising potential off-target effects.
    ii. *Tissue-Penetrating Peptide (TPP) Conjugation:* For enhanced cell permeability and intracellular delivery, a tissue-penetrating peptide (TPP), such as L-arginine-rich TPP (R8), will be conjugated to the surface of the nanoparticles. TPPs have been shown to improve the penetration of nanoparticles into tissues, facilitating the delivery of therapeutic agents to target cells.
    iii. *Targeting Ligands:* To further enhance targeted delivery to bone-forming cells, targeting ligands, such as antibodies or peptides, will be conjugated to the surface of the nanoparticles. These ligands will specifically bind to cell surface receptors expressed on osteoblasts and MSCs, promoting nanoparticle uptake and intracellular delivery.

Vector Diagram

LTR - Promoter - CRISPR/Cas9 - BMP2 Gene - Polyadenylation Signal - LTR

- *LTRs:* Long terminal repeats, essential for lentiviral vector integration into the host cell genome.
- *Promoter:* Osteocalcin promoter, driving bone-specific expression of BMP2 gene.
- *CRISPR/Cas9:* CRISPR/Cas9 components, enabling gene editing of BMP2.
- *BMP2 Gene:* Bone morphogenetic protein 2 gene, promoting bone formation and regeneration.
- *Polyadenylation Signal:* Signals for polyadenylation, stabilising mRNA transcripts (Table 1).

Table 1. Vector components and their functions.

Component	Function
LTR	Lentiviral vector integration
Osteocalcin Promoter	Bone-specific BMP2 expression
CRISPR/Cas9	Gene editing of BMP2

BMP2 Gene	Bone formation and regeneration
Polyadenylation Signal	mRNA transcript stability
TPP	Enhanced cell permeability and intracellular delivery
Targeting Ligands	Specific binding to bone-forming cells

Plasmid and Gene

The engineered lentiviral vector will be cloned into a bacterial plasmid for propagation and amplification. The plasmid will contain the entire vector sequence, including the LTRs, promoter, CRISPR/Cas9 components, BMP2 gene, polyadenylation signal, TPP, and targeting ligands. The BMP2 gene will be the target gene for CRISPR/Cas9-mediated editing.

Cell Culture and Transfection

1. Culture osteoblasts, mesenchymal stem cells (MSCs), and macrophages in appropriate culture media and conditions.
2. Transfect osteoblasts and MSCs with the lentiviral vector encoding CRISPR/Cas9 components and BMP2 gene.
3. Evaluate transfection efficiency and gene expression using reporter gene assays and qPCR.

Nanoparticle Loading and Delivery

1. Encapsulate the lentiviral vector encoding CRISPR/Cas9 components and BMP2 gene into the functionalized nanoparticles.
2. Optimise nanoparticle loading conditions to maximise gene delivery efficiency while minimising cytotoxicity.
3. Deliver the nanoparticle-encapsulated vector to bone defects in animal models using appropriate injection methods.

In Vivo Evaluation

1. Select an appropriate animal model, such as mice or rats, for in vivo evaluation of the nanocarrier-mediated CRISPR/Cas9 system for BMP2 delivery in bone defects.
2. Create standardised bone defects in the animal model using surgical procedures to induce bone healing and regeneration.
3. Administer the nanoparticle-encapsulated vector to the bone defects using appropriate injection methods.
4. Evaluate bone healing and regeneration process using radiographic, histological, and biomechanical methods:
    a. *Radiographic Evaluation:* Assess bone healing progress using radiography at different time points to observe bone formation and remodelling.
    b. *Histological Evaluation:* Analyse bone tissue regeneration at the defect site using histological staining techniques, such as H&E and Masson's trichrome staining, to evaluate bone morphology, cell infiltration, and matrix deposition.
    c. *Biomechanical Evaluation:* Assess the biomechanical strength of the regenerated bone using biomechanical testing methods, such as four-point bending or push-to-failure tests.

CONCLUSION

The utilisation of a lentiviral vector with a bone-specific promoter, TPP conjugation, and targeting ligands enhances the targeted delivery of CRISPR/Cas9 components and the BMP2 gene to bone-forming cells, resulting in significant therapeutic benefits. This approach promotes improved bone healing and regeneration in bone defects by enabling sustained BMP2 expression at the injury site, thereby reducing the need for repeated administration. Enhanced bone formation and mineralization occur as BMP2 induces the differentiation of mesenchymal stem cells (MSCs) into osteoblasts, leading to stronger, denser bones with superior biomechanical properties. The use of a bone-specific promoter and targeted delivery system minimizes off-target effects and systemic toxicity, ensuring BMP2 is

expressed only in bone-forming cells. This specificity reduces the likelihood of unintended genetic modifications in non-target tissues, thereby increasing the safety and efficacy of the treatment. Collectively, this strategy represents a promising advancement in bone regeneration therapies, addressing limitations of current approaches and offering a safer, more effective solution for treating bone defects and fractures.

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