RNAi Delivery

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Bone-related injury and disease constitute a significant global burden both socially and economically. Current treatments have many limitations and thus the development of new approaches for bone-related conditions is imperative. Gene therapy is an emerging approach for effective bone repair and regeneration, with notable interest in the use of RNA interference (RNAi) systems to regulate gene expression in the bone microenvironment. Calcium phosphate nanoparticles represent promising materials for use as non-viral vectors for gene therapy in bone tissue engineering applications due to their many favorable properties, including biocompatibility, osteoinductivity, osteoconductivity, and strong affinity for binding to nucleic acids. However, low transfection rates present a significant barrier to their clinical use. This article reviews the benefits of calcium phosphate nanoparticles for RNAi delivery and highlights the role of surface functionalization in increasing calcium phosphate nanoparticles stability, improving cellular uptake and increasing transfection efficiency. Currently, the underlying mechanistic principles relating to these systems and their interplay during in vivo bone formation is not wholly understood. Furthermore, the optimal microRNA targets for particular bone tissue regeneration applications are still unclear. Therefore, further research is required in order to achieve the optimal calcium phosphate nanoparticles-based systems for RNAi delivery for bone tissue regeneration.

bone tissue engineering | calcium phosphates | gene therapy | nanoparticles | non-viral vectors | RNA interference | surface functionalization

1. Introduction

Despite bone's intrinsic ability to repair itself without scarring, 5–10% of all bone fractures result in delayed or non-union fractures [1], causing chronic pain for patients. This impacts significantly on patient quality of life and places a significant burden on the health system with the cost of treating non-union fractures in the USA expected to rise from $19 billion (2005) per annum to $25 billion by 2025 [2]. Autologous bone grafts, the "gold standard" currently employed to treat delayed or non-union fractures, exhibit a high incidence of failure and numerous limitations, including donor site morbidity, lack of tissue availability, and invasive surgery [3]. Similar drawbacks, such as unfavorable immune responses, rejection rates and lack of graft availability, are found in the use of allografts and xenografts, whereas synthetic bone graft substitutes often lack biocompatibility and osteogenic potential [4]. The combination of protein therapy with synthetic bone grafts has demonstrated promising early results. However, the low retention rate and high concentration of protein required to obtain a biological effect is a cause for concern [5]. Consequently, there is an increasing need for and interest in the development of new, effective therapies for bone regeneration.

2. Calcium Phosphate Nanoparticles-Based Systems for RNAi Delivery

Gene therapy is considered as the latest approach for the repair of challenging bone defects-delivering exogenous deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) to obtain controlled and sustained protein expression at the fracture site [6]. In particular over the last two decades, the important role played by RNA interference (RNAi) mediated gene repression/silencing in bone metabolism through the regulation of the proliferation, differentiation and function of bone cells has been recognized and RNA-based therapies have shown promise for bone regeneration [7]. In contrast to DNA-based technologies, RNA-based approaches offer the advantage of utilizing a cell's own internal machinery to alter the gene expression. Furthermore, as RNA-based therapeutics do not require nuclear entry, these molecules completely avoid the risk of insertional mutagenesis and therefore present a safer and more viable alternative [8]. However, the delivery of RNA is often hampered by its susceptibility to degradative enzymes, which present a significant limitation to its use. [8],[9]. Furthermore, its poor capacity to penetrate the host cell membrane and selectively distribute to the desired tissues or cells within the body presents a significant barrier to clinical translation [7]. Thus, the establishment of a carrier that would increase protection, intracellular release and expression of genetic material is imperative. Calcium phosphate nanoparticles hold particular potential in this regard for bone-related conditions as they have a strong affinity for binding to nucleic acids [10][11]. They are also well-accepted by the body and have a significant surface-to-volume ratio that allows for a higher driving force for diffusion, increased particle solubility and adhesion to specific proteins [12]. Furthermore, calcium phosphate-based systems are osteoconductive, osteoinductive, and the majority are considered bioresorbable [13]. This review provides a synopsis of the current state-of-the-art relating to the design of calcium phosphate nanoparticles as non-viral vectors and their application in RNA-based therapy for bone tissue regeneration. The potential for surface functionalization methods to improve the stability, transfection and safety of calcium phosphate will also be discussed.

Recently, the application of gene therapy for bone tissue regeneration has attracted significant attention, offering the possibility to guide cellular fate without the administration of high quantities of drugs or protein. The use of calcium phosphate nanoparticles has been shown to facilitate the delivery of miRNAs and siRNAs, through the formation of stable complexes that can efficiently be endocytosed by targeted cells, allowing for the cytoplasmic release of miRNAs and siRNAs. This feature, in tandem with the natural biocompatibility of calcium phosphate and its promotion of bone mineralization, highlights the potential use of calcium phosphate nanoparticles as non-viral vectors for the treatment of a wide variety of bone diseases. However, reduced transfection efficacy of calcium phosphate nanoparticles remains a significant barrier to their use in clinical applications. For increased therapeutic response and transfection efficacy, calcium phosphate nanoparticles must demonstrate long-term stability under physiological conditions, reduced agglomerate formation, a mean particle size ≤200 nm and positive zeta potential for better interaction with the cell membrane. Surface functionalization of calcium phosphate nanoparticles offers the potential to overcome transfection limitations through surface modification of the nanoparticle to achieve the desired physical properties. The overall goal of these surface functionalization techniques is to maximize transfection efficiency by striking the optimal balance between protection of the genetic cargo and efficient release. Of the approaches discussed here PEG-ylation has been shown to offer improved CaP is more stable due to the

colloidal stability of PEG, while generally cationic liposome coating methods result in an improved transfection efficiency due to the cationic characteristics of the lipids. Novel approaches have investigated the possibility of combining more than one functionalization method, e.g., an initial surface modification process to ensure calcium phosphate stabilization and then a further surface treatment method to improve intracellular endosomal escape. The majority of research has been directed towards the stabilization of calcium phosphate-RNAi nanoparticles for applications within the fields of cancer and bioimaging. However, the concepts proven for such applications also have potential for bone tissue regeneration applications, since both the bonding interactions between the calcium phosphate and RNAi and the molecular interactions between the functionalized calcium phosphate surface and the cell membrane would be similar. Specifically, the application of CPP-based systems to improve calcium phosphate transfection efficiency seems extremely promising-though the underlying mechanisms of the peptide-cell interaction are not yet well understood and require further investigation.

Finally, limited clinical translation of calcium phosphate-based gene delivery is primarily due to the lack of understanding of their behavior in vivo. As bone-related disease and bone injuries are generally not life-threatening conditions, risk-benefit concerns relating to the use of gene therapy in the treatment of these conditions requires consideration. A deeper understanding of the mechanistic principles of the in vivo bone formation process is required, especially concerning the role of miRNAs and their interplay during bone physiology. Furthermore, in order to design efficacious and clinically relevant therapies, a greater understanding of the role and target of each miRNA is required, along with identification of the optimal time frame for therapeutic delivery.

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