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Bone Targeting Systems to Systemically Deliver Therapeutics to Bone Fractures for Accelerated Healing

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Abstract

Purpose of Review—Compared to the current standard of implanting bone anabolics for fracture repair, bone fracture targeted anabolics would be more effective, less invasive, less toxic and would allow for control over what phase of fracture healing is being affected. We therefore sought to identify the optimal bone targeting molecule to allow for systemic administration of therapeutics to bone fractures.

Recent Findings—We found that many bone targeting molecules exist, but most have been developed for the treatment of bone cancers, osteomyelitis, or osteoporosis. There are a few examples of bone targeting ligands that have been developed for bone fractures that are selective for the bone fracture over the body and skeleton.

Summary—Acidic oligopeptides have the ideal half-life, toxicity profile, and selectivity for a bone fracture targeting ligand and are the most developed and promising of these bone fracture targeting ligands. However, many other promising ligands have been developed that could be used for bone fractures.

Keywords

Bone fracture targeting; Systemic administration; Bone fracture drugs; Acidic oligopeptides; Bisphosphonates; Drug targeting

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Compliance with Ethical Standards

Conflict of Interest

Jeffery Nielsen reports having patents pending.

Dr. Stewart Low reports that he is an employee of Novosteo, which works on targeting bone anabolics to fractures. This is primarily why he understands the field in such depth, qualifying him to discuss the topic.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Introduction

The 18.3 million bone fractures treated annually in the US represent one of the greatest cost to the US healthcare system (176.1 billion dollars annually)(1) and result in over 216.5 million days of work lost. Patients with comorbidities (e.g., diabetes or osteoporosis) are up to six times as likely to experience fractures and are also likely to experience twice as slow healing rates.(2–5) Patients over 65 who break hips have a 33% chance of dying within a year from complications associated with fracture-induced immobility.(6) For patients over 85, these rates can reach as high as 65%.(1) Those who survive often never walk unaided. Common morbidities associated with months of such immobility result in heart attacks, strokes, embolisms, pressure ulcers, muscle and bone loss, increased risk of infection, depression, and wage loss.(7)

Despite these ramifications and the dramatic need to accelerate bone fracture repair to minimize associated morbidities and mortality, most fracture treatments still consist solely of stabilization and opioid pain treatment. (7,8) Little has been done to improve fracture healing from a pharmacological standpoint.

Systemically administered anabolics show promise for accelerating bone healing but would benefit from targeting systemic administration. Such targeting can be accomplished using compounds that home to the hydroxyapatite in bone that is exposed to the bloodstream when bone is damaged. To aid other researchers in identifying which of these targeting compounds are best suited to treat various bone conditions, this review analyzes which bone-targeting molecules have been applied to treat various forms of bone damage in recent years.

Current Bone Fracture Therapeutics

Efforts have been made to improve fracture repair by augmenting the differentiation and activity of osteoblasts. Currently, the only clinically approved (US) pharmaceutical interventions are BMP2 and BMP7(9), which have been approved for open tibia trauma. BMPs activate BMP receptors, which signal through the SMAD pathway to elicit their osteogenic effects on mesenchymal stem cells (MSCs) and osteoblasts.(10) Their use is relatively limited because of ectopic bone growth side effects caused by leakage from the administration site (11), cancer risks, and invasiveness of drug administration.(12)

Many researchers have ramified the clinical success of BMP fracture repair, elucidating interesting biological and chemical factors that promote MSC differentiation, angiogenesis, collagen production, and mineralization needed to reach the injury site to elicit their effects there.(13) Most investigative strategies have looked to deliver therapeutics to the injury site in the form of a drug-eluting hydrogel,(14) drug-impregnated cement,(15) or as part of biocompatible scaffolds.(16) Many such strategies have potential to improve on some of the negative side effects produced with BMP administration. However, each relies on an invasive application at the fracture site during surgery. Surgery is highly invasive and can have poor outcomes in geriatric patients.(17) Furthermore, it is difficult to maintain a pharmacological effect from a single drug dose, and doing so often requires using a huge bolus of drug during its single administration. Additionally, there is no temporal control over which fracture

healing phase is affected. These therapeutics are therefore too invasive and insufficiently effective as fracture repair therapies.

Systemic Anabolics Currently in Use or Being Tested

Systemically administrable anabolic agents represent a more promising step in the right direction toward accelerated bone healing, but they still present challenges when it comes to treating non-systemic conditions, such as fractures. On the one hand, such anabolics allow physicians to control how long a drug is delivered, as well as which phase of fracture repair it affects. They also improve upon the single bolus by allowing for multiple smaller injections. Several systemically administered anabolics such as teriparatide, abaloparatide, and antisclerostin antibodies (e.g., romosozumab) are quite successful in treating osteoporosis.(18) However, several attempts have been made to bring teriparatide to the clinic for fracture repair, resulting in conflicting studies(19–22). Although systemically administrable anabolics safely improve bone density in osteoporosis, achieving a local fracture concentration capable of improving repair would be blunted by dose-limiting toxicities (such as hypercalcemia(12)). Other anabolics, if not locally implanted like BMP2, lead to possible ectopic mineralization.(23) Many of the biochemical pathways involved in tissue repair are shared between tissues, and anabolics involved in bone repair will affect other tissues as well. Therefore, a targeting platform is needed to allow these potent anabolics to be selectively delivered directly—and exclusively—to the fracture.

Targeting Systemic Administration

Such a systemically administered drug that achieves anabolic concentrations at the fracture site without reaching side-effect-inducing blood concentrations can be achieved by the means of targeting. Targeting a bone anabolic agent concentrates it at the fracture site, thus resulting in the following benefits: fewer side effects, noninvasive administration, reduced ectopic bone formation from anabolic leakage, an expanded range of fractures that can benefit from its use. Attempts to achieve such bone targeting has revolved primarily around targeting the crystallin component of bone hydroxyapatite (HA), which makes up more than 70% of the bone.(24) Though HA remains unexposed in unfractured bone, a fracture reveals the HA, exposing it to the bloodstream. With this exposure, a targeting system can be designed to localize to increased-capillary-supply regions where bone is forming, resorbing, or damaged. This targeting allows the treatment to localize according to the needs of different orthopedic indications. In addition, different HA-binding ligands have preferences for different exposed HA surfaces relative to the others to allow skeletal selectivity. A variety of such compounds have been investigated for their ability to localize therapeutics to damaged HA to treat orthopedic diseases. The remainder of this review compares these compounds in order to elucidate instances in which each might prove useful for a pharmaceutical scientist.

Bisphosphonates

Due to their high affinity for HA, bisphosphonates are likely the most utilized drug in the toolbox for homing molecules to bone to treat bone disease. They have been around since the 1960s, and a number of bisphosphonate products are currently available. These

molecules were originally developed as water softeners that could pull the calcium out of hard water. Their high affinity for HA inhibits farnesyl pyrophosphate (FFPS) synthase in OC to shut down bone turnover. Their structure is derived from a stabilized pyrophosphate. Pyrophosphate naturally regulates bone mineralization with an unstable, rapidly-cleaved anhydride bond. The carbon-phosphate part of bone is very stable, giving bisphosphonates up to 20 years' residence time in the bone.(54) The deprotonated hydroxyls in the two phosphates that are in a bisphosphonate molecule are separated by approximately 2.9 to 3.1 Å.(55) This is similar to the native spacing of oxygen atoms in HA. The hydroxyl group that extends from the geminal bisphosphonate carbon also interacts with HA and further provides affinity to the bone.(55) The other R group (see table 1), usually a nitrogen-containing carbon chain or aromatic ring, influences the molecule's ability to inhibit FFPS and thus determines its potency and contribute to its calcium binding. Bisphosphonates have been used extensively to target treatments for cancer, osteomyelitis, osteoporosis, dental diseases, and other mineral-based diseases. Recently, one of the first bisphosphonate-based therapeutics completed a successful phase I clinical trial for delivering cytarabine to cancer-induced bone lesions. The safety profile has improved upon targeting, and over half the bone lesions treated had reduced activity.(29) Other therapeutics have been developed to deliver a bortezomib bisphosphonate conjugate with a special boron linker that will not cleave in the bloodstream to treat multiple myeloma.(28) The bisphosphonate targeted bortezomib was twice as effective as the untargeted bortezomib at reducing the tumor burden in multiple myeloma, and the targeted form prevented most of the side effects from bortezomib including paralysis and loss of platelets. A special effort was made to use a bisphosphonate that would not prevent resorption via FFPS inhibition once the bortezomib was released. However, the pyrophosphate-like structure of bisphosphonates has proven to inhibit mineralization without any interaction with osteoclasts.(56) It remains unknown whether these non-active bisphosphonates would be effective for targeting bone fractures as they might inhibit mineralization, thus disrupting the basic bone unit in the long run.

Bisphosphonates have also been used to deliver radionuclides such as ^{177}Lu for a targeted radiotherapy of bone metastasis. The bisphosphonates have allowed for highly-selective delivery of radionuclides to the metastasis, thus minimizing the soft tissue damage during radiotherapy.(30,57) Other work has focused on delivering nanoparticles and micelles with chemotherapeutics to bone cancers.(58–62) Bisphosphonates have proven effective at improving the pharmacokinetics of chemotherapeutics and a number of antibiotics in treating osteomyelitis.(63–65)

More relevant to fracture treatment is using bisphosphonates to target anabolics for osteoporosis.(66) Young et al. have worked on using bisphosphonates to deliver prostaglandin E2 agonists via a releasable ester linkage for the treatment of osteoporosis. Upon cleavage and hydrolysis, the bone conjugate works to deliver both an antiresorptive bisphosphonate and an anabolic prostaglandin EP2 agonist.(31,67,68) This combination completely restored the bone lost due to ovariectomy and improved the overall mechanical properties of the bone. The targeted combination therapy was better than its constituents delivered simultaneously but not tethered together, giving credence to the power of bone targeting.(69) The group found that identifying the right linkage is critical to the success of

these conjugates. Other osteoporosis treatments have focused on localizing estrogens(70) or other anabolics to the bone.(27,71)

Bisphosphonates have also been used to deliver anabolics for the treatment of periodontal disease.(72,73) With limited success, some studies have even attempted to use bisphosphonates to localize proteins and cells to bone surfaces.(74,75) Though bisphosphonates are effective tools for localizing compounds of various sizes to HA, they have also been shown to lead to osteonecrosis of the jaw (BRONJ), atypical sub-trochanteric fractures in the femur, and esophageal cancer in fairly high frequencies.(25,76) Though arguments can be made that concentrations of BP for targeting are far lower than concentrations that induce side effects, the association with BRONJ has prevented most bisphosphonate-based drugs from fully developing(77). Most research today in this field focuses on non-active bisphosphonates as targeting ligands or dual-action targeting compounds where the skeletal activity of the bisphosphonate is desired.

Tetracycline

Tetracycline is a broad-spectrum antibiotic that blocks protein synthesis in bacteria. It is originally derived from the bacterial genus *streptomyces* and has been used as a therapeutic agent for decades.(78,79) Its use has drastically declined due to its unexpected skeletal side effects of discoloring bones and teeth and preventing normal tooth development in children, because of its affinity for calcium. Tetracycline should therefore be taken without milk in order to prevent it from having reduced bioavailability resulting from its binding to calcium ions.(80) These same properties that result in skeletal side effects enable its use as a biomarker for dynamic histomorphometry measurements in bone, as it emits fluorescence at 390 nM. The β -diketone system at positions 1 and 2, the enol system at positions 4 and 6, and the carboxamide group at position 5 are responsible for the chelating behavior.(55) Additionally, the chelation of tetracycline is permanent, meaning the unwanted side effects are also permanent.(81)

The ability to bind and label primarily growing bone have led people to localize anabolics to the bone surface by conjugating them to tetracycline and tetracycline-derived analogs. Tetracyclines have been used to create bone-targeted PLGA nanoparticle systems to deliver the drug simvastatin to osteoporotic bone with the localization only minimal success.(33) The tetracycline nanoparticles primarily localized to the liver and spleen with less than 3% accumulating in the bone, which moderately improved the effects of simvastatin on ovariectomized rats. There are concerns with the toxicities associated with tetracycline in that its presence in the body leads not only to undesirable skeletal effects, but also several organ toxicities. Tetracyclines have been shown to decrease collagenase levels and osteoblast activity levels,(80) which has led some groups to make attempts at modifying the structure of tetracycline to reduce some of its organ toxicities. Neale et al. found that they could retain 50% of the full tetracycline bone-binding ability with just one of the rings and use it to localize estrogen for the treatment of osteoporosis which was able to separate the skeletal effects of estrogen from its uterine effects thus improving its safety profile.(34) Tetracycline preferentially binds to areas of low crystallinity where bone is forming, rather than to resorption surfaces.(82) This and its oral bioavailability make it attractive as a targeting

ligand. However, its toxicity and skeletal side effects have prevented it from being fully developed. The osteoblastic toxicities associated with tetracyclines and the strong affinity towards growing bone make it a far better targeting ligand for osteosarcoma and bone metastases over the previous work in osteoporosis. Similarly, tetracyclines could target the growing bone on of bone fractures but it would be important to use a modified version such as described in Neale et al.

Acidic Oligopeptides

Acidic oligopeptides are the means by which nature homes protein to bone. Proteins such as bone sialoprotein and osteopontin have highly acidic stretches of their sequences in which up to 22% of their sequences are glutamic acid.(83) These proteins serve a role in localizing to bone, nucleating HA, and mineralizing collagen.(84) These amino acids contain side chains with carboxylic acid that are believed to chelate the calcium component of the bone. (55,85) Affinity for the hydroxyapatite in the bone increases as the number of repeating units of aspartic acid or glutamic acid increase. Typically, affinity towards bone plateaus around 6–8 repeated units.(38) However, longer chains improve the amount of compound delivered and retained *in vivo*.(40,41) The interaction between acidic oligopeptides and HA is a non-chiral interaction as L and D enantiomers of amino acids have similar affinities to HA. However, *in vivo*, the D enantiomer degrades more slowly, leading to greater accumulation. (38,41,86,87) Acidic oligopeptides are very attractive as targeting ligands for bone due to their lack of obvious toxicities, especially compared to bisphosphonates and tetracyclines. (60) Acidic oligopeptides also have more desirable half-lives for treating acute bone disorders than tetracyclines and bisphosphonates, which can have half-lives in the bone for years rather than hours or days for acidic oligopeptides.(76,81,87) Acidic oligopeptides are also tunable and can localize a nanoparticle using as few as three aspartic acid molecules all the way out to much longer polymers to generate the desired half-life.(50)

Acidic oligopeptides have also been used to target therapy for a number of bone-related diseases. The clinically-approved asfotase alfa relies on a chain of 10 aspartic acids to localize tissue-nonspecific alkaline phosphatase to bone for treating hypophosphatasia. Acidic oligopeptides have also been used preclinically to localize antibiotics to treat osteomyelitis,(88) as well as to deliver liposomes and micelles carrying chemotherapy agents for osteosarcomas and bone metastasis.(42,89) They have also been shown to effectively deliver radionucleotides for imaging(41,90) and nanoparticles for photothermally treating bone tumors.(91)

Furthermore, there is evidence that acidic oligopeptides can be used for more than targeting bone infections and the large osteolytic lesions that form in bone cancer. They have been reported to improve treatment in osteoporosis when localizing anabolics such as EPI agonists to bone via acidic oligopeptides.(82) Our group, Low et al., have reported that acidic oligopeptides can be used to localize compounds to bone fractures specifically (Fig. 1).(35) This was a remarkable result because acidic oligopeptides tend to target longer, mature, HA crystals rather than the short growing HA crystals in a fracture. We further reported that these compounds can be used to improve the efficacy and safety of bone anabolics such as 6-BIO.(36) Our group has demonstrated that this platform can localize

small molecules in the absence of a micelle, liposome, or nanoparticle through direct conjugation to the small molecule as with the conjugation to dasatinib. This promising subcutaneously-injected drug was well tolerated and reduced the time for fractured femurs to return to full strength by greater than 50%.(37)

Acidic oligopeptides are a promising potential targeting platform for bone fractures. They have exceptional safety profiles and half-lives for treating acute skeletal damage such as bone fracture, their propensity to adsorb to mature HA does not inhibit their affinity towards fractures, and they themselves are osteogenically inert, allowing for natural healing. Still, they are peptides and can be degraded; therefore, work will need to be done to identify the appropriate enantiomeric configurations and lengths to provide the proper pharmacokinetics for bone fracture repair.

Tartrate-Resistant Acid Phosphate (TRAP)

A newer method that has been used to target bone fractures specially uses tartrate-resistant acid phosphate (TRAP), a protein deposited on bone resorptive surfaces. Benoit et al. have demonstrated that nanoparticles targeted using a peptide that binds to TRAP have increased localization in bone fractures.(92) Though the particles do have a fairly low target-to-background ratio for the spleen and liver, there is about a threefold selectivity for fractured femurs relative to unfractured femurs.(93) Benoit et al. were able to use TRAP to deliver GSK-3 β inhibitors to elicit moderate improvements in density and strength of the bone fractures. TRAP has only moderate bone delivery capacities and will preferentially deliver payloads to resorption pits, not bone formation surfaces such as those that are present in the early stages of bone fractures. Localizing TRAP to the fractures is only slightly better than using untargeted nanoparticles, which preferential localization has been attributed to ELVIS (Extravasation through Leaky Vasculature and Inflammatory cell-mediated Sequestration) mechanism.(94,95)

Pyrophosphate

A promising new targeting technique for bones is that of pyrophosphorylated cholesterol or bone-targeting liposomes, which, in their bone-targeting capacity, are comparable to bisphosphonates. Wang et al. demonstrated that these bone-targeted liposomes could selectively localize to the fracture more than would be predicted by the ELVIS effect. They used them to selectively deliver salvianolic acid A, a potent bone anabolic, to mice in a glucocorticoid-induced delayed fracture healing model.(44) The targeted agent saw significant improvement relative to the untargeted free therapeutic. Though it retained the targeting capacity of a bisphosphonate, the pyrophosphorylated cholesterol had the advantage of a shorter half-life than a bisphosphonate. Pyrophosphate is metabolized into one of the main components of HA and is a safe additive. It is widely used in the food and oral care industry. This helps it keep the best of both worlds: a shorter-lived (like acidic oligopeptides) but higher-affinity (like bisphosphonates) targeting ligand. More work needs to be done to validate this new targeting technology and tune its pharmacokinetics, but it could be useful in delivering therapeutics to fractures.

(DSS)₆

The tripeptide repeat of aspartate-serine-serine (DSS)_n was identified as an important region for HA binding in dentin phosphoprotein (DPP). DPP is one of the main non-collagen proteins found in the dentin extracellular matrix and plays an important role in the nucleation of HA during dentin mineralization. DPP's HA binding region contains a large number of repeats of the sequence Asp-Ser-Ser, which are heavily phosphorylated. It has been shown that unphosphorylated repeats of DSS can be used to localize to HA. The binding does not require the serines to be phosphorylated. Furthermore, replacing the serines with alanines did not diminish the binding affinity, but only affected the maximum monolayer DSS repeat concentration deemed to be optimal. It was shown that increasing the number of repeats proportionally increased the targeting capacity until about 6 repeats.(45) DSS peptide has been shown to preferentially bind to bone-forming surfaces over resorption pits where there is low crystallinity HA.(96) DSS₆ was found to favorably bind to mantle dentin, which consists of small and randomly-oriented crystals, rather than the enamel surface, which consists of elongated and well-oriented HA crystal. These properties make it attractive as a targeting ligand in terms of its abilities to localize to newly growing bone rather than to existing bone.

DSS has been shown to be a successful delivery platform for the treatment of osteoporosis. Saidak et al showed that (DSS)₆ could be used to deliver an osteogenic cyclic peptide ligand for $\alpha_5\beta_1$ integrin. This $\alpha_5\beta_1$ integrin priming stimulates the differentiation of MSC into osteoblasts. The targeting of this compound allows its effect to be constrained to the desired regions and improves long bone mass and microarchitecture.(46) (DSS)₆ has also proven to be effective at localizing liposomes with siRNAs to bone-forming surfaces. Dioleoyl trimethylammonium propane (DOTAP)-based cationic liposomes encapsulate siRNAs and achieve 10x greater delivery than untargeted liposomes. This liposomal system has been able to achieve almost a two-fold greater knockdown in PLEK 01 RNA relative to untargeted liposomes.(97) The system has potential to make siRNA more commonly used in the treatment of bone diseases. However, it does have relatively high liver and kidney uptake. (DSS)₆ does not have the highest affinity relative to bisphosphonates but is biocompatible, nontoxic, and prefers bone-forming surfaces.

VTK and Other Phage-Display Derived Peptides

Phage-display techniques have led to the development of new targeting ligands that are specific to HA or to osteoblasts. One of the most studied of these is VTKHLNQLSQSY (VTK) a 12mer that was identified as having strong and specific affinity toward HA and bone-like material.(47) Surprisingly, it does not contain any aspartic acids or glutamic acids and is positively charged. However, follow-up studies have shown that VTK has dramatically improved the phosphorylation of its serines.(48) VTK-modified biomaterial has been shown to improve osteogenic differentiation of MSCs and biomineral deposition.(49) However, there are reports that VTK can inhibit osteoblast mineralization, which could generate undesired skeletal side effects as a targeting ligand. (50) Between this and its poorly understood adsorption mechanism, VTK requires additional preliminary work before being fully developed as a targeting platform for bone fractures.

Phage-display has been used to identify other HA-specific peptides, such as EHBP7(NNHYLPR), based on its ability to bind enamel.(51) It has been successfully used to localize KSLW (a broad-spectrum antimicrobial) to tooth surfaces to reduce biofilm formation.(98) Not only has phage-display allowed for the development of HA-specific ligands, but it has also been used to develop a number of ligands specific to different components of the bone.(79) Targeting ligands that specifically interact with osteoblasts are of particular interest, because they allow for a more specific stimulation of the desired cells in the bone. Two ligands that have allowed osteoblast-specific interaction are SDSSD and CH6. SDSSD was discovered via phage-display and binds to osteoblasts via periostin.(52) SDSSD has been used to localize nanomicelles encapsulating siRNA and microRNA to osteoblasts. The biodistribution of the compound suggests a broad uptake of the SDSSD targeting ligand in the brain, heart, liver, and lung in addition to the bone. This undesirable localization in other tissues makes SDSSD a poor targeting ligand for treating bone fractures because it could lead to off-target side effects in many tissues. The CH6 aptamer is a single-stranded oligonucleotide modified with 2'-O-methyl-nucleotide that can directly target osteoblasts at the cellular level. It was discovered via aptamer exponential enrichment (cell-SELEX) and has been shown to selectively localize lipid nanoparticles loaded with siRNA to osteoblasts.(53) These aptamer biodistributions are much more specific to the bone than SDSSD, and after 12 hours has most of its accumulation in the bone. It appears to achieve greater localization and therapeutic effects than (DSS)6 does with the same payload. CH6 seems to stimulate micropinocytosis and helps to facilitate lysosomal escape for its payload. However, nucleotide-based aptamers such as CH6 do suffer from nuclease degradation and are expensive to make. Nevertheless, CH6 is a promising osteoblast-specific aptamer that could be used to deliver osteoblast-specific gene therapies.

Conclusions

The above targeting systems have allowed for the development of systemic administration for bone disease treatments, many of which can be or have already been applied to localize compounds specifically to bone fractures. Their cargo has been delivered in the form of targeting ligands linked to nanoparticles, liposomes, and micelles loaded with therapeutic small molecules or siRNA. Additionally, these targeting ligands have been used to directly localize peptides, proteins, or small molecules through releasable or non-releasable conjugation. Overall, the safety of drugs is dramatically improved when the localization of a drug is confined to the desired location. The efficacy of the compound is increased because the concentration at the desired location is amplified relative to the administered systemic dose due to accumulation. Each targeting ligand has its pros and cons but their use has helped to further develop systemic noninvasive medicines for the treatment of bone diseases. This development allows for a noninvasive course of drug administration rather than surgically implanting therapeutics. It also allows for repeated therapeutic administration such that the therapeutic index can be maintained with much less drug and for longer periods of time. This temporal control over when in the bone fracture treatment the compound is delivered allows compounds that affect the different phases of fracture healing to differentially be administered during the ideal time of fracture repair.

Bone fracture targeting is a step in the direction of making more precise medicines and helping to bring pharmacological solutions into the treatment regime of fractures. Further developments need to be made to identify the ideal targeting ligand for bone fractures. There is most likely not a one-size-fits-all solution, because the therapeutic payload significantly influences the biodistribution. In some cases, an aptamer like CH6 might make the most sense for gene therapy, whereas acidic oligopeptides might be appropriate for peptide-based therapeutics due to their ease of synthesis, half-life, and biocompatibility. Future work can also focus on targeting components of bone fractures other than just the mineral aspect. Most targeting platforms have revolved entirely around targeting HA. However, collagen, fibronectin, or even osteoblast targeting could also be developed to localize compounds to bone fractures.(79,99,100). Targeting therapeutics to bone fractures is only half the battle. Once the ideal targeting platforms are identified, choosing the compounds that best exert their anabolic effects when locally delivered from a systemic injection is the next half of the battle. Developing these targeting strategies has great potential to help get people moving faster and to reduce the enormous global burden bone fractures place on humanity.

Abbreviations

6BIO	6-Bromoindirubin-3'-oxime
EP1	Prostaglandin E2 receptor 1
HA	Hydroxy apatite
BMP	Bone morphogenetic proteins
MSC	Mesenchymal stem cells
LIPUS	low-intensity pulsed ultrasound
BRONJ	osteonecrosis of the jaw
FFPS	farnesyl pyrophosphate
TRAP	Tartrate-resistant acid phosphate
ELVIS	Extravasation through Leaky Vasculature and Inflammatory cell-mediated Sequestration

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* of importance

** of high importance

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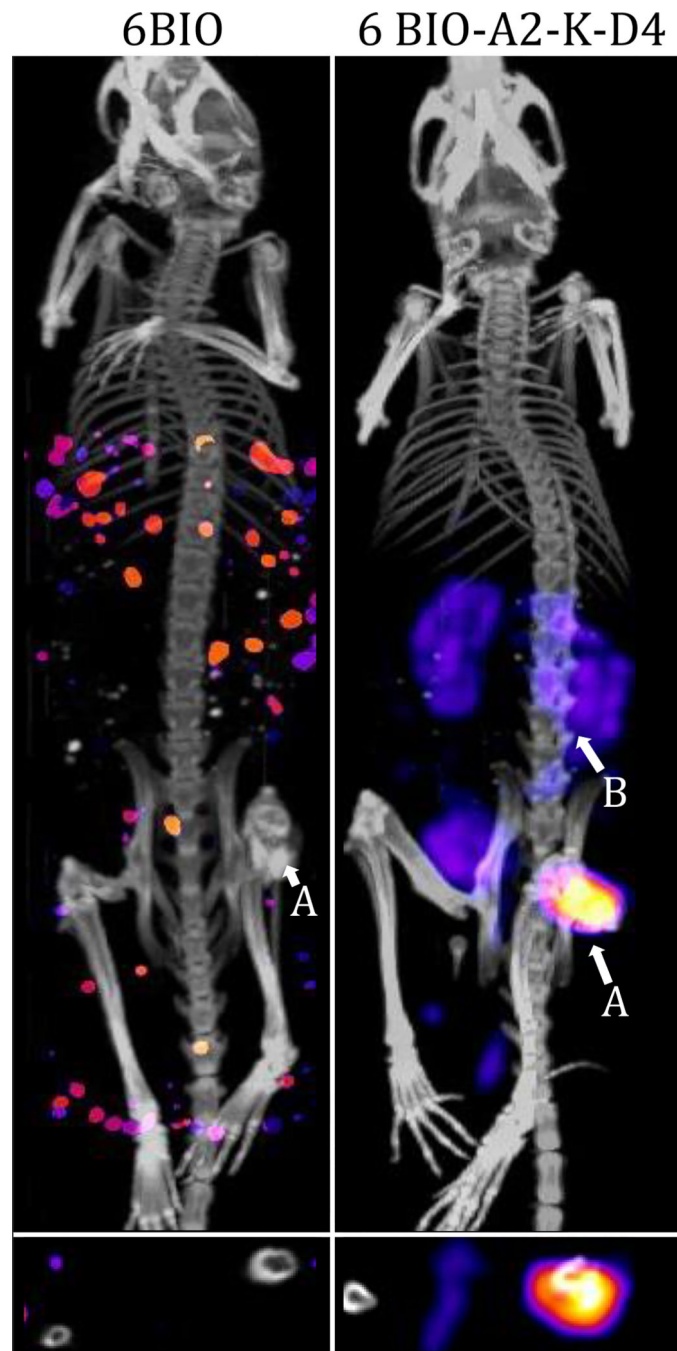
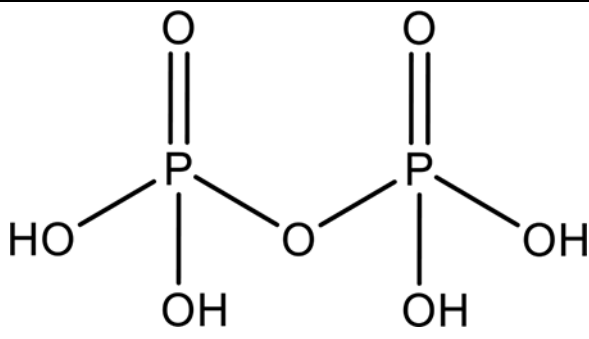
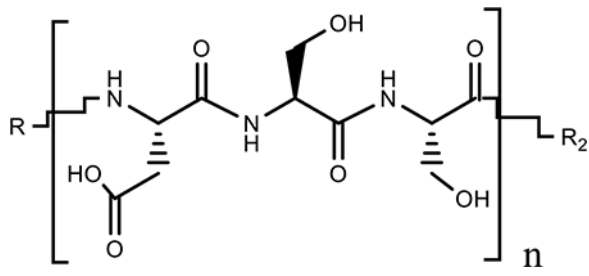
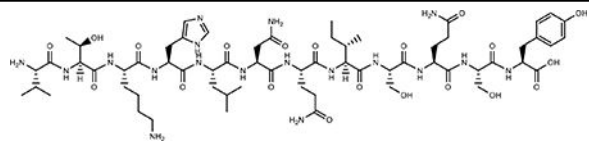
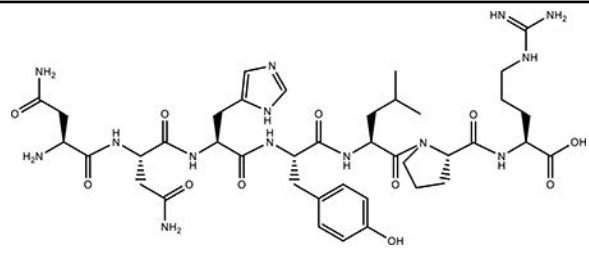
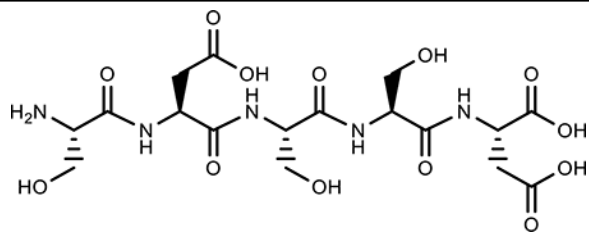


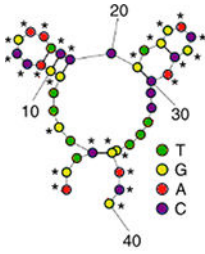
Figure 1. Femoral fracture targeting of ^{125}I labeled 6-BIO vs targeted 6- BIO. Uptake in the kidneys (B) is likely due to amino acid reuptake receptors. Adapted from Low SA, Galliford C V, Yang J, Low PS, City SL, Chemistry P, et al. Biodistribution of fracture-targeted GSK3 β inhibitor-loaded micelles for improved fracture healing. Vol. 16, *Biomacromolecules*. 2015. 3145–3153 p. Copyright (2015) American Chemical Society. Used with permission.

Table 1:

Overview of common bone targeting ligands

Name	Structure	Cons	Pros	Select Citations
Bisphosphonate		Selectivity Half life Skeletal Side effects	Affinity Dual action Targeting for osteoporosis	(25–32)
Tetracycline		Toxicity Poor Selectivity	Binds growing bone	(33,34)
Acidic Oligopeptide		Peptide	Half life Selectivity Tunable Nontoxic	(35–42)
TRAP binding Peptide		Peptide Poor Specificity	Nontoxic	(43)

Name	Structure	Cons	Pros	Select Citations
Pyrophosphoric Acid		Liposomal formulation and leakage 20-day half-life	High affinity Nontoxic	(44)
(DSS)n		Peptide Poor Affinity Poor Specificity	Specific for bone forming surfaces Nontoxic	(11,45,46)
VTK		Peptide Requires Phosphoserines can inhibit osteoblast mineralization	nontoxic	(47-50)
EHBP7		Peptide Enamel specific	Nontoxic	(51)
SDSSD		Peptide Poor Specificity	Osteoblast specific	(52)

Name	Structure	Cons	Pros	Select Citations
CH6	 <p data-bbox="625 514 954 548">* 2'-O-methoxy nucleotides</p>	Nucleotide Expensive	Nontoxic Cell penetrating Good for gene delivery	(53)

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