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REVIEW ARTICLE

Therapeutic approaches to activate the canonical Wnt pathway for bone regeneration

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Abstract

Activation of the canonical Wingless-related integration site (Wnt) pathway has been shown to increase bone formation and therefore has therapeutic potential for use in orthopedic conditions. However, attempts at developing an effective strategy to achieve Wnt activation has been met with several challenges. The inherent hydrophobicity of Wnt ligands makes isolating and purifying the protein difficult. To circumvent these challenges, many have sought to target extracellular inhibitors of the Wnt pathway, such as Wnt signaling pathway inhibitors Sclerostin and Dickkopf-1, or to use small molecules, ions and proteins to increase target Wnt genes. Here, we review systemic and localized bioactive approaches to enhance bone formation or improve bone repair through antibody-based therapeutics, synthetic Wnt surrogates and scaffold doping to target canonical Wnt. We conclude with a brief review of emerging technologies, such as mRNA therapy and Clustered Regularly Interspaced Short Palindromic Repeats technology, which serve as promising approaches for future clinical translation.

KEYWORDS
biomaterials, bone regeneration, canonical Wnt, drug delivery, therapeutics

1 | INTRODUCTION

This article provides an overview of Wingless-related integration site (Wnt) signaling pathways and summarizes therapeutic strategies to activate the canonical Wnt pathway in order to enhance bone formation. We first review traditional and novel molecules, ions, antibodies, and proteins that have been used to activate the Wnt pathway, then we discuss how tissue engineering approaches designed to achieve local delivery may improve both safety and efficacy. Engineered materials such as hydrogels, liposomes, or scaffold doping offer strategies to integrate Wnt-pathway activators to mitigate some of the potential limitations seen with systemic administration.

Three Wnt signaling pathways have been described: the canonical Wnt pathway (β-catenin-dependent) and two non-canonical (β-catenin-independent) pathways, including, the Wnt-Planar Cell Polarity pathway and the Wnt/Ca^{2+} pathway (Hosseini et al., 2019). The canonical Wnt pathway is a highly conserved pathway which plays a central role in tissue development, regeneration and serves as a key anabolic regulator of bone repair and homeostasis (Kirstetter et al., 2006; Komiya & Habas, 2008; Wang & Wynshaw-Boris, 2004). Canonical Wnt signaling modulates cytosolic transcription factor β-catenin to activate genes involved in osteoblast differentiation and bone matrix maintenance (Manolagas, 2014; Schupbach et al., 2020). Canonical Wnt pathway reduces osteoclast differentiation through secretion of osteoclast receptor antagonist, osteoprotegerin (Lacey et al., 1998; Yasuda et al., 1998). While some studies have indicated that bone healing is also modulated by the non-canonical Wnt pathways, it is unknown if these pathways serve as positive or negative modulators (Schupbach et al., 2020). Due to its established role in osteogenesis, activation of the canonical Wnt pathway is a promising approach to promote bone regeneration or accelerate bone healing.

There are a total of 19 Wnt ligands (secreted glycoproteins which activate Wnt) which target 10 different receptors in the frizzled (Fzd) protein family with multiple co-receptors required to activate a downstream response (Liu et al., 2013; Siman-Tov et al., 2021). Each Wnt ligand may stimulate different Wnt pathways depending on the receptor and co-receptor combination, yet there are several ligands strongly associated with canonical Wnt (Wnt1, Wnt3a) (Liu et al., 2013; Siman-Tov et al., 2021). Wnt ligands undergo extensive post translational modifications with one of the most significant modifications to these proteins being lipidation. Due to canonical Wnt's involvement in stem cell maintenance, stem cells require an excess supply of the enzymes catalyzing fatty acylation (Hosseini et al., 2019). In fact, dysregulations in the fatty acyl chains of Wnts are associated with various embryonic developmental abnormalities (Ng et al., 2019; Nile & Hannoush, 2016). Lipid modifications serve to anchor the Wnt ligands to the endoplasmic reticulum membrane, prevent misfolding, regulate signal transduction and facilitate long range signaling (Hosseini et al., 2019). While essential for function, the hydrophobic nature of the Wnt proteins makes them difficult to engineer and deliver therapeutically using conventional strategies.

The canonical Wnt pathway is stimulated through binding of a Wnt ligand to the Fzd receptor, a seven transmembrane-span receptor, and the low density lipoprotein receptor-related proteins (LRP5/6) co-receptor (Brown et al., 1998; Kato et al., 2002; Wodarz & Nusse, 1998) (Figure 1a). Once the ligand binds to the receptor complex, the cytoplasmic tail of the LRP5/6 co-receptor is phosphorylated, opening a binding site for Axin (Tamai et al., 2004; Zeng et al., 2005). This co-cluster of proteins (LRP5/6, frizzled and Disheveled (Dsh/Dvl)) recruit Axin and GSK3 complex to form the destruction complex, which is key in regulating the stability of β-catenin (Dajani et al., 2003; He et al., 2004; Mao et al., 2001). Following Axin's recruitment, the destruction complex is disassembled and β-catenin enters the nucleus to stimulate transcription of targeted genes (Ikeda et al., 2000; Xing et al., 2003). Specifically, β-catenin modulates the T-cell factor/lymphoid enhancer factor family of transcription factors regulating multiple pathways associated with proliferation and differentiation (Bienz & Clevers, 2003; Cong et al., 2003; Tolwinski & Wieschaus, 2004). Relative to bone formation, target genes that are activated upon stimulation of canonical Wnt include transcriptional cascade *RUNX2* (*runt-related transcription factor 2*) and *Osterix*, which have been shown to transcribe osteoblast markers *Collagen 1*, *Osteopontin* (*OPN*), *Osteocalcin* (*OCN*) and *Alkaline phosphatase* (*ALP*) (Felber et al., 2015). In the absence of Wnt ligands, β-catenin is phosphorylated and sent to the proteosome for proteolytic degradation (Aberle et al., 1997; Orford et al., 1997).

Given the canonical Wnt pathway's central role in promoting bone formation, it is an attractive therapeutic target for bone regeneration (Liu et al., 2013; Minear et al., 2010; Xu et al., 2014). However, there are several challenges associated with therapeutic activation of the Wnt pathway, predominantly with the lipid modifications of Wnt ligands that make isolating and purifying the protein difficult and expensive (Cadigan & Liu, 2006; Liu et al., 2013). Currently available clinical interventions target the inhibitors of the canonical Wnt pathway in age-related diseases, such as osteoporosis and cancer (Baron & Rawadi, 2007; Krishnamurthy & Kurzrock, 2018). Although there is more recent evidence that demonstrates an antibody-based treatment modality can improve bone formation (Haffner-Luntzer, 2021), this review highlights a broader range of strategies to activate the canonical Wnt pathway using engineering approaches.

Dysregulation within the canonical Wnt signaling pathway has been associated with various age-related conditions. Age-associated aberrations within Wnt/β-catenin signaling pathway are tissue dependent. For example, an increase in Wnt/β-catenin signaling has been associated with age-related pathology of muscle, specifically sarcopenia (Brack et al., 2007). Conversely, a decrease in Wnt/β-catenin signaling is correlated with age-related disorders of bone, such as osteoporosis (Harada & Rodan, 2003). Dysregulation of Wnt signaling pathways in age-related diseases will need to be further studied to understand effects of systemic and local Wnt activation therapies across the lifespan in order to elucidate best practices for clinical use.

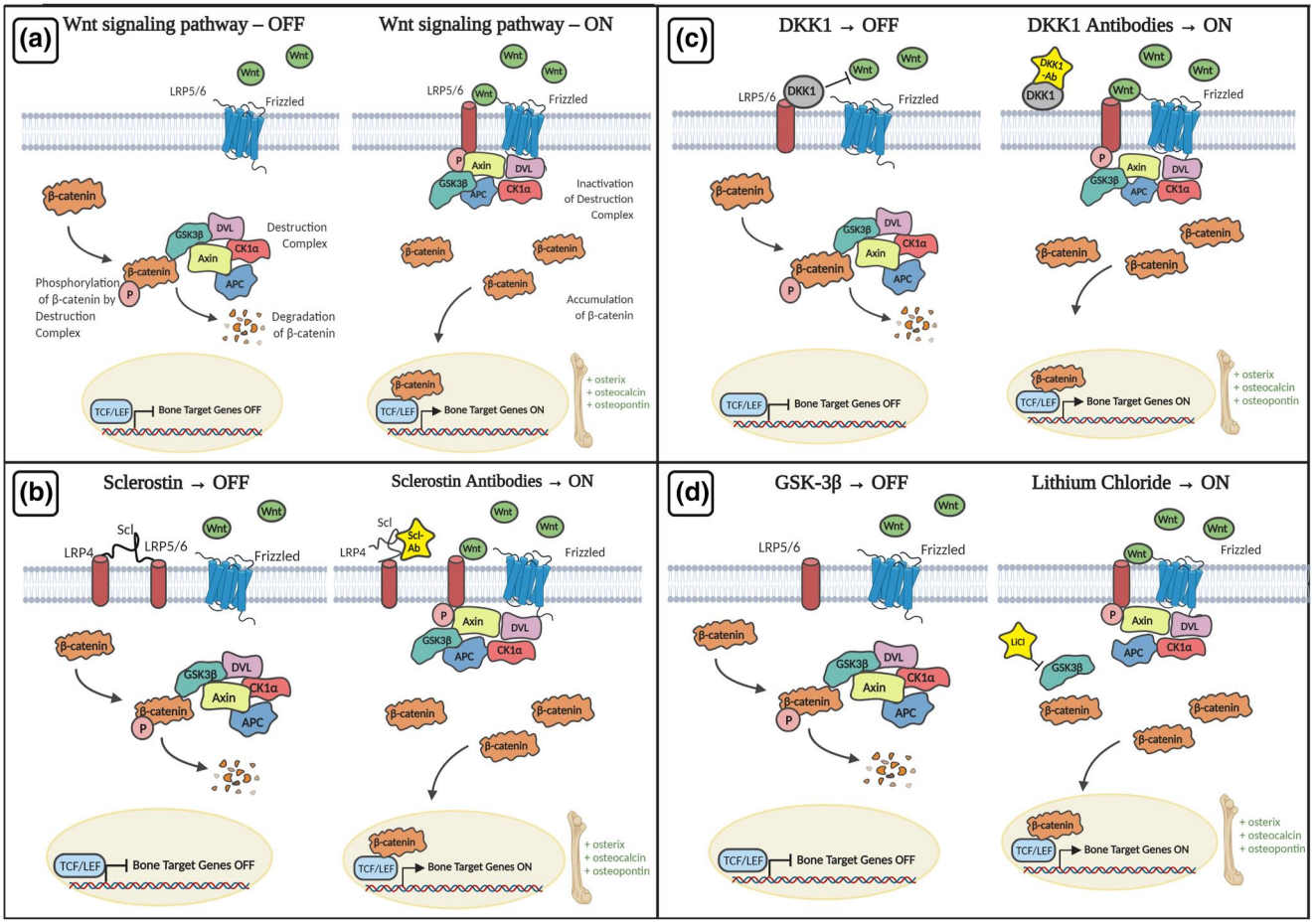

FIGURE 1 Schematic of the canonical Wingless-related integration site (Wnt) signaling pathway and mechanistic approaches of how various bioactive agents target the canonical Wnt pathway. (a) In the absence of Wnt ligands, β-catenin is phosphorylated, triggering ubiquitination by the destruction complex. In the presence of Wnt ligands, destruction complex disassembles. This allows for β-catenin to accumulate in the cytoplasm and translocate to the nucleus, stimulating transcription of target genes. (b) Antibodies targeting Sclerostin, an inhibitor of Wnt, allow for Wnt ligands to bind and activate the pathway. (c) Dickkopf-related protein 1 (Dkk1) binds to the LRP receptor inhibiting Wnt ligands from activating the pathway. Antibodies specific to Dkk1 allow for Wnt ligands to bind to the LRP receptor and stimulate the pathway. (d) Lithium Chloride, an inhibitor of GSK-3β, facilitates disassembly of the destruction complex [Colour figure can be viewed at wileyonlinelibrary.com]

2 | WNT ACTIVATING THERAPEUTICS

In terms of specificity and therapeutic potential, protein delivery is typically superior to small molecule drugs. However, proteins have low stability in physiological environments (Boraiah et al., 2009). For this reason, therapeutic efficacy in protein delivery is usually achieved by delivering supraphysiological doses of proteins, causing several side-effects (Wang et al., 2018). This hampers the clinical translation of protein delivery and is the main reason why the vast majority of FDA-approved drugs for clinical use are small molecule drugs instead (New Drugs at FDA: CDER's New Molecular Entities and New Therapeutic Biological Products, 2021). Moreover, specific to the Wnt pathway, since the ligand is lipidated, unmodified therapeutic delivery of this protein is ineffective. As such, alternative approaches have been tested to achieve targeted activation of canonical Wnt signaling. Re-purposing some of these compounds to target the Wnt pathway could accelerate clinical translation. In this section, we discuss bioactive agents which have been used in pre-clinical and clinical studies to activate Wnt and stimulate bone regeneration. In the following section, we assess tissue engineering strategies to deliver these bioactive agents.

2.1 | Sclerostin antibodies

Sclerostin is a well-defined inhibitor of the canonical Wnt signaling pathway. Sclerostin is a small glycoprotein transcribed from the *SOST* gene secreted by osteocytes. Sclerostin binds to the LRP5/6 co-receptor on the cell surface of osteoblasts preventing association with the Frz receptor and thereby inhibiting canonical Wnt pathway activation (Figure 1b) (Li et al., 2005; Semënov et al., 2005) Sclerostin antibodies activate canonical Wnt signaling by inhibiting the inhibitor of the Wnt pathway to enhance bone formation and are one of the most translational strategies to date. Their use has been shown in several preclinical and clinical studies for the treatment of osteoporosis and is elegantly reviewed by Clarke (Becker, 2014; Clarke, 2014;

Li et al., 2009; Markham, 2019; McClung et al., 2014; Ominsky et al., 2017; Yang et al., 2011).

Romosozumab (EVENITY™) is an FDA-approved monoclonal antibody that inhibits sclerostin. EVENITY™ is approved for the treatment of severe osteoporosis in postmenopausal women at high risk of fracture in 37 countries, including the U.S., Japan, and Canada (Markham, 2019). The approved EVENITY™ dose is 210 mg administered subcutaneously once a month for 12 months. In addition to a systemic effect in osteoporosis, the sclerostin antibody has also been tested as a bioactive approach to improve fracture healing. As recently reviewed, most preclinical studies suggest that systemic administration of EVENITY™ enhances bone formation and may accelerate fracture healing (Alaee et al., 2014; Cui et al., 2013; Ke et al., 2012; Ominsky et al., 2011, 2017; Virk et al., 2013). Based on these pre-clinical successes, an international, Phase-2, randomized and placebo-controlled clinical trial tested the efficacy of EVENITY™ on fracture patients with open reduction and internal fixation of intertrochanteric or femoral neck hip fractures. No significant differences were found in the median time to radiographic evidence of fracture healing between EVENITY™ and placebo treated groups (Schemitsch et al., 2020). Similarly, in a study evaluating the effect of EVENITY™ on tibial diaphyseal fractures, radiographic evaluation after 6 months revealed no significant differences in the time to radiographic and clinical healing between treatment groups (Bhandari et al., 2020). EVENITY™ is considered an osteoanabolic treatment yet it has been reported to only have transient effects on increasing bone formation (Chavassieux et al., 2019). Thus, it has been proposed that the therapeutic efficacy derives from its anti-resorptive properties, which result in decreased bone turnover rate and an increase in bone density (Chavassieux et al., 2019). Taken together, these data suggest systemic delivery of sclerostin antibodies are effective in building bone to treat osteoporosis, but this therapeutic approach is not effective for bone repair applications.

2.2 | Other antibody-based approaches to modulate Wnt

Based on the translational success of EVENITY™, multiple other antibodies to Wnt pathway inhibitors have been tested for treating osteoporosis or as a fracture healing therapeutic. Here, we focus on summarizing data utilizing these antibodies for bone repair. One approach utilizes an antibody to Dickkopf-related protein 1 (Dkk1), which, like sclerostin, is a soluble protein that binds to LRP5/LRP6 co-receptor to inhibit canonical Wnt signaling (Figure 1c). As Dkk1 overexpressing mice are characterized with osteopenia, preclinical models have shown that the inhibition of Dkk1 with anti-Dkk1 antibodies has increased bone mass (Pinzone et al., 2009; Yaccoby et al., 2007). Other studies analyzing levels of Dkk1 in cells surrounding a fracture callus report elevated levels in human nonunion fractures (Jin et al., 2015). To test the use of anti-Dkk1 antibodies as a potential anabolic agent in fracture healing, murine fracture models were treated with anti-Dkk1 antibodies and showed radiographic evidence of enhanced callus formation (Bajada et al., 2009). Dickkopf-related protein 1 treatment appears to necessitate a critical window for administration to enhance fracture healing, with studies showing that treatment is not effective unless started immediately post-fracture (Komatsu et al., 2010). Importantly, osteoanabolic effects of Dkk1-inhibition through treatment with anti-Dkk1 antibodies only results in bone formation when Sclerostin is deactivated (Witcher et al., 2018). Using this information, groups have tested therapeutic potential using combinatorial treatments of Wnt inhibitors, Dkk1 and Sclerostin (Choi et al., 2021). Choi et al. determined significantly enhanced therapeutic efficacy using this dual antibody treatment on cancellous bone, but not for cortical bone (Choi et al., 2021).

Additional approaches for targeting Wnt/β-catenin pathway inhibitors using antibody-based therapeutics include targeting the growth factor midkine or secreted frizzled-receptor 1 (sFRP1). Antagonizing midkine, which targets LRP family of receptors, has helped accelerate fracture healing in mice by increasing bone volume density, bone formation, and osteoblast activity (Haffner-Luntzer et al., 2016; Liedert et al., 2014). However, more research is needed to determine whether midkine represents a viable bioactive target to enhance fracture healing. Secreted frizzled-receptor 1 is a glycoprotein that negatively modulates Wnt signaling through binding and inhibition of Wnt ligands (Komiya & Habas, 2008). Mice deficient in sFRP1 resulted in slower age-related bone loss and decreased apoptosis in osteoblasts (Ohnaka et al., 2009; Yao et al., 2010). Secreted frizzled-receptor 1 antagonists could serve as an additional therapeutic target for osteoporosis and the ability of sFRP1 to mitigate its progression is currently being studied preclinically (Bodine et al., 2009). Additionally, it has been reported that the loss of sFRP1 expression improves fracture healing *in vivo* (Gaur et al., 2010; Xu et al., 2014). While antibody-based therapeutics show promising results in increasing bone mass, further tests on the dosing, timing, and route of delivery of these antibodies for fracture repair need to be executed in both pre-clinical and clinical studies.

The four R-spondin proteins, derived from roof-plate specific spondin (*Rspo*) gene, are implicated in various biological functions including skeletal repair (Kamata et al., 2004; Kazanskaya et al., 2004). Within the last decade, there have been several breakthroughs in R-spondin signaling and stimulation of canonical Wnt pathway activation (Binnerts et al., 2007; Kazanskaya et al., 2004; Nam et al., 2006). Specifically, several groups reported that R-spondins amplify Wnt activation when co-delivered with Wnt ligands (Binnerts et al., 2007; Wei et al., 2007). The mechanism of how R-spondins synergistically activate the canonical Wnt pathway has yet to be elucidated, yet it is generally thought that R-spondins do not bind directly with Fzd receptor (Jin & Yoon, 2012a; Wei et al., 2007). Despite limited mechanistic details, many groups show that R-spondins synergistically stimulate bone repair through enhanced osteoblast differentiation following treatment with R-spondin1 and Wnt3a *in vitro* (Lu et al., 2008; Nagano, 2019; Sharma et al., 2013). Additionally, R-spondin1 disrupted osteoclast expansion, reduced bone erosion, and enhanced cartilage integrity

following intraarticular injections into an osteoarthritic mouse model (Krönke et al., 2010). R-spondin2 has also been shown to promote osteoblastogenesis during skeletal repair by modulating bone morphogenetic protein (BMP) signaling (Friedman et al., 2009; Knight et al., 2018). Despite these published roles in potentiating Wnt activity, fundamental details addressing R-spondins role in activating canonical Wnt and bone repair are still needed to effectively harness its therapeutic potential (Jin & Yoon, 2012b; Knight & Hankenson, 2014).

2.3 | Synthetic Wnts

Recent interest has been geared toward developing surrogate Wnt ligands for the activation of canonical Wnt pathway (Chen et al., 2020; Janda et al., 2017). Janda et al. generated synthetic Wnt surrogates to be non-lipidated, water-soluble Wnt ligands which induce Fzd-LRP5/6 receptor heterodimerization, identified as a key molecular regulator for canonical Wnt pathway activation. Treatment of synthetic Wnt surrogates resulted in accumulation of nuclear β-catenin and upregulation of ALP, suggesting activation of canonical Wnt, in a murine hepatomegaly model (Janda et al., 2017). Despite these advances in generating synthetic Wnt surrogates, many groups have questioned the surrogate specificity for activating the Fzd pathway since Wnt ligands are cross-reactive for multiple receptors (Tao et al., 2019). To combat this selectivity concern, Tao et al. has developed tetravalent synthetic antibodies specific for any Fzd receptor. Further, they reported that activation of both binding sites within LRP6 resulted in greater intracellular signaling (Tao et al., 2019). Future directions in synthetic Wnt development entail elucidating the specific mechanisms involved in Wnt receptor activation, and determining the therapeutic capacity of synthetic Wnts for bone repair applications.

2.4 | Lithium Chloride

In addition to extracellular modulation of the Wnt pathway with antibodies to Wnt inhibitors, Wnt pathway activation can be modulated at the intracellular level by targeting the destruction complex. Lithium is a chemical element that has been compounded with salts and given orally as a psychoactive medication to treat bipolar disorder by increasing mTOR phosphorylation (O'Connell et al., 1991; Xiao et al., 2020). Dysregulation of mTOR pathway has been found in patients who suffer from bipolar disorder and once mTOR signaling is activated, the production of synaptic proteins and inhibition of autophagy result in nerve growth and synaptic transmission (Park et al., 2022). Interestingly, the pleiotropic effects from lithium was shown through its change in patient bone mineral density and was subsequently discovered that lithium activates canonical Wnt signaling. Lithium specifically inhibits GSK-3β in the destruction complex, enabling β-catenin to stimulate Wnt-responsive genes (Figure 1d). (Clément-Lacroix et al., 2005; Klein & Melton, 2019) Numerous preclinical studies confirm that lithium has significantly enhanced fracture healing by improving bone mass, volume, and formation when administered several days after fracture (Chen et al., 2007). Due to its success preclinically, a double blind, randomized controlled clinical trial has begun evaluating the clinical efficacy of lithium treatment in long-bone fractures, but have yet to report any conclusions (Nam et al., 2020).

2.5 | Fluoride

Fluoride is the ion of the highly reactive element fluorine and it can influence the Wnt signaling pathway by inhibiting the destruction complex (Grynpas et al., 2019; Pan et al., 2014). Specifically, fluoride stimulates phosphorylation of Akt and GSK-3β, thereby blocking activity of the destruction complex and increasing nuclear localization of β-catenin (Pan et al., 2014). Additionally, exposure to fluoride was shown to decrease the secretion of other inhibitors of the WNT pathway, such as Dkk-1 and SOST, in a time and concentration-dependent manner (Liu et al., 2012). In murine models, fluoride increased the enzymatic activity of ALP and decreased activity of osteoclast-derived serum tartrate-resistant acid phosphatase, indicating enhanced osteoblastic differentiation and reduced bone resorption (Li et al., 2016). However, fluoride has displayed adverse effects, including potentially dangerous levels of toxicity and decreased gene expression levels of bone morphogenetic protein 2 and type I collagen (*COL1A1*) when treated at high doses (Grynpas et al., 2019; Li et al., 2016). Additionally, fluoride has been shown to have pleiotropic effects and it has been characterized to suppress mTOR pathway, significantly downregulating mTOR related genes, inhibiting cell proliferation and increasing autophagy when delivered at high doses (Kuang et al., 2018; Ma et al., 2021). While fluoride demonstrates a promising approach to enhancing bone formation both *in vitro* and *in vivo*, there has been no published research evaluating its effects on fracture repair (Grynpas et al., 2019).

2.6 | Strontium

It has been found that strontium can substitute calcium in bone, increasing bone formation (Buehler et al., 2001). Mechanistically, strontium substitution serves as an osteoanabolic by promoting osteoblastic differentiation (Stefanic et al., 2018) and proliferation (Wu et al., 2017), increasing ALP activity (Khan et al., 2016; Moghanian et al., 2017), promoting angiogenesis (al Qaysi et al., 2015), and increasing calcium deposition (Su et al., 2015) and mineralization (Ehret et al., 2017; Sato et al., 2016). Strontium also enhances osteoblast activity through calcium-sensing receptors which activate the Ras/mitogen-activated protein kinase signaling pathway and trigger cell replication (Peng et al., 2009). Furthermore, strontium acts directly on the Wnt pathway by decreasing the expression of sclerostin (Rybchyn et al., 2011) and increasing the expression of Wnt11 and Wnt3a (Fromigue et al., 2010; Saidak & Marie, 2012).

Independent studies have demonstrated links between strontium and the Wnt pathway, showing that strontium-induced osteogenesis were managed by the canonical Wnt pathway through regulation of sFRP1 and DKK1 (Fromigue et al., 2010; Wang et al., 2021). Strontium ranelate (StRan), PROTELOS®, is a newly-approved drug for lowering the risk of vertebral fracture in postmenopausal women. In addition to treating osteoporosis, preclinical studies have shown that StRan can promote bone repair by accelerating osteogenesis and improving bone formation in a calvarial defect (Yang et al., 2011). Similarly, in an ovariectomized rat model of osteoporotic fractures, 2 months of treatment with StRan increased bone volume within the fracture callus (al Qaysi et al., 2015) and improved callus strength (Habermann et al., 2010). While clinical trials of StRan report its efficacy in reducing the risk of new vertebral fractures in postmenopausal women, no clinical data has shown a benefit in fracture repair (Deeks & Dhillon, 2010).

2.7 | Other Wnt modifiers

Several other families of Wnt modulators have recently been determined to antagonize and deactivate Wnt proteins, such as Tiki, while other modulators serve as an agonist of canonical Wnt pathway, like Porcupine. Tiki proteins have been found to act as metalloproteases by cleaving and deactivating Wnt ligands (Zhang et al., 2016). These glycosylphosphatidylinositol-anchored proteins (GPI-Aps) are localized to the plasma-membrane through the GPI moiety and inhibit canonical Wnt ligands (Li et al., 2022). Porcupine is another protein which post-translationally modifies Wnt proteins through catalyzing their lipidation, imperative for Wnt ligand secretion (Proffitt & Virshup, 2012). Specifically, both Wnt1 and Wnt3a have been found to be lipidated through porcupine, promoting their Wnt activity (Galli et al., 2007). While these proteins have been implicated in the canonical Wnt pathway, few studies have determined their therapeutic efficacy in bone repair. As porcupine inhibitors have been studied for use as a cancer treatment, *Funck-Brentano et al.* examined potential adverse effects on bone health finding deleterious amounts of bone loss and bone resorption (Funck-Brentano et al., 2018; Lung et al., 2021).

3 | TISSUE ENGINEERING STRATEGIES TO ACHIEVE DELIVERY

The two main obstacles that drug delivery strategies must overcome to ensure therapeutic efficacy are: (i) the stability of the cargo and (ii) targeting the area of interest. While some of the molecular modulators of the Wnt pathway discussed above have demonstrated pharmaceutical benefits for treatment of osteoporosis (e.g., increased bone density following EVENITY™ treatment), few of these modulators have been tested in fracture repair. Although systemic approaches are often simple, the non-selective nature of their delivery results in variable concentrations delivered to the region of interest. As an example, the systemic delivery of strontium ranelate without proper targeting strategies results in biodistribution of less than 1% of the drug to the bone (Wang et al., 2018). Due to the vast nature of cellular responses in which Wnt plays a role, systemic administration of bioactive agents targeting Wnt to enhance bone formation poses the risk of off-target effects (Krishnamurthy & Kurzrock, 2018). For example, lithium has been shown to enhance bone mass through the inhibition of GSK-3β enzymes, but studies now reveal that its long-term usage is associated with elevated incidence ratios of renal cancer (Kahn, 2014). Even sclerostin monoclonal antibodies, like the newly approved EVENITY™, have reported adverse events such as injection-site erythema, hemorrhage, headaches, and arthralgia (Padhi et al., 2011). While these therapies can be further designed to circumvent adverse events, side effects are an inherent risk with systemic delivery.

To circumvent the limitations associated with systemic delivery, including poor biodistribution and aberrant side effects, there is an opportunity to engineer more effective approaches to activate the Wnt pathway at a local level. An overview of the potential applications for systemic versus localized Wnt-activation strategies can be found in Figure 2 (Erdine & de Andrés, 2006). Localized delivery of a bioactive agent involves integrating the drug with a drug administering device or process to control the rate of release and target a specific tissue type. Various approaches have been used to activate canonical Wnt pathway locally, including embedding bioactive agent into a delivery carrier, using bioactive agents as a dopant for scaffolds, or incorporating them in hydrogel platforms. A schematic of these tissue engineered approaches to deliver Wnt-activating therapeutics is shown in Figure 3. While these approaches have been successful *in vitro* and *in vivo* applications, they have yet to be translated to the clinic. This section highlights local approaches for targeting canonical Wnt pathway.

3.1 | Liposomes

The most direct pathway to locally activate canonical Wnt signaling would be through delivery of the ligand. Lipid nanoparticles, inspired by the lipid bilayer of liposomes, can be an effective delivery vehicle to circumvent the limitations of delivering hydrophobic Wnt proteins. Liposomal Wnt-ligand therapies serve as a promising clinically translatable approach due to its preclinical successes (Leucht et al., 2013; Popelut et al., 2010). In one embodiment, Chen et al. sought to transiently activate Wnt in murine bone grafts by adding a liposomal Wnt3a formulation (Chen et al., 2018). Following treatment, the cells in the bone graft showed elevated Wnt signaling, increased cell proliferation and reduced apoptosis, resulting in three-fold increase in bone formation as compared to bone grafts alone (Chen et al., 2018). Similarly, other studies have reported that liposomal Wnt delivery injected into skeletal defects enhanced bone regeneration three times more than the control (Minear et al., 2010). Leucht et al. has highlighted the therapeutic potential for transiently upregulating canonical Wnt in patients with reduced skeletal healing

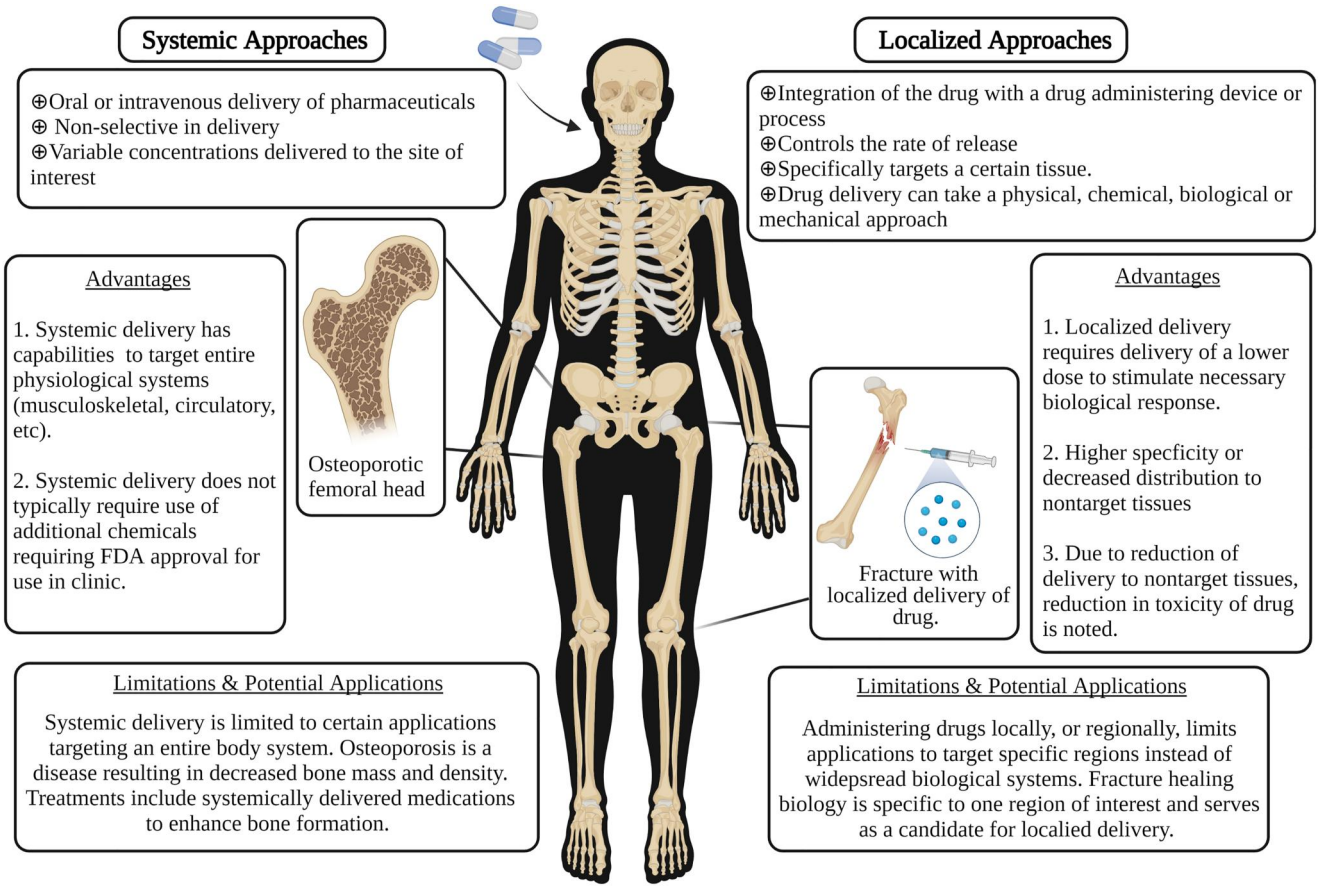

FIGURE 2 Clinical overview of systemic versus localized drug or small molecule delivery approaches (Erdine & de Andrés, 2006). [Colour figure can be viewed at wileyonlinelibrary.com]

potential, such as an aged demographic (Leucht et al., 2013; Minear et al., 2010).

3.2 | Scaffold dopants

A promising approach to deliver ions is to use them as a dopant in biomaterials. This approach enables the local delivery of small Wnt-activating ions, such as strontium or fluoride, while utilizing the biomaterial scaffold to interact with the adjacent tissues. Mineralization of biomaterials, typically through the addition of synthetic hydroxyapatite or calcium phosphate, is a common procedure used to increase scaffold strength and promote osseointegration with the surrounding microenvironment (Kavitha et al., 2015; Kim & Kim, 2021; Lee et al., 2019; Liu et al., 2019; Müller et al., 2017; Park et al., 2016). Moreover, mineralization of metal-based implants obtained through coating with hydroxyapatite or bioglass was found to alleviate inflammation caused by the corrosion of the implant (Cui et al., 2020).

Strontium is one of the most used dopants in mineralized biomaterials and strontium-doped biomaterials consistently promote improved osteogenic effects *in vitro* and *in vivo* (al Qaysi et al., 2015; Meka et al., 2016; Weng et al., 2017). For example, strontium-doped hydroxyapatite induced osteogenic differentiation of MSCs through upregulation of β-catenin expression to produce new bone formation both *in vitro* and *in vivo* (Cui et al., 2020; Müller et al., 2017; Yang et al., 2011). Based on the successes of these strontium-doped hydroxyapatite systems, Kavitha et al. has studied the synthesis parameters of strontium doped hydroxyapatite powder for the purpose of scaling up the process to maintain desired powder characteristics and optimal strontium concentrations to fabricate in bulk (Kavitha et al., 2015). Similarly, bioactive borate glass cement containing strontium was also found to enhance osteogenesis *in vivo* and *in vitro* (Cui et al., 2020). Another strontium containing cement comprised of calcium phosphate was reported to have new bone formation and enhanced osseointegration at the bone-implant area of the cements *in vivo* as compared to the strontium-free bone cements (Pina et al., 2010; Thormann et al., 2013). While there only a few published studies for the use of strontium-doped materials *in vivo*, they have promising results for the treatment of osteoporotic-related fractures (Schumacher & Gelinsky, 2015).

As with strontium, biomaterials can be doped with fluoride to enhance osteogenesis. Cooper et al. studied fluoride's capabilities in accentuating osseointegration of sandblasted titanium implants (Cooper et al., 2006). They found that fluoride modified titanium implants resulted in increased osteoblast differentiation capacity *in vitro* and twice as much bone formation on the implant *in vivo* (Borkowski et al., 2020). Hydroxyapatite scaffolds have also been

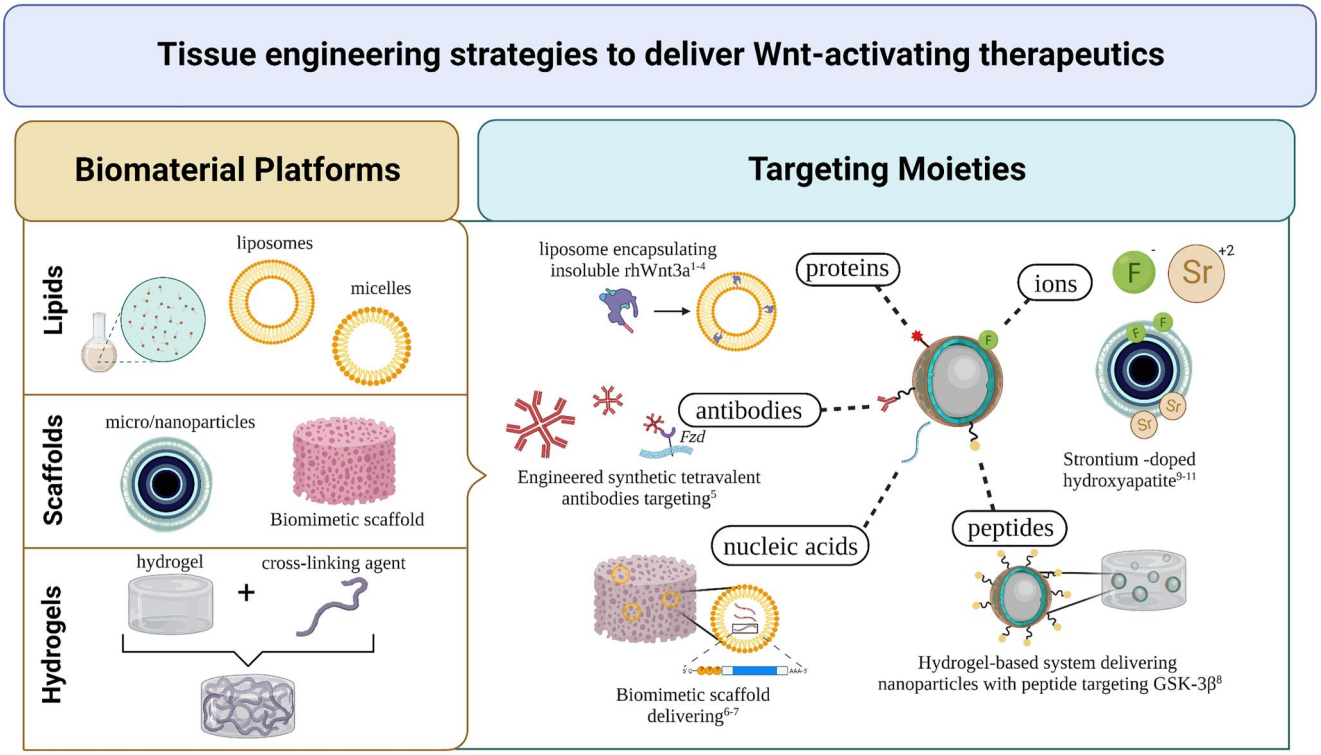

FIGURE 3 Tissue engineering strategies to achieve delivery of Wingless-related integration site (Wnt)-activating therapeutics. Localized, engineered approaches frequently involve the use of biomaterial platforms to deliver targeted therapeutics. This schematic depicts tissue engineering strategies integrating biomaterial platforms and targeting moieties which are reviewed in this manuscript. References: 1. Minear et al., 2010; 2. Popelut et al., 2010; 3. Leucht et al., 2013; 4. Chen et al., 2018; 5. Tao et al., 2019; 6. Balmayor et al., 2016; 7. Khorsand et al., 2017; 8. Yuchen Wang et al., 2016; 9. Kavitha et al., 2015; 10. Müller et al., 2017; 11. Yang et al., 2011. [Colour figure can be viewed at wileyonlinelibrary.com]

engineered to release fluoride ions at a controlled rate, increasing proliferation and osteogenesis of MC3T3 cells *in vitro* (Borkowski et al., 2020). Similarly, titanium containing fluoride-doped phosphate nanobioglasses enhanced osteogenesis *in vitro* resulting in higher amounts of bone formation *in vivo* (Sankaralingam et al., 2021).

3.3 | Hydrogels

Hydrogels, networks of hydrophilic polymers, have been used in a large number of studies to target bone repair as they can be absorbable, integrated with adjacent tissues and do not require surgical removal (Bai et al., 2018; Gresham et al., 2021). Hydrogels can be synthesized from natural or synthetic materials and have a wide range of applications due to their flexibility and potential for injectability (Ahmed, 2015). Many studies have employed hydrogel systems to deliver growth factors and/or cells for the promotion of bone regeneration, but few have used this approach specifically to activate Wnt signaling (Alaohali et al., 2021; Bai et al., 2018; Gresham et al., 2021). Alaohali et al. used a hyaluronic acid-based hydrogel to deliver a GSK-3 inhibitor to promote bone formation in dental applications (Alaohali et al., 2021). In this application, the hydrogel was injected into a murine molar defect, crosslinked using UV light to facilitate drug encapsulation, and the drug was control released through degradation, stimulating dentine formation (Alaohali et al., 2021). Similarly, Wang et al. utilized a hydrogel-based system to deliver bone targeting nanoparticles with a peptide targeting GSK-3β, showing that fracture healing was accelerated in a murine fracture model (Wang et al., 2016). Other groups utilized a thermo-responsive hydrogel platform as a scalable, cost-effective strategy to enhance the production of Wnt3a protein (Li et al., 2018).

4 | FUTURE DIRECTIONS

There remains an unmet clinical need developing strategies to activate the canonical Wnt pathway in bone repair and bone regeneration. Emerging technologies, such as mRNA, have had exciting developments leading to clinical translation. To date, few of these technologies have been applied to activating canonical Wnt pathway. However, these new strategies can be used to navigate the current limitations seen in developing Wnt-activating therapeutics.

4.1 | mRNA delivery

Delivery of mRNA is an attractive new bioactive approach as it does not require genomic integration (Mukherjee & Thrasher, 2013; Zohra et al., 2007). Protein expression through mRNA delivery is sustained for a limited time which is ideal to stimulate bone formation and

minimize adverse events for application to fracture healing (Agholme et al., 2010). Until recently, the use of mRNA as a therapeutic has been limited due to challenges associated with mRNA stability, cytotoxicity of the delivery platform, and induction of innate inflammation (Mockey et al., 2006; Ramunas et al., 2015; Sultana et al., 2017; Zohra et al., 2007). New technology to mitigate these undesirable effects through modification of mRNA constructs and delivery platforms has recently led to the successful mRNA COVID-19 vaccines (Pilishvili et al., 2021; Roy, 2021). Recent work pioneering mRNA therapies to promote bone regeneration through coding for BMP2/9 has shown promise, yet all studies use a biomimetic scaffold which requires surgical implantation (Balmayor et al., 2016; Khorsand et al., 2017). An alternative would be to use microparticles as an injectable delivery vehicle for therapeutic mRNA. Recent studies have shown that delivering mRNA via mineral-coated microparticles increased transfection and cell survival *in vitro* (Fontana et al., 2019) and *in vivo* (Khalil et al., 2020). Using mRNA-based approaches to target the Wnt pathway will circumvent the solubility challenges encountered when delivering Wnt proteins and capitalize on the endogenous cellular machinery to add the post-translational modifications essential to ligand function.

4.2 | CRISPR gene editing

As with mRNA strategies, gene editing technology has vast potential in tissue engineering. The newest of the genome editing technologies, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9), has accelerated translation from bench to clinic. Many groups have proposed various methods for leveraging this technology for the regeneration of bone, yet this approach has yet to be fully explored. While CRISPR genome editing has been used to develop osteoporotic murine models (Lambert et al., 2016; Liu et al., 2019; Ubels et al., 2020), several investigators are currently exploring the therapeutic capacity of using CRISPR technology in therapies. The most current clinically effective CRISPR-Cas9 applications involve genetic engineering of cells *ex vivo*. One group recently used CRISPR/Cas9 and single guide RNAs as a platform to restore the expression of type I collagen to combat osteogenesis imperfecta, a genetic disorder characterized by bone fragility and repeat fractures (Jung et al., 2021). Another group has employed CRISPR technology to engineer stem cells as an alternative therapeutic approach to combat osteoarthritis (Brunger et al., 2017). *Brunger et al.* engineered stem cells to maintain resistance toward IL-1-induced degradation and inflammation. This approach implies that when cells are inserted in diseased or injured tissues the host inflammatory response may compromise the therapeutic potential of the implant. Importantly, *ex vivo* CRISPR gene therapy has now moved into human clinical trials as an immunotherapy treatment and these trials will help to establish safety and efficacy of this technology (Hsu et al., 2020; Khalaf et al., 2020; Uddin et al., 2020).

The RNA targeting CRISPR-Cas technologies may be the most promising *in vivo* therapeutic approach for bone repair and/or trauma. The RNA-targeting Cas9 platform (RCas9) works by cleaving ssDNA strands, and can also be designed to specifically target RNA sequences using sgRNAs (O'Connell et al., 2014; Pickar-Oliver & Gersbach, 2019). Therapies aiming to reduce expression of toxic or potentially lethal RNAs have been employed in patient cells *ex vivo* (Batra et al., 2017). While RCas9 shows promise in treating nongenetic derived injuries, it has yet to be employed for use in bone repair but could be possibly be used to silence inhibitors of Wnt/β-catenin.

Despite the most clinically effective CRISPR-Cas9 applications involving genetic engineering of cells *ex vivo*, the first human clinical trials using *in vivo* genome editing have recently been performed to treat various genetic diseases (Frangoul et al., 2021; Maeder et al., 2019). Further studies utilizing *in vivo* CRISPR technologies need to establish a robust safety profile of the developed therapies and their capacity in acquiring off-target effects in order to employ *in vivo* technologies using CRISPR-based platforms (Brunger et al., 2017; Pickar-Oliver & Gersbach, 2019).

5 | CONCLUSIONS

While the canonical Wnt signaling proves to be a complex pathway to target therapeutically, this review presents promising engineering approaches to circumvent limitations in activating Wnt specifically for bone regeneration. First, we reviewed Wnt-activating therapeutics designed to stimulate canonical Wnt when delivered systemically. Systemic delivery of bioactive agents requires targeting the entire skeletal system, limiting the clinical application to diseases like osteoporosis. We subsequently discussed strategies to deliver bioactive molecules and ions locally, which may be a preferable strategy for bone regeneration and fracture repair applications. Local delivery approaches, including liposomes, scaffold doping, and hydrogel-based systems show promise, but these strategies have not yet been translated clinically. In addition to these approaches, several emerging methods should be considered as novel strategies to activate the canonical Wnt pathway.

AUTHOR CONTRIBUTIONS

Anna Laura Nelson, GianLuca Fontana and Chelsea Bahney conceptualized the review article. William Murphy and Johnny Huard provided financial support. Anna Laura Nelson, GianLuca Fontana, Elizabeth Miclau, Nicole Ehrhart and Chelsea Bahney wrote the manuscript, with critical revisions from all authors. Anna Laura Nelson and Elizabeth Miclau designed graphics using BioRender. All authors have read and approved the final submitted manuscript.

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CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

Aberle, H., Bauer, A., Stappert, J., Kispert, A., & Kemler, R. (1997). Beta-catenin is a target for the ubiquitin-proteasome pathway. *The EMBO Journal*, *16*(13), 3797–3804. https://doi.org/10.1093/emboj/16.13.3797

Agholme, F., Li, X., Isaksson, H., Ke, H. Z., & Aspenberg, P. (2010). Sclerostin antibody treatment enhances metaphyseal bone healing in rats. *Journal of Bone and Mineral Research*, *25*(11), 2412–2418. https://doi.org/10.1002/jbmr.135

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research*, *6*(2), 105–121. https://doi.org/10.1016/j.jare.2013.07.006

Alaee, F., Virk, M. S., Tang, H., Sugiyama, O., Adams, D. J., Stolina, M., Dwyer, D., Ominsky, M. S., Ke, H. Z., & Lieberman, J. R. (2014). Evaluation of the effects of systemic treatment with a sclerostin neutralizing antibody on bone repair in a rat femoral defect model. *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society*, *32*(2), 197–203. https://doi.org/10.1002/jor.22498

Alaohali, A., Salzlechner, C., Zaugg, L. K., Suzano, F., Martinez, A., Gentleman, E., & Sharpe, P. T. (2021). GSK3 inhibitor-induced dentinogenesis using a hydrogel. *Journal of Dental Research*, *101*, 1–8. https://doi.org/10.1177/00220345211020652

al Qaysi, M., Walters, N. J., Foroutan, F., Owens, G. J., Kim, H. W., Shah, R., & Knowles, J. C. (2015). Strontium- and calcium-containing, titanium-stabilised phosphate-based glasses with prolonged degradation for orthopaedic tissue engineering. *Journal of Biomaterials Applications*, *30*(3), 300–310. https://doi.org/10.1177/0885328215588898

Bai, X., Gao, M., Syed, S., Zhuang, J., Xu, X., & Zhang, X. (2018). Bioactive Materials Bioactive hydrogels for bone regeneration. *Bioactive Materials*, *3*(4), 401–417. https://doi.org/10.1016/j.bioactmat.2018.05.006

Bajada, S., Marshall, M. J., Wright, K. T., Richardson, J. B., & Johnson, W. E. B. (2009). Decreased osteogenesis, increased cell senescence and elevated Dickkopf-1 secretion in human fracture non union stromal cells. *Bone*, *45*(4), 726–735. https://doi.org/10.1016/j.bone.2009.06.015

Balmayor, E. R., Geiger, J. P., Aneja, M. K., Berezhanskyy, T., Utzinger, M., Mykhaylyk, O., Rudolph, C., & Plank, C. (2016). Chemically modified RNA induces osteogenesis of stem cells and human tissue explants as well as accelerates bone healing in rats. *Biomaterials*, *87*, 131–146. https://doi.org/10.1016/j.biomaterials.2016.02.018

Baron, R., & Rawadi, G. (2007). Minireview: Targeting the Wnt/β-catenin pathway to regulate bone formation in the adult skeleton. *Endocrinology*, *148*(6), 2635–2643. https://doi.org/10.1210/en.2007-0270

Batra, R., Nelles, D. A., Pirie, E., Blue, S. M., Marina, R. J., Wang, H., Chaim, I. A., Thomas, J. D., Zhang, N., Nguyen, V., Aigner, S., Markmiller, S., Xia, G., Corbett, K. D., Swanson, M. S., & Yeo, G. W. (2017). Elimination of toxic microsatellite repeat expansion RNA by RNA-targeting Cas9. *Cell*, *170*(5), 899–912. e10. https://doi.org/10.1016/j.cell.2017.07.010

Becker, C. B. (2014). Sclerostin inhibition for osteoporosis — A new approach. *New England Journal of Medicine*, *370*(5), 476–477. https://doi.org/10.1056/NEJMe1315500

Bhandari, M., Schemitsch, E. H., Karachalios, T., Sancheti, P., Poolman, R. W., Caminis, J., Daizadeh, N., Dent-Acosta, R. E., Egbuna, O., Chines, A., & Miclau, T. (2020). Romosozumab in skeletally mature adults with a fresh unilateral tibial diaphyseal fracture: A randomized phase-2 study. *Journal of Bone and Joint Surgery American Volume*, *102*(16), 1416–1426. https://doi.org/10.2106/JBJS.19.01008

Bienz, M., & Clevers, H. (2003). Armadillo/β-catenin signals in the nucleus – proof beyond a reasonable doubt? *Nature Cell Biology*, *5*(3), 179–182. https://doi.org/10.1038/ncb0303-179

Binnerts, M. E., Kim, K.-A., Bright, J. M., Patel, S. M., Tran, K., Zhou, M., Leung, J. M., Liu, Y., Lomas, W. E., Dixon, M., Hazell, S. A., Wagle, M., Nie, W.-S., Tomasevic, N., Williams, J., Zhan, X., Levy, M. D., Funk, W. D., & Abo, A. (2007). R-Spondin1 regulates Wnt signaling by inhibiting internalization of LRP6. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(37), 14700–-14705. https://doi.org/10.1073/pnas.0702305104

Bodine, P. V. N., Stauffer, B., Ponce-de-leon, H., Bhat, R. A., Mangine, A., Seestaller-wehr, L. M., Moran, R. A., Billiard, J., Fukayama, S., Komm, B. S., Pitts, K., Krishnamurthy, G., Gopalsamy, A., Shi, M., Kern, J. C., Commons, T. J., Woodworth, R. P., Wilson, M. A., Welmaker, G. S., & Moore, W. J. (2009). A small molecule inhibitor of the Wnt antagonist secreted frizzled-related protein-1 stimulates bone formation. *Bone*, *44*(6), 1063–1068. https://doi.org/10.1016/j.bone.2009.02.013

Boraiah, S., Paul, O., Hawkes, D., Wickham, M., & Lorich, D. G. (2009). Complications of recombinant human BMP-2 for treating complex tibial plateau fractures: A preliminary report. *Clinical Orthopaedics and Related Research*, *467*(12), 3257–3262. https://doi.org/10.1007/s11999-009-1039-8

Borkowski, L., Przekora, A., Belcarz, A., Palka, K., Jozefaciuk, G., Lübek, T., Jojczuk, M., Nogalski, A., & Ginalska, G. (2020). Fluorapatite ceramics for bone tissue regeneration: Synthesis, characterization and

assessment of biomedical potential. *Materials Science and Engineering: C*, *116*(June), 111211. https://doi.org/10.1016/j.msec.2020.111211

Brack, A. S., Conboy, M. J., Roy, S., Lee, M., Kuo, C. J., Keller, C., & Rando, T. A. (2007). Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science (New York, N.Y.)*, *317*(5839), 807–810. https://doi.org/10.1126/science.1144090

Brown, S. D., Twells, R. C. J., Hey, P. J., Cox, R. D., Levy, E. R., Soderman, A. R., Metzker, M. L., Caskey, C. T., Todd, J. A., & Hess, J. F. (1998). Isolation and characterization ofLRP6, a novel member of the low density lipoprotein receptor gene family. *Biochemical and Biophysical Research Communications*, *248*(3), 879–888. https://doi.org/10.1006/bbrc.1998.9061

Brunger, J. M., Zutshi, A., Willard, V. P., Gersbach, C. A., & Guilak, F. (2017). CRISPR/Cas9 editing of murine induced pluripotent stem cells for engineering inflammation-resistant tissues. *Arthritis & Rheumatology*, *69*(5), 1111–1121. https://doi.org/10.1002/art.39982

Buehler, J., Chappuis, P., Saffar, J. L., Tsouderos, Y., & Vignery, A. (2001). Strontium ranelate inhibits bone resorption while maintaining bone formation in alveolar bone in monkeys (Macaca fascicularis). *Bone*, *29*(2), 176–179. https://doi.org/10.1016/S8756-3282(01)00484-7

Cadigan, K. M., & Liu, Y. I. (2006). Wnt signaling: Complexity at the surface. *Journal of Cell Science*, *119*(3), 395–402. https://doi.org/10.1242/jcs.02826

Chavassieux, P., Chapurlat, R., Portero-Muzy, N., Roux, J. P., Garcia, P., Brown, J. P., Libanati, C., Boyce, R. W., Wang, A., & Grauer, A. (2019). Bone-Forming and antiresorptive effects of romosozumab in postmenopausal women with osteoporosis: Bone histomorphometry and microcomputed tomography analysis after 2 and 12 Months of treatment. *Journal of Bone and Mineral Research*, *34*(9), 1597–1608. https://doi.org/10.1002/jbmr.3735

Chen, H., Lu, C., Ouyang, B., Zhang, H., Huang, Z., Bhatia, D., Lee, S. J., Shah, D., Sura, A., Yeh, W. C., & Li, Y. (2020). Development of potent, selective surrogate WNT molecules and their application in defining frizzled requirements. *Cell Chemical Biology*, *27*(5), 598–609. e4. https://doi.org/10.1016/j.chembiol.2020.02.009

Chen, T., Li, J., Córdova, L. A., Liu, B., Mouraret, S., Sun, Q., Salmon, B., & Helms, J. (2018). A WNT protein therapeutic improves the bone-forming capacity of autografts from aged animals. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-017-18375-x

Chen, Y., Whetstone, H. C., Lin, A. C., Nadesan, P., Wei, Q., Poon, R., & Alman, B. A. (2007). Beta-catenin signaling plays a disparate role in different phases of fracture repair: Implications for therapy to improve bone healing. *PLoS Medicine*, *4*(7), 1216–1229. https://doi.org/10.1371/journal.pmed.0040249

Choi, R. B., Bullock, W. A., Hoggatt, A. M., Loots, G. G., Genetos, D. C., & Robling, A. G. (2021). Improving bone health by optimizing the anabolic action of wnt inhibitor multitargeting. *JBMR Plus*, *5*(5). https://doi.org/10.1002/jbm4.10462

Clarke, B. L. (2014). Anti-sclerostin antibodies: Utility in treatment of osteoporosis. *Maturitas*, *78*(3), 199–204. https://doi.org/10.1016/j.maturitas.2014.04.016

Clément-Lacroix, P., Ai, M., Morvan, F., Roman-Roman, S., Vayssière, B., Belleville, C., Estrera, K., Warman, M. L., Baron, R., & Rawadi, G. (2005). Lrp5-independent activation of Wnt signaling by lithium chloride increases bone formation and bone mass in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(48), 17406–17411. https://doi.org/10.1073/pnas.0505259102

Cong, F., Schweizer, L., Chamorro, M., & Varmus, H. (2003). Requirement for a nuclear function of β-catenin in wnt signaling. *Molecular and Cellular Biology*, *23*(23), 8462–8470. https://doi.org/10.1128/mcb.23.23.8462-8470.2003

Cooper, L. F., Zhou, Y., Takebe, J., Guo, J., Abron, A., Holmén, A., & Ellingsen, J. E. (2006). Fluoride modification effects on osteoblast behavior and bone formation at TiO2 grit-blasted c.p. titanium endosseous implants. *Biomaterials*, *27*(6), 926–936. https://doi.org/10.1016/j.biomaterials.2005.07.009

Cui, L., Cheng, H., Song, C., Li, C., Simonet, W. S., Ke, H. Z., & Li, G. (2013). Time-dependent effects of sclerostin antibody on a mouse fracture healing model. *Journal of Musculoskeletal and Neuronal Interactions*, *13*(2), 178–184.

Cui, X., Zhang, Y., Wang, J., Huang, C., Wang, Y., Yang, H., Liu, W., Wang, T., Wang, D., Wang, G., Ruan, C., Chen, D., Lu, W. W., Huang, W., Rahaman, M. N., & Pan, H. (2020). Strontium modulates osteogenic activity of bone cement composed of bioactive borosilicate glass particles by activating Wnt/β -catenin signaling pathway. *Bioactive Materials*, *5*(2), 334–347. https://doi.org/10.1016/j.bioactmat.2020.02.016

Dajani, R., Fraser, E., Roe, S., Yeo, M., Good, V., Thompson, V., Dale, T., & Pearl, L. (2003). Structural basis for recruitment of glycogen synthase kinase 3 to the axin APC scaffold. *The EMBO Journal*, *22*(3), 494–501. https://doi.org/10.1093/emboj/cdg068

Deeks, E. D., & Dhillon, S. (2010). Spotlight on strontium ranelate: In postmenopausal osteoporosis. *Drugs & Aging*, *27*(9), 771–773. https://doi.org/10.2165/11206440-000000000-00000

Ehret, C., Sagardoy, T., Siadous, R., Bareille, R., Rey, S., Pechev, S., Etienne, L., Kalisky, J., de Mones, E., Letourneur, D., & Amedee Vilamitjana, J. (2017). Strontium-doped hydroxyapatite polysaccharide materials effect on ectopic bone formation. *PLoS One*, *12*(9), 1–21. https://doi.org/10.1371/journal.pone.0184663

Erdine, S., & de Andrés, J. (2006). Drug delivery systems. *Pain Practice*, *6*(1), 51–57. https://doi.org/10.1111/j.1533-2500.2006.00059.x

Felber, K., Elks, P. M., Lecca, M., & Roehl, H. H. (2015). Expression of osterix is regulated by FGF and Wnt/β-catenin signalling during osteoblast differentiation. *PLoS One*, *10*(12), 1–17. https://doi.org/10.1371/journal.pone.0144982

Fontana, G., Martin, H. L., Lee, J. S., Schill, K., Hematti, P., & Murphy, W. L. (2019). Mineral-coated microparticles enhance mRNA-based transfection of human bone marrow cells. *Molecular Therapy - Nucleic Acids*, *18*(December), 455–464. https://doi.org/10.1016/j.omtn.2019.09.004

Frangoul, H., Altshuler, D., Cappellini, M. D., Chen, Y.-S., Domm, J., Eustace, B. K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., Ho, T. W., Kattamis, A., Kernytsky, A., Lekstrom-Himes, J., Li, A. M., Locatelli, F., Mapara, M. Y., de Montalembert, M., Rondelli, D., & Corbacioglu, S. (2021). CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *New England Journal of Medicine*, *384*(3), 252–260. https://doi.org/10.1056/nejmoa2031054

Friedman, M. S., Oyserman, S. M., & Hankenson, K. D. (2009). Wnt11 promotes osteoblast maturation and mineralization through R-spondin 2. *Journal of Biological Chemistry*, *284*(21), 14117–14125. https://doi.org/10.1074/jbc.M808337200

Fromigue, O., Haÿ, E., Barbara, A., & Marie, P. J. (2010). Essential role of Nuclear Factor of Activated T Cells (NFAT)-mediated Wnt signaling in osteoblast differentiation induced by strontium ranelate. *Journal of Biological Chemistry*, *285*(33), 25251–25258. https://doi.org/10.1074/jbc.M110.110502

Funck-Brentano, T., Nilsson, K. H., Brommage, R., Henning, P., Lerner, U. H., Koskela, A., Tuukkanen, J., Cohen-Solal, M., Movérare-Skrtic, S., & Ohlsson, C. (2018). Porcupine inhibitors impair trabecular and cortical bone mass and strength in mice. *Journal of Endocrinology*, *238*(1), 13–23. https://doi.org/10.1530/JOE-18-0153

Galli, L. M., Barnes, T. L., Secrest, S. S., Kadowaki, T., & Burrus, L. W. (2007). Porcupine-mediated lipid-modification regulates the activity and distribution of Wnt proteins in the chick neural tube. *Development (Cambridge, England)*, *134*(18), 3339–3348. https://doi.org/10.1242/dev.02881

Gaur, T., Wixted, J. J., Hussain, S., Connell, S. O., Morgan, E. F., Ayers, D., Komm, B. S., Bodine, P. v, Stein, G. S., & Lian, J. B. (2010). Secreted frizzled related protein 1 is a target to improve fracture healing.

Journal of Cellular Physiology, *220*(1), 174–181. https://doi.org/10.1002/jcp.21747.Secreted

Gresham, R. C. H., Bahney, C. S., & Leach, J. K. (2021). Bioactive Materials Growth factor delivery using extracellular matrix-mimicking substrates for musculoskeletal tissue engineering and repair. *Bioactive Materials*, *6*(7), 1945–1956. https://doi.org/10.1016/j.bioactmat.2020.12.012

Grynpas, M. D., Chachra, D., & Limeback, H. (2019). The action of fluoride on bone (pp. 318–330).

Habermann, B., Kafchitsas, K., Olender, G., Augat, P., & Kurth, A. (2010). Strontium ranelate enhances callus strength more than PTH 1-34 in an osteoporotic rat model of fracture healing. *Calcified Tissue International*, *86*(1), 82–89. https://doi.org/10.1007/s00223-009-9317-8

Haffner-Luntzer, M. (2021). Experimental agents to improve fracture healing: Utilizing the WNT signaling pathway. *Injury*, *52*, S44–S48. https://doi.org/10.1016/j.injury.2020.11.051

Haffner-Luntzer, M., Heilmann, A., Rapp, A. E., Roessler, R., Schinke, T., Amling, M., Ignatius, A., & Liedert, A. (2016). Antagonizing midkine accelerates fracture healing in mice by enhanced bone formation in the fracture callus. *British Journal of Pharmacology*, *173*(14), 2237–2249. https://doi.org/10.1111/bph.13503

Harada, S., & Rodan, G. A. (2003). Control of osteoblast function and regulation of bone mass. *Nature*, *423*(6937), 349–355. https://doi.org/10.1038/nature01660

He, X., Semenov, M., Tamai, K., & Zeng, X. (2004). LDL receptor-related proteins 5 and 6 in Wnt/β-catenin signaling: Arrows point the way. *Development*, *131*(8), 1663–1677. https://doi.org/10.1242/dev.01117

Hosseini, V., Dani, C., Geranmayeh, M. H., Mohammadzadeh, F., Nazari Soltan Ahmad, S., & Darabi, M. (2019). Wnt lipidation: Roles in trafficking, modulation, and function. *Journal of Cellular Physiology*, *234*(6), 8040–8054. https://doi.org/10.1002/jcp.27570

Hsu, M. N., Huang, K. L., Yu, F. J., Lai, P. L., Truong, A. V., Lin, M. W., Nguyen, N. T. K., Shen, C. C., Hwang, S. M., Chang, Y. H., & Hu, Y. C. (2020). Coactivation of endogenous Wnt10b and foxc2 by CRISPR activation enhances BMSC osteogenesis and promotes calvarial bone regeneration. *Molecular Therapy*, *28*(2), 441–451. https://doi.org/10.1016/j.ymthe.2019.11.029

Ikeda, S., Kishida, M., Matsuura, Y., Usui, H., & Kikuchi, A. (2000). GSK-3β-dependent phosphorylation of adenomatous polyposis coli gene product can be modulated by β-catenin and protein phosphatase 2A complexed with Axin. *Oncogene*, *19*(4), 537–545. https://doi.org/10.1038/sj.onc.1203359

Janda, C. Y., Dang, L. T., You, C., Chang, J., de Lau, W., Zhong, Z. A., Yan, K. S., Marecic, O., Siepe, Di., Li, X., Moody, J. D., Williams, B. O., Clevers, H., Piehler, J., Baker, D., Kuo, C. J., & Garcia, K. C. (2017). Surrogate Wnt agonists that phenocopy canonical Wnt and β-catenin signalling. *Nature*, *545*(7653), 234–237. https://doi.org/10.1038/nature22306

Jin, H., Wang, B., Li, J., Xie, W., Mao, Q., Li, S., Dong, F., Sun, Y., Ke, H. Z., Babij, P., Tong, P., & Chen, D. (2015). Anti-DKK1 antibody promotes bone fracture healing through activation of β-catenin signaling. *Bone*, *71*, 63–75. https://doi.org/10.1016/j.bone.2014.07.039

Jin, Y. R., & Yoon, J. K. (2012a). The R-spondin family of proteins: Emerging regulators of WNT signaling. *The International Journal of Biochemistry & Cell Biology*, *44*(12), 2278–2287. https://doi.org/10.1016/j.biocel.2012.09.006

Jin, Y. R., & Yoon, J. K. (2012b). The R-spondin family of proteins: Emerging regulators of WNT signaling. *The International Journal of Biochemistry & Cell Biology*, *44*(12), 2278–2287. https://doi.org/10.1016/j.biocel.2012.09.006

Jung, H., Rim, Y. A., Park, N., Nam, Y., & Ju, J. H. (2021). Restoration of osteogenesis by crispr/cas9 genome editing of the mutated col1a1 gene in osteogenesis imperfecta. *Journal of Clinical Medicine*, *10*(14), 3141. https://doi.org/10.3390/jcm10143141

Kahn, M. (2014). Can we safely target the WNT pathway? *Nature Reviews Drug Discovery*, *13*(7), 513-532. https://doi.org/10.1038/nrd4233

Kamata, T., Katsube, K. I., Michikawa, M., Yamada, M., Takada, S., & Mizusawa, H. (2004). R-spondin, a novel gene with thrombospondin type 1 domain, was expressed in the dorsal neural tube and affected in Wnts mutants. *Biochimica et Biophysica Acta - Gene Structure and Expression*, *1676*(1), 51–62. https://doi.org/10.1016/j.bbaexp.2003.10.009

Kato, M., Patel, M. S., Levasseur, R., Lobov, I., Chang, B. H. J., Glass, D. A., Hartmann, C., Li, L., Hwang, T. H., Brayton, C. F., Lang, R. A., Karsenty, G., & Chan, L. (2002). Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. *Journal of Cell Biology*, *157*(2), 303–314. https://doi.org/10.1083/jcb.200201089

Kavitha, M., Subramanian, R., Vinoth, K. S., Narayanan, R., Venkatesh, G., & Esakkiraja, N. (2015). Optimization of process parameters for solution combustion synthesis of Strontium substituted Hydroxyapatite nanocrystals using Design of Experiments approach. *Powder Technology*, *271*(December 2014), 167–181. https://doi.org/10.1016/j.powtec.2014.10.046

Kazanskaya, O., Glinka, A., del Barco Barrantes, I., Stannek, P., Niehrs, C., & Wu, W. (2004). R-Spondin2 is a secreted activator of Wnt/beta-catenin signaling and is required for Xenopus myogenesis. *Developmental Cell*, *7*(4), 525–534. https://doi.org/10.1016/j.devcel.2004.07.019

Ke, H. Z., Richards, W. G., Li, X., & Ominsky, M. S. (2012). Sclerostin and dickkopf-1 as therapeutic targets in bone diseases. *Endocrine Reviews*, *33*(5), 747–783. https://doi.org/10.1210/er.2011-1060

Khalaf, K., Janowicz, K., Dyszkiewicz-Konwińska, M., Hutchings, G., Dompe, C., Moncrieff, L., Jankowski, M., Machnik, M., Oleksiewicz, U., Kocherova, I., Petitte, J., Mozdziak, P., Shibli, J. A., Iżycki, D., Józkowiak, M., Piotrowska-Kempisty, H., Skowroński, M. T., Antosik, P., & Kempisty, B. (2020). CRISPR/Cas9 in cancer immunotherapy: Animal models and human clinical trials. *Genes*, *11*(8), 1–19. https://doi.org/10.3390/genes11080921

Khalil, A. S., Yu, X., Umhoefer, J. M., Chamberlain, C. S., Wildenauer, L. A., Diarra, G. M., Hacker, T. A., & Murphy, W. L. (2020). Single-dose mRNA therapy via biomaterial-mediated sequestration of overexpressed proteins. *Science Advances*, *6*(27), 1–13. https://doi.org/10.1126/sciadv.aba2422

Khan, P. K., Mahato, A., Kundu, B., Nandi, S. K., Mukherjee, P., Datta, S., Sarkar, S., Mukherjee, J., Nath, S., Balla, V. K., & Mandal, C. (2016). Influence of single and binary doping of strontium and lithium on in vivo biological properties of bioactive glass scaffolds. *Scientific Reports*, *6*(September), 1–18. https://doi.org/10.1038/srep32964

Khorsand, B., Elangovan, S., Hong, L., Dewerth, A., Kormann, M. S. D., & Salem, A. K. (2017). A comparative study of the bone regenerative effect of chemically modified RNA encoding BMP-2 or BMP-9. *The AAPS Journal*, *19*(2), 438–446. https://doi.org/10.1208/s12248-016-0034-8

Kim, H. W., & Kim, Y. J. (2021). Fabrication of strontium-substituted hydroxyapatite scaffolds using 3D printing for enhanced bone regeneration. *Journal of Materials Science*, *56*(2), 1673–1684. https://doi.org/10.1007/s10853-020-05391-y

Kirstetter, P., Anderson, K., Porse, B. T., Jacobsen, S. E. W., & Nerlov, C. (2006). Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nature Immunology*, *7*(10), 1048–1056. https://doi.org/10.1038/ni1381

Klein, P. S., & Melton, D. A. (2019). A molecular mechanism for the effect of lithium on development. *Bipolar Disorder: The Science of Mental Health*, *93*(August), 235–240. https://doi.org/10.4324/9781315054308-24

Knight, M. N., & Hankenson, K. D. (2014). R-spondins: Novel matricellular regulators of the skeleton. In *Matrix biology* (Vol. 37, pp. 157–161). Elsevier B.V. https://doi.org/10.1016/j.matbio.2014.06.003

Knight, M. N., Karuppaiah, K., Lowe, M., Mohanty, S., Zondervan, R. L., Bell, S., Ahn, J., & Hankenson, K. D. (2018). R-spondin-2 is a Wnt agonist that regulates osteoblast activity and bone mass. *Bone Research*, *6*(1), 24. https://doi.org/10.1038/s41413-018-0026-7

Komatsu, D., Mary, M., Schroeder, R., Robling, A., Turner, C., & Warden, S. (2010). Modulation of wnt signaling influences fracture repair. *Journal of Orthopaedic Research*, *23*(7), 928–936. https://doi.org/10.1002/jor.21078

Komiya, Y., & Habas, R. (2008). Wnt signal transduction pathways. *Organogenesis*, *4*(2), 68–75. https://doi.org/10.4161/org.4.2.5851

Krishnamurthy, N., & Kurzrock, R. (2018). Targeting the Wnt/beta-catenin pathway in cancer: Update on effectors and inhibitors. *Cancer Treatment Reviews*, *62*, 50–60. https://doi.org/10.1016/j.ctrv.2017.11.002

Krönke, G., Uderhardt, S., Kim, K. A., Stock, M., Scholtysek, C., Zaiss, M. M., Surmann-Schmitt, C., Luther, J., Katzenbeisser, J., David, J. P., Abdollahi-Roodsaz, S., Tran, K., Bright, J. M., Binnerts, M. E., Akhmetshina, A., Böhm, C., Distler, J. H., Joosten, L. A. B., Schett, G., & Abo, A. (2010). R-spondin 1 protects against inflammatory bone damage during murine arthritis by modulating the Wnt pathway. *Arthritis & Rheumatism*, *62*(8), 2303–2312. https://doi.org/10.1002/art.27496

Kuang, P., Deng, H., Liu, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2018). Sodium fluoride induces splenocyte autophagy via the mammalian targets of rapamycin (mTOR) signaling pathway in growing mice. *Aging*, *10*(7), 1649–1665. https://doi.org/10.18632/aging.101499

Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., … Boyle, W. J. (1998). Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell*, *93*(2), 165–176. https://doi.org/10.1016/s0092-8674(00)81569-x

Lambert, L. J., Challa, A. K., Niu, A., Zhou, L., Tucholski, J., Johnson, M. S., Nagy, T. R., Eberhardt, A. W., Estep, P. N., Kesterson, R. A., & Grams, J. M. (2016). Increased trabecular bone and improved biomechanics in an osteocalcin-null rat model created by CRISPR/Cas9 technology. *DMM Disease Models and Mechanisms*, *9*(10), 1169–1179. https://doi.org/10.1242/dmm.025247

Lee, J., Byun, H., Madhurakkat Perikamana, S. K., Lee, S., & Shin, H. (2019). Current advances in immunomodulatory biomaterials for bone regeneration. *Advanced Healthcare Materials*, *8*(4), 1–20. https://doi.org/10.1002/adhm.201801106

Leucht, P., Jiang, J., Cheng, D., Liu, B., Dhamdhere, G., Fang, M. Y., Monica, S. D., Urena, J. J., Cole, W., Smith, L. R., Castillo, A. B., Longaker, M. T., & Helms, J. A. (2013). Wnt3a reestablishes osteogenic capacity to bone grafts from aged animals. *Journal of Bone and Joint Surgery - Series A*, *95*(14), 1278–1288. https://doi.org/10.2106/JBJS.L.01502

Li, M., Zheng, J., He, X., & Zhang, X. (2022). Tiki proteins are glycosylphosphatidylinositol-anchored proteases. *FEBS Letters*, *596*(8), 1037–1046. https://doi.org/10.1002/1873-3468.14320

Li, Q., Wang, Q., Wang, O., Shao, K., Lin, H., & Lei, Y. (2018). A simple and scalable hydrogel-based system for culturing protein-producing cells. *PLoS One*, *13*(1), 1–14. https://doi.org/10.1371/journal.pone.0190364

Li, X., Ominsky, M. S., Warmington, K. S., Morony, S., Gong, J., Cao, J., Gao, Y., Shalhoub, V., Tipton, B., Haldankar, R., Chen, Q., Winters, A., Boone, T., Geng, Z., Niu, Q.-T., Ke, H. Z., Kostenuik, P. J., Simonet, W. S., Lacey, D. L., & Paszty, C. (2009). Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis*. *Journal of Bone and Mineral Research*, *24*(4), 578–588. https://doi.org/10.1359/jbmr.081206

Li, X., Zhang, Y., Kang, H., Liu, W., Liu, P., Zhang, J., Harris, S. E., & Wu, D. (2005). Sclerostin binds to LRP5/6 and antagonizes canonical wnt signaling. *Journal of Biological Chemistry*, *280*(20), 19883–19887. https://doi.org/10.1074/jbc.M413274200

Li, Y., Bian, S., Wang, J., & Wang, J. (2016). Effects of fluoride and chitosan on the gene expressions of bone morphogenic protein 2 and collagen type-1 alpha 1 chain in the mouse femur. *Fluoride*, *49*(March), 47–55.

Liedert, A., Schinke, T., Ignatius, A., & Amling, M. (2014). The role of midkine in skeletal remodelling. *British Journal of Pharmacology*, *171*(4), 870–878. https://doi.org/10.1111/bph.12412

Liu, D., Nie, W., Li, D., Wang, W., Zheng, L., Zhang, J., Zhang, J., Peng, C., Mo, X., & He, C. (2019). 3D printed PCL/SrHA scaffold for enhanced bone regeneration. *Chemical Engineering Journal*, *362*, 269–279. https://doi.org/10.1016/j.cej.2019.01.015

Liu, X., Kim, J. H., Wang, J., Chen, X., Zhang, H., Kim, S. H., Cui, J., Ruidong, L., Zhang, W., Kong, Y., Zhang, J., Shui, W., Lamplot, J., Rogers, M. R., Zhao, C., Wang, N., Tomal, J., Statz, J., Wang, N., & Rajan, P. (2013). Wnt signaling in bone formation and its therapeutic potential for bone diseases. *Therapeutic Advances in Musculoskeletal Disease*, *5*(1), 13–31. https://doi.org/10.1177/1759720X12466608

Liu, X. L., Li, C. C., Liu, K. J., Cui, C. Y., Zhang, Y. Z., & Liu, Y. (2012). The influence of fluoride on the expression of inhibitors of Wnt/β-catenin signaling pathway in rat skin fibroblast cells. *Biological Trace Element Research*, *148*(1), 117–121. https://doi.org/10.1007/s12011-012-9333-9

Liu, Y., Wang, J., Liu, S., Kuang, M., Jing, Y., Zhao, Y., Wang, Z., & Li, G. (2019). A novel transgenic murine model with persistently brittle bones simulating osteogenesis imperfecta type I. *Bone*, *127*, 646–655. https://doi.org/10.1016/j.bone.2019.07.021

Lu, W., Kim, K. A., Liu, J., Abo, A., Feng, X., Cao, X., & Li, Y. (2008). R-spondin1 synergizes with Wnt3A in inducing osteoblast differentiation and osteoprotegerin expression. *FEBS Letters*, *582*(5), 643–650. https://doi.org/10.1016/j.febslet.2008.01.035

Lung, H., Moody, T., Wentworth, K., Kang, M., Ho, S., & Hsiao, E. (2021). Global wnt inhibition with a porcupine inhibitor decreases established trabecular bone in a mouse model of fibrous dysplasia. *Journal of the Endocrine Society*, *5*(Supplement 1), A234. https://doi.org/10.1210/jendso/bvab048.475

Ma, L., Zhang, R., Li, D., Qiao, T., & Guo, X. (2021). Fluoride regulates chondrocyte proliferation and autophagy via PI3K/AKT/mTOR signaling pathway. *Chemico-Biological Interactions*, *349*, 109659. https://doi.org/10.1016/j.cbi.2021.109659

Maeder, M. L., Stefanidakis, M., Wilson, C. J., Baral, R., Barrera, L. A., Bounoutas, G. S., Bumcrot, D., Chao, H., Ciulla, D. M., DaSilva, J. A., Dass, A., Dhanapal, V., Fennell, T. J., Friedland, A. E., Giannoukos, G., Gloskowski, S. W., Glucksmann, A., Gotta, G. M., Jayaram, H., & Jiang, H. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nature Medicine*, *25*(2), 229–233. https://doi.org/10.1038/s41591-018-0327-9

Manolagas, S. C. (2014). Wnt signaling and osteoporosis. *Maturitas*, *78*(3), 233–237. https://doi.org/10.1016/j.maturitas.2014.04.013

Mao, J., Wang, J., Liu, B., Pan, W., Farr, G. H., Flynn, C., Yuan, H., Takada, S., Kimelman, D., Li, L., & Wu, D. (2001). Low-density lipoprotein receptor-related protein-5 binds to axin and regulates the canonical wnt signaling pathway. *Molecular Cell*, *7*(4), 801–809. https://doi.org/10.1016/S1097-2765(01)00224-6

Markham, A. (2019). Romosozumab: First Global approval. *Drugs*, *79*(4), 471–476. https://doi.org/10.1007/s40265-019-01072-6

McClung, M. R., Grauer, A., Boonen, S., Bolognese, M. A., Brown, J. P., Diez-Perez, A., Langdahl, B. L., Reginster, J.-Y., Zanchetta, J. R., Wasserman, S. M., Katz, L., Maddox, J., Yang, Y. C., Libanati, C., & Bone, H. G. (2014). Romosozumab in postmenopausal women with low bone mineral density. *New England Journal of Medicine*, *370*(5), 412–420. https://doi.org/10.1056/NEJMoa1305224

Meka, S. R. K., Jain, S., & Chatterjee, K. (2016). Strontium eluting nanofibers augment stem cell osteogenesis for bone tissue regeneration.

Colloids and Surfaces B: Biointerfaces, *146*, 649–656. https://doi.org/10.1016/j.colsurfb.2016.07.012

Minear, S., Leucht, P., Jiang, J., Liu, B., Zenga, A., Fuerer, C., Nusse, R., & Helms, J. A. (2010). Wnt proteins promote bone regeneration. *Science Translational Medicine*, *2*(29). https://doi.org/10.1126/scitranslmed.3000231

Mockey, M., Gonçalves, C., Dupuy, F. P., Lemoine, F. M., Pichon, C., & Midoux, P. (2006). mRNA transfection of dendritic cells: Synergistic effect of ARCA mRNA capping with Poly(A) chains in cis and in trans for a high protein expression level. *Biochemical and Biophysical Research Communications*, *340*(4), 1062–1068. https://doi.org/10.1016/j.bbrc.2005.12.105

Moghanian, A., Firoozi, S., & Tahriri, M. (2017). Characterization, in vitro bioactivity and biological studies of sol-gel synthesized SrO substituted 58S bioactive glass. *Ceramics International*, *43*(17), 14880–14890. https://doi.org/10.1016/j.ceramint.2017.08.004

Mukherjee, S., & Thrasher, A. J. (2013). Gene therapy for PIDs: Progress, pitfalls and prospects. *Gene*, *525*(2), 174–181. https://doi.org/10.1016/j.gene.2013.03.098

Müller, W. E. G., Tolba, E., Ackermann, M., Neufurth, M., Wang, S., Feng, Q., Schröder, H. C., & Wang, X. (2017). Fabrication of amorphous strontium polyphosphate microparticles that induce mineralization of bone cells in vitro and in vivo. *Acta Biomaterialia*, *50*, 89–101. https://doi.org/10.1016/j.actbio.2016.12.045

Nagano, K. (2019). R-spondin signaling as a pivotal regulator of tissue development and homeostasis. In *Japanese dental science review* (Vol. 55, pp. 80–87). Elsevier Ltd. https://doi.org/10.1016/j.jdsr.2019.03.001

Nam, D., Balasuberamaniam, P., Milner, K., Kunz, M., Vachhani, K., Kiss, A., & Whyne, C. (2020). Lithium for fracture treatment (LiFT): A double-blind randomised control trial protocol. *BMJ Open*, *10*(1), e031545. https://doi.org/10.1136/bmjopen-2019-031545

Nam, J. S., Turcotte, T. J., Smith, P. F., Choi, S., & Jeong, K. Y. (2006). Mouse cristin/R-spondin family proteins are novel ligands for the frizzled 8 and LRP6 receptors and activate β-catenin-dependent gene expression. *Journal of Biological Chemistry*, *281*(19), 13247–13257. https://doi.org/10.1074/jbc.M508324200

New Drugs at FDA:*CDER's New Molecular Entities and New Therapeutic Biological Products*. (2021). *FDA*. Retrieved from https://www.fda.gov/drugs/development-approval-process-drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products

Ng, L. F., Kaur, P., Bunnag, N., Suresh, J., Sung, I. C. H., Tan, Q. H., Gruber, J., & Tolwinski, N. S. (2019). WNT signaling in disease. *Cells*, *8*(8), 826. NLM (Medline). https://doi.org/10.3390/cells8080826

Nile, A. H., & Hannoush, R. N. (2016). Fatty acylation of Wnt proteins. In *Nature chemical biology* (Vol. 12, pp. 60–69). Nature Publishing Group. https://doi.org/10.1038/nchembio.2005Issue 2.

O'Connell, M. R., Oakes, B. L., Sternberg, S. H., East-Seletsky, A., Kaplan, M., & Doudna, J. A. (2014). Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature*, *516*(7530), 263–266. https://doi.org/10.1038/nature13769

O'Connell, R. A., Mayo, J. A., Flatow, L., Cuthbertson, B., & O'Brien, B. E. (1991). Outcome of bipolar disorder on long-term treatment with lithium. *British Journal of Psychiatry*, *159*(JUL), 123–129. https://doi.org/10.1192/bjp.159.1.123

Ohnaka, K., Yamamoto, K., Nakamura, K., Adachi, M., Kawate, H., Kono, S., & Takayanagi, R. (2009). Association of single nucleotide polymorphisms in secreted frizzled-related protein 1 gene with bone mineral density in Japanese women. *Geriatrics and Gerontology International*, *9*(3), 304–309. https://doi.org/10.1111/j.1447-0594.2009.00540.x

Ominsky, M. S., Boyd, S. K., Varela, A., Jolette, J., Felx, M., Doyle, N., Mellal, N., Smith, S. Y., Locher, K., Buntich, S., Pyrah, I., & Boyce, R. W. (2017). Romosozumab improves bone mass and strength while maintaining bone quality in ovariectomized cynomolgus monkeys. *Journal of Bone and Mineral Research*, *32*(4), 788–801. https://doi.org/10.1002/jbmr.3036

Ominsky, M. S., Li, C., Li, X., Tan, H. L., Lee, E., Barrero, M., Asuncion, F. J., Dwyer, D., Han, C. Y., Vlasseros, F., Samadfam, R., Jolette, J., Smith, S. Y., Stolina, M., Lacey, D. L., Simonet, W. S., Paszty, C., Li, G., & Ke, H. Z. (2011). Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. *Journal of Bone and Mineral Research*, *26*(5), 1012–1021. https://doi.org/10.1002/jbmr.307

Orford, K., Crockett, C., Jensen, J. P., Weissman, A. M., & Byers, S. W. (1997). Serine phosphorylation-regulated ubiquitination and degradation of β-catenin. *Journal of Biological Chemistry*, *272*(40), 24735–24738. https://doi.org/10.1074/jbc.272.40.24735

Padhi, D., Jang, G., Stouch, B., Fang, L., & Posvar, E. (2011). Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *Journal of Bone and Mineral Research*, *26*(1), 19–26. https://doi.org/10.1002/jbmr.173

Pan, L., Shi, X., Liu, S., Guo, X., Zhao, M., Cai, R., & Sun, G. (2014). Fluoride promotes osteoblastic differentiation through canonical Wnt/ -catenin signaling pathway. *Toxicology Letters*, *225*(1), 34–42. https://doi.org/10.1016/j.toxlet.2013.11.029

Park, S. W., Seo, M. K., Webster, M. J., Lee, J. G., & Kim, S. (2022). Differential expression of gene co-expression networks related to the mTOR signaling pathway in bipolar disorder. *Translational Psychiatry*, *12*(1), 184. https://doi.org/10.1038/s41398-022-01944-8

Park, S. Y., Kim, K. il, Park, S. P., Lee, J. H., & Jung, H. S. (2016). Aspartic acid-assisted synthesis of multifunctional strontium-substituted hydroxyapatite microspheres. *Crystal Growth & Design*, *16*(8), 4318–4326. https://doi.org/10.1021/acs.cgd.6b00420

Peng, S., Zhou, G., Luk, K. D. K., Kenneth, M. C., Li, Z., Lam, W. M., Zhou, Z., & Lu, W. W. (2009). Strontium promotes OSteogenic differentiation of mesenchymal stem cells through the Ras/MAPK signaling pathway. *Cellular Physiology and Biochemistry*, *23*(1–3), 165–174. https://doi.org/10.1159/000204105

Pickar-Oliver, A., & Gersbach, C. A. (2019). The next generation of CRISPR–Cas technologies and applications. In *Nature reviews molecular cell biology* (Vol. 20, pp. 490–507). Nature Publishing Group, 8. https://doi.org/10.1038/s41580-019-0131-5

Pilishvili, T., Gierke, R., Fleming-Dutra, K. E., Farrar, J. L., Mohr, N. M., Talan, D. A., Krishnadasan, A., Harland, K. K., Smithline, H. A., Hou, P. C., Lee, L. C., Lim, S. C., Moran, G. J., Krebs, E., Steele, M. T., Beiser, D. G., Faine, B., Haran, J. P., Nandi, U., & Schrag, S. J. (2021). Effectiveness of mRNA Covid-19 vaccine among U.S. Health care personnel. *New England Journal of Medicine*, *385*(25), e90. https://doi.org/10.1056/NEJMoa2106599

Pina, S., Vieira, S., Rego, P., Torres, P., da Cruz e Silva, O., da Cruz e Silva, E., & Ferreira, J. (2010). Biological responses of brushite-forming Zn- and ZnSr-substituted beta-tricalcium phosphate bone cements. *European Cells and Materials*, *20*(May 2014), 162–177. https://doi.org/10.22203/ecm.v020a14

Pinzone, J. J., Hall, B. M., Thudi, N. K., Vonau, M., Qiang, Y.-W., Rosol, T. J., & Shaughnessy, J. D. (2009). The role of Dickkopf-1 in bone development, homeostasis, and disease. *Blood*, *113*(3), 517–525. https://doi.org/10.1182/blood-2008-03-145169

Popelut, A., Rooker, S. M., Leucht, P., Medio, M., Brunski, J. B., & Helms, J. A. (2010). The acceleration of implant osseointegration by liposomal Wnt3a. *Biomaterials*, *31*(35), 9173–9181. https://doi.org/10.1016/j.biomaterials.2010.08.045

Proffitt, K. D., & Virshup, D. M. (2012). Precise regulation of porcupine activity is required for physiological Wnt signaling. *Journal of Biological Chemistry*, *287*(41), 34167–34178. https://doi.org/10.1074/jbc.M112.381970

Ramunas, J., Yakubov, E., Brady, J. J., Corbel, S. Y., Holbrook, C., Brandt, M., Stein, J., Santiago, J. G., Cooke, J. P., & Blau, H. M. (2015).

Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells. *Federation of American Societies for Experimental Biology Journal*, *29*(5), 1930–1939. https://doi.org/10.1096/fj.14-259531

Roy, B. (2021). Effects of mRNA modifications on translation: An overview. In *RNA modifications. Methods in molecular biology* (Vol. 2298, pp. 327–356). Humana Press. https://doi.org/10.1007/978-1-0716-1374-0_20

Rybchyn, M. S., Slater, M., Conigrave, A. D., & Mason, R. S. (2011). An akt-dependent increase in canonical wnt signaling and a decrease in sclerostin protein levels are involved in strontium ranelate-induced osteogenic effects in human osteoblasts. *Journal of Biological Chemistry*, *286*(27), 23771–23779. https://doi.org/10.1074/jbc.M111.251116

Saidak, Z., & Marie, P. J. (2012). Strontium signaling: Molecular mechanisms and therapeutic implications in osteoporosis. *Pharmacology & Therapeutics*, *136*(2), 216–226. https://doi.org/10.1016/j.pharmthera.2012.07.009

Sankaralingam, P. P., Sakthivel, P., Andinadar Subbiah, P., Rahumathulla, J., & Chinnaswamy Thangavel, V. (2021). Preparation and physical-biological characterization on titanium doped fluorophosphate nanobioglass: Bone implants. *International Journal of Ceramic Engineering & Science*, *3*(1), 37–48. https://doi.org/10.1002/ces2.10078

Sato, M., Chen, P., Tsutsumi, Y., Shiota, M., Hanawa, T., & Kasugai, S. (2016). Effect of strontium ions on calcification of preosteoblasts cultured on porous calcium-and phosphate-containing titanium oxide layers formed by micro-arc oxidation. *Dental Materials Journal*, *35*(4), 627–634. https://doi.org/10.4012/dmj.2016-032

Schemitsch, E. H., Miclau, T., Karachalios, T., Nowak, L. L., Sancheti, P., Poolman, R. W., Caminis, J., Daizadeh, N., Dent-Acosta, R. E., Egbuna, O., Chines, A., Maddox, J., Grauer, A., & Bhandari, M. (2020). A randomized, placebo-controlled study of romosozumab for the treatment of hip fractures. *Journal of Bone and Joint Surgery American Volume*, *102*(8), 693–702. https://doi.org/10.2106/JBJS.19.00790

Schumacher, M., & Gelinsky, M. (2015). Strontium modified calcium phosphate cements - approaches towards targeted stimulation of bone turnover. *Journal of Materials Chemistry B*, *3*(23), 4626–4640. https://doi.org/10.1039/c5tb00654f

Schupbach, D., Comeau-Gauthier, M., Harvey, E., & Merle, G. (2020). Wnt modulation in bone healing. *Bone*, *138*, 115491. https://doi.org/10.1016/j.bone.2020.115491

Semënov, M., Tamai, K., & He, X. (2005). SOST is a ligand for LRP5/LRP6 and a wnt signaling inhibitor. *Journal of Biological Chemistry*, *280*(29), 26770–26775. https://doi.org/10.1074/jbc.M504308200

Sharma, A. R., Choi, B.-S., Park, J.-M., Lee, D.-H., Lee, J.-E., Kim, H.-S., Yoon, J.-K., Song, D.-K., Nam, J.-S., & Lee, S.-S. (2013). Rspo 1 promotes osteoblast differentiation via Wnt signaling pathway. *Indian Journal of Biochemistry & Biophysics*, *50*(1), 19–25.

Siman-Tov, R., Zelikson, N., Caspi, M., Levi, Y., Perry, C., Khair, F., Stauber, H., Sznitman, J., & Rosin-Arbesfeld, R. (2021). Circulating wnt ligands activate the wnt signaling pathway in mature Erythrocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *41*(5), E243–E264. https://doi.org/10.1161/ATVBAHA.120.315413

Stefanic, M., Peroglio, M., Stanciuc, A. M., Machado, G. C., Campbell, I., Kržmanc, M. M., Alini, M., & Zhang, X. (2018). The influence of strontium release rate from bioactive phosphate glasses on osteogenic differentiation of human mesenchymal stem cells. *Journal of the European Ceramic Society*, *38*(3), 887–897. https://doi.org/10.1016/j.jeurceramsoc.2017.08.005

Su, W. T., Wu, P. S., & Huang, T. Y. (2015). Osteogenic differentiation of stem cells from human exfoliated deciduous teeth on poly(ε-caprolactone) nanofibers containing strontium phosphate. *Materials Science and Engineering: C*, *46*, 427–434. https://doi.org/10.1016/j.msec.2014.10.076

Sultana, N., Magadum, A., Hadas, Y., Kondrat, J., Singh, N., Youssef, E., Calderon, D., Chepurko, E., Dubois, N., Hajjar, R. J., & Zangi, L. (2017). Optimizing cardiac delivery of modified mRNA. *Molecular Therapy*, *25*(6), 1306–1315. https://doi.org/10.1016/j.ymthe.2017.03.016

Tamai, K., Zeng, X., Liu, C., Zhang, X., Harada, Y., Chang, Z., & He, X. (2004). A. *Molecular Cell*, *13*(1), 149–156. https://doi.org/10.1016/s1097-2765(03)00484-2

Tao, Y., Mis, M., Blazer, L., Ustav, M., Steinhart, Z., Chidiac, R., Kubarakos, E., O'Brien, S., Wang, X., Jarvik, N., Patel, N., Adams, J., Moffat, J., Angers, S., & Sidhu, S. S. (2019). Tailored tetravalent antibodies potently and specifically activate Wnt/Frizzled pathways in cells, organoids and mice. *Elife*, *8*. https://doi.org/10.7554/eLife.46134

Thormann, U., Ray, S., Sommer, U., El Khassawna, T., Rehling, T., Hundgeburth, M., Henß, A., Rohnke, M., Janek, J., Lips, K. S., Heiss, C., Schlewitz, G., Szalay, G., Schumacher, M., Gelinsky, M., Schnettler, R., & Alt, V. (2013). Bone formation induced by strontium modified calcium phosphate cement in critical-size metaphyseal fracture defects in ovariectomized rats. *Biomaterials*, *34*(34), 8589–8598. https://doi.org/10.1016/j.biomaterials.2013.07.036

Tolwinski, N. S., & Wieschaus, E. (2004). A nuclear function for armadillo/β-catenin. *PLoS Biology*, *2*(4), e95. https://doi.org/10.1371/journal.pbio.0020095

Ubels, J. L., Diegel, C. R., Foxa, G. E., Ethen, N. J., Lensing, J. N., Madaj, Z. B., & Williams, B. O. (2020). Low-density lipoprotein receptor-related protein 5-deficient rats have reduced bone mass and abnormal development of the retinal vasculature. *CRISPR Journal*, *3*(4), 284–298. https://doi.org/10.1089/crispr.2020.0009

Uddin, F., Rudin, C. M., & Sen, T. (2020). CRISPR gene therapy: Applications, limitations, and implications for the future. *Frontiers in Oncology*, *10*(August). https://doi.org/10.3389/fonc.2020.01387

Virk, M. S., Alaee, F., Tang, H., Ominsky, M. S., Ke, H. Z., & Lieberman, J. R. (2013). Systemic administration of sclerostin antibody enhances bone repair in a critical-sized femoral defect in a rat model. *The Journal of Bone and Joint Surgery*, *95*(8), 694–701. https://doi.org/10.2106/JBJS.L.00285

Wang, B., Guo, H., Geng, T., Sun, K., Zhang, L., Lu, Z., & Jin, Q. (2021). The effect of strontium ranelate on titanium particle-induced periprosthetic osteolysis regulated by WNT/β-catenin signaling in vivo and in vitro. *Bioscience Reports*, *41*(1), 1–18. https://doi.org/10.1042/BSR20203003

Wang, J., & Wynshaw-Boris, A. (2004). The canonical Wnt pathway in early mammalian embryogenesis and stem cell maintenance/differentiation. *Current Opinion in Genetics & Development*, *14*(5), 533–539. https://doi.org/10.1016/j.gde.2004.07.013

Wang, Y., Newman, M. R., Ackun-Farmmer, M., Baranello, M. P., Sheu, T.-J. J. E. P., & Benoit, D. S. W. (2016). Fracture-targeted delivery of β-catenin agonists via peptide- functionalized. *Physiology & Behavior*, *176*(1), 139–148. https://doi.org/10.1021/acsnano.7b05103.Figure

Wang, Y., Newman, M. R., & Benoit, D. S. W. (2018). Development of controlled drug delivery systems for bone fracture-targeted therapeutic delivery: A review. *European Journal of Pharmaceutics and Biopharmaceutics*, *127*, 223–236. https://doi.org/10.1016/j.ejpb.2018.02.023

Wei, Q., Yokota, C., Semenov, M. v., Doble, B., Woodgett, J., & He, X. (2007). R-spondin1 is a high affinity ligand for LRP6 and induces LRP6 phosphorylation and β-catenin signaling. *Journal of Biological Chemistry*, *282*(21), 15903–15911. https://doi.org/10.1074/jbc.M701927200

Weng, L., Boda, S. K., Teusink, M. J., Shuler, F. D., Li, X., & Xie, J. (2017). Binary doping of strontium and copper enhancing osteogenesis and angiogenesis of bioactive glass nanofibers while suppressing osteoclast activity. *ACS Applied Materials and Interfaces*, *9*(29), 24484–-24496. https://doi.org/10.1021/acsami.7b06521

Witcher, P. C., Miner, S. E., Horan, D. J., Bullock, W. A., Lim, K. E., Kang, K. S., Adaniya, A. L., Ross, R. D., Loots, G. G., & Robling, A. G. (2018). Sclerostin neutralization unleashes the osteoanabolic effects of Dkk1 inhibition. *JCI Insight*, *3*(11). https://doi.org/10.1172/jci.insight.98673

Wodarz, A., & Nusse, R. (1998). Mechanisms of WNT signaling in development. *Annual Review of Cell and Developmental Biology*, *14*(1), 59–88. https://doi.org/10.1146/annurev.cellbio.14.1.59

Wu, J., Wang, P., Jiang, D., Li, H., Luo, C., Liu, X., Qu, X., Cao, Y., & Li, M. (2017). In vitro and in vivo characterization of strontium-containing calcium sulfate/poly(amino acid) composite as a novel bioactive graft for bone regeneration. *RSC Advances*, *7*(86), 54306–54312. https://doi.org/10.1039/c7ra10523a

Xiao, Y., Fan, M., Jin, W., Li, W., Jia, Y., Dong, Y., Jiang, X., Xu, J., Meng, N., & Lv, P. (2020). Lithium chloride ameliorated spatial cognitive impairment through activating mTOR phosphorylation and inhibiting excessive autophagy in the repeated cerebral ischemia-reperfusion mouse model. *Experimental and Therapeutic Medicine*, *20*(5), 1. https://doi.org/10.3892/etm.2020.9237

Xing, Y., Clements, W. K., Kimelman, D., & Xu, W. (2003). Crystal structure of a β-catenin/Axin complex suggests a mechanism for the β-catenin destruction complex. *Genes & Development*, *17*(22), 2753–2764. https://doi.org/10.1101/gad.1142603

Xu, H., Duan, J., Ning, D., Li, J., Liu, R., Yang, R., Jiang, J. X., & Shang, P. (2014). Role of Wnt signaling in fracture healing. *BMB Reports*, *47*(12), 666–672. https://doi.org/10.5483/BMBRep.2014.47.12.193

Yaccoby, S., Ling, W., Zhan, F., Walker, R., Barlogie, B., & Shaughnessy, J. D. (2007). Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. *Blood*, *109*(5), 2106–2111. https://doi.org/10.1182/blood-2006-09-047712

Yang, F., Yang, D., Tu, J., Zheng, Q., Cai, L., & Wang, L. (2011). Strontium enhances osteogenic differentiation of mesenchymal stem cells and in vivo bone formation by activating wnt/catenin signaling. *Stem Cells*, *29*(6), 981–991. https://doi.org/10.1002/stem.646

Yao, W., Cheng, Z., Shahnazari, M., Dai, W., Johnson, M. L., & Lane, N. E. (2010). Overexpression of secreted frizzled-related protein 1 inhibits bone formation and attenuates parathyroid hormone bone anabolic effects. *Journal of Bone and Mineral Research*, *25*(2), 190–199. https://doi.org/10.1359/jbmr.090719

Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N., & Suda, T. (1998). Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proceedings of the National Academy of Sciences*, *95*(7), 3597–3602. https://doi.org/10.1073/pnas.95.7.3597

Zeng, X., Tamai, K., Doble, B., Li, S., Huang, H., Habas, R., Okamura, H., Woodgett, J., & He, X. (2005). A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature*, *438*(7069), 873–877. https://doi.org/10.1038/nature04185

Zhang, X., MacDonald, B. T., Gao, H., Shamashkin, M., Coyle, A. J., Martinez, R. v., & He, X. (2016). Characterization of tiki, a new family of wnt-specific metalloproteases. *Journal of Biological Chemistry*, *291*(5), 2435–2443. https://doi.org/10.1074/jbc.M115.677807

Zohra, F. T., Chowdhury, E. H., Tada, S., Hoshiba, T., & Akaike, T. (2007). Effective delivery with enhanced translational activity synergistically accelerates mRNA-based transfection. *Biochemical and Biophysical Research Communications*, *358*(1), 373–378. https://doi.org/10.1016/j.bbrc.2007.04.059

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