Published in final edited form as:

Trends Biotechnol. 2014 February ; 32(2): 74–81. doi:10.1016/j.tibtech.2013.12.002.

Small Molecule based Musculoskeletal Regenerative Engineering

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Abstract

Clinicians and scientists working in the field of regenerative engineering are actively investigating a wide range of methods to promote musculoskeletal tissue regeneration. Small molecule-mediated tissue regeneration is emerging as a promising strategy for regenerating various musculoskeletal tissues and a large number of small molecule compounds have been recently discovered as potential bioactive molecules for musculoskeletal tissue repair and regeneration. In this review, we summarize the recent literature encompassing the past four years in the area of small bioactive molecule for promoting repair and regeneration of various musculoskeletal tissues including bone, muscle, cartilage, tendon, and nerve.

Keywords

tissue regeneration; regenerative engineering; musculoskeletal tissue; small molecules

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Small molecules in regenerative medicine

Regenerative engineering is an emerging interdisciplinary field in which classical tissue engineering converges with advanced materials science, stem cell technology, and developmental biology toward the regeneration of complex tissues and organs (1). In regenerative engineering, growth factors, synthetic scaffolds, and cells combine to form a construct that will be structurally, functionally, and mechanically similar to the native tissue that requires repair and regeneration (Figure 1) (2). Growth factors are signaling polypeptides that guide cells during tissue development and have received considerable clinical interest (3). Although promising, a significant limitation associated with therapeutic growth factors is immunogenicity which can induce pleiotropic effects including the development of a high affinity B cell-mediated humoral response directed against the therapeutic agents (4). Therapeutic small molecules (< 1000 Da) have unique advantages over and are an important alternative to growth factors (5, 6) (7). Small molecules are unlikely to induce an immune response in the host because they are too small to do so (8). Unlike polypeptides, higher order structure is not a factor for small molecule function (9). Additionally, the manufacturing cost and the risk of cross-species contamination can be significantly minimized with the use of small molecules versus recombinant protein-based growth factors (6, 10). Therefore, small molecules with therapeutic potential may represent the next generation of regenerative engineering for regenerative medicine and other applications. This review article focuses on recent developments of small molecules associated with musculoskeletal regeneration.

Small molecule mediated bone formation

Skeletal injuries are major bone disorders in human clinics. An estimated 1.5 million skeletal injuries require tissue graft reconstruction in the U.S. each year (11). Thus, the development of effective strategies to regenerate bone tissue defects is a significant medical need. Stimulation of bone repair and regeneration using growth factors is a promising approach to these complications (12, 13). For instance, U.S. Food and Drug Administration (FDA) has approved several recombinant human bone morphogenetic proteins (rhBMPs)-based products for use in various bone disorders including long bone fractures, spinal fusion, and oral surgeries (12, 13). Although the clinical results are usually promising, there is an increasing demand for osteogenic small molecules because the rhBMPs raised concerns about safety and effectiveness in clinical setting (5, 6, 13, 14). In order to improve the effectiveness of BMPs, doses on the order of 1 million times the concentrations found in nature are used, potentially causing serious side effects. For instance, Taghavi *et al.* noted inflammatory reactions and ectopic bone formation associated with high doses of BMP (15). Another study by Lad *et al.* concluded that the usage of BMP in lumbar spinal fusions to be associated with increased rates of benign neoplasia. (16). In addition, the high cost for manufacturing BMP creates barriers for routine clinical use. A small molecule that could activate BMP signaling in the cell would be beneficial since it could reduce the patient's dose of BMP (thus minimizing the side effects) as well as the treatment cost (10, 17).

BMP signaling is a tightly regulated process. Binding of BMP to the BMP receptor (BMPR) will lead to the activation of intracellular signal transduction (Figure 2). BMPs bind to type I and type II serine/threonine kinase receptors and activate their kinase activities. The activated complex recruits and phosphorylates the transcription factors Smad1, Smad5, and Smad8. In turn, the phosphorylated proteins then form a complex with Smad4, translocate from the cytoplasm to the nucleus, and induce the expression of the osteoblast-related genes (18). Interestingly, a number of extracellular and intracellular proteins have been identified as specific negative regulators of the BMP signaling. For example, the E3 ubiquitin ligase for BMP-specific Smad proteins (Smurf1), secreted BMP antagonists (noggin, gremlin, cerberus, and chordin), and antagonistic Smads (Smad6 and Smad7) have been implicated as negative regulators of this signaling pathway (Figure 2) (19, 20). In contrast to negative regulators of BMP, activators of BMP signaling have not been widely reported. Identification of a small molecule activator targeting these negative regulators is a promising strategy to potentiate the BMP signaling.

Several small molecule based BMP signaling activators have been recently discovered using a variety of methods. In one study, using a customized cell-based reporter assay system, the small molecule SVAK-12 was identified as a novel BMP signaling activator for potentiating BMP-2 induced trans-differentiation of muscle cells into osteogenic lineages by interrupting the function of Smurf1 (21). A single-dose of SVAK-12 (100, 250, or 500 µg) combined with a low-dose of exogenous BMP-2 (1.5 µg) enhanced ectopic bone formation when both therapeutics were subcutaneously implanted in rats. More importantly, SVAK-12 can improve the quality of bone-healing in a femoral fracture model in the presence of endogenous BMP-2 (17). Collectively, these observations suggested the potential therapeutic uses of SVAK-12 as a novel BMP activator in bone formation. Similar to SVAK-12, the small molecule phenamil, an analog of amiloride, has been shown to induce *in vitro* bone formation through BMP/Smad signaling in different cell types including mouse osteoprogenitor MC3T3-E1 cells and mouse mesenchymal stem cells (22-24). Intriguingly, the molecular mechanism underlying phenamil-induced osteogenesis is mediated by the expression of Tribbles homolog 3 (Trb3), which activates the BMP signaling pathway by down-regulating the negative regulator Smurf1. Therefore, the expression of Trb3 stabilizes the Smad transcription factors, which in turn bind to BMP responsive elements on the promoters of specific target genes, including osteoblast-associated genes (24). The *in vivo* bone formation effect of phenamil is currently under investigation in our group.

High-throughput screens are becoming increasingly more common for identifying novel osteogenic compounds as therapeutics. Using a high-throughput screen of a large library of small molecules (5405 compounds) along with several cell-based functional assays, Darcy *et al* identified 45 small molecules that were capable of promoting osteoblast differentiation (25). Two immunosuppressants identified in this screen (rapamycin and FK-506), either alone or in combination with exogenous BMP-2, promoted the phosphorylation of Smad 1/5/8 and transcription of osteoblast specific genes including *Osx*, *Runx2*, and *Smad-7*. The results were consistent with the small molecules having a role in promoting BMP-signaling mediated osteoblastic differentiation. However, only FK-506 was able to promote *in vitro* bone formation (25). Using similar technology in a screen of ~5600 compounds, Vrijens *et*

al. identified 2 small molecules (isoliquiritigenin and 4'-hydroxychalcone) that were able to induce Smad1/5/8 phosphorylation in a dose-dependent manner (18). Interestingly, in a very recent study, Lepparanta *et al.* found that an antiviral drug, tilorone, could induce BMP signaling by increasing the expression of a BMP target gene, *Id3* (inhibitor of differentiation-3) as well as BMP-7 in a lung epithelial cell line (26). However, *in vitro* and *in vivo* studies of these three small molecule compounds on bone formation were not reported in either paper.

Synthetic organic chemistry has generated bioactive small molecules for therapeutic purposes (27). Using an organic synthesis method, the novel chemical compound 11, 2-((1-(benzyl(2-hydroxy-2-phenylethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzoic acid, reportedly induced osteoblast differentiation in rat calvarial osteoblasts, and this effect was blocked by the BMP inhibitor noggin. The new compound also caused dose-dependent increases in nascent bone formation at the fracture site in rats (28).

Small molecule mediated skeletal muscle regeneration

Although muscles typically regenerate themselves, the regenerative property can become compromised as people age, if they suffer from a muscular degenerative disease (29), or if they experience a large, traumatic injury, in which the natural repair mechanism is unable to cope with the large muscle loss (30). Small molecules can offer an attractive option to satisfy the clinical need for muscle recovery therapeutics (10, 31).

Normal skeletal muscle recovery begins with the activation of satellite cells, a series of motile, self-renewing myoblast precursor cells. These cells, once activated, proliferate to the site of the injury and begin a process of terminal differentiation which ends in the formation of multinucleated myocytes that replace the lost or injured muscle cells (32)(Box 1). Figure 3 shows the molecular mechanism of muscle regeneration in adult. Interestingly, a number of studies have recently shown the capability of small molecules to aid in muscle regeneration. One such approach utilizes the modulation of sphingosine-1-phosphate (S1P) levels within the cell. S1P has been implicated in processes ranging from cell motility and survival to angiogenesis (33). In a recent study, Danieli-Betto *et al.* showed that direct injection of S1P into the injured area promoted significantly greater mean muscle fiber cross sectional area when compared to control in their mouse model (34). Another study published by Ieronimakis *et al.* showed that mice treated with the small molecule 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI), which they had previously proven to increase S1P expression, had a 4 fold increase in the number of cell expressing *myf5*, a protein plays a key role in regulating myogenesis (35). More importantly, THI treated cells showed a 3.6 fold increase in the number of muscle cell fibers as revealed by an embryonic myosin heavy chain (eMyHC) stain (35). These observations indicate an increase in muscle proliferation and growth, suggesting muscle healing.

Inducing stem cell differentiation is another promising means to aid in muscle regeneration. Several studies have demonstrated the capability of small molecules to induce stem cells to differentiate into a myoblastic lineage. In one study, embryonic stem cells treated with low-level doses of retinoic acid showed increased expression of the myoblastic markers (Pax3,

Meox1 and myogenin) over control, which suggests the formation of myoblast progenitor cells (MPC), which ultimately differentiate into myoblasts (36). Interestingly, various groups have demonstrated that the dedifferentiation of myoblasts can be beneficial to muscular regeneration. Under certain conditions, multinucleated myoblasts can dedifferentiate into mononuclear proliferatory cells, satellite cells, which then have potential to differentiate into multinucleated cells at another site (29). Recently, Kim *et al.* induced myoblast dedifferentiation using the small molecules BIO (glycogen synthase-3 kinase inhibitor), SB203580 (p38 MAP kinase inhibitor), and lysophosphatidic acid. Additionally, they showed that these compounds did not affect the potential of the dedifferentiated cells to redifferentiate into myoblasts at a later date (37).

Small molecule mediated cartilage formation

Healthy hyaline cartilage contributes to a smooth articular surface and is essential for normal joint functions. Articular cartilage lesions, resulting from joint diseases such as osteoarthritis (OA) or trauma, affect millions of individuals. In the United States alone, approximately 27 million people live with OA in different joints. Due to its avascular nature, articular cartilage of adults has limited ability to self-regenerate. A number of surgical treatment options are available for damaged or lost articular cartilage including articular debridement, microfracture, mosaicplasty, autologous chondrocyte transplantation, and allograft osteochondral transplantation. Each of the treatments has provided great benefits to patients, however, they suffer from a variety of limitations (38). Autologous chondrocyte transplantation has been recently regarded as the gold standard for cartilage repair, however, it faces a number of challenges such as maintaining the chondrocyte phenotype before and after cell transplantation and maintaining the transplanted chondrocytes in the damaged area following implantation (39). Therefore, alternative biological solutions involving the use of small molecules to attenuate the progression of OA or to promote cartilage formation have attracted great research interest.

Cartilage tissue consists of zonal structures from the superficial zone to calcified cartilage. Subchondral bone is the zone of epiphyseal bone beneath the calcified cartilage. Growing evidence has suggested that subchondral bone remodeling plays an important role in joint cartilage degeneration and pathogenesis OA (40, 41). Therefore, subchondral bone is a key target for OA treatment. As a consequence, small molecules that prevent bone resorption and/or promote bone formation have also been investigated as disease-modifying drugs for treating OA. A number of bisphosphonates such as alendronate, risedronate, etidronate, clodronate, pamidronate, tiludronate, neridronate, and zoledronate have been studied for OA treatment (42, 43) (44, 45). Bisphosphonates are thought to reduce cartilage degeneration by inhibiting subchondral bone turnover, but the mechanism is not fully understood. Bisphosphonates can have direct effects, such as promoting chondrocyte proliferation, inhibiting matrix metalloproteinases (MMPs), inducing chondrocyte apoptosis and endochondral ossification, or indirect effects such as altering the distribution of mechanical stress (45). In one study, Hayami *et al.* showed that alendronate suppressed subchondral bone resorption and reduced the incidence of osteophytes in a dose-dependent manner (46). Alendronate also inhibited vascular invasion into the calcified cartilage in a rat cruciate ligament transection model. In addition, alendronate was shown to be chondroprotective as

evidenced by histology and collagen degradation markers. Research has shown that alendronate treatment prevented proteoglycan loss and reduced the expression of MMP-13 and active transforming growth factor β (TGF- β) in rat with surgically induced OA (46). Two other small molecule drugs, licofelone and balicatib, have also been investigated in preclinical studies and phase II and III clinical trials to manage OA disease (47-49). Both drugs have been shown to be effective inhibitors for cathepsin K, a protease involved in bone resorption that cleaves type I and II collagen and degrades aggrecans. However, only licofelone significantly reduced knee cartilage loss measured by MRI over 12 and 24 months (49).

Strontium ranelate, a strontium (II) salt of ranelic acid marketed as Protelos or Protos, is an antiosteoporotic agent which both increases bone formation and inhibits bone resorption. Henrotin *et al.* showed that treating human chondrocytes with 10^{-3} M strontium ranelate strongly increased cartilage matrix formation by ionic stimulation of chondrocyte anabolism without affecting cartilage resorption, providing a preclinical basis for *in vivo* testing of strontium ranelate in OA (50). The therapeutic effect of strontium ranelate was then tested in different animal models. In one study, dogs with surgically-induced OA received oral treatment of strontium ranelate 25, 50 or 75 mg/kg per day, for 12 weeks (51). The most favorable effect was observed at the dosages of 50 or 75 mg/kg, leading to a reduction in the expression levels of key catabolic factors involved in osteoarthritic structural changes such as interleukin (IL) 1 β and C-terminal telopeptides of type II collagen (CTX-II). In a rat medial meniscal tear OA model, a high dose of strontium ranelate oral administration at 1800 mg/kg/day significantly attenuated the loss of cartilage matrix and chondrocytes, decreased chondrocyte apoptosis, and increased the expression of transcription factor SOX9, a critical transcription factor responsible for the expression of anabolic genes for type II collagen and aggrecan (52). The effect of strontium ranelate on the cartilage degradation marker CTX-II has also been evaluated in a phase III clinical trial involving 2617 postmenopausal osteoporotic women. An analysis on the expression of this cartilage degradation marker in these subjects showed that strontium ranelate-treated patients had significant decreased urinary level of CTX-II (53, 54).

Small molecule mediated ligament formation

Regeneration or replacement of a ruptured/torn ligament or tendon is one of the most common procedures performed by sports medicine surgeons. Anterior cruciate ligament (ACL) rupture is one of the most common injuries of the knee due to trauma or disease. During the past three decades, the bone-patella tendon-bone autograft has been considered the gold standard for ACL reconstruction, while the use of other graft choices such as hamstring tendon autografts or allografts has also become popular (55). Although the success rate of these grafts is high, their use is associated with some limitations such as donor site pain and morbidity with autografts, as well as possible disease transmission with allografts (56). Therefore, alternative approaches to repairing torn ACL have been greatly desired.

Matrix metalloproteinases (MMPs), a family of zinc-dependent proteolytic enzymes that play an important role in tissue remodeling and repair, are involved in the development of

tendinopathy (57). Collectively, MMPs are able to degrade all kinds of extracellular matrix proteins. The regulation of tendon remodeling requires an appropriate balance between the activities of MMPs and their natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs), which prevent tissue degradation. However, tendon or ligament ruptures significantly increase the production and activation of MMPs and in turn, the resulted imbalance between the activities of MMPs and TIMPs produces collagen dysregulation and disturbances in tendons and ligaments such as alterations in collagen and proteoglycan structures. Therefore, blockage of MMPs by MMP-inhibitors may provide a variable therapy to treat tendinopathy or regenerate ligaments and tendons (58).

Lui *et al.* investigated the effects of systematic administration of alendronate on the healing of tendon-bone interface in a rat ACL reconstruction model. Results showed that alendronate treatment reduced the expression of MMP1, MMP3 and CD68-positive cells (CD68: marker for the various cells of the macrophage lineage, including osteoclasts precursors and osteoclasts) at the region of peri-tunnel region as well as the interface of graft-bone tunnel (59). In addition, Lui *et al.* (59, 60) and Thomopoulos *et al.* (61) reported that local and/or systemic alendronate administration reduced bone resorption and increased mineralized tissue formation inside the bone tunnel. Thus, the capacity of alendronate to protection from tendon deterioration and also reduce of bone loss may act synergistically to improve tendon-bone integration. In another study, Hays *et al.* found that intraperitoneal injections of liposomal clodronate selectively induced macrophage apoptosis, leading to tendon-to-bone healing with less scar formation, more organized collagen deposition, and improved pull-out strengths (62). Other TIMPs such as Bay-11-7082 have also been shown to significantly decrease MMP activities in injured ACL fibroblasts and thus hold promise as small molecule drugs to facilitate ACL repair (63, 64). These recent efforts have remarkably improved the understanding of the effects of small molecules on ACL repair and tendon-to-bone interface healing. Future work in the form of well-designed randomized controlled trials should be conducted to explore whether the results of these basic science and preclinical studies can be extrapolated to clinical practice.

Small molecule mediated peripheral nervous regeneration

Unlike the neurons in the central nervous system, neurons in the peripheral nervous system have an innate capacity to regenerate themselves after injury. However, the successful innervation of peripheral neurons back into the CNS depends upon the environment in which they are located (65). Sometimes, surgical intervention is required to aid in this process, and in 2005, approximately 200,000 patients required surgical procedures for this purpose (66). A small molecule capable of expediting natural peripheral nervous regeneration could potentially lessen the need for surgical intervention.

Polysialic acid (PSA) is a glycan which has been found to be upregulated following nerve injury. This compound has been implicated in preferential motor reinnervation, as well as axon path finding in regenerating peripheral neurons (67). Glycans are complex to synthesize and have a short half-life *in vivo*, making them minimally useful for *in vivo* applications (68). Glycan mimetics, however, have a great potential for achieving the same effect without these complications (69). One small molecule PSA mimetic, 5-

nonyloxytryptamine oxalate (5-NOT) has been shown to be effective *in vitro*. 5-NOT promotes *in-vitro* neuritogenesis of motor neurons, as well as *in vitro* myelination and Schwann cell motility, all of which are critical to peripheral nervous repair (70).

Another small molecule, dibutyl cyclic-AMP (db-cAMP) has a significant effect on neural regeneration. Exogenous administration of db-cAMP into the injured area significantly improved motor recovery in a rat ischemia model (71). Additionally, the db-cAMP treated rat groups showed increased expression of growth associated protein 43 (GAP-43), a protein associated with axon regeneration and neural regeneration (71). Treatment with db-cAMP also effectively induces neuronal differentiation of neural stem/progenitor cells (NSPCs), resulting in a 67.5 fold increase of cells expressing beta III tubulin, a neural precursor protein, over control (72). Additionally, implanting a chitosan scaffold seeded with NSPCs that were pretreated with db-cAMP into a completely transected spinal cord caused an increase in motor function of hind limbs in the rat model (72). Though small molecule manipulation of peripheral nervous repair is still a growing field, it is clear that small molecules hold a tremendous amount of repair potential in the peripheral nervous system.

Concluding remarks and future directions

The value of small molecules for clinical applications in musculoskeletal tissue regeneration is enormous and many small molecules have been investigated (Table 1). Although promising, it is important to point out that the major concern associated with small molecule therapeutics is their non-specific side effects (6, 10, 73). Due to their small size, small molecules can easily enter non-target cells and elicit unwanted physiological responses (74). To overcome the aforementioned issues, small molecules can be delivered directly to the target site by a controlled release drug delivery method so that the systemic side effects can be minimized (75). Beyond precise spatial control of small molecule release, advanced material designs can dictate release kinetics to ensure biological or chemical cues are manifested at the appropriate times. This coordination between timing and location of small molecules could be optimized for both specific tissue types and tissue interfaces (1). In addition, reducing the frequency of administration of small molecule drugs has been recently proposed as a promising strategy to minimize their side effects (22, 76). Nevertheless, more research work needs to be done to unravel the role of small molecules in musculoskeletal tissue regeneration prior to its clinical utility, including studying dosage requirements and effective delivery strategies (Box 3).

Numerous strategies for treatment in the regenerative engineering of multiple tissue types stand to benefit greatly from the introduction of more innovative applications of small molecules. New techniques and concepts including chemical screening in zebrafish (77), drug affinity responsive target stability method (78), and advances in high performance computing (79) are poised to push the entire field of regenerative medicine forward. As clinicians begin to appreciate the negative systemic effects of protein-based growth factors, the dream of therapeutic “magic bullets” theorized by Paul Ehrlich more than a century ago may come to fruition.

Acknowledgments

This work was supported by funding from the NIH (R21-AR060480) and the Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences to Dr. Cato Laurencin, a grant from the State of Connecticut Stem Cell Research Foundation to Dr. Kevin Lo. Dr. Laurencin was the recipient of the Presidential Faculty Fellowship Award from President William Clinton and the Presidential Award for Excellence in Science, Mathematics, and Engineering Mentorship from President Barack Obama.

Glossary

Aggrecan	a protein that is encoded by the ACAN gene in human. The encoded protein is an integral part of the extracellular matrix of cartilage.
Bisphosphonate	a group of carbon-substituted analogs of pyrophosphate that are potent inhibitors of osteoclast-mediated bone resorption.
Bone Morphogenetic Proteins (BMPs)	BMPs belong to the larger family of proteins known as the transforming growth factor-beta (TGF- β) superfamily. BMPs are low-molecular weight secretory proteins which are active signaling molecules that influence proliferation, differentiation, and extracellular matrix synthesis of many different cell types.
Chondrocyte	The only type of cells found in healthy cartilage. They are contained in cavities called cartilage lacunae in the cartilage matrix and produce and maintain the cartilaginous matrix.
Embryonic Myosin Heavy Chain	a protein found in muscle fibers that is used as a marker of developing muscle fibers.
Epiphyseal bone	The rounded end of a long bone.
Glycan	a carbohydrate polymer consisting of sugar subunits.
Hyaline cartilage	Hyaline cartilage is a type of cartilage found on many joint surfaces. It appears glassy, provides a low friction surface, participates in lubrication in synovial joints, and distributes applied forces to the underlying bone. Articular cartilage refers to hyaline cartilage on the articular surfaces of bones.
Neuritis	formation of neuritis.
Myf5	Myogenic Factor 5, a transcription factor plays a key role in myogenesis.
Myoblasts	cells responsible for muscle growth.
Myogenesis	formation of muscular tissue.
Osteoarthritis	a painful disease of joints characterized by degeneration of healthy joint tissue including articular cartilage.
Osteoblasts	cells responsible for bone growth.
Osteoclasts	cells responsible for bone resorption.

Osteoporosis	a disease of unbalanced, natural bone turnover where too much bone is broken down and not enough new bone is created.
Rat calvarial osteoblasts	osteoblasts isolated from the calvaria bone of rats.
Sphingosine-1-phosphate	A lipid that has been implicated in different cellular signaling pathways.

References

1. Laurencin CT, Khan Y. Regenerative Engineering. *Sci Transl Med*. 2012; 4:160ed9.
2. El-Amin SF, Thomas JWL, Ihekweazu UN, Woods MD, Gupta A. Regenerative engineering: the future of medicine. Regenerative Engineering CRC Press. 2013:1–20.
3. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *Journal of the Royal Society, Interface / the Royal Society*. 2010; 8:153–170.
4. Baker M, Carr F. Pre-clinical considerations in the assessment of immunogenicity for protein therapeutics. *Current drug safety*. 2010; 5:308–313. [PubMed: 20615174]
5. Egusa H, Saeki M, Doi M, Fukuyasu S, Matsumoto T, Kamisaki Y, Yatani H. A Small-molecule Approach to Bone Regenerative Medicine in Dentistry. *J. Oral Biosci*. 2010; 52:107–118.
6. Lo KW, Ulerly BD, Deng M, Ashe KM, Laurencin CT. Current Patents on Osteoinductive Molecules for Bone Tissue Engineering. *Recent Patents on Biomedical Engineering*. 2011; 4:153–167.
7. Segar CE, Ogle ME, Botchwey EA. Regulation of angiogenesis and bone regeneration with natural and synthetic small molecules. *Current pharmaceutical design*. 2013; 19:3403–3419. [PubMed: 23432670]
8. Blaich, G.; Janssen, B.; Roth, G.; Salfeld, J. *Handbook of Pharmaceutical Biotechnology*. John Wiley & Sons, Inc.; Hoboken, NJ, USA: 2006. Overview: Differentiating Issues in the Development of Macromolecules Compared with Small Molecules; p. 89-123.doi: 10.1002/9780470117118.ch1c
9. Fu K, Klivanov AM, Langer R. Protein stability in controlled-release systems. *Nat Biotechnol*. 2000; 18:24–25. [PubMed: 10625383]
10. Lo KW-H, Ashe KM, Kan HM, Laurencin CT. The Role of Small Molecules in the Musculoskeletal Regeneration. *Regen Med*. 2012; 7:535–549. [PubMed: 22817627]
11. Bishop GB, Einhorn TA. Current and future clinical applications of bone morphogenetic proteins in orthopaedic trauma surgery. *International orthopaedics*. 2007; 31:721–727. [PubMed: 17668207]
12. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). *Journal of tissue engineering and regenerative medicine*. 2008; 2:81–96. [PubMed: 18383454]
13. Lo KW-H, Ulerly BD, Ashe KM, Laurencin CT. Studies of Bone Morphogenetic Protein based Surgical Repair. *Adv. Drug Deliv. Rev*. 2012; 64:1277–1291. [PubMed: 22512928]
14. Chrastil J, Low JB, Whang PG, Patel AA. Complications associated with the use of the recombinant human bone morphogenetic proteins for posterior interbody fusions of the lumbar spine. *Spine*. 2013; 38:E1020–1027. [PubMed: 23629484]
15. Taghavi CE, Lee KB, He W, Keorochana G, Murray SS, Brochmann EJ, Uludag H, Behnam K, Wang JC. Bone morphogenetic protein binding peptide mechanism and enhancement of osteogenic protein-1 induced bone healing. *Spine (Phila Pa 1976)*. 2010; 35:2049–2056. [PubMed: 20581758]

16. Lad SP, Bagley JH, Karikari IO, Babu R, Ugiliweneza B, Kong M, Isaacs RE, Bagley CA, Gottfried ON, Patil CG, Boakye M. Cancer after spinal fusion: the role of bone morphogenetic protein. *Neurosurgery*. 2013; 73:440–449. [PubMed: 23756740]
17. Wong E, Sangadala S, Boden SD, Yoshioka K, Hutton WC, Oliver C, Titus L. A novel low-molecular-weight compound enhances ectopic bone formation and fracture repair. *The Journal of bone and joint surgery*. 2013; 95:454–461. [PubMed: 23467869]
18. Vrijens K, Lin W, Cui J, Farmer D, Low J, Pronier E, Zeng FY, Shelat AA, Guy K, Taylor MR, Chen T, Roussel MF. Identification of small molecule activators of BMP signaling. *PloS one*. 2013; 8:e59045. [PubMed: 23527084]
19. Chan MC, Nguyen PH, Davis BN, Ohoka N, Hayashi H, Du K, Lagna G, Hata A. A novel regulatory mechanism of the bone morphogenetic protein (BMP) signaling pathway involving the carboxyl-terminal tail domain of BMP type II receptor. *Molecular and cellular biology*. 2007; 27:5776–5789. [PubMed: 17576816]
20. Bandyopadhyay A, Yadav PS, Prashar P. BMP signaling in development and diseases: a pharmacological perspective. *Biochemical pharmacology*. 2013; 85:857–864. [PubMed: 23333766]
21. Kato S, Sangadala S, Tomita K, Titus L, Boden SD. A synthetic compound that potentiates bone morphogenetic protein-2-induced transdifferentiation of myoblasts into the osteoblastic phenotype. *Molecular and cellular biochemistry*. 2011; 349:97–106. [PubMed: 21110071]
22. Lo K-WH, Kan HM, Laurencin CT. Short term treatment of small molecule phenamil induced a protracted osteogenic effect on osteoblast-like MC3T3-E1 cells. *Journal of tissue engineering and regenerative medicine*. 2013 Epub ahead of print.
23. Lo KW-H, Ulerly BD, Ashe KM, Kan HM, Laurencin CT. Evaluating the Feasibility of Utilizing Small Molecule Phenamil as a Novel Biofactor Factor for Bone Regenerative Engineering. *Journal of tissue engineering and regenerative medicine*. 2012 Epub ahead of print (doi: 10.1002/term.1573).
24. Park KW, Waki H, Kim WK, Davies BS, Young SG, Parhami F, Tontonoz P. The Small Molecule Phenamil Induces Osteoblast Differentiation and Mineralization. *Molecular and cellular biology*. 2009; 29:3905–3914. [PubMed: 19433444]
25. Darcy A, Meltzer M, Miller J, Lee S, Chappell S, Ver Donck K, Montano M. A novel library screen identifies immunosuppressors that promote osteoblast differentiation. *Bone*. 2012; 50:1294–1303. [PubMed: 22421346]
26. Leppäranta O, Tikkanen JM, Beshpalov MM, Koli K, Myllärniemi M. Bone morphogenetic protein-inducer tilorone identified by high-throughput screening is antifibrotic in vivo. *Am J Respir Cell Mol Biol*. 2013; 48:448–455. [PubMed: 23258233]
27. Schreiber SL. Organic synthesis toward small-molecule probes and drugs. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:6699–6702. [PubMed: 21464328]
28. Balaramnavar VM, Khan IA, Siddiqui JA, Khan MP, Chakravarti B, Sharan K, Swarnkar G, Rastogi N, Siddiqui HH, Mishra DP, Chattopadhyay N, Saxena AK. Identification of novel 2-((1-(benzyl(2-hydroxy-2-phenylethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl) benzoic acid analogues as BMP-2 stimulators. *Journal of medicinal chemistry*. 2012; 55:8248–8259. [PubMed: 22978808]
29. Jung DW, Williams DR. Reawakening atlas: chemical approaches to repair or replace dysfunctional musculature. *ACS chemical biology*. 2012; 7:1773–1790. [PubMed: 23043623]
30. Grasman JM, Page RL, Dominko T, Pins GD. Crosslinking strategies facilitate tunable structural properties of fibrin microthreads. *Acta biomaterialia*. 2012; 8:4020–4230. [PubMed: 22824528]
31. Laurencin CT, Lo KW. Bone morphogenetic proteins for bone regeneration and their alternatives. *Current pharmaceutical design*. 2013; 19:3353. [PubMed: 23432681]
32. Yoshida T, Galvez S, Tiwari S, Rezk BM, Semprun-Prieto L, Higashi Y, Sukhanov S, Yablonka-Reuveni Z, Delafontaine P. Angiotensin II Inhibits Satellite Cell Proliferation and Prevents Skeletal Muscle Regeneration. *The Journal of biological chemistry*. 2013; 288:23823–23832. [PubMed: 23831688]

33. Bernacchioni C, Cencetti F, Blescia S, Donati C, Bruni P. Sphingosine kinase/sphingosine 1-phosphate axis: a new player for insulin-like growth factor-1-induced myoblast differentiation. *Skeletal muscle*. 2012; 2:15. [PubMed: 22788716]
34. Danieli-Betto D, Peron S, Germinario E, Zanin M, Sorci G, Franzoso S, Sandona D, Betto R. Sphingosine 1-phosphate signaling is involved in skeletal muscle regeneration. *Am J Physiol Cell Physiol*. 2010; 298:C550–558. [PubMed: 20042733]
35. Ieronimakos N, Pantoja M, Hays AL, Dosey TL, Qi J, Fischer KA, Hoofnagle AN, Sadilek M, Chamberlain JS, Ruohola-Baker H, Reyes M. Increased sphingosine-1-phosphate improves muscle regeneration in acutely injured mdx mice. *Skeletal muscle*. 2013; 3:20. [PubMed: 23915702]
36. Kennedy KA, Porter T, Mehta V, Ryan SD, Price F, Peshdary V, Karamboulas C, Savage J, Drysdale TA, Li SC, Bennett SA, Skerjanc IS. Retinoic acid enhances skeletal muscle progenitor formation and bypasses inhibition by bone morphogenetic protein 4 but not dominant negative beta-catenin. *BMC biology*. 2009; 7:67. [PubMed: 19814781]
37. Kim WH, Jung DW, Kim J, Im SH, Hwang SY, Williams DR. Small molecules that recapitulate the early steps of urodele amphibian limb regeneration and confer multipotency. *ACS chemical biology*. 2012; 7:732–743. [PubMed: 22270490]
38. Dhinsa BS, Adesida AB. Current clinical therapies for cartilage repair, their limitation and the role of stem cells. *Current stem cell research & therapy*. 2012; 7:143–148. [PubMed: 22023635]
39. O'Driscoll SW, Saris DBF. Articular cartilage repair. *AAOS*. 2007
40. Buckwalter JA, Martin JA. Osteoarthritis. *Advanced drug delivery reviews*. 2006; 58:150–167. [PubMed: 16530881]
41. Goldring MB, Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Annals of the New York Academy of Sciences*. 2010; 1192:230–237. [PubMed: 20392241]
42. Strassle BW, Mark L, Leventhal L, Piesla MJ, Jian Li X, Kennedy JD, Glasson SS, Whiteside GT. Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of degenerative joint disease. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2010; 18:1319–1328.
43. Kadri A, Funck-Brentano T, Lin H, Ea HK, Hannouche D, Marty C, Liote F, Geoffroy V, Cohen-Solal ME. Inhibition of bone resorption blunts osteoarthritis in mice with high bone remodelling. *Annals of the rheumatic diseases*. 2010; 69:1533–1538. [PubMed: 20525838]
44. Moreau M, Rialland P, Pelletier JP, Martel-Pelletier J, Lajeunesse D, Boileau C, Caron J, Frank D, Lussier B, del Castillo JR, Beauchamp G, Gauvin D, Bertaim T, Thibaud D, Troncy E. Tiludronate treatment improves structural changes and symptoms of osteoarthritis in the canine anterior cruciate ligament model. *Arthritis research & therapy*. 2011; 13:R98. [PubMed: 21693018]
45. Castañeda S, Roman-Blas JA, Largo R, Herrero-Beaumont G. Subchondral bone as a key target for osteoarthritis treatment. *Biochemical pharmacology*. 2012; 83:315–323. [PubMed: 21964345]
46. Hayami T, Pickarski M, Wesolowski GA, McLane J, Bone A, Destefano J, Rodan GA, Duong le T. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. *Arthritis and rheumatism*. 2004; 50:1193–1206. [PubMed: 15077302]
47. Pelletier JP, Boileau C, Brunet J, Boily M, Lajeunesse D, Reboul P, Laufer S, Martel-Pelletier J. The inhibition of subchondral bone resorption in the early phase of experimental dog osteoarthritis by licofelone is associated with a reduction in the synthesis of MMP-13 and cathepsin K. *Bone*. 2004; 34:527–538. [PubMed: 15003800]
48. Manicourt D, Beaulieu A, Garnerio P, Peterfy C, Bouisset F, Haemmerle S. Effect of treatment with the cathepsin-k inhibitor, balicatib, on cartilage volume and biochemical markers of bone and cartilage degradation in patients with painful knee osteoarthritis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2007; 15:C130.
49. Raynauld JP, Martel-Pelletier J, Bias P, Laufer S, Haraoui B, Choquette D, Beaulieu AD, Abram F, Dorais M, Vignon E, Pelletier JP. Protective effects of licofelone, a 5-lipoxygenase and cyclooxygenase inhibitor, versus naproxen on cartilage loss in knee osteoarthritis: a first multicentre clinical trial using quantitative MRI. *Annals of the rheumatic diseases*. 2009; 68:938–947. [PubMed: 18653484]

50. Henrotin Y, Labasse A, Zheng SX, Galais P, Tsouderos Y, Crielard JM, Reginster JY. Strontium ranelate increases cartilage matrix formation. *J Bone Miner Res.* 2001; 16:299–308. [PubMed: 11204430]
51. Pelletier JP, Kapoor M, Fahmi H, Lajeunesse D, Blesius A, Maillet J, Martel-Pelletier J. Strontium ranelate reduces the progression of experimental dog osteoarthritis by inhibiting the expression of key proteases in cartilage and of IL-1 β in the synovium. *Annals of the rheumatic diseases.* 2013; 72:250–257. [PubMed: 23065732]
52. Yu DG, Ding HF, Mao YQ, Liu M, Yu B, Zhao X, Wang XQ, Li Y, Liu GW, Nie SB, Liu S, Zhu ZA. Strontium ranelate reduces cartilage degeneration and subchondral bone remodeling in rat osteoarthritis model. *Acta pharmacologica Sinica.* 2013; 34:393–402. [PubMed: 23334238]
53. Alexandersen P, Karsdal MA, Qvist P, Reginster JY, Christiansen C. Strontium ranelate reduces the urinary level of cartilage degradation biomarker CTX-II in postmenopausal women. *Bone.* 2007; 40:218–222. [PubMed: 17010685]
54. Alexandersen P, Karsdal MA, Byrjalsen I, Christiansen C. Strontium ranelate effect in postmenopausal women with different clinical levels of osteoarthritis. *Climacteric.* 2011; 14:236–243. [PubMed: 20726814]
55. Mariscalco MW, Magnussen RA, Mehta D, Hewett TE, Flanigan DC, Kaeding CC. Autograft Versus Nonirradiated Allograft Tissue for Anterior Cruciate Ligament Reconstruction: A Systematic Review. *The American journal of sports medicine.* 2013
56. Sherman OH, Banffy MB. Anterior cruciate ligament reconstruction: which graft is best? *Arthroscopy.* 2004; 20:974–980. [PubMed: 15525931]
57. Del Buono A, Oliva F, Osti L, Maffulli N. Metalloproteases and tendinopathy. *Muscles, ligaments and tendons journal.* 2013; 3:51–57.
58. Demirag B, Sarisozen B, Ozer O, Kaplan T, Ozturk C. Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blockage of matrix metalloproteinases. *The Journal of bone and joint surgery.* 2005; 87:2401–2410. [PubMed: 16264114]
59. Lui PP, Lee YW, Mok TY, Cheuk YC, Chan KM. Alendronate reduced peri-tunnel bone loss and enhanced tendon graft to bone tunnel healing in anterior cruciate ligament reconstruction. *European cells & materials.* 2013; 25:78–96. [PubMed: 23325540]
60. Lui PP, Lee YW, Mok TY, Cheuk YC. Local administration of alendronate reduced peri-tunnel bone loss and promoted graft-bone tunnel healing with minimal systemic effect on bone in contralateral knee. *J Orthop Res.* 2013 (in press).
61. Thomopoulos S, Matsuzaki H, Zaegel M, Gelberman RH, Silva MJ. Alendronate prevents bone loss and improves tendon-to-bone repair strength in a canine model. *J Orthop Res.* 2007; 25:473–479. [PubMed: 17205555]
62. Hays PL, Kawamura S, Deng XH, Dagher E, Mithoefer K, Ying L, Rodeo SA. The role of macrophages in early healing of a tendon graft in a bone tunnel. *The Journal of bone and joint surgery.* 2008; 90:565–579. [PubMed: 18310707]
63. Tang Z, Yang L, Xue R, Zhang J, Wang Y, Chen PC, Sung KL. Differential expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in anterior cruciate ligament and medial collateral ligament fibroblasts after a mechanical injury: involvement of the p65 subunit of NF- κ B. *Wound Repair Regen.* 2009; 17:709–716. [PubMed: 19769723]
64. Wang Y, Tang Z, Xue R, Singh GK, Lv Y, Shi K, Cai K, Deng L, Yang L. TGF- β 1 promoted MMP-2 mediated wound healing of anterior cruciate ligament fibroblasts through NF- κ B. *Connect Tissue Res.* 2011; 52:218–225. [PubMed: 20932173]
65. Chen ZL, Yu WM, Strickland S. Peripheral regeneration. *Annu Rev Neurosci.* 2007; 30:209–233. [PubMed: 17341159]
66. Zhang M, Yannas I. Peripheral Nerve Regeneration. *Regenerative Medicine II.* 2005; 94:67–89.
67. Mehanna A, Mishra B, Kurschat N, Schulze C, Bian S, Loers G, Irintchev A, Schachner M. Polysialic acid glycomimetics promote myelination and functional recovery after peripheral nerve injury in mice. *Brain.* 2009; 132:1449–1462. [PubMed: 19454531]
68. Masand SN, Perron II, Schachner M, Shreiber DI. Neural cell type-specific responses to glycomimetic functionalized collagen. *Biomaterials.* 2012; 33:790–797. [PubMed: 22027596]

69. Masand SN, Chen J, Perron IJ, Hammerling BC, Loers G, Schachner M, Shreiber DI. The effect of glycomimetic functionalized collagen on peripheral nerve repair. *Biomaterials*. 2012; 33:8353–8362. [PubMed: 22917737]
70. Loers G, Saini V, Mishra B, Papastefanaki F, Lutz D, Chaudhury S, Ripoll DR, Wallqvist A, Gul S, Schachner M, Kaur G. Nonyloxytryptamine mimics polysialic acid and modulates neuronal and glial functions in cell culture. *Journal of neurochemistry*. 2013 in press.
71. Niu L, Zhou J, Huang Y, Chen Y, Li C. db-Cyclic adenosine monophosphate promotes axon regeneration and motor function recovery in cerebral ischemia-reperfusion rats. *Neurology India*. 2010; 58:195–200. [PubMed: 20508335]
72. Kim H, Zahir T, Tator CH, Shoichet MS. Effects of dibutyl cyclic-AMP on survival and neuronal differentiation of neural stem/progenitor cells transplanted into spinal cord injured rats. *PloS one*. 2011; 6:e21744. [PubMed: 21738784]
73. Weiss WA, Taylor SS, Shokat KM. Recognizing and exploiting differences between RNAi and small-molecule inhibitors. *Nat Chem Biol*. 2007; 3:739–744. [PubMed: 18007642]
74. Brouwers L, Iskar M, Zeller G, van Noort V, Bork P. Network neighbors of drug targets contribute to drug side-effect similarity. *PloS one*. 2011; 6:e22187. [PubMed: 21765950]
75. Tayalia P, Mooney DJ. Controlled Growth Factor Delivery for Tissue Engineering. *Advanced Materials*. 2009; 21:3269–3285. [PubMed: 20882497]
76. Lo K-WH, Kan HM, Gagnon KA, Laurencin CT. One-day Treatment of Small Molecule 8-Bromo-cyclic AMP Analogue Induces Cell-based VEGF Production for In Vitro Angiogenesis and Osteoblastic Differentiation. *Journal of tissue engineering and regenerative medicine*. 2013 in press. doi: 10.1002/term.1839.
77. Taylor KL, Grant NJ, Temperley ND, Patton EE. Small molecule screening in zebrafish: an in vivo approach to identifying new chemical tools and drug leads. *Cell Commun Signal*. 2010; 8:11. [PubMed: 20540792]
78. Lomenick B, Olsen RW, Huang J. Identification of direct protein targets of small molecules. *ACS chemical biology*. 2011; 6:34–46. [PubMed: 21077692]
79. Okimoto N, Futatsugi N, Fuji H, Suenaga A, Morimoto G, Yanai R, Ohno Y, Narumi T, Taiji M. High-performance drug discovery: computational screening by combining docking and molecular dynamics simulations. *PLoS computational biology*. 2009; 5:e1000528. [PubMed: 19816553]
80. Kugimiya F, Yano F, Ohba S, Igawa K, Nakamura K, Kawaguchi H, Chung UI. Mechanism of osteogenic induction by FK506 via BMP/Smad pathways. *Biochem Biophys Res Commun*. 2005; 338:872–879. [PubMed: 16246307]
81. Deng M, James R, Laurencin CT, Kumbar SG. Nanostructured polymeric scaffolds for orthopaedic regenerative engineering. *IEEE transactions on nanobioscience*. 2012; 11:3–14. [PubMed: 22275722]
82. Parker MH, Seale P, Rudnicki MA. Looking back to the embryo: defining transcriptional networks in adult myogenesis. *Nature reviews*. 2003; 4:497–507.

Box 1

Normal Muscular Regeneration

- Skeletal muscle contains satellite cells, a group of self-renewing stem cells normally in a quiescent state.
- Upon muscle injury, satellite cells proliferate to the site of the injury.

Satellite cells begin differentiating down a myoblastic lineage, ultimately producing a multinucleate myocyte to replace the damaged cell.

Box 2**Connective tissue**

Connective tissue is one of the four types of biological tissue that consists of cells and extracellular matrix including fibers, ground substances, and tissue fluid. Different types of connective tissue are responsible for various functions that are reflected in the types of cells and fibers present within the tissue and the components of the ground substance in the extracellular matrix.

Cartilage is a form of connective tissue composed of chondrocytes and a highly specialized extracellular matrix which differentiate the tissue into hyaline cartilage, elastic cartilage, and fibrocartilage. Hyaline cartilage is composed of chondrocytes located in spaces called lacunae in cartilage matrix. Hyaline cartilage matrix consists of predominantly type II collagen, proteoglycans, noncollagenous proteins, and glycoproteins. Osteoarthritis (OA), also referred to as degenerative joint disease, is a group of diseases and abnormalities involving the degeneration of articular cartilage and the subchondral bone. Most commonly used surgical treatment methods for OA are as follows:

Articular debridement: a procedure involves the use of small arthroscopic instruments to remove damaged cartilage and smooth cartilage edges. The procedure is primarily used for treating small cartilage lesions.

Microfracture: a procedure involves removal of calcified cartilage and subsequent perforation of the subchondral plate with specially designed arthroscopic awls. By creating tiny fractures in the subchondral bone plate, blood and bone marrow diffuse out of the fracture and aid in developing new cartilage.

Mosaicplasty: a grafting procedure performed by harvesting one or more small cylindrical osteochondral autografts from a low weight-bearing area of the knee such as the superolateral trochlea and transplanting them towards the defective site in a mosaic pattern.

Autologous chondrocyte transplantation: a cell-based articular cartilage repair procedure involves the isolation, *in vitro* expansion of autologous chondrocytes, and implantation of those cells back to the cartilage defect in combination with a membrane such as periosteum or a scaffold.

Allograft osteochondral transplantation: transplantation of fresh or processed allogenic osteochondral grafts into a cartilage defect. The osteochondral allografts are usually harvested from cadaver tissue.

In preclinical research, animal models of OA are frequently used to study the pathogenesis of cartilage degeneration and to evaluate potential antiarthritic drugs for clinical use. Several commonly used surgically induced OA models include meniscal tear in rats, partial meniscectomy in rabbits, and anterior cruciate ligament transection in dogs and rats.

Ligament is a tough, fibrous dense connective tissue that connects bones to other bones. A ligament is composed of dense fibrous bundles of collagenous fibers (mainly type I

collagen) and spindle-shaped fibroblasts. The anterior cruciate ligament (ACL) is one of the four major ligaments of human knee. ACL tears are one of the most common knee injuries. In clinic, bone-patellar tendon-bone autograft has been considered gold standard for ACL reconstruction while other autograft and allograft options are available. In preclinical studies, an ACL reconstruction model in different animals such as rat or rabbit has been frequently used. These models involve replacing the native ACL with synthetic material grafts or other tissue grafts, inserting the grafts through bone tunnels created in tibia and femoral condyles, and fixing the grafts with sutures or other fixation devices. Mechanically, pull-out test (pulling the soft tissue out of the bone tunnel) is commonly performed and pull-out strength can be measured according to ASTM standards and used as an indication of level of interface regeneration between ligament and bone.

Box 3

Outstanding questions:

- How do small molecules elicit cellular responses?
- How can we minimize the off-target effects of small molecules?
- How can we combine small molecules with advanced materials for regenerative engineering?

How can we develop an effective drug discovery program in order to identify novel small molecules with regenerative potential?

Highlights

- Regenerative engineering has been recently proposed to regenerate complex tissue
- Therapeutic proteins have many limitations for their use in regenerating tissues
- Small molecule-based therapeutics is emerging as an alternative strategy
- Small molecules may represent the next generation in musculoskeletal regenerative medicine

Figure 1.

Schematics representation of the field of regenerative engineering. The field of regenerative engineering has been defined as “the integration of tissue engineering with advanced material science, stem cell science, and areas of developmental biology” (1). Regenerated tissues/organs via the regenerative engineering approach comprised on novel advanced biomaterials integrated with stem cells or biofactors, alone or in combination. Adapted with permission from (81).

Figure 2.

Schematic of canonical BMP signaling pathway. Binding of BMP ligands promotes dimerization of type I and type II BMP receptors (BMPR). The BMPR-II phosphorylates BMPRI which in turn phosphorylates the downstream effector proteins (Smad1/5/8). The phosphorylated Smad1/5/8 forms a complex with Smad4 and translocate from cytoplasm to nucleus and induce the expression of the osteoblast-related genes. Several extracellular protein including Chordin and Noggin, Samd6/7, and smurf anatagonize the BMP signaling pathway. Adapted with permission from (18).

Figure 3.

Molecular pathways of muscle regeneration in adult. Pax7 is an important transcriptional factor for the satellite cell lineage specification, other transcription factors including Dach2, Eya2, and Six1 may play a role in activating genes associated with myogenesis. During muscle injury or exercise, satellite cells are activated and committed to the myogenic lineage as myogenic precursor cells (MPCs). Myogenic regulatory factors (MRFs) such as MyoD and Myf5 are upregulated. In addition, several signaling pathways including Notch, BMP, Wnt, and Shh were found to play crucial roles in regulating myogenesis. Note that small molecule retinoic acid has been shown to promote myogenesis in stem cells via the Wnt signaling cascade. Adapted with permission from Macmillan Publishers Ltd: Nature Reviews Genetics (82).

Table 1

Small molecules mediated musculoskeletal tissues regeneration.

Small Molecules	Mechanism of action	References
<i>Small molecules mediated bone regeneration</i>		
SVAK-12	Enhance BMP signaling by interrupting the function of Smad ubiquitin regulatory factor 1 (Smurf1)	(17)
Phenamil	Enhance BMP signaling by negatively regulating Smurf1	(24)
FK-506	Activate BMP/ signaling by activating the BMP receptors (BMPR-1)	(80)
Isoliquiritigenin & 4'-hydroxychalcone	Activate BMP pathway by inducing Smad1, 5 phosphorylation	(18)
Tilorone	Induce BMP signaling and increase the expression of BMP-7	(26)
Compound 11	Unknown	(28)
<i>Small molecules mediated muscle regeneration</i>		
SIP	Promote muscle regeneration through SIP signaling pathway	(33)
THI	Function as an SIP lyase inhibitor that raise endogenous SIP levels systemically	(35)
Retinoic acid	Enhance myogenesis via the Wnt signaling pathway	(36)
BIO, SB203580, & LPA	Mechanism is unclear; the first article to demonstrate that small molecule is able to de-differentiate muscle cell and re-differentiate into myoblasts	(37)
<i>Small molecules mediated cartilage regeneration</i>		
Bisphosphonates	Mechanism is not fully understood; these molecules are thought to reduce cartilage degradation by inhibiting subchondral bone turnover.	(42) (43) (44) (45)
Licofelone	Inhibit two major proteases responsible for bone resorption: cathepsin K and MMP-13.	(47) (49)
Strontium ranelate	Increase the expression of transcription factor <i>SOX9</i> which is essential for cartilage formation	(52)
<i>Small molecules mediated ligament regeneration</i>		
Alendronate	Promote ligament healing by decreasing the expression of MMP1 and MMP13 expression as well as inhibiting osteoclasts formation.	(59, 60)
Clodronate	Promote ligament healing by depleting of macrophage at the tendon-bone interface.	(62)
Bay-11-7082	Promote ligament healing by Inhibiting MMP activities	(64) (63)
<i>Small molecules mediated peripheral nerve regeneration</i>		
5-NOT	Mimic the functions of polysialic acid for neuron regeneration	(70)
db-cAMP	Promote axon regeneration by promoting the expression of neuronal growth associated protein-43 (GAP-43)	(71)