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EDITORIAL

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The Bone, the Joints and the Balm of Gilead

The skeletal system is uniquely adapted to fulfill multiple complex biological, physiological and mechanical functions. These properties of bone can be attributed to its structural organization and importantly to its chemical composition, which consists of an organic matrix and inorganic mineral phase of hydroxyapaptite (HA) in the form of nanosized needle-like crystals.[1] The mineral is spatially distributed in an orderly fashion in the organic matrix, the major component of which is type I collagen. It is this natural HA composite that imparts the strength and toughness to the bone tissue, enabling it to provide mechanical support of the body and protection to the internal organs. In addition, the skeleton also provides a reservoir of mineral ions that play a critical role in calcium homeostasis, which is critically important for maintenance of diverse physiologic functions.[2]

The skeleton is organized into multiple separate components that are linked together and stabilized by a system of ligaments and tendons. These tissues provide the structural framework for the network of joints that are essential for locomotion and movement. The surfaces of the bones that form the articulations are lined by cartilage that provides a low friction surface that facilitates motion. The joint cavity is lined by a specialized membrane, the synovium, which regulates the transport of nutrients to the cartilage and is a source of factors that help to lubricate the articular cartilage surfaces.[3] Despite its remarkable strength and durability, the skeletal system and joint structures are susceptible to a broad spectrum of pathologic processes. The strength of bone is dependent on the activity of specialized cells that remodel and repair the bone. There are multiple endocrine, inflammatory and neoplastic processes that can adversely affect the activities of these cells, resulting in loss of bone and the development of generalized or focal osteoporosis, which is associated with the risk for disabling mechanical failure and fracture. Battlefield and sports injuries or surgical trauma are additional processes that can disrupt the integrity of bone and interfere with its capacity to heal. In addition, there are multiple inflammatory and autoimmune disorders that may attack the bone directly, but also may target the synovium that lines the joint tissues. Inflammation at these sites may extend into the periarticular bone and cartilage leading to joint destruction and crippling arthritis. The skeleton is particularly susceptible to invasion by malignant cells based on the composition of the bone tissue that provides a "fertile soil" for the growth and proliferation of cancer cells.

Because of the extensive morbidity and mortality associated with the diverse array of pathological disorders that affect the musculoskeletal system, there has been an intense interest in the pursuit of better treatments for musculoskeletal diseases. During recent decades, numerous novel therapeutic targets have been identified and many new drugs have been introduced to successfully treat these conditions.[4] One gap in the general drug development strategy, however, is that the unique biological, physiological and pathophysiological processes that affect the musculoskeletal system are rarely considered in the drug design paradigm.[5] The general drug development approach has focused on specific molecules or molecular pathways, with minimum attention on strategies to optimize the tissue specificity of the drug. As a consequence, many therapeutic candidates were shelved at late stages of development not just due to the lack of efficacy but importantly related to "off target" toxicity that is associated with systemic or nontarget organ toxicity. Indeed, there is increasing awareness that the capacity to manipulate the drug concentration at the desired sites of action is as important as the identification of the right molecule for molecular intervention or targeting a particular pathway. In consideration of this concept, the field of drug delivery has much to contribute.

In this issue, van den Hoven et al. provide a comprehensive review of the development of liposomal formulations for improving the treatment of inflammatory arthritis.[6] The review discusses the efficacy of encapsulation of different antirheumatic drugs in liposomes; the impact of administration routes (intravenous versus local injection); and the influence of targeting approaches (passive versus active). The authors conclude that, as a flexible delivery platform, liposomal formulations can significantly improve the therapeutic index of many antirheumatic drugs. Another advantage of liposomes is their nature of being a formulation rather than a new chemical entity, which makes the pathway for regulatory approval (when an approved drug is encapsulated) straightforward. A critical issue related to employing the liposome delivery system for inflammatory arthritis is its passive targeting mechanism, which the authors briefly mention in their introduction. Different from the conventional enhanced permeability and retention (EPR) effect observed in solid tumors,[7] the passive targeting of colloidal vesicles to the sites of inflammation likely involves a different retention mechanism.

Using a *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer delivery system, Quan et al.[8] have found that its passive targeting and retention at the site of inflamed joints is related to its **e**xtravasation through **l**eaky **v**asculature and subsequent **i**nflammatory cell-mediated **s**equestration, a process that is termed, "ELVIS". Publications from the Storm laboratory and others provide support for this mechanism, in which different colloidal systems were retained in the inflamed synovium by type A or type B synoviocytes.[9,10] Encouraged by this novel inflammation targeting mechanism, Ren et al. extended its application for use as a theranostic for orthopedic wear particle-induced osteolysis. Particle-induced inflammation is considered to be the major cause of aseptic implant loosening and clinical failure after total joint replacement. In the paper presented in this issue, Ren et al. developed a HPMA copolymer-based optical imaging contrast

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agent (P-IRDye) for the detection of wear particle-induced inflammation in a murine calvaria osteolysis model.[11] They showed that the particle-induced inflammation could be detected one day after introduction of the particles, prior to detection of osteolysis by μ-CT. An acid-labile HPMA copolymer−dexamethasone conjugate (P-Dex) was prepared and shown to completely block the particle-induced inflammation and bone damage in the calvaria osteolysis model. Potentially, this novel targeting mechanism can be adapted for use in other inflammatory diseases that affect other organs and organ systems.

Both rheumatoid arthritis and osteoarthritis have the capacity to produce significant damage to the articular cartilage and periarticular bone, resulting in joint destruction and impairment of mobility. No therapeutic interventions are available to completely reverse structural damage to the joint cartilage once it occurs. The most promising options for restoration of the structural and functional properties of cartilage are through tissue engineering approaches. Toh et al. presented a comprehensive review of this research area in this issue.[12] After a brief introduction of articular cartilage structure and past development of biomaterials for cartilage regeneration, the authors focused their efforts on the use of biomaterials as delivery systems for microenvironmental cues (e.g., geometric, mechanical, adhesive or soluble) to guide the transplanted cells and to orchestrate the host cell response to participate in the repair of cartilage defects. Not only did the authors outline the particular challenges in delivery of these microenvironmental cues, they also provided their perspective on the future developments in this area. As discussed in the manuscript, a particular challenge that may complicate the development of this cartilage repair approach is the potential adverse effects of the surgical procedures that are involved in this intervention. The powerful inflammatory response associated with the surgery and healing process may significantly alter the local microenvironmental cues that are required for cartilage regeneration.

Another important application of biomaterials for regenerative medicine is for use in repair of bone defects. Battlefield trauma, sports injuries, tumors and surgical procedures are among the many conditions that may be associated with the production of bone defects that are difficult to heal naturally. In this issue, Wang et al. described the development of a scaffolding material for bone regeneration.[13] While the overall concept of using scaffolds is not new, the authors provided a novel approach for incorporation of therapeutic agents and prolonging their release from the scaffolds by using a novel osteotropic liposome formulation. To anchor the liposome within the collagen/hydroxyapatite (Col/HA) scaffolds, the liposomes were modified with a bisphosphonate (BP). The BP-decorated liposomes (BP-liposomes) were shown to display a strong affinity for the HA-containing scaffolds. Compared to controls, the model drugs entrapped in BP-liposomes showed a slower release from the Col/HA scaffolds, supporting their potential use to provide a sustained drug release platform for bone regeneration and repair. The platform also has the flexibility to allow combined delivery of multiple therapeutic agents simultaneously.

In the development of pharmaceutical tools for bone regeneration, it is essential to take into consideration the diversity and complexity of the skeletal anatomy and the variety of clinical needs. Compared to the surgical repair of skeletal defects in the rest of the body, oral bone regeneration through open gum surgery has a higher probability of infection and other complications. Therefore, minimally invasive approaches such as direct injection of bone anabolic agents to the defective sites would be ideal. In this issue, Lee et al. presented the concept of local delivery of simvastatin (Sim) for bone regeneration in the oral cavity and investigated the mechanisms involved in drug targeting and efficacy.[14] Off-label use of Sim (a popular cholesterol-lowering drug) for local bone regeneration (a concept that is still controversial via systemic delivery) has been suggested as a viable strategy. However, the clinical application of Sim is hampered by several challenges: the anabolic mechanism of Sim; the solubilization of Sim; and the retention of newly formed bone after Sim treatment. In the paper, the authors provide the first direct evidence of the involvement of the prostaglandin pathway in Sim-induced bone regeneration. These findings are very significant, as the most common pain medications prescribed following dental bone augmentation procedures are cyclooxygenase (COX) inhibitors (e.g., aspirin, ibuprofen, etc). They also found that an alendronate−β-cyclodextrin conjugate not only could be used as an osteotropic drug carrier to solubilize Sim but also has the capability of preventing the newly formed bone from being removed.

For the development of a delivery system for musculoskeletal diseases, most investigators have chosen a therapeutic agent with established treatment efficacy. Wang et al. have chosen to pursue new drug therapy by exploiting siRNA to "knock down" farnesyl pyrophosphate synthase (FPPS) in osteoclasts and osteoblasts.[15] This being the first report on siRNA knockdown of FPPS, the authors' focus is not on the delivery system itself, but rather, it focuses on the biological effects of the siRNA treatment compared with the bisphosphonates that are the major clinically used FPPS inhibitors (i.e., alendronate). The authors found that siRNA treatment may increase bone mass by its effects on both osteoblasts and osteoclasts. Given the emerging concern over the long-term side effects of bisphosphonates, the use of siRNA knockdown of FPPS to inhibit bone resorption and enhance bone formation holds potential for clinical application in managing osteoporosis and related conditions associated with bone loss. siRNA delivery technology is a topic of great interest in the field of pharmaceutical science. However, the delivery of siRNA to the entire musculoskeletal system may not be easily achieved within the foreseeable future. Nevertheless, local delivery of siRNA to specific sites in the skeletal system or the application of siRNA within tissue engineering constructs has more immediate clinical applicability.

The potential of macromolecular prodrug development for the treatment of different skeletal diseases has been thoroughly reviewed in the past.[5] Several pathological conditions (e.g., osteomyelitis, fracture, cancer bone metastasis) are particularly amenable to the macromolecular prodrug approach due to the altered vasculature and increased local bone metabolism associated with the specific clinical conditions. In this issue, Miller et al. reported the use of HPMA copolymer for the osteotropic delivery of the antiangiogenic agent paclitaxel (PTX) for the treatment of breast cancer bone metastasis. Bisphosphonate was incorporated into the delivery system to provide osteotropicity.[16] The results presented in the paper showed that the alendronate (ALN)-containing HPMA copolymer−PTX conjugate had the greatest antitumor efficacy compared with PTX alone or in combination with ALN in a model involving 4T1 mammary adenocarcinoma inoculation into the tibia. From the safety perspective, the treatment was also better tolerated and more easily administered intravenously than the clinically used PTX formulated in Cremophor/ethanol. Based on a similar concept, Clementi et al. developed a novel dendritic polyethylene glycol

(PEG)-based osteotropic delivery system for PTX.[17] Different from the HPMA copolymer structural design by Miller et al., a heterofunctional PEG was used to conjugate with PTX at one chain terminus and the other modified with four ALN molecules through a β-Glu-based dendritic structure. This amphiphilic construct self-assembles in aqueous solution into a nanosized structure with the highly water-soluble ALN exposed on the surface. The conjugate has strong HA-binding affinity, and its IC_{50} is comparable to that of the free drug concentration in human adenocarcinoma of the prostate (PC3) cells, which is probably due to the easily cleavable ester linker that connects the PTX to PEG. Due to the increase of molecular weight and the self-assembly, the conjugate shows a much longer half-life in the circulation. The solubility of the conjugate in aqueous media is also excellent. Clearly, these two papers show great potential in developing osteotropic macromolecular therapeutics for the improved treatment of cancer bone metastasis (either osteolytic, for breast cancer, or osteoblastic, for prostate cancer). In addition to further exploration of therapeutic agents and molecular constructs, an additional focus for future development in this area should be the evaluation of these promising nanomedicines in primary tumor models or spontaneous bone metastasis models, in which the bone structure is not compromised during the model development.

The publication of this series of papers this year is particularly timely, since 2011 marks the end of the "Bone and Joint Decade".[18] Examination of the burden of musculoskeletal diseases in our country reveals that more than 1 in 4 Americans have a musculoskeletal condition requiring medical attention. Annual direct and indirect costs for bone and joint health are at 849 billion: 7.7% of the gross domestic product (GDP); and yet the current funding for research in this area is less than 2% of the NIH budget. Importantly, the burden of musculoskeletal conditions is expected to escalate in the next 10−20 years due to the aging population and sedentary lifestyles. Indeed, as the first group of baby boomers (~2.8 million) becomes eligible this year, Medicare costs are expected to skyrocket due to these musculoskeletal conditions.

In addition to the significant effort and resources that the public and the private agencies have invested in identification of new therapeutic targets for musculoskeletal diseases, there remains a significant gap in the development of molecular pharmaceutical approaches that address the challenges of tissue-specific drug targeting. Several leading groups have presented their ideas and exciting progress in this issue of *Molecular Pharmaceutics*. It is hoped that they may serve as a framework for encouraging more colleagues in the field to join this community of scientists in exploring this exciting new research frontier. Through collective and synergistic efforts, it is hoped that one day the application of these technologies will find the "Balm of Gilead" for the diverse array of crippling musculoskeletal diseases that burden our society.

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REFERENCES

(1) Posner, A. S. Crystal chemistry of bone mineral. *Physiol. Rev.* **1969**, *49*, 760–92.

(2) Marks, S. C., Jr.; Odgren, P. R. Structure and Development of the Skeleton. In Bilezikian, J. P., Raisz, L. G., Rodan, G. A., Eds.; *Principles of Bone Biology*, 2nd ed.; Academic Press: San Diego, 2002; pp 3−15.

(3) Goldring, S. R.; Goldring, M. B. Biology of the Normal Joint. In *Kelly's Text Book Rheumatology*, 8th ed.; Firestein, G. S., Budd, R. C., Harris, E. D., Jr., McInnes, I. B., Ruddy, S., Sergent, J. S., Eds.; Saunders Elsevier: Philadelphia, 2009; pp 1−22.

(4) Rodan, G. A.; Martin, T. J. Therapeutic approaches to bone diseases. *Science* **2000**, *289*, 1508–14.

(5) Wang, D.; Miller, S. C.; Kopeček, J. Targeted drug delivery for musculoskeletal diseases. *Adv. Drug Delivery Rev.* **2005**, *57*, 935–7.

(6) van den Hoven, J. M.; Van Tomme, S. R.; Metselaar, J. M.; Nuijen, B.; Beijnen, J. H.; Storm, G. Liposomal drug formulations in the treatment of rheumatoid arthritis. *Mol. Pharmaceutics* **2011**10.1021/mp2000742.

(7) Matsumura, Y.; Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **1986**, *46*, 6387–92.

(8) Quan, L. D.; Purdue, P. E.; Liu, X. M.; Boska, M. D.; Lele, S. M.; Thiele, G. M.; Mikuls, T. R.; Dou, H.; Goldring, S. R.; Wang, D. Development of a macromolecular prodrug for the treatment of inflammatory arthritis: mechanisms involved in arthrotropism and sustained therapeutic efficacy. *Arthritis Res. Ther.* **2010**, *12*, R170.

(9) Metselaar, J. M.; van den Berg, W. B.; Holthuysen, A. E.; Wauben, M. H.; Storm, G.; van Lent, P. L. Liposomal targeting of glucocorticoids to synovial lining cells strongly increases therapeutic benefit in collagen type II arthritis. *Ann. Rheum. Dis.* **2004**, *63*, 348–53.

(10) Fiehn, C.; Muller-Ladner, U.; Gay, S.; Krienke, S.; Freudenberg-Konrad, S.; Funk, J.; Ho, A. D.; Sinn, H.; Wunder, A. Albumin-coupled methotrexate (MTX-HSA) is a new anti-arthritic drug which acts synergistically to MTX. *Rheumatology (Oxford)* **2004**, *43*, 1097–105.

(11) Ren, K.; Purdue, P. E.; Burton, L.; Quan, L.-d.; Fehringer, E. V.; Geoffrey M.Thiele, G. M.; Goldring, S. R.; Wang, D. Early detection and treatment of wear particle-induced inflammation and bone loss in a mouse calvarial osteolysis model using HPMA copolymer conjugates. *Mol. Pharmaceutics* **2011**10.1021/mp2000555.

(12) Toh, W. S.; Spector, M.; Lee, E. H.; Cao, T. Biomaterial-mediated delivery of microenvironmental cues for repair and regeneration of articular cartilage. *Mol. Pharmaceutics* **2011**10.1021/mp100437a.

(13) Wang, G.; Babadağli, M. E.; Uludağ, H. Bisphosphonate-derivatized liposomes to control drug release from collagen/hydroxyapatite scaffolds. *Mol. Pharmaceutics* **2011**10.1021/mp200028w.

(14) Lee, Y.; Liu, X.; Nawshad, A.; Marx, D. B.; Wang, D.; Reinhardt, R. A. Role of prostaglandin pathway and alendronate-based carriers to enhance statin-induced bone. *Mol. Pharmaceutics* **2011**10.1021/mp200045p.

(15) Wang, Y.; Panasiuk, A.; Grainger, D. W. Small interfering RNA knocks down the molecular target of alendronate, farnesyl pyrophosphate synthase, in osteoclast and osteoblast cultures. *Mol. Pharmaceutics* **2011**10.1021/mp100374n.

(16) Miller, K.; Eldar-Boock, A.; Polyak, D.; Segal, E.; Benayoun, L.; Shaked, Y.; Satchi-Fainaro, R. Antiangiogenic antitumor activity of HPMA copolymer−paclitaxel−alendronate conjugate on breast cancer bone metastasis mouse model. *Mol. Pharmaceutics* **2011**10.1021/mp200083n.

(17) Clementi, C.; Miller, K.; Mero, A.; Satchi-Fainaro, R.; Pasut, G. Dendritic poly(ethylene glycol) bearing paclitaxel and alendronate for targeting bone neoplasms. *Mol. Pharmaceutics* **2011**10.1021/mp2001445.

(18) Official website of US Bone and Joint Decade: http://www.usbjd.org/.