Bone Tissue Engineering and Regeneration: From Discovery to the Clinic—An Overview

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A National Institutes of Health sponsored workshop “Bone Tissue Engineering and Regeneration: From Discovery to the Clinic” gathered thought leaders from medicine, science, and industry to determine the state of art in the field and to define the barriers to translating new technologies to novel therapies to treat bone defects. Tissue engineering holds enormous promise to improve human health through prevention of disease and the restoration of healthy tissue functions. Bone tissue engineering, similar to that for other tissues and organs, requires integration of multiple disciplines such as cell biology, stem cells, developmental and molecular biology, biomechanics, biomaterials science, and immunology and transplantation science. Although each of the research areas has undergone enormous advances in last decade, the translation to clinical care and the development of tissue engineering composites to replace human tissues has been limited. Bone, similar to other tissue and organs, has complex structure and functions and requires exquisite interactions between cells, matrices, biomechanical forces, and gene and protein regulatory factors for sustained function. The process of engineering bone, thus, requires a comprehensive approach with broad expertise. Although *in vitro* and pre-clinical animal studies have been pursued with a large and diverse collection of scaffolds, cells, and biomolecules, the field of bone tissue engineering remains fragmented up to the point that a clear translational roadmap has yet to emerge. Translation is particularly important for unmet clinical needs such as large segmental defects and medically compromised conditions such as tumor removal and infection sites. Collectively, manuscripts in this volume provide luminary examples toward identification of barriers and strategies for translation of fundamental discoveries into clinical therapeutics.

A ONE-DAY WORKSHOP “Bone Tissue Engineering and Regeneration: From Discovery to the Clinic” was convened before the Tissue Engineering and Regenerative Medicine International Society–North America meeting held in December 2010. The goal of the workshop was to bring together a group of thought leaders to define the state of art in the field and to discuss strategies to overcome the scientific barriers preventing realization of new treatments for bone defects. Bone is comprised of a variety of cell populations, extracellular matrix, and other proteins as well as inorganic components that work synergistically to sustain physical forces, molecular signals, and systemic hormone networks. The workshop presentations and discussions focused on the state of the understanding of various components of bone structure and function and on integration of this knowledge for engineering physiologically and mechanically competent bone tissue. Barriers for translation of bone regeneration products toward clinical therapeutics and corresponding strategies to address them were also identified and discussed in the workshop.

The Clinical Need for Bone Tissue Engineering

In his Introductory Keynote Lecture, “The Importance of Translating Bone Tissue Engineering to the Clinic,” Dr. Randy Rosier described the variety of clinical conditions that result in major structural defects in bone and which would benefit from tissue-engineering advances. Severe trauma can result in open fractures with tissue devascularization and bone loss. Delayed unions and non-unions contribute to the morbidity of fractures and are another area in need for improved treatments. Bone tumors and resulting surgical defects are a special challenge due to local radiation and/or chemotherapy. Spine fusions and arthrodesis of joints require adjuvant treatment with bone grafts or substitutes. Finally, bone loss with failed hip and knee replacements is an expanding problem due to increased number of joint replacements in the United States. Undoubtedly, there is a large and essentially unmet clinical need for highly effective bone substitutes.

Bone grafts for injuries, tumors, infections, and degenerative diseases represent an enormous cost. The magnitude of

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the problem includes approximately 15 million fractures annually (\$45B); 1.6 million patients with trauma with hospital admission (\$27B); 2 million osteoporotic fractures (\$24B); 500,000 knee, 350,000 hip replacements, and 90,000 revision arthroplasty procedures (\$30B); 300,000 spinal fusions, 20,000 revision spine fusions (\$18B); 2400 primary bone malignancies; and 4500 benign tumors (\$100M). Collectively, approximately 1.6 million bone grafts are performed each year in the United States alone.

Although bone autografts are the gold standard, their supply is limited, and there are many potential drawbacks associated with their use including donor site morbidity, danger of infection, and pain. Moreover, autografts often have limited ability to accelerate normal morphogenic and cellular events of fracture healing and remodeling. Allografts represent an alternative, particularly in areas with massive bone defects. However, allografts are not free of drawbacks, because structural bone allografts have limited potential to remodel and, therefore, have a tendency to accumulate mechanical stress and frequently undergo fractures. In addition, they are expensive and carry risks of infection and/or disease transmission. The ideal characteristics of a bone substitute includes a material or composite that is non-immunogenic, nontoxic, controllable, inexpensive, and readily available. It should possess structure, architecture, and pore sizes that would facilitate broad application for a variety of conditions including the replacement of structural versus nonstructural bone. It is also important for the composite to contain a minimal number of components, in order to reduce its complexity and potential cost and to simplify the regulatory approval process.

A number of bone graft substitute components is currently available. These include inorganic materials (calcium phosphate and calcium sulfates in ceramics, pellets, and injectable cements); organic materials (demineralized bone matrix, bone morphogenetic proteins); mesenchymal stem cells (autologous bone marrow derived and selected populations of bone marrow stem cells); and platelet gels and plasma.

Through control of a variety of different but inter-related parameters, there is the potential to develop novel and increasingly advanced composites. This is also a major challenge, because clear strategies for optimizing and combining the various components are currently not available. Important issues to be addressed are as follows: (1) scaffold (material, geometry, and architecture); (2) cell sources (endogenous autologous, transplanted autologous, allogeneic, systemic versus locally delivered, type of cell such as stem cell, osteoblast, chondrocyte, endothelial cells, and pericytes); (3) growth factors and signaling pathways (bone morphogenetic protein [BMP], transforming growth factor β [TGF- β], Prostaglandins, hedgehog proteins, Wnt/beta-catenin, osteix, parathyroid hormone, and others); and (4) material/host interactions (mechanotransduction, cell matrix interactions, vascularization, and immune response).

Dr. Rosier also discussed clinical outcome assessment and comparative effectiveness to determine the efficacy of the tissue-engineered bone. This was identified as an area in dire need of advancement. Many of the preclinical models to test efficacy use methods that are invasive or not readily translated to the assessment of human engineered tissues. Outcomes such as histology and biomechanical evaluation require sacrifice of the animal and tissue harvest. Micro-

computed tomography scanning is not feasible in humans due to technical issue and radiation dosing. Radiographic determination of union is imprecise and is primarily semi-quantitative. The insertion of hardware further complicates the assessment of bone healing. Finally, unlike assessment of bone healing in relatively homogenous populations of laboratory animals with standard types of injuries, assessment of patient responses is a significantly more complex task due to heterogeneity of genetic background and dissimilarity of fracture types and bone loss patterns. This leads to increased variability in human studies compared with those in animal models. Improved noninvasive assessment tools are needed to monitor bone healing to allow effective comparisons of the relative efficacy of different materials and composites. It will also be necessary to address real-life questions, such as whether and when patients can return safely to partial or full activity and the location of the fracture on the healing continuum.

Dr. Rosier's introduction provided a strong rationale for the workshop and the various components of tissue-engineered bone that had been presented throughout the workshop. The subsequent presentations covering the subtopic areas important for bone tissue engineering appear in review form in this special edition and are a part of this compendium. Dr. Robert Guldberg moderated the first scientific session, "Building Blocks"; and Dr. George Muschler moderated the second session, "Biological Processes." Panel discussions for these sessions were led by the National Institutes of Health (NIH) program directors, Rosemarie Hunziker of National Institute of Biomedical Imaging and Bioengineering (NBIB) and by Nadia Lumelsky of National Institute of Dental and Craniofacial Research (NIDCR), respectively.

Scaffolds and Bone Tissue Engineering

Dr. Scott Hollister provided an overview of scaffolds, which serve as the fundamental framework for bone tissue engineering. He introduced Form, Function, Fixation, and Formation, the 4Fs that are critical consideration in the design of an effective scaffold. Form refers to the ability to conform to the three-dimensional shape of the defect. Function refers to the mechanical properties of the scaffold that are optimally similar to the missing bone which it replaces. Fixation refers to the ability of the scaffold to integrate and attach to the existing neighboring bone and soft tissues. Formation refers to the osteoconductive properties of the bone substitute, features that are related to factors such as porosity, permeability, diffusivity, and the integration of bioactive molecules or cells. Although it is optimal to include each of the 4Fs, in combination they markedly increase the complexity of the scaffold. This complexity challenges tissue-engineered bone formulations to extend beyond simple bone void fillers and also leads to more challenging, lengthy, and costly approval for translational products. To best address these challenges, Dr. Hollister presented the concept of modular scaffold systems as a potential model to solve the complexities of integrating different design features for bone tissue engineering. In this model, a bone void filler with an FDA approval and established current Good Manufacturing Practice (cGMP) manufacturing processes can be combined with approved biologics to overcome some of the development and regulatory issues.

Cells and Bone Tissue Engineering

Dr. Pamela Robey and Dr. Celine Colnot addressed another essential building block—cells involved in repair. Dr. Robey presented a comprehensive overview of exogenous cell sources potentially available to regenerate bone and features of these cells that are optimal for bone tissue engineering. Cells from a variety of sites, such as bone marrow stromal cells, trabecular bone cells, periosteal cells, circulating skeletal cells, cord blood cells, amniotic fluid cells, adipose derived cells, and cells from virtually any connective tissue, have been shown to undergo osteoblast differentiation *in vitro*. However, the ability of cell populations to undergo *in vitro* differentiation may not be the best predictor of *in vivo* behavior of the cell populations. Dr. Robey also discussed human embryonic stem cells and induced pluripotent stem cells, and their potential to engineer bone and other tissues as well as concerns about their unlimited ability to self-renew and their safe use in humans.

Dr. Colnot described the variable potential of the local stem/progenitor cell population to participate in bone repair and the potentially significant contribution of developmental and regenerative biology to tissue engineering. Stem cell populations in the bone microenvironment are particularly important. Injury or ablation of periosteal stem cells on the surface of the bone limit or prevent bone healing in mice. Periosteal cells in the injury environment have the potential to differentiate into both chondrocytes and osteoblasts. In contrast, cells lining the endosteal surface of the bone have limited capacity to undergo chondrogenesis, but readily undergo osteoblast differentiation. Circulating cells are recruited to the injury site, but have limited ability to differentiate into bone forming cells. Instead, they influence the repair process by secreting regulatory factors. An important source of cells during the maturation and remodeling phase of bone healing are the peri-vascular cells (pericytes) that enter the healing environment during vascularization of the tissue. Dr. Colnot also discussed systemic delivery and local recruitment of cells, and their implication to bone repair.

Growth Factors and Bone Tissue Engineering

Dr. Vicki Rosen and Dr. Benjamin Alman discussed the role of growth factors and signaling pathways in bone tissue engineering and endogenous regeneration. Optimizing use of growth factors and signaling molecules in bone tissue engineering requires more advanced understanding of how these signals interact and are inter-related in repairing tissues. Numerous potentially available tools include parathyroid hormone (PTH), BMPs, Hedgehogs, insulin-like growth factors, fibroblast growth factors, Wnts/beta-catenin, TGF- β , platelet-derived growth factor, and prostaglandins. Although all these factors have been effective in preclinical models, further insights regarding how their signals are coordinated and their temporal effects on bone repair will enable translation.

A successful translation example, BMP-2, is approved for clinical use in spine fusion and in tibia non-union. However, large doses of BMP-2 are required and concerns have emerged, including reports of inflammatory reactions, retrograde ejaculation, and questions about efficacy. Although use of the BMP-2 product is supported by numerous trials and clinical reports, there is mounting concern regarding

whether some clinical reports may have been influenced by conflict of interest. Since BMP-2 is an imperfect solution, work has proceeded with other factors, such as Wnt/b-catenin signals. Although this pathway modulates bone formation, effects are variable and dependent on the stage of fracture healing and target cell population, and the timing, delivery, and targeting of factors that regulate beta-catenin signaling will be critically important.

PTH₁₋₃₄ (Forteo) is approved for the treatment of metabolic bone disease. Preclinical trials of intermittent PTH demonstrate an increase in size of fracture callus and enhanced healing. Experimental findings suggest that the anabolic effect of PTH on bone repair might be related to an induction in BMP-2 expression. Several human studies are suggestive of an anabolic effect on fracture healing but are not definitive. The heterogeneity of human fractures and challenges of associated with the design of human clinical trials make data interpretation challenging. Use of these factors in a scaffold combined with cells remains an even more difficult challenge.

Regulation of Gene Expression and Bone Tissue Engineering

Dr. Christopher Evans provided an update on the status of gene therapy or modulation and its potential role in bone tissue engineering. The enormous anticipated promise of gene therapy in 1990s has not been realized, mainly due to safety concerns related to adverse immune responses that have led to some well-publicized patient deaths in clinical trials. For clinical applications, gene therapy requires extensive information regarding the safety and efficacy of the expressed target gene as well as its delivery vehicles. BMP-2 is a potential target gene, because the recombinant protein has been in clinical use for a decade. Other methods to control gene expression, such as siRNA techniques, are emerging and may be more easily translated to clinical use. Further, Dr. Evans identified some of the barriers to translate bone tissue engineering research into clinics, including testing in large animals to accelerate “the first use in humans.”

Mechanical Forces and Bone Tissue Engineering

Mechanical properties play a very important role in skeletal tissues. The significance of mechanobiology for bone healing was discussed at the workshop by Dr. Dennis Carter. He presented mechanobiological parameters at different levels, such as cytoskeletal changes, stretch activated ion channels, and growth factors at the molecular level; shape changes, cell-matrix interactions, and temperature at the cellular level; stress, strain, and fatigue damage at the tissue level; and displacement, stiffness, and failure force at the organ level. Mechanical cues regulate cell and matrix biology and, hence, bone remodeling and regeneration.

Tissue and Organ Responses to Bone Tissue Engineering

Dr. Edward Botchwey, Dr. Laura Calvi, and Drs. Kurtis Kasper and Antonios Mikos described the integration of bone healing with other tissues/organs. Dr. Botchwey showed the close inter-relationship of bone healing/formation with the vascular compartment. *In vitro*, endothelial cells

stimulate the differentiation of osteoprogenitor cells. Similarly three-dimensional cultures have demonstrated that osteoblasts drive vasculogenesis and formation of a mature vascular network. Bone cells influence endothelial cells by secreting growth factors and vice versa. In keeping with a theme present in a number of other presentations, Dr. Botchwey underscored the importance of the vasculature as a supply of pericytes that are an important source of osteoblasts in the repair tissue.

Dr. Calvi reviewed the interactions between the hematopoietic cell populations and osteoblasts. These two important organs, bone and hematopoietic tissues, share the bone marrow microenvironment. Recent studies show the enormous influence that these tissues exert on one another. Osteoblastic cells are in direct contact with the long term-hematopoietic stem cell population (LT-SC) in "the hematopoietic stem cell niche." Bone marrow also contains short term-hematopoietic stem cells (ST-SC) and more differentiated hematopoietic precursors. Osteoblasts regulate the maintenance of LT-SC populations and keep them in a stem cell state. In contrast, Wnt/ β -catenin signaling stimulates differentiation of hematopoietic stem cells.

Inflammation and Bone Tissue Engineering

Drs. Kasper and Mikos examined the role of the inflammatory process and its influence on bone formation in areas of injury. Inflammation is a key component of the early response to bone injury. Inflammatory cells are recruited to the damaged bone and release cytokines, chemokines, and growth factors that amplify the process. Inflammation in the early phase of fracture repair is associated with enhanced healing. However, chronic inflammation, such as observed in inflammatory arthritis, has a very deleterious effect on healing. In mice with inflammatory arthritis, treatment with a tumor necrosis factor- α (TNF- α) inhibitor enhances healing. In addition to actual inflammatory cells, it appears that chondrocytes and bone cells in the healing tissues are important sources of inflammatory factors such as TNF- α and interleukin-6. Controlling inflammatory signals is a promising strategy for bone regeneration. Drs. Kasper and Mikos suggested designing rational strategies to control these signals for bone tissue engineering and regeneration.

Regulatory Issues and Bone Tissue Engineering

In his Closing Keynote Lecture, "Translation and Product Development," Dr. Anthony Ratcliffe detailed the challenges associated with product development and translation towards human therapies. He noted that a careful assessment of the market for a new product is essential and so is a comparison of the costs of development versus the potential financial benefit. Product approval process is often dependent on the nature of the product design. Some of the approval pathways are more detailed, costly, and time consuming than others. Extensive use of pre-clinical models, including large animal models, followed by multiple clinical trials is required. Due to the complexity of a tissue-engineering product associated with multiple different components, cells, growth factors, and scaffolds, the approval process is particularly complex and lengthy. There-

fore, investigators need to have a realistic understanding of commercial world in order to successfully bring their research into the marketplace for clinical applications.

Summary

Despite the challenges in bone tissue engineering, there remains tremendous optimism concerning the potential to replace damaged and degenerated structures and tissues, among the workshop participants. Continued progress in the individual scientific fields that are components of bone tissue engineering and the collective commitment and collaboration of scientists with expertise in material sciences, genetic, cell biology, physiology, immunology, and others are essential. Strategic funding of initiatives designed to bring together talented scientists from the variety of scientific disciplines that constitute tissue engineering will enable improved treatment of skeletal defects in the future.

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