Translational Models for Musculoskeletal Tissue Engineering and Regenerative Medicine

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The National Institutes of Health–sponsored workshop “Translational Models for Musculoskeletal Tissue Engineering and Regenerative Medicine” was held to describe the utility of various translational models for engineered tissues and regenerative medicine therapies targeting intervertebral disc, cartilage, meniscus, ligament, tendon, muscle, and bone. Participants included leaders in the various topics, as well as National Institutes of Health and Food and Drug Administration. The Food and Drug Administration representatives provided perspectives and needs for studies supported by animal models. Researchers described animal models for specific tissues and addressed the following questions: (1) What are the unmet musculoskeletal clinical needs that may be addressed by tissue engineering and regenerative medicine? (2) Are there appropriate models available? (3) Are there needs to develop standardized animal models? (4) What are the translational pathways that lead to clinical trials and therapeutic development? The workshop provided an effective and succinct summary of the status of various animal models in musculoskeletal regenerative medicine. Although many models are available and serve well to answer a variety of questions, the general consensus was that there is a substantial need for improved and standardized animal models for tissue engineering and regenerative medicine of the musculoskeletal system, and that animal models, especially large animal models, are critical to the preclinical step of translating research from bench to bedside.

THE WORKSHOP “Translational Models for Musculoskeletal Tissue Engineering and Regenerative Medicine” was held before the Tissue Engineering and Regenerative Medicine International Society–North America meeting held December 2008. The purpose of the workshop was to describe the utility of various translational models, as well as research and development pathways, for engineered tissues and regenerative medicine therapies that target intervertebral disc, cartilage, meniscus, ligament, tendon, muscle, and bone. Food and Drug Administration (FDA) representatives provided perspectives and needs for studies supported by animal models, and researchers addressing animal models for specific tissues were asked to address the following questions with respect to specific musculoskeletal tissues:

1. From the clinical perspective, what are unmet clinical needs and opportunities for injury and disease of musculoskeletal tissues and organs that may be amenable to tissue engineering and regenerative medicine therapies?
2. Are there appropriate models (e.g., animal) for particular injuries and diseases to address efficacy, safety, and mechanism of action?

3. Are there needs to develop standardized animal models? If so, is it possible to develop such models, and what would be the best ways to achieve the goal?
4. What are the translational pathways for engineered tissues and regenerative medicine therapies for these injuries and diseases that successfully lead to clinical trials and therapeutic development?

Selected papers derived from this workshop are provided in this issue of *Tissue Engineering Part B*.

Mark Lee *et al.* provide perspectives from the FDA, presented by Drs. Charles Durfor and Richard McFarland at the Workshop, on the review process before clinical studies for investigational products in musculoskeletal tissue engineering and regenerative medicine. They stressed the availability to investigators of the FDA for early feedback when initiating key animal studies. They provided a review of FDA-regulated product categories, including biologics, devices, drugs, human tissue, and combination products. Issues to be considered early on in development of musculoskeletal products based on cells and/or scaffolds include composition and design, intended use, mechanism of action, and the intended role in the clinical practice. Important experimental

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design factors include the length of time for the animal study, and the utility of early predictors that reliably predict long-term outcomes.

Drs. Jeffrey Lotz and Koichi Masuda discussed animal models for regenerative medicine therapies of the intervertebral disc. The nature of human disc disease is chronic, whereas animal models are typically acute, and this distinction provides challenges. Although the clinical situation often presents with late-stage disease, models to study preclinical phases are useful for interventions at earlier stages. The etiology of pain associated with disc degeneration is poorly understood, and pain, inflammation, and degeneration together provide complexity to models and may alone and in combination have adverse effects on stem cells and tissue regeneration. No gold-standard models for disc repair are currently available. Cell-based approaches would likely benefit from a source of healthy disc cells capable of regeneration. Selection of animal models depends on the questions asked and on the research stage. Small animal models, such as mice, rats, and rabbits, are commonly used at the discovery stage, whereas dogs, sheep and other large animal models are used for preclinical studies. All common animal models utilize quadruped animal species, and the effect of gravity on the course of disease progression and recovery may not be fully accounted for in such studies. Challenges in creating animal models include the health and age of animals, and challenges in using such models include consistency in methods for assessing treatment efficacy (imaging, biomechanics, and biochemistry). There is a need for standardization of surgical techniques, of assessment methods, and for high standards in preclinical studies to support clinical trials.

Articular cartilage animal models were discussed by Drs. Constance Chu, Timothy Simon, and Lori Setton. Dr. Chu reviewed animal models for studying repair and regeneration of focal defects in articular cartilage. Treatments of acute knee trauma focus primarily on meniscus, ligament, and tendon, whereas articular cartilage injuries are often not apparent shortly after injury. Only at the end stage of cartilage disease are radiographic changes evident, and even then the patient's subjective pain assessment and knee function are poorly correlated with the results of diagnostic imaging. Although histological and biomechanical measurements are used widely to assess the success of therapeutic interventions (functional) magnetic resonance imaging and also optical coherence tomography (OCT) are being studied especially for assessing early disease stages. Here also, the utility of various animal models depends on the question being investigated and the research stage. The mouse (transgenic, knockout, and athymic) is useful for mechanistic early-stage studies. The rat is more versatile and useful for study of injections (whether chondroprotective or chondrotoxic) as well as local gene therapy and osteochondral defects. The rabbit has a propensity for self-repair. The dog is used especially in rehabilitation studies, but often not in endpoint studies due to their pet status. The goat is useful for osteochondral defects but difficult for arthroscopy. The pig is typically portly, which impedes maneuvering. The horse has thick cartilage, has a large joint that permits knee arthroscopy, can have defined postoperative rehabilitation, and is increasingly used as a preclinical model, even though its large size and knee anatomy impedes magnetic resonance imaging.

Dr. Setton elaborated on rodent models for studying treatment of osteoarthritis by intraarticular delivery of therapeutic agents. Rat and mouse animal models allow investigations of therapeutic drug distribution and activity as well as the disease outcome. An attractive approach to therapy is the local intraarticular delivery of agents to attain high local concentrations. Drug distribution studies in small animal models should be performed in such small animal models before transitioning to larger animal models such as the goat.

Dr. Simon provided additional perspectives on animal models for cartilage repair, especially the goat model. Large animals are mostly farm animals such as pigs, cows, sheep, and goats. The thickness of the articular cartilage is sufficient for osteochondral and even chondral repair. However, the bone is somewhat stiffer than that of humans. Goats do not successfully self-repair an osteochondral defect. Rapid indentation testing has been used as a sensitive indicator of the load-bearing quality of repair cartilage. Thus, commonly used models are sheep and goats because of their size and lack of repair. In addition, they offer the possibility of studying age-related changes.

Drs. Steven Arnoczky and Thomas Carter discussed meniscus repair and regeneration. Dr. Carter described the clinical challenges for meniscus repair and regeneration, as well as possible interventions (including partial meniscectomy) and their pros and cons for meniscus repair. He noted that cartilage is at risk and that, in terms of regeneration, collagen meniscus implants function better with chronic than with acute conditions. Dr. Arnoczky provided a comprehensive summary of animal models for meniscus repair. When selecting an animal model, it is important to consider size, age, sex, and activity. He discussed the necessity to distinguish "models of disease" versus "models for research." Rehabilitation strategy is also an important consideration, especially for large animals. There exists a need for a model of meniscus function. Currently, there is not a gold-standard model to study meniscus repair.

Drs. Kurt Spindler and Martha Murray then addressed repair of the anterior cruciate ligament (ACL). Dr. Spindler addressed bedside-to-bench research strategies. There are needs to identify reliable predictors for ACL and meniscus tears, and needs to identify factors that predictably modify outcome. He emphasized that the important role of animal models in translational research is to compare different treatment strategies in the same model, and not necessarily to try to replicate human disease conditions with such models. Dr. Murray presented the approach of primary repair and the use of platelets to enhance repair. She addressed the requirements for animal models for ACL research. She contrasted the medial collateral ligament's (MCL's) innate ability to heal on its own with suturing of ACL with a high failure rate. Regarding species to be used, Yorkshire pigs are economical, but grow quickly, whereas minipigs are better for studies of ACL reconstruction and repair, but less economical.

Dr. Kathleen Derwin discussed the use of rabbit and canine animal models for rotator cuff tendon tears. She indicated that small animals are better suited to mechanistic studies, and that either small or large animals can be used for studies of repair. Large animal models are needed for work involving fixation. There is a need for better clinical outcome measures, and there is the need to keep the tendon close to the bone when implementing therapeutic fixation strategies.

In the discussion it was also pointed out that the limitations in extrapolating the results obtained in animal models to human disease include the paucity of animal models recapitulating chronic conditions and repeated injuries, which are frequent factors in human disease.

Dr. Herman Vandenburg presented his work on development of bioartificial muscle mini-fibers for high-throughput drug screening. He described the three-dimensional tissue model concept as an alternative to the use of animal models. A challenge for the future is scaling up the bioartificial muscle fibers and increasing their force-generating capability, which may be limited by the lack of vasculatures and innervation.

Drs. George Muschler, Edward Schwartz, and Dietmar Hutmacher addressed the tissue engineering and regenerative medicine of bone. Dr. Muschler provided a comprehensive summary of animal models for bone research. Appropriate models should meet functional and operational demands. Recently, a significant success has been achieved with developing effective bone graft materials.

Dr. Schwarz summarized animal models used for gene therapy studies of large bone defect repair. He demonstrated the use of microcomputed tomography to assess the outcome of allograft bone implantation. Since bone repair is a mature field, future studies need an active comparator arm. *In vivo* imaging mechanical testing are critical outcome measures.

Dr. Hutmacher discussed his group's work on bone regeneration. One of the important challenges in scaffold design is the need to control scaffold degradation. Normally, for optimal bone regeneration, the scaffold should degrade over 3–8 months. He presented an overview of a variety of new approaches for manipulating the scaffold turnover.

Overall, the workshop provided an effective and succinct summary of the status of various animal models in musculoskeletal regenerative medicine. Although many models are available and serve well to answer a variety of questions, the general consensus was that there is a substantial need for

improved and standardized animals models, as well as accompanying outcome measures at defined timepoints, throughout the field for tissue engineering and regenerative medicine of the musculoskeletal system, and that animal models, especially large animal models, are a critical pre-clinical step for translating research from bench to bedside.

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