Evaluation Criteria for Musculoskeletal and Craniofacial Tissue Engineering Constructs: A Conference Report

Functional Tissue Engineering Conference Group[†]

Over the past 20 years, tissue engineering (TE) has evolved into a thriving research and commercial development field. However, applying TE strategies to musculoskeletal (MSK) and craniofacial tissues has been particularly challenging since these tissues must also transmit loads during activities of daily living. To address this need, organizers invited a small group of bioengineers, surgeons, biologists, and material scientists from academia, industry, and government to participate in a 2½-day conference to develop general and tissue-specific criteria for evaluating new concepts and tissue-engineered constructs, including threshold values of success. Participants were assigned to four breakout groups representing commonly injured tissues, including tendon and ligament, articular cartilage, meniscus and temporomandibular joint, and bone and intervertebral disc. Working in multidisciplinary teams, participants first carefully defined one or two important unmet clinical needs for each tissue type, including current standards of care and the potential impact of TE solutions. The groups then sought to identify important parameters for evaluating repair outcomes in preclinical studies and to specify minimally acceptable values for these parameters. The importance of *in vitro* TE studies was then discussed in the context of these preclinical studies. Where data were not currently available from clinical, preclinical, or culture studies, the groups sought to identify important areas of preclinical research needed to speed the development process. This report summarizes the findings of the conference.

Introduction

SINCE FIRST COINED ALMOST 20 YEARS ago by Y.C. Fung,¹ tissue engineering (TE) has evolved into a thriving research field, with active parallel commercial development. Academic and industry researchers have sought to repair a wide array of tissues using many approaches, with progress in some areas like skin graft substitutes, but more uneven progress in others. The early promise of success in TE has been tempered by the realism that tissue regeneration is complex, requiring innovative approaches to design individual tissue and organ repairs.

Applying TE strategies to musculoskeletal (MSK) and craniofacial tissues is particularly challenging, since these tissues must also transmit loads during activities of daily living (ADLs). Tissues like the anterior cruciate ligament (ACL), articular cartilage (AC), long bones, intervertebral disc (IVD), and temporomandibular joint (TMJ) can experience large and recurring *in vivo* forces in challenging environments during these ADLs. The tissue engineer thus faces mechanical and chemical demands in designing tissues that can carry load and remain functional during the entire healing and remodeling process after implantation.

Although TE has, thus far, led to few clinically successful products for patients with MSK and craniofacial disorders, the field has many potential bioengineered technologies currently under development. However, investigators seeking to evolve these technologies into clinical application are hindered by the lack of a coherent strategy to evaluate the relative merits of competing TE designs. While some areas like bone have a well-developed science base and preclinical models, experts still have not reached agreement on which preclinical models, parameters, and threshold values of success to use in their design strategy. This conference was intended to address these issues.

Rationale and Conference Design

To address this deficiency in TE evaluation criteria, two bioengineers (Butler and Lewis) and one surgeon (Frank) from academia organized a unique multidisciplinary meeting to begin to define and reach consensus on evaluation criteria for TE constructs. The small group invited to participate came from academia, industry, government, and so on (see Appendix). This group was asked to relate what they believe should be measured to evaluate success or failure for

The conference was held April 26–29, 2007, at the Hilton Oceanfront Resort in Hilton Head, South Carolina.

[†]The coauthors are the group of conference organizers and participants listed in the Appendix.

relevant MSK and craniofacial TE constructs. With current knowledge from the literature and input from other experts in the field, the group was charged to identify suitable controls and standards of comparison, parameters to measure in preclinical models, and values for these parameters that would constitute a successful outcome. The organizers initially assumed that practicing tissue engineers would follow a standard research and development process where *in vitro* studies are performed first to optimize the TE component, and then constructs are implanted and evaluated in suitable animals. This conference thus focused on the clinical problem, evaluation of the construct in the animal model, and the *in vitro* studies needed to support these animal studies.

Members split into four stakeholder groups with the following perspectives on TE goals and objectives.

Basic researchers (bioengineers, biologists, and material scientists): Researchers brought a spectrum of perspectives to the discussions. Many of the biologists and some of the engineers and material scientists emphasized the importance of understanding basic mechanisms and fundamental properties of the TE constructs and repairs like restoring normal cellularity and matrix composition as well as construct material quality. Several individuals stressed the value and challenges in designing constructs that could be translated in a more practical fashion into actual products to help patients. Nearly all members of this “stakeholder” group recognized the difficulties in bridging these large gaps between *in vitro*, preclinical, and clinical studies.

Clinicians: Surgeons brought clinical and translational perspectives to the discussions. They emphasized factors like ease of clinical use, including handling and *in vivo* fixation at surgery; reproducibility; safety; short- and long-term effectiveness; identifying risk/benefit ratio versus existing approaches (efficacy); and minimizing complications after surgical implantation. These stakeholders also recognized that researchers must perform *in vitro* and preclinical studies to identify the most promising treatments, but that translating these results to the clinic remains challenging.

Industry: Industry representatives contributed important practical perspectives to the discussions. They pointed out that TE must have a specific market size with limited competition to be economically viable. They cited other practical considerations for TE, including its complex regulatory and reimbursement pathways, its risk/benefit ratio (particularly for constructs involving cells), the relatively high costs of investigation, challenges in manufacturing and scale-up, and the complex analytical methods required to define its effectiveness. They also noted the need to judge clinical outcomes (efficacy and cost) against other current standard treatments and reminded the group of important intellectual property ownership and safety issues.

Funding agencies: Funding agency representatives brought key perspectives about the need for scientific excellence in the field of TE and the potential returns on investment to society associated with a successful design. It was noted that “NIH supports high quality basic, applied and clinical research that has the potential to improve the Nation’s health” and “NIH feeds a pipeline for R&D work ... and recognizes the translational nature of TE science.” Representatives also shared the fact that NIH’s “portfolio” differs from that of the private

FIG. 1. Inverted pyramids showing opportunities and needs by the National Institutes of Health and the private sector. Slide originates from Dr. Zerhouni’s presentation, “NIH at the crossroads: Strategies for the future.”

sector (Fig. 1) and that various funding options exist (through special emphasis panels and study sections) to pursue TE research. These include the Bioengineering Research Partnerships and Bioengineering Research Grants.

One to three members from a stakeholder team joined a “tissue specific” breakout group. These groups included (1) tendon and ligament, (2) bone and IVD, (3) AC, and (4) meniscus and TMJ. We selected two breakout groups based on the similar structure, composition, and function of ligaments and tendons as well as meniscus and TMJ. We assigned AC to its own group because of its unique character. We grouped bone and IVD because they both sustain compression and because of their proximity in the spine. Less emphasis was placed on common technologies within groups. Each group focused on one or two clinical problems in order to complete the exercise during the conference (see “Defining the Clinical Problem” below). We intentionally mixed members of different groups (1) to provide complementary input and expertise, (2) to allow broader discussions about how to conduct basic research and product development, and (3) to highlight the needs of patient populations.

The Approach Used to Define Evaluation Criteria

Conference organizers (Butler, Lewis, and Frank) requested that participants work backward from the clinical problem to the preclinical models and then to *in vitro* development (Fig. 2). This strategy ensured that groups first clearly focused on the clinical goal and unmet medical need as the primary rationale for new development. Groups then identified evaluation criteria of success in preclinical studies that could be better controlled so as to provide clear benefits before starting expensive human clinical trials. *In vitro* studies were only addressed briefly on the last half day, since these would depend on the specific clinical and preclinical questions and evaluation criteria.

FIG. 2. Process followed in developing evaluation criteria recommendations.

Defining the clinical problem

Each breakout team, including surgeons, first identified one or two high-priority clinical problems for each tissue type. While any clinical problem could have been selected, team members rapidly reached consensus on their choices. Organizers reasoned that by successfully completing the clinical-to-preclinical-to-*in vitro* exercise for one or two problems, the paradigm could be repeated for other clinical problems in the future. Clinical problems were selected based on

- Clinical relevance (common clinical problems) in the MSK and craniofacial fields;
- Prevalence of the disease and its projected incidence in the future;
- Potential for TE to safely and effectively address unmet clinical needs, where current treatments (1) are not effective enough in the short or long term, (2) result in excessively slow recovery times, and/or (3) induce excessive morbidity;
- Existing evidence supporting strong potential for TE success over time (progressive improvements over existing standards).

Attendees generally agreed that successful TE constructs should be better than current standards of care by one or more of the following outcome measures. Patients should

- Demonstrate faster recovery time;
- Show better short-term and long-term functional outcomes (e.g., reduced pain or effusion; improved joint stability, range of motion, and overall function; return to preinjury activity; return to work, etc.);
- Exhibit more durable functional improvements in specific patient-related factors (e.g., by delaying disease progression and/or the need for later, more aggressive options);
- Show little or no morbidity (even when outcomes are no better, patients could dramatically benefit from decreasing morbidity and side effects associated with some existing treatments);
- Need less-expensive treatment in the longer term, resulting in fewer surgeries (for example, one surgical procedure rather than two required for Carticel).

Clinical evaluation issues

Although the conference was not primarily designed to discuss/debate current clinical evaluation problems, the group recognized that few validated clinical evaluation tools are currently available for the clinical problems to be addressed by TE. This problem was universally identified at the end of the conference as an important current and future research need. Specifically, the group noted the importance of

establishing tools and methods for clinical evaluation of specific procedures, including better noninvasive diagnostics (imaging) as well as better joint-specific and condition-specific functional evaluation methods in patients.

Clinical target populations: tissue targets, intent of TE constructs, and their potential advantages and constraints

The groups then identified specific clinical needs for each tissue. For each clinical problem, pathological conditions were cited along with current standards of care, potential advantages of a TE approach over an existing standard, and any limitations and constraints that might result from a TE solution. Results of this process for specific clinical problems are shown in Table 1 for AC,²⁻⁵ ACL,⁶⁻⁸ rotator cuff,⁹ bone,^{10,11} IVD,^{12,13} TMJ,¹⁴⁻¹⁷ and meniscus.¹⁸⁻²⁰

As an example, the AC group identified two clinical problems: late-stage osteoarthritis (OA) and focal cartilage defects.²⁻⁵ Late-stage OA, and the attending diffuse, usually multicompartamental cartilage loss, was cited as an enormous clinical problem with few surgical treatment options before total joint replacement. Focal defects in AC, which the group defined as areas of acute partial or full-thickness chondral loss, usually restricted to an isolated area in one compartment of the knee, were judged to be less common. However, the group did recognize the important aspects of this clinical problem and its value as a “proving ground” for potential solutions for OA. The cartilage breakout team concluded that the acute focal defect may not be common enough to be commercially viable by itself, but its treatment could serve as an intermediate step toward the ultimate goal of treating larger, more diffuse, and opposing-surface OA lesions. The current clinical standard of care for focal defects is microfracture, a simple, fast, and fairly uncomplicated procedure. The team concluded that TE solutions after cartilage injury offer several potential advantages, including more natural-like tissue with improved short- and long-term function, delay of total joint replacement, and better compatibility with surrounding joint structures. However, the group also recognized the potentially high cost of TE repairs of focal defects as well as the increased technical demands at surgery and longer rehabilitation time needed to protect the early repairs. A similar process was followed for each tissue type by respective groups.

Conference participants then heard reports on all tissue types and identified common advantages and constraints. Potential advantages included normal or quasinormal tissue quality, reduced patient morbidity (e.g., compared to harvesting autografts), and increased durability of the treatment. The group also agreed that several constraints must be overcome for all of the tissue types examined. For example, the field has still not identified the clinical populations and “at-risk” subpopulations that should be treated. This limitation is, in part, due to the fact that not all clinical (or preclinical) standards of care have validated outcomes. In addition, many clinical and preclinical studies can be quite lengthy and costly, suggesting the need for short-term predictors of long-term outcome in animals and ultimately in patients.

Conference participants also heard about practical constraints facing companies seeking to bring products to market. These industry participants emphasized that the product

TABLE 1. TISSUES, TARGETS, CLINICAL TREATMENTS, AND POTENTIAL ADVANTAGES AND CONSTRAINTS OF TE

<i>Tissue type</i>	<i>Clinical target</i>	<i>Current standard clinical treatment(s) & any special considerations</i>	<i>Potential advantages of TE over existing standards</i>	<i>Potential constraints of TE for this application</i>	<i>References to clinical conditions and clinical demographics</i>
AC	OA	OA: treat symptoms, osteotomy, unicompartamental, or total joint replacement	Improved tissue quality, more natural tissue	Cost	Aroen <i>et al.</i> , ² Arthritis Foundation, ³ American Academy of Orthopaedic Surgeons, ⁴ and American College of Rheumatology ⁵
	Focal defects (partial and full thickness)	Focal defects: microfracture	Improved short- and long-term function Delayed TJR Better compatibility	Increased technical demands at surgery Rehabilitation time Identifying clinical population Morbidity	
ACL	Complete tear	Autograft or allograft tendon graft	No secondary morbidity site No disease transmission Improved knee mechanics Retention of proprioceptive function	Ability to resist initial loads Synovial environment Reestablishment of bone–ligament insertion site Ability to restore complex ACL geometry Loss of neurologic function	Myer <i>et al.</i> , ⁶ Spindler <i>et al.</i> , ⁷ and Von Porat <i>et al.</i> ⁸
Rotator cuff	Tendon tear	Suture repair	Normal insertion sites Less invasive Reduced OA Faster healing Stronger repairs Lower failure rates Less invasive	Large tears may be hard to close Multidimensional loads Muscle atrophy Healing to bony insertion sites Synovial environment Cost	Galatz <i>et al.</i> ⁹
Bone	Large defects Spine fusion Fracture nonunions	Autograft Allograft BMPs	No secondary morbidity site Faster, more consistent repair Engineering bone–soft tissue interfaces	High-functional loads Inflammatory/immune response	Kneser <i>et al.</i> ¹⁰ and Swiontkowski <i>et al.</i> ¹¹
IVD	Disc degeneration	Fusion Disc replacement	Less disruptive at surgery Physiologically and structurally closer to normal	Filling complex defect geometries Lack of knowledge of what is “normal” Unknown pain pathophysiology	Garfin ¹² and Johannessen <i>et al.</i> ¹³
TMJ	Pain, crepitus, clicking, opening deviation	Conservative treatment including diet modification; condyle replacement by prosthesis or autologous grafting (e.g., rib grafts)	Integration with host tissue; remodeling with host bone	Ability to resist initial loads Retention of implant Cost Complexity of joint structures	Friction ¹⁴ Kapila, ¹⁵ Oke-son, ¹⁶ and Sindelar and Herring ¹⁷
Meniscus	Avascular zone tear Nonrepairable meniscus tears	Direct surgical repair Excision None	Delay knee OA Restore a stable and functional meniscus	High initial functional loads Complex geometry to prevent knee OA Synovial environment	Lozano <i>et al.</i> , ¹⁸ Matava, ¹⁹ and Ryzewicz <i>et al.</i> ²⁰

TJR, total joint replacement.

must be manufactured and delivered at a competitive price that generates profits for the company that must incur the development costs. Companies will not invest in technologies that pose too great a risk with too small a profit margin. Specific factors that enter into this position include the potential market size for each tissue-engineered clinical condition; specification of well-defined, necessary, and sufficient design requirements for these biologics and devices; and the ability of surgeons to implant these structures into their patients using common surgical technologies that do not introduce risk or safety issues. Industry experts also emphasized that although the biologic or device may be appealing on other grounds, ultimately it must be economically viable and worth the expenditure of additional research funds. This evaluation should be made as early as possible in the research and development process.

Identifying Evaluation Criteria in Preclinical Models: What Should Be Measured?

Given the previous considerations, then, what should be measured in a preclinical model and how should the experiment be designed? Critical elements of concern to this question are the aim of the experiment, model selection, appropriate controls and standard of care, and specific variables to measure.

To address these concerns and identify parameters, the entire group was given a common mission. They were challenged not to be "model-specific" in their comments, but rather to identify an idealized set of outcome measures that would capture important aspects of biology, integrity, structure, and/or function. They were also asked to identify differences between experimental arms and distinguish between competing TE approaches, including appropriate controls. The groups were also instructed to prioritize their overall results for individual tissues and models. These results are presented below.

Evaluation considerations in preclinical models—model selection

Animal models of relevance are required for each tissue application. Tissue engineers should consider models that actually simulate, as closely as possible, diseases, pathology, and age of interest. Investigators should also recognize the limitations of the various available models and that they often do not simulate the human clinical condition (e.g., in age, degenerative disease, avascularity, and pain). Researchers should clearly understand that when an acute injury is repaired in an otherwise normal young animal, it likely does not simulate the "real case." These models also introduce a series of "technical issues," including cost, size (bigger = better for some measures; worse for others), reproducibility, ease of surgical implantation, and similarity to clinical techniques regarding implantation and fixation. The group also concluded that less-invasive insertion methods would be best and that the model should ideally allow some type of realistic rehabilitation after surgery. The animal and tissue model selected should allow repairs to be examined over longer time intervals after surgery and provide adequate tissue for multiple, well-controlled analyses. Yet, the choice of animal and tissue models obviously depends on the question being asked and how the model relates to that question. For example,

empty or "blank" defects might be useful to assess the natural healing (i.e., the absence of treatment; see next section).

Evaluation considerations in preclinical models—controls and standard of care

The question of "controls" and "standard of care" was a central issue debated in breakout groups and in plenary session. As with any experiment, appropriate controls are critical. Participants discussed the need for internal controls (two or more treatments within the same joint or animal), and the use of "empty defect" controls. The groups also debated the value of nonoperated controls (e.g., no operation on the opposite leg) versus simulations of clinical standard of care.

The groups examined the advantages and disadvantages of running internal controls. If carefully performed with minimal influence or interaction, treatments could be contrasted among multiple locations in an individual joint or after contralateral surgeries in paired limbs (including the use of sham operations). These controls can avoid statistically underpowered observations that commonly occur when inter-animal differences exceed intra-animal differences for specific evaluation measures. Such inter-animal, biological variance can be a significant barrier that masks our ability to identify a successful TE construct and then to publish the results.

A treatment might also be contrasted against whatever is appropriate for the specific question being addressed. For example, the comparison might be to a "blank" or empty defect control (no treatment) to demonstrate the natural healing ability in the model of interest. As noted above, this approach could be used to demonstrate that the TE solution does better than no treatment, especially if this blank control does not heal on its own. However, this control would be incomplete on its own, since available treatments are expected to be better than the blank control.

Participants also questioned if a tissue-engineered repair should be compared to a nonoperated, normal tissue. "Normal" is certainly an "ideal" standard, but few TE solutions achieve this goal and normal may not be necessary for clinical success. Normal, within a functional loading range, might be more achievable, however, especially in the early stage of development. If normal was the only control and goal of the experiment, one might judge a TE treatment to have failed, especially since few TE solutions achieve normal properties under abnormal conditions. Over time, this target can hopefully be approached (where required), and for this reason, we have included some of the key features, target parameters, and evaluation measures (with a few references) in Table 2 for the interested reader (AC,^{21–26} ACL,²⁷ rotator cuff,^{28–30} bone,³¹ IVD,^{32–39} TMJ,^{40–44} and meniscus^{45–48}).

Instead, treatments could be validated against a "simulation" of the current clinical standard of care for that condition, if that option was available and could be tested in the animal model of choice. This comparison would probably be required at some point, since the purpose of developing a new TE construct is to improve on the "existing" clinical options (both in terms of clinical efficacy and cost).

Participants eventually recommended that investigators use multiple controls and seek to show positive effects of the treatment relative to normal and simulations of standard of care. The choice of control(s) will depend on the specific purpose of the experiment and the sensitivity of the

TABLE 2. NORMAL CHARACTERISTICS OF CANDIDATE TISSUES CLASSIFIED BY SOME POTENTIAL "KEY STRUCTURAL AND FUNCTIONAL CONSIDERATIONS" FOR TE REPLACEMENTS, WITH A FEW SELECTED REFERENCES TO THESE FEATURES

MSK and craniofacial tissues with TE potential	Normal targets— imaging, gross appearance, and some key characteristics	Normal histology; cell types, matrix organization	Normal tissue functions and biomechanical properties	Normal biochemistry (markers of normal)	Key references to normal structures and functions
Cartilage	Thin, uniform thickness (3–6 mm), white, avascular, aneural, low friction	Chondrocytes (no cloning), zonal, matrix highly ordered, dense proteoglycan staining, surface intact	Nearly frictionless, firm viscoelastic, low permeability, fracture resistant	Collagen II, aggrecan, matrix metalloproteinases	Cohen <i>et al.</i> , ²¹ Faber <i>et al.</i> , ²² Hasler <i>et al.</i> , ²³ Mankin <i>et al.</i> , ²⁴ Mow and Hayes, ²⁵ and Treppo <i>et al.</i> , ²⁶ Murray and Spector ²⁷
ACL	Organized collagen fascicles, low signal on T2 imaging, vascular, innervated, strong in tension, TE construct stiffness?	Fibroblasts, collagen in parallel bundles making fascicles with vessels and nerves running along the fascicles, crimp, synovial layer covering ligament, highly specific ligament insertion site	Very strong in tension	Collagen I, collagen I/III ratio, matrix metalloproteinases, decorin, proteoglycans	
Rotator cuff	Organized collagen fascicles, low signal on T2 imaging, vascular, innervated, strong in tension	Fibroblasts, collagen in parallel bundles making fascicles with vessels and nerves running along the fascicles, crimp, synovial layer covering tendon, highly specific tendon insertion site	Toe region in stress-strain curve to allow normal motion Equivalent tangent stiffness to peak <i>in vivo</i> forces plus safety factor Very strong in tension	Collagen I, collagen I/III ratio, matrix metalloproteinases, decorin, proteoglycans	Blevins <i>et al.</i> , ²⁸ Clark and Harryman, ²⁹ and Soslowky <i>et al.</i> ³⁰
Bone	High radiodensity Integrated with adjacent bone	Osteoblasts, osteocytes, and osteoclasts Woven bone initially but remodeled into highly organized lamellar bone	Very small toe region in stress-strain curve to allow efficient transfer of muscle force to bone Equivalent tangent stiffness to peak <i>in vivo</i> forces plus safety factor Very strong in compression High fracture toughness and fatigue resistance	Collagen I, hydroxyapatite mineral	Taylor <i>et al.</i> ³¹

IVD	Vascularized and remodeling Normal disc height by radiog- raphy	Fibroblast-like in annulus fibrosus, chondrocytic in inner annulus and nucleus pulposus, noto- chordal cells in young human, chondrocytic cells in adult	Pliant, viscoelastic, stiff in compression (approx- imately 4 MPa)	Opposing gradients of col- lagen I (outer annulus) and collagen II (nucleus)	Adams <i>et al.</i> , ³² Ferguson and Steffen, ³³ Guerin and Elliott, ³⁴ Johan- nessen and Elliott, ³⁵ Nerurkar <i>et al.</i> , ³⁶ Roberts <i>et al.</i> , ³⁷ and Schnake <i>et al.</i> , ³⁸ and Setton and Chen ³⁹
	MRI normal signal on T2 imaging, no herniation		Compressive strength to exceed ADL estimates (approximately three to five times body weight) Flexible in bending	Aggrecan gradient from outer annulus to nucleus	
	Fibrous annular rings, gelati- nous nucleus in younger individuals, crabmeat like in older individuals Hyaline cartilage endplate across entire disc in young, extending to inner annulus in adult		Exhibiting a “neutral zone”	Aggrecan and collagen II in endplate	
				Low cytokine levels	
TMJ	Disc is fibrocartilage and/or fibrous tissue; articular condyle is fibrocartilage; age variation; CT and MR used in diagnosis; bilater- ally linked and must func- tion in unison	Disc is mostly type I colla- gen, although type II collagen can be found; both disc and articular condyle consist of large and small proteoglycans, including aggrecan, ver- sican, decorin, biglycan, etc.; age variation Meniscus cells viable in entire tissue	Withstands tensile, com- pressive, and shear forces; fibrocartilage’s compressive properties between fibrous tissue and hyaline cartilage; regional differences in mechanical properties	Low lactate levels Type I and type II colla- gens; regional dif- ferences in the distribu- tion of proteoglycans; adaptation with func- tional demands in matrix composition	Alhadlaq and Mao, ⁴⁰ Allen and Athana- siou, ⁴¹ Hu <i>et al.</i> , ⁴² and Mao <i>et al.</i> ^{43,44}
	Intact circumferential collagen fibrils and attachments		Act like normal meniscus the loads on AC in that specific compartment	Types I and II collagen	Hauger <i>et al.</i> , ⁴⁵ Kambic and McDevitt, ⁴⁶ McDevitt and Web- ber, ⁴⁷ and Upton <i>et al.</i> ⁴⁸
Meniscus	Simulate the normal collagen/ PG distribution	Spatial organization of collagen-circumferential and radial PG matrix Vascular outer rim	Similar tensile and com- pression properties	Type VI collagen	
	No extrusion or tears			Aggrecan PGs Matrix genes	

MRI, magnetic resonance imaging; PG, proteoglycan.

evaluation measures being used. Showing positive effects of a treatment, especially without a suitable clinical standard of care, might still be useful in pursuing a treatment option.

Evaluation parameters and values in preclinical models for TE constructs—issues leading to summary and recommendations

With these considerations in mind, specific evaluation parameters in preclinical models were identified for each tissue (AC,^{49,50} ACL,^{7,51–54} rotator cuff,^{28–30,55,56} bone,^{10,57–62} IVD,^{12,32,33,38,63–70} TMJ,^{14–17,40–44} and meniscus^{18–20,45–48,71–76}), and then specific parameter values were chosen that were considered to be successful relative to a specific standard (Table 3). The group determined that an extensive list of criteria must be considered when assessing the potential “success” of any TE construct. The group focused more on the clinical and research areas of interest rather than the late-stage development and marketing issues such as the ability to pass regulatory requirements, ensure quality, etc., introduced at the beginning of the conference.

Some participants voiced concern that by expressing “common evaluation issues,” we would overlook specific criteria that are unique to individual tissues. Therefore, these “generic” evaluation suggestions are presented only as a summary of what the group felt should be “minimum expectations” and recommendations for those considering TE research. Evaluation criteria common to all tissues were identified as follows. These are divided into three sections: must have, should have, and nice to have, as follows.

“Must have” criteria: (1) Be able to be implanted and retained under appropriate early loading conditions. (2) Meet or exceed current “best” treatment for that tissue in the model of interest according to one or more quantitative measures. These measurement tools could include implant integrity, imaging, structure, mechanics, or biochemistry over time. Specific examples might include imaging and noninvasive assessments (ideally quantitative); joint function (inflammation, pain, etc.), since it assesses more than the tissue itself; tissue mechanics if possible (acutely, locally, and over a reasonable time period after surgery); individual tissue assessment (gross histology, etc.) as well as assessment of other tissues in or around the joint; geometry; other relevant time-related properties like durability, longevity, and recovery; biological measures like cellularity, viability, organization, vascularity, and matrix composition; and molecular measures to understand mechanisms leading to iterative and relevant improvements in repair outcome. (3) Be viable (cellular) after implantation (cell-based therapy). (4) Be safe (no signs of adverse reaction *in vivo*). And (5) be functionally integrated into surrounding tissues and/or be replaced by functional host tissue.

“Should have” criteria: (1) Be evaluated by more than one validated quantitative outcome measure; (2) aspire to achieve normal tissue properties by as many measures as possible.

“Nice to have” criteria: Promote improved endogenous repair or ingrowth of surrounding injured or degenerated host tissues. This criterion would enhance fixation and/or longevity of the replacement.

Based on this list and on tissue-specific needs, evaluation parameters were then identified for each tissue and values were known (Table 3).

For AC, the primary clinical problem is repair of focal defects and cartilage resurfacing as a treatment for OA. The current standard of care for focal defects is microfracture, and the current standard of care for OA is treatment of symptoms, osteotomy, or joint replacement, depending on stage of disease. At animal sacrifice, TE constructs for both repair of focal defects and cartilage resurfacing as a treatment for OA should be evaluated by noninvasive imaging, gross examination, biochemistry, histology, histochemistry, and mechanics. Noninvasive imaging could include plain film radiography with characterization of radiographic features of OA, including narrowing of the cartilage space, marginal osteophytes, subchondral sclerosis, and breaking of the tibial spines. Imaging could also include magnetic resonance imaging (MRI) techniques designed specifically for cartilage evaluation. Function of the animal should also be evaluated using validated outcome instruments, which would ideally include indices of pain and motor function. Parameters from these measurements should be assembled into one of the available composite scoring systems (e.g., Outerbridge or India Ink staining for gross score, O'Driscoll for histology, and International Cartilage Repair Society (ICRS) for composite appearance) and should be found to improve over values for those in knees undergoing the current standard of treatment for the target condition.

For the ACL, the primary clinical problem is treatment after traumatic rupture. The current standard of care after ACL rupture (when surgery is indicated) is removal of the ligament and replacement or reconstruction with autologous patellar tendon or hamstring tendons, or with allograft tissues. New methods for treating ACL rupture using TE techniques should be evaluated using functional methods and mechanical assessments, as well as biologic measures that indicate continuation of function over time. Evaluations should be carried out in a large animal model, such as goat, pig, sheep, or dog (rabbit and smaller models not recommended). Observations should be recorded at 1–3 months for biologic and histologic changes and between 3 and 12 months for biomechanical properties or development of AC changes. Biologic parameters include gross inspection parameters (cartilage, synovium, effusion, and India ink staining of cartilage), as well as microscopic evaluations of the bone–ligament interface; presence of inflammatory cells; vascularization; histology of the ACL tissue, graft material, and joint cartilage; and the appearance of the ACL on noninvasive imaging. Functional evaluations include measurements of anteroposterior (AP) laxity of the knee, the stiffness and maximum load of the ACL construct, the condition of the AC and overall joint function measures, including joint range of motion, gait abnormalities, and activity monitoring. The target values for these outcome measures should compare favorably with the current gold standard of treatment (ACL reconstruction with autograft tendon). The mechanical properties should have 100% of the stiffness of the normal ACL in low load regions (up to 20% of the intact ACL maximum load) in addition to stiffness and strength similar to ACL reconstruction. The joint function measures should also have values similar to knees undergoing ACL reconstruction in terms of range of motion, effusion, AC changes, gait abnormalities, and activity changes.

For the rotator cuff, the primary clinical problem is tendon tears that are unresponsive to nonsurgical treatment. The current standard of care for these injuries is suture repair of

TABLE 3. PRECLINICAL MODELS, MEASURES, AND VALUES

<i>Tissue type</i>	<i>Preclinical model options</i>	<i>Models that are not recommended (if any)</i>	<i>Assessment times based on condition and/or model(s)</i>	<i>Proposed evaluation parameters</i>	<i>Target values—specify controls (%N:%C)</i>	<i>References</i>
Cartilage	Focal defect (into bone or partial, depending on target condition) or larger defect in a medium-sized animal model (e.g.,sheepanddog)	Immature animals	Depends on question(s)	Joint function (surrogate of pain)	Should exceed "treatment controls"—e.g., C×110%, where C = microfracture for focal defects, or appropriate treatment for target stage for OA resurfacing Should not just exceed "blank controls"	Lee <i>et al.</i> , ⁴⁹ and Troken <i>et al.</i> , ⁵⁰
ACL	Central defect	Models that cannot assess joint function	More than one time point At least months (to understand processes) 1–3 months for biological and histologic changes	Cartilage integrity and composition OA scores	Similar to laxity for ACL autograft reconstruction	Spindler <i>et al.</i> , ⁷ Butler <i>et al.</i> , ⁵¹ Hunter <i>et al.</i> , ⁵² Rasmussen <i>et al.</i> , ⁵³ and Schwartz <i>et al.</i> , ⁵⁴
Rotator cuff	Complete tear	Goat, sheep, dog, and pig are acceptable	3–12 months for biomechanical and joint changes	Stiffness and strength (max. load) of ACL construct	100% of stiffness up to 20–30% of maximum force for normal ACL; similar to stiffness and strength of ACL autograft reconstruction Similar AC condition and joint function to ACL reconstruction	Blevins <i>et al.</i> , ²⁸ Clark and Harryman, ²⁹ Soslowsky <i>et al.</i> , ³⁰ Derwin <i>et al.</i> , ⁵⁵ and Glaser <i>et al.</i> , ⁵⁶
Delayed repair	Complete tear	Animal size—rabbit or smaller not recommended	3–12 months	Overall joint function Recurrence of tear	Rate similar to suture repair	(continued)

TABLE 3. (CONTINUED)

Tissue type	Preclinical model options	Models that are not recommended (if any)	Assessment times based on condition and/or model(s)	Proposed evaluation parameters	Target values—specify controls (%N:%C)	References
Bone	Large segmental defects	Animals where cuff tear is not intrasy-novial (unless capsular defect made as well)		Muscle strength and histology	Appearance and function similar to repair	Kneser <i>et al.</i> , ¹⁰ Bruder and Fox, ⁵⁷ Cancedda <i>et al.</i> , ⁵⁸ Einhorn, ⁵⁹ Guldberg <i>et al.</i> , ⁶⁰ Laurencin <i>et al.</i> , ⁶¹ and Liebschner ⁶²
	Nonhealing craniofacial and long bone defects	Noncritically sized defects	1–6 months for bone bridging	Bone bridging by CT	30% of intact or similar to suture repair Rapid return to full and symmetrical weight bearing	
	Spine fusion	Large (dog, sheep, and goat) animal models to test therapeutic angiogenesis strategies	3–6 months for biomechanical properties	Biology (revascularization and remodeling by histology) Integration strength by biomechanical testing	Restoration of full-intact bone strength and work to failure Accelerated repair compared to current standards	
	Fracture nonunion Compromised models (irradiated, aged, immunodeficient) No ideal model					
IVD		Stab models made with large wounds through the annulus	Depends on question(s)	Disc height	>90% of normal height	Garfin, ¹² Adams <i>et al.</i> , ³² Ferguson and Steffen, ³³ Schnake <i>et al.</i> , ³⁸ An and Masuda, ⁶³ An <i>et al.</i> , ⁶⁴ Anderson <i>et al.</i> , ⁶⁵ Lotz, ⁶⁶ Lotz and Kim, ⁶⁷ Lotz and Ulrich, ⁶⁸ O'Halloran and Pandit, ⁶⁹ and Paesold <i>et al.</i> ⁷⁰
	Large animal preferred to mimic nutritional, surgical constraints	Animals with notochordal cells predominant in nucleus pulposus	More than one time point	MRI	Normal T2-weighted image on MRI	

TMJ	Effective small and large animal models of disease to be established such as OA	Multiple time points	At least months (to understand processes)	ECM production	Normal kinematics of spine
			Cytokine levels	Cytokine levels	Normal ECM and cytokine composition
			Delamination	Delamination	Normal compressive strength and pressure-volume relationship
			Motion segment testing	Motion segment testing	No nerves or vessels in nucleus pulposus
Meniscus	Repair	Not rodents	Repair: 3–6 months	Compressive strength	Should restore function over time; range of motion and mastication need to be restored over time
				Pressure-volume testing	
				Histologic assessment of nucleus pulposus	
				Joint function including range of motion, bite forces; tissue quality	
Scaffold Transplantation	Repair	Maybe rabbit Recommended: dog, goat/sheep, pig	Repair: 3–6 months	All: (1) Protection against OA compartment; (2) avoid extrusion	Friction, ¹⁴ Kapla, ¹⁵ Okeson, ¹⁶ Sindelar and Herring, ¹⁷ Alhadlaq and Mao, ⁴⁰ Allen and Athanasiou, ⁴¹ Hu <i>et al.</i> , ⁴² and Mao <i>et al.</i> , ^{43,44}
				Repair: 3–6 months	Lozano <i>et al.</i> , ¹⁸ Matava, ¹⁹ Ryzewicz <i>et al.</i> , ²⁰ Hauger <i>et al.</i> , ⁴⁵ Kambic and McDevitt, ⁴⁶ McDevitt and Webber, ⁴⁷ Upton <i>et al.</i> , ⁴⁸ Arnoczky <i>et al.</i> , ⁷¹ Cook <i>et al.</i> , ^{72,73} Peretti <i>et al.</i> , ⁷⁴ Valiava-veettil <i>et al.</i> , ⁷⁵ and Webber <i>et al.</i> ⁷⁶
				Scaffolds: 6–12 months	All: (1) no tears or "loose body" formation; (2) delay in time by 30–50% onset OA
			Transplantation: 12 months	Repair/scaffold: (1) integration with normal; (2) repair site strength; (3) scaffold–meniscus interface strength	

the failed ends of the tendon. Repair using a TE construct in an animal model should be evaluated by both functional and biologic measures. The animal model should be larger than a rabbit to assure similar requirements of tissue healing to the human case. The animal model should also have an intraarticular location of the torn tendon, either by the naturally occurring anatomy or by creating a capsular defect so that the tear is exposed to synovial fluid during the healing process. Outcomes should be evaluated at 3–12 months for recurrence of tear, muscle strength, and histologic changes, as well as repair strength. Functional measures include activity monitoring and altered joint motions relative to the normal shoulder, as well as mechanical assessments of *in vivo* and *in vitro* joint laxity versus time as well as stiffness and failure loads from load–displacement tests in the laboratory. Biologic measures encompass gross inspection (size of defect or gap between tendon and repair site on the bone and degree of muscle contraction), histology (presence of inflammatory cells, vascularization, histology of repair, and insertions into bone and muscle), as well as appearance of the tendon and muscle on noninvasive imaging. The healing musculotendinous unit should be carefully evaluated at times up to 3 months and possibly at 6 and 12 months postsurgery as well. The target values for these outcome measures should compare favorably with the current gold standard of treatment (rotator cuff suture repair). The maximum load of the repairs should be at least 30% of the intact cuff tendon, or similar to suture repair values. The joint function measures should also have values similar to shoulders undergoing suture repair in terms of range of motion, effusion, AC changes, gait abnormalities for quadrupeds, and activity changes.

For bone, the main clinical problems are large segmental defects, nonhealing craniofacial bone defects, bone–soft tissue interfaces, spine fusion, and fracture nonunions. The current standard of care is autograft or allograft, although the use of BMPs is increasing rapidly. Restoration of full mechanical properties is a realistic goal for bone repair, so bone TE constructs should be compared to normal intact bone as well as allograft and collagen sponge/BMP standards. Construct integration should be evaluated as a function of time by morphology (CT or micro-CT), biology (revascularization by histology, osteoclast/osteoblast remodeling, and physiologic Ca/P ratios to lamellar bone by x-ray photoelectron spectroscopy/Fourier transform infrared spectroscopy (XPS/FTIR), and mechanics (torsion testing and correlation of three-dimensional bone volume/distribution with integration strength).

For IVD, the main clinical problem is painful disc degeneration. This is typically attributed to unstable spinal biomechanics or abnormal disc biochemistry. The goal of clinical treatment is pain-free motion. There is no current gold standard of care. Treatments include physical therapy, local anesthetic injection, and surgery as a last resort (typically spinal arthrodesis, although total disc replacement is gaining in popularity). The TE goal should be restoration of the disc's physical and biochemical properties, with both fusion and the "normal" disc as suitable comparison standards in a large animal model. At animal sacrifice, the group recommended that tissues should be evaluated for structural integrity from MRI (normal T2-weighted image with at least 90% of normal disc height as compared to adjacent levels and absence of adjacent segmental degeneration), biochemistry (ECM mo-

lecular ratios and cytokine levels restored to normal, inhibition of innervation and vascularization into the nucleus pulposus), and biomechanics (adequate initial fixation strength under functional loads, *in vitro* strength and concentric range of motion of the motion segment, and restoration of normal pressure–volume relationships).

For the TMJ, the primary clinical problems are pain, clicking and opening deviation, degenerative joint disease, OA and rheumatoid arthritis, and ankylosis. The current standards of care are conservative treatment, pain management, intraarticular injections, and surgical replacement with metallic implants and tissue grafts that are condition specific. Clinical outcome of current treatments is not well documented, leading to difficulty in predicting a successful prognosis. There are few valid animal models of TMJ arthritis. Some of the mechanical, structural, and biochemical properties of the TMJ, including the disc, ligaments, and condyle, have been documented but are nonetheless incomplete. Outcome measures should include functional assessment (e.g., range of motion, chewing capacity, and bite force) and, at animal sacrifice, the tissue should be evaluated by structure/morphology (biological fixation and integration, biochemistry, histology, mechanical properties, and imaging).

For the meniscus, the primary clinical problems are direct repair of the tissue in the avascular zone and replacement of irreparably damaged meniscus after partial meniscectomy. The rationale for performing these procedures is that loss of meniscus function leads to premature OA. A TE meniscus construct for either repair or replacement in an animal should be evaluated by structure/morphology measures (imaging, integration with suitable tissue, and histology), biochemistry, and mechanics (contact pressure and extrusion under compression). The articular surfaces in the compartment where the TE meniscus is inserted must be evaluated by histologic, biochemical, and mechanical assessments because the primary goal of inserting a TE meniscus is to prevent arthritis.

***In Vitro* Models of TE—Rationale and Evaluation**

In vitro TE studies can be pursued once the clinical problem has been carefully defined and the preclinical model, measures, and values have been identified. While these models are not intended to simulate the *in vivo* situation (and thus direct extrapolations should be avoided), they do offer certain advantages. (1) They can be especially useful if the effects of certain treatments on *in vitro* response measures directly correlate with repair outcomes after surgery.⁷⁷ Treatment effects can then be rapidly screened *in vitro* to identify and even optimize promising cell, matrix, or stimulus methods to ultimately pursue at surgery. (2) These models are also beneficial from a scientific and ethical standpoint by minimizing preclinical surgery and reducing time and cost of development. (3) Investigators can also perform studies to define mechanisms of action (e.g., to study cell behavior with and without matrices or the action of specific growth factors and cytokines on cell phenotype). (4) Others may seek to test different strategies of construct enhancement or augmentation at surgery (e.g., by crosslinking biomaterials to enhance construct stiffness while also preserving cell phenotype).

This meeting was not intended to identify all *in vitro* TE evaluation issues for MSK and craniofacial applications. How-

ever, the concept of preengineering or preconditioning a TE construct before surgery remains attractive in accelerating the development process toward preclinical and clinical design goals. Better understanding the relationship between the state of the engineered construct before surgery and the final repair outcome remains an important goal.

Institutional Tools, Priority Programs, and Partnerships

In addition to addressing these research needs, conference participants recognized that academic, government, and industry barriers are slowing the effective and efficient evaluation of TE constructs. For example, multidisciplinary teams typically evaluate the complex, interactive factors noted above. However, most universities do not fully promote and reward the individual talents in "multidisciplinary" teams. In a similar way, funding mechanisms and funding programs at granting agencies are only beginning to be created that encourage teams of researchers to pursue these difficult translational studies. Industrial partners must also be engaged early in the process to facilitate and ensure "practical" development toward a clinical solution.

In the final plenary session, delegates were asked to identify possible models and partnerships as well as funding mechanisms that could enhance the rate of investigation and development. (1) Models and partnerships: Participants suggested that TE evaluation centers or networks be created as well as multiple principal investigator programs in TE. They also recommended that leaders in TE seek to form University-Corporate and Federal-Corporate (i.e., public-private) partnerships in TE. The FDA should also be engaged as a partner. The group suggested the formation of a Clinician-Scientist program in TE to encourage physicians to participate early in the development process and a Translational Scientist program for researchers to more fully appreciate the end product of the TE process. Several organizations are examining these possibilities at this time. (2) Funding mechanisms: TE research has usually relied on government, foundation, and private funding and this should continue. However, longer-term, high-risk funding programs from agencies like NIH (e.g., extended R-21 grants) would be most useful during the early TE development phase. Tissue engineers should also pursue corporate and venture capital funding, particularly when developing solutions for specific problems that must be addressed in a targeted research and development program. For TE to be optimally developed, funding must be available at the critical, albeit early stage research activities, and tissue engineers must better appreciate the challenges of delivering the final product to the surgeon.

All are encouraged to seriously consider these programs and funding strategies in the short term if TE is to match the potential that many claimed it would have when the field was just beginning. Only then can truly novel methods be taken from the "benchtop to the bedside."

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