The Rationale for Identifying Clinical Predictors Modifiable by Tissue Engineering for Translational Models

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This article proposes a “bedside-to-bench” approach as a model to improve clinical outcomes for patients through functional tissue engineering (TE). The link between the highest level of clinical research and evaluation criteria for musculoskeletal TE is in identifying clinically proven predictors that are amenable to functional TE. The TE solutions developed in the laboratory should then be tested in translational models to evaluate efficacy and safety prior to controlled clinical trials. The best available evidence for potentially decreasing the incidence of radiographically confirmed osteoarthritis after anterior cruciate ligament injury is preservation of meniscus function. Meniscus tears occur concurrently in ~50% of anterior cruciate ligament tears. TE could promote repair of torn meniscus and/or replacement of meniscus loss because meniscus tear is a proven predictor of clinically relevant outcomes (such as osteoarthritis) in patients and is amenable to a potential TE solution.

Introduction

THIS REPORT DEFINES the relationship between clinical research and evidence-based medicine for physician decision making, tissue engineering (TE), and choice of engineering variables, and it establishes the primary value of preclinical comparative studies to evaluate the biology for efficacy and safety. For details of the highest level of evidence on clinical research of anterior cruciate ligament (ACL) tears, the reader is referred to the work of Spindler.¹ For a comprehensive discussion of evaluation criteria for musculoskeletal TE, see Butler’s conference report.² Finally, our goal is to design TE and translational models to explore proven predictors of clinically relevant outcomes (i.e., “bedside-to-bench”). This is in sharp contrast to the traditional “bench-to-bedside” approach. The proposed bedside-to-bench approach would optimize the probability of TE improving clinical outcomes.

Physician Decision Making

Table 1 outlines the three stages in the evolution of the current ongoing shift in physician decision making. Incorporating evidence-based medicine into clinical practice means evaluating controlled clinical trials for outcomes, complications, risk–benefit ratio, and cost–benefit ratio. The problem with establishing high evidence in prospective studies is the hundreds of thousands of dollars it costs to answer a specific question. Ideally, treatment studies and levels of evidence would reveal systematic reviews or meta-

analyses that are exclusive to level 1 randomized controlled trials or level 2 prospective cohort studies. Ultimately, the goal of a physician is to establish evidence-based treatment guidelines based upon the highest level of evidence, preferably levels 1 and 2 for a given injury type. For example, in anterior shoulder dislocation, the specific reduction anesthesia technique, rehabilitation versus arthroscopy, type of rehabilitation, and arthroscopic versus open repair can all be based on level 1 and level 2 studies. For an ACL tear in sports, whether to reconstruct versus just rehabilitate,^{3,4} the choice of autograft,⁵ whether to use an autograft or allograft, the specific surgical technique (one- versus two-incision),⁶ the type of meniscal repair, and rehabilitation can all be based on level 1 and level 2 studies. In practice, systematic reviews,⁵ meta-analyses,^{7–9} and clinical practice guidelines leveled by evidence should help guide the physician in his or her current practice. The medical research for the clinical practice of ACL tears has recently been published.¹

In summary, the goal of clinical research is to improve patient care. This can be thought of as a three-step process: (1) establishment of a scientific truth through the use of statistics, epidemiology, and appropriate study design; (2) determining whether this truth or difference is clinically meaningful or clinically relevant; and (3) evaluating whether this clinically meaningful difference and scientific truth is worth the cost to society to improve the said outcome. The final step can be exemplified by having two treatments with the same effect size or improvement but with varying different costs, such as the use of physician-provided hyaluronic acid injections versus over-the-counter glucosamine

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TABLE 1. PHYSICIAN DECISION MAKING

Prescientific method (<1900)	• Apprenticeship, eminence based, anecdotal, or experience based
Biologically plausible (modern era)	• <i>In vitro</i> science: biomechanical, cell, test tube
	• <i>In vivo</i> : translational or animal models
Evidence-based medicine (last 20+ years)	• Application of scientific method in clinical studies (epidemiology, statistics, patient-reported outcomes)

Courtesy of Robert S. Dittus, M.D., M.P.H., Assistant Vice Chancellor for Public Health, Vanderbilt University Medical Center, and Associate Dean for Population Health Sciences, Vanderbilt School of Medicine, Nashville, TN.

chondroitin for knee osteoarthritis (OA). The relationship between using the highest level of evidence, TE, and translational animal models is reinforced through modifiable predictors of clinical outcomes that are amenable to TE.

Predictors of Clinical Outcomes

Clinical predictors are variables often statistically evaluated in multivariable models that are shown to significantly influence the outcome or the result desired. Perhaps the most famous cohort study was the Framingham cohort¹⁰ which determined the predictors of cardiovascular disease. The Framingham cohort identified age, hypertension, elevated cholesterol, diabetes, elevated weight, low physical activity, and family history as predictors of both myocardial infarction and mortality. Some of the listed predictors are modifiable and could be altered in randomized trials to lower the incidence of cardiovascular disease. For example, there are now medications to lower hypertension, medications to lower cholesterol, insulin to treat diabetes, and exercise programs to promote healthy physical activity. However, in orthopedics the predictors of outcomes have not been established for many common diseases. Table 2 shows outcomes and predictors (identified in level 1 and 2 clinical studies) using ACL reconstruction as a model for both initiation and development of OA, as well as returning a patient back to peak function. One can think of the outcome as the desired result, and the predictor of each of these outcomes may not be the same. For example, the return to the highest activity level at 2 years, which is what most patients desire, could be the most important predictor of arthritis in the

operated knee at 6–10 years following surgery, the rationale being that the highest activity on a knee with abnormal kinematics increases the physiological loads much more than lower activity levels (i.e., cutting and pivoting) but this must be evaluated in longitudinal cohorts.

TE should be focused on modifiable predictors of clinically relevant outcomes that are amenable to repair or replacement and ideally on adequate translational models to study these predictors. For example, if the goal is to improve clinical outcomes, which is one of the stated missions of functional TE and is extensively discussed in Butler's "white paper" on TE, one needs to take a bedside-to-bench approach. First, we should determine which modifiable predictors from prospective cohorts are amenable to TE. The next step would be to tissue-engineer a solution in the laboratory, to be evaluated in human controlled clinical trials. This bedside-to-bench approach should optimize the probability of success (i.e., improving clinical outcomes). Two specific examples would be promoting repair where it does not occur, such as in the ACL, and replacing lost or severely damaged tissue, such as meniscus—both of these will be discussed later in this article. For example, meniscus tears and injuries occur in ~50% of ACL tears and preservation of meniscus through promoting repair or replacement is amenable to TE. Another example is the high failure rate of allografts in young patients undergoing ACL reconstruction; TE techniques could be designed to enhance the healing capability of this graft. Another example would diminish the need for replacement ACL graft by engineering repair of torn ACL. This TE strategy could promote intraarticular healing and repair, therefore preserving the complex attachments of the native ACL which cannot be

TABLE 2. ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION OUTCOMES AND PREDICTORS, 2009

Measurement	Outcomes	Predictors
Instrumented laxity (KT)	Avg. 1–2 mm	Not autograft (HG vs. BTB), approach (1 vs. 2 incision), or fixation methods
Isokinetic strength	Avg. >90%	Not HG vs. BTB vs. allograft
AROM	~ Normal	Unknown (rehabilitation)
Marx activity level	Decrease by 4 at 2 years	Yes to Marx, sport, ethnicity, etc.
IKDC Composite score	<40% normal	Unknown
Osteoarthritis	~ 0–48% at 6–10 years	Meniscus injury (tear)
Graft failure	1–20%	Allograft: younger and highest Marx
Contralateral ACL tear	Equal to ACLR	Unknown
Infection	0.9%	Not HG versus BTB
Meniscus repair success	87–96%	Not all-in or inside-out

Data from level 1 systematic reviews and meta-analyses.

KT, Knee Ligament Arthrometer®; Avg., average; HG, hamstring graft; BTB, bone-patellar tendon-bone graft; AROM, active range of motion; IKDC, International Knee Documentation Committee; ACL, anterior cruciate ligament; ACLR, ACL reconstruction.

duplicated in reconstructions. Thus, in the ideal world, prospective longitudinal cohorts such as Framingham's could be performed for major orthopedic conditions such as OA following joint injury, ACL tears, and rotator cuff tears. The modifiable predictors of clinically relevant outcomes would be identified, and those amenable to functional TE could be explored in the laboratory and in translational models. The new TE solutions would be tested in future prospective comparative clinical trials. This approach should optimize the chance of improving important patient outcomes.

ACL Reconstruction as a Clinical and Translational Model

The goal of ACL reconstruction is to restore function to the patient's knee so he or she can participate in their desired activities. How one measures function is open to debate. Other outcomes also important to patients include activity level as measured by the Marx Activity Level score, return to play, prevention of OA, and avoidance of infection and graft failure. Surrogate measures such as instrumented laxity and isokinetic strength, believed in the past to relate to function, have been shown to correlate poorly with function. The middle column of Table 2 lists the outcomes believed important to the field and to patients, and the far right column shows the most important predictors of these outcomes. Tissue engineers should identify predictors that are amenable to a TE approach to potentially improve these clinically relevant outcomes. It is obvious that the predictors of these outcomes are not always modifiable. For example, in a large multicenter cohort with multivariable modeling, we could predict the future activity level of patients at 2 years after ACL reconstruction. Overall, their activity declined four points on a 16-point scale at 2 years, and their prior activity, sport, ethnicity, sex, and body mass index were all found to have an association with future activity level.¹¹ These predictors are not modifiable through TE. Failure of the ACL reconstruction graft is a devastating complication to patients, and a longitudinal study with 94% follow-up of 1000 ACLs found that failure was predicted by graft type, usually allograft greater than autograft, and was also very much dependent upon age. For example, an allograft in an 18-year old had a failure rate of over 20% versus an autograft failure rate of 6%, whereas in a 40-year old the allograft failure rate was 3% and autograft failure rate was 1%.¹² In both of these clinically relevant outcomes of activity level and graft failure, there is not an obvious modifiable TE construct except for the ability to potentially enhance healing of an allograft ACL reconstruction.

Meniscus tears, which are common in ACL reconstructions and are treated by either repair or excision, are hypothesized to be a major outcome variable for future OA and potential patient-reported knee outcomes at more than 2-year follow-up.

A recent systematic review of knee OA after ACL injury demonstrated that prevalence of OA was dependent on meniscus injury.¹³ In isolated ACL tears without a meniscus tear, the radiographic prevalence of OA was 0–13% as opposed to 21–48% in ACL tears with a meniscus tear.¹³ Thus, the best available clinical evidence establishes meniscus tears as a potential modifiable predictor amenable to functional TE. A large cohort¹⁴ predicts that the potential U.S. market for meniscus treatment is based upon the type of tear, cur-

TABLE 3. TOTAL POTENTIAL U.S. MARKET^a
MENISCUS TREATMENT WITH ANTERIOR CRUCIATE
LIGAMENT RECONSTRUCTION

<i>Future treatment</i>	<i>Annual U.S. market</i>
Biological repair	29,260
Advance zone repair	46,500
Replacement scaffold	72,700

^aBased on 200,000 ACLR annually.¹³

rent treatment, and 200,000 ACL reconstructions. Table 3 shows the current treatment strategies in the U.S. market. There is need to replace the mechanical repair strategies with an all-biological repair in almost 30,000; there is the ability to advance the zone of repair where these tears were taken out rather than undergo repairs in over 46,000; and finally, the most common potential operation would be to replace the badly damaged meniscus with a scaffold in over 72,000. Thus, there are three potential TE strategies that could be investigated in the laboratory and in translational models. To improve the clinical outcome, one assumes that treatment of a meniscus tear concurrent with ACL tear will improve ACL reconstruction outcomes. Future longitudinal follow-up of several ACL cohorts or randomized clinical trials will determine whether meniscus treatment can improve clinical outcomes such as patient-reported outcomes scores, activity level, and most importantly, OA. An important caveat in determining the role of meniscus in clinically relevant outcomes is that one would need to evaluate the meniscus predictor in a clinically relevant fashion. For example, it would be least helpful to know that meniscus excision is a risk factor but it would be most helpful to know if the medial or lateral meniscus excision was a factor because of the diminished blood supply (popliteal hiatus) in the lateral meniscus and it has twice the anterior–posterior mobility of the medial meniscus. This knowledge could contribute to better and more focused TE approaches.

TE Evaluation Criteria

Butler outlined the Tissue Engineering Evaluation Criteria in the extensive white paper based upon a National Institutes of Health (NIH) conference. Using ACL repair as a model, Table 4 presents the tissue type, preclinical model, assessment

TABLE 4. ANTERIOR CRUCIATE LIGAMENT REPAIR

Tissue type	Central defect, transection, delayed repair
Preclinical model	Goat, sheep, canine, pig
Assessment times	1–3 months biology and histology; 3–12 months biomechanics and joint
Evaluation	AP laxity, ACL construct strength and stiffness, articular cartilage, "joint"
Target values	100% stiffness and 20–30% strength of normal ACL; similar to ACLR on stiffness, strength, joint function, and OA and meniscus

AP, anterior-posterior; OA, osteoarthritis.

TABLE 5. COMPARISON OF HUMAN VERSUS ANIMAL MODEL

<i>Major evaluation</i>	<i>Specific evaluation</i>	<i>Human</i>	<i>Animal</i>
Clinical function	Gait	RTP, Marx	Lameness
	Meniscus	Reoperation tear	Tear
	Articular cartilage	Chondromalacia OA	Chondromalacia OA
Knee "kinematics"	AP laxity	<3 mm negative pivot shift	200–250% normal
	Rotation	Unknown	Unknown
Biomechanics "ACL construct"	Load at yield	Unknown	≤50%
	Stiffness	Unknown	Inferior
	Energy	Unknown	Inferior
	Material properties	Unknown	Inferior = scar

RTP, return to play.

times, evaluation, and target values to outline a strategy for investigating potential ACL repairs in translational models. The reader is referred to this paper for details of each of the individual constructs. More important is to look at the comparison between the human versus the animal model to find common outcome parameters that would give more relevance to any given result in an animal model (Table 5). One can consider evaluating the major functions as clinical, kinematic, and biomechanic (Table 5). In the human model, we can measure gait and activity level, identify reoperation for the meniscus, and evaluate the articular cartilage. In some animal models, we can measure lameness, evaluate a retear of the meniscus, and observe articular cartilage. However, animal models are extremely ACL dependent in contrast to human where normal gait is not an ACL-dependent function. In evaluating knee kinematics clinically, a pivot shift test measures knee rotation in ACL function, and a KT-1000 provides an instrumented measurement of anterior–posterior displacement which is subject to significant interrater variability. In animal models, postmortem laxity measurements reveal significant increases as opposed to normal, which appear much greater in laxity than that seen in the human condition. Neither human nor animal model has an accurate way to quantify rotational changes or even to determine what degree of rotational changes are important. The biomechanical properties of an ACL construct in animal models can be measured postmortem. The direct measurement of the load at yield, stiffness, energy, and material properties of a human graft are not completely understood. Thus, in summary, there really is no single direct measure in an animal model that will directly correlate with a condition in the human model. Therefore, the best expected outcome of a translational animal model is really the comparison of two treatments within one model to evaluate for efficacy and, in particular, the direction of the effect. Additionally, the animal model can document that the treatment applied, either a repair or a replacement, is biologically safe for that joint in that animal.

Summary—Clinical Problem to TE Solution to Clinical Solution

In summary, we should transition TE from the traditional bench-to-bedside approach to a more applicable bedside-to-bench approach. This requires evaluating the modifiable predictors of clinical problems in humans for predictors that are amenable to a potential TE repair strategy. These functional tissue-engineered solutions would be useful in trans-

lational animal models for determining initial efficacy and safety and could then be followed by a human control trial to prove outcome. This multistep process develops from the need to treat a human condition. The first step would be to identify modifiable predictors of important clinical outcomes that are amenable to TE (i.e., such as meniscus tears). Second would be to pilot the TE construct to establish proof of concept and reproducibility in a translational model. Third would be to perform comparative and safety studies in a larger animal experiment over a longer time period (i.e., translational model). The fourth step would be a pilot study in a small group of humans to confirm evidence of effect and safety. Fifth would be an efficacy trial, either a randomized trial or a human cohort. Sixth would be postmarket surveillance for safety in a large human cohort. Thus, there is a complete circle including identifying appropriate high-level predictors in clinical studies, a focused TE approach to the most amenable modifiable predictors that can improve outcome, the clinical study proving that these are efficacious, and then evaluation of its risk–benefit and cost–benefit. Unfortunately, at this point we are in our infancy and limited by lack of proven major predictors of clinically relevant outcomes. Thus, there is a definite need for funding of large prospective cohorts for major orthopedic conditions by the NIH analogous to the Framingham study on cardiovascular disease. The evidence thus far shows that meniscus tears in conjunction with an ACL tear and the resulting surgical excision are predictors of future arthritis after ACL reconstruction and correlate with poor patient-reported outcomes. Thus, a reasonable approach at this point based on the best available evidence would be for TE to focus strategies on both enhancing meniscus repair and promoting replacements or scaffolds.

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