Large Animal Models of Meniscus Repair and Regeneration: A Systematic Review of the State of the Field

Sonia Bansal, BS,^{1-3,**} Niobra M. Keah, MS,^{1,3,**} Alexander L. Neuwirth, MD,^{1,3,**} Olivia O'Reilly, BS,¹ Feini Qu, PhD, VMD,¹⁻³ Breanna N. Seiber, BS,^{1,3} Sai Mandalapu, BA,¹ Robert L. Mauck, PhD,¹⁻³ and Miltiadis H. Zgonis, MD¹

Injury to the meniscus is common, but few viable strategies exist for its repair or regeneration. To address this, animal models have been developed to translate new treatment strategies toward the clinic. However, there is not yet a regulatory document guiding such studies. The purpose of this study was to carry out a systematic review of the literature on meniscus treatment methods and outcomes to define the state of the field. Public databases were queried by using search terms related to animal models and meniscus injury and/or repair over the years 1980–2015. Identified peer-reviewed manuscripts were screened by using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. One of nine reviewers read each manuscript and scored them based on whether the publication described a series of predefined study descriptors and outcome measures. Additional data were extracted to identify common assays used. A total of 128 full-length peer-reviewed manuscripts were identified. The number of publications increased over the time frame analyzed, with 48% focused on augmented repair. Rabbit was, by far, the most prevalent species utilized (46%), with dog (21%) and sheep (20%) being the next most common. Analysis of study descriptors revealed that most studies appropriately documented details of the animal used, the surgical approach, and defect and implant characteristics (e.g., 63% of studies identified clearly the defect size). In terms of outcome parameters, most studies carried out macroscopic (85%), histologic (90%), and healing/integration (83%) analyses of the meniscus. However, many studies did not provide further analysis beyond these fundamental measures, and less than 40% reported on the adjacent cartilage and synovium, as well as joint function. There is intense interest in the field of meniscus repair. However, given the current lack of guidance documentation in this area, preclinical animal models are not performed in a standardized fashion. The development of a “Best Practices” document would increase reproducibility and external validity of experiments, while accelerating advancements in translational research. Advancement is of paramount importance given the high prevalence of meniscal injuries and the paucity of effective repair or regenerative strategies.

Keywords: meniscus, large animal model, systematic review, repair

Introduction

SURGICAL INTERVENTION TO address meniscus injury is one of the most common orthopedic procedures performed worldwide.¹ Historically, surgical interventions involved complete removal (i.e., total meniscectomy), but have ever since evolved toward tissue preservation approaches using arthroscopic sub-total (or partial) meniscectomy. This procedure addresses immediate symptomatic pain associated with mechanical instability of the meniscus, and it seeks to preserve as much native tissue function as possible by limiting

the amount of tissue removed.² These minimal approaches recognize the importance of the meniscus in distributing load, absorbing shock, and stabilizing the knee joint.^{2,3} However, sub-optimal outcomes can still occur when the extent of tissue damage or degeneration requires that a significant volume of the tissue be removed.

Indeed, although partial meniscectomy can address acute symptoms, this procedure is in no way reparative or regenerative. To overcome these limitations, meniscus repair technologies have evolved and improved considerably over the past two decades. These innovations include complex

¹McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

²Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania.

³Translational Musculoskeletal Research Center, Philadelphia Veterans Administration Medical Center, Philadelphia, Pennsylvania.

**These three authors contributed equally to this work.

*This article is part of a special focus issue on Animal Models in Tissue Engineering. Part I.

suturing and fixation devices, some of which can be used arthroscopically. However, the successful application of these “repair” devices is limited by the poor endogenous healing capacity of the native tissue, with repair generally only attempted in regions that are well supplied by the vasculature (the outer 1/3), and it is contraindicated in regions that lack a vascular supply.⁴ Building on early repair efforts, new devices have emerged that provide for “augmented repair,” potentially expanding the indications for such procedures. In augmented repair, re-approximation of torn meniscal surfaces can be accompanied by application of biologic agents (e.g., cells, growth factors, or their combination) to improve on the endogenous healing capacity of the tissue. When repair is not possible (even with augmentation), due to substantial loss and/or degradation of the meniscus tissue, allotransplantation is an option. However, allotransplanted tissues have proved to be less durable than native tissue, likely given the acellular nature of these implants and their application in joints that have already been significantly compromised. To better address this clinical need, tissue engineers have developed materials for complete and/or partial replacement via cell-based tissue-engineered constructs and acellular scaffolds that are intended for restoration of meniscus function.⁵

As new technologies and surgical interventions are developed, they are generally first evaluated in small and then large animal *in vivo* systems.⁶ These model systems have played an important role in advancing our understanding of injury and disease processes⁷ and can be used to optimize candidate therapeutics and to ensure the safety of these interventions before their use in human clinical trials.⁸ To harmonize approaches in this translational process, research of some areas, most notably articular cartilage repair,⁹ have guidance documents that provide investigators with a standard for best practices by which to carry out experimental studies by using these animal models. These documents, informed by lead researchers in the field and published by various regulatory agencies (e.g., Food and Drug Administration [FDA], European Medicines Agency [EMA], American Society for Testing and Materials [ASTM]), include specific suggestions for study design and execution, and make recommendations regarding the most appropriate outcome measures to assess. Though these guidance documents are not binding,¹⁰ they can be useful in establishing “best practices” across preclinical studies, in terms of both study design and outcomes. To date, however, no such document exists to guide the evaluation of new meniscus repair or regeneration strategies.

In the absence of guidance documentation, fields tend to develop based on either historical precedence or individual preferences regarding the most appropriate and important methods and outcomes to assess. Over time and without specific guidelines, methods may diverge, resulting in a lack of coherence in methods and approaches. Likewise, the advent and adoption of new technology makes a comparison between studies difficult and dependent on the timing and degree of penetrance of a new evaluation technology across the field. Given that guidance documents for large animal models of meniscus repair and regeneration are not currently publicly available (though efforts are underway toward that end), our goal here was to identify the primary outcomes reported over the past 35 years in studies performed in this area. We were specifically interested in determining the design and outcome parameters that were the most com-

monly used in large animal models of meniscus repair, and how these have changed as the field has developed. The goal of this systematic review was to identify those features that are the most prevalent and, in doing so, contribute to the ongoing discussion of guidance documentation relevant to the injury, repair, and regeneration of the knee meniscus.

Materials and Methods

Identification of peer-reviewed manuscripts for analysis

Literature was identified and screened by using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.¹¹ PubMed Plus, PubMed Central, Scopus, and Embase were searched on June 1, 2016 for full-length peer-reviewed journal articles on meniscus repair/injury that were published between December 31, 1979 and December 31, 2015 (Fig. 1). The following search criteria were agreed on before executing the search: “meniscus repair” AND; “meniscus injury” AND; “animal model” NOT; “rat” NOT; “mouse.” Mice and rats were excluded from our analysis given their small size. Initial results yielded a total of 629 articles across the databases. Titles and abstracts were then screened to exclude repeats, unavailable articles, and studies in languages other than English. In addition, studies whose focus was not *in vivo* (i.e., *ex vivo* and *in vitro*) meniscus injury/repair were excluded. Based on the application of these inclusion/exclusion criteria, a total of 128 full-length peer-reviewed manuscripts were identified.

Data extraction and analysis

Before reviewing the identified manuscripts, we defined specific categories for analysis related to descriptors of study design and study outcome measures. This was based on categories previously employed to assess the cartilage repair field.⁹ Study descriptors included: species characterization (age, sex, weight), intervention type, defect characterization (size, geometry, relative size), implant geometry and composition, and surgical approach. We also noted meniscus laterality (medial or lateral), and whether the intervention was assessed in a unilateral or bilateral fashion in the animal model. Study outcomes were categorized separately for the meniscus, cartilage, and the joint/synovium. For each, outcomes included: histologic imaging, analysis, and scoring; macroscopic imaging, analysis, and scoring; assessment of integration, healing, and/or inflammation; immunohistochemical (IHC) and biochemical analysis; molecular/gene expression analysis; mechanical testing; magnetic resonance (MR) imaging; and arthroscopic assessment of joint health (noted as “arthroscopy”). In addition, outcome measures specific to cartilage included assessment of subchondral bone and radiographic imaging. Outcome measures specific to joint/synovium included assessment of gait, range of motion, and force plate analysis, as well as radiographic imaging.

Each outcome category was assigned a “yes/no” binary value of 1 or 0, respectively, such that a “1” indicated that the study in question had reported on this category. When scored as a “1,” details on the specific methods of analysis performed were also noted (e.g., type of histological stain utilized). If an outcome was noted that did not fall into the categories mentioned earlier, it was assigned as an “Other Outcome,” and its details were noted. Manuscripts were

FIG. 1. Diagram depicting literature search, exclusion process, and eligibility testing according to PRISMA guidelines for the execution of a systematic review/meta-analysis.¹¹ Based on our review of the literature, 128 articles were identified for consideration in the period spanning 1980–2015. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

randomly assigned to a total of nine independent reviewers, who read each paper in detail and noted responses for each category. When questions arose (i.e., a measure was not clearly defined), the manuscript was reviewed by multiple readers to arrive at a consensus on the appropriate score. Data were tabulated in Microsoft Excel, and graphical representations were developed in MathWorks MATLAB (Natick, MA) and GraphPad Prism (San Diego, CA).

Results

Overall findings

Query of the databases initially identified 297 unique manuscripts for consideration. Review of the titles/abstracts reduced the number to 153, 25 of which were subsequently removed from consideration based on language, availability, or relevance to meniscus injury/repair. The remaining 128 manuscripts were included for analysis by the nine reviewers (the authors of this article). These manuscripts are listed in alphabetical order in the Supplementary Data (Supplementary Data are available online at www.liebertpub.com/tec).

Trends in research activity and species selection

Review of these 128 manuscripts and analysis revealed interesting trends in the field of large animal models of meniscus repair and regeneration. First, the distribution of published articles was not uniform over the time window analyzed. Rather, we noted a general increase in peer-reviewed publications during each year (Fig. 2) except for 2008–2009, where there were fewer papers published. In terms of species, we identified manuscripts reporting on procedures performed in rabbits (lapine), dogs (canine), sheep (ovine), goat (caprine), pig (porcine), and monkey

(simian). Of these, rabbits were, by far, the most common, accounting for 46% of all studies (Fig. 3). The next most prominent species utilized were ovine and canine, with each accounting for ~20% of studies identified. The remaining species were much less commonly employed, with caprine and porcine each at 6–7%. Only 1 out of the 128 studies used a monkey model. With respect to trends in utilization, some species showed a stable positive slope in utilization over time, whereas others were stable or decreased in utilization rate over this time window. For example, the highest slopes (i.e., highest rate of utilization) over the past 5 years were seen for rabbit and sheep models, whereas the rate of publications using canine models appeared to plateau during this same period. This may suggest a growing consensus in the field as to the use of sheep and rabbits for such studies.

Analysis of reported study design and treatment descriptors

In addition to characterization of animal models, our data offer insight into the evolution of the field of meniscus repair with respect to how these studies are being performed and described. Beginning with the oldest article analyzed, which featured an augmented repair followed by observation in New Zealand white rabbits,¹² augmented repair studies have dominated the field in each year (Fig. 4). Indeed, the frequency of studies investigating methods for augmented repair continued to increase over this time frame, accounting for 48% of all studies identified. Other intervention types included complete replacement (15%), repair (13%), partial replacement (12%), and injection/other (7%). From 2010 to 2015, there has been an increase in the number of studies focused on complete or partial replacement and/or injection, likely reflecting emerging technology in these areas. Conversely, those studies categorized as

FIG. 2. Histogram showing the number of meniscus-repair focused articles identified as a function of time. Each bar represents a 2-year increment starting with the listed year, spanning from 1980 to 2015. Color images available online at www.liebertpub.com/tec

“repair” began to plateau in the mid-to-late 1990s, perhaps reflecting the growing consensus that additional measures are needed to promote repair and regeneration.

Further analysis also revealed important details and trends on how meniscus repair studies have been performed over this time window. For example, more than 75% of all studies focused exclusively on the medial meniscus, only 4% of studies used both medial and lateral menisci, and 2% of studies did not report which meniscus was examined (Fig. 5A). Nearly half of all studies (41%) performed procedures in a bilateral fashion, where occasionally the contralateral joint was used as a sham or control condition (Fig. 5B). With respect to descriptors of

the animals and procedures (Fig. 5C), 40% of all studies reported on age, 55% reported on sex, and 68% reported on the weight of study animals. Interestingly, these descriptors were variably reported across species. In terms of meniscal defects, 69% of studies commented on defect size, and 89% dealt with defect geometry, but only 21% described the relative size of the defect (most commonly reported as a percentage of meniscal width). When applicable, the geometry of implants was described in 56% of studies and their composition was detailed in 83% of studies. The surgical approach was described in most papers, with 91% of all studies reporting on this metric.

FIG. 3. Line graph showing the (cumulative) number of studies utilizing specific animal models as a function of time. *Inset* shows aggregated percent utilization by species over this same time frame. Color images available online at www.liebertpub.com/tec

FIG. 4. Line graph describing the (cumulative) number of studies utilizing specific methods of intervention as a function of time. Inset shows percentage of each intervention type over this time frame. Color images available online at www.liebertpub.com/tec

Analysis of reported outcome measures

Although study descriptors were broadly described, study outcomes were much less commonly reported on and more variable in their reporting, especially for intra-articular tissues other than the meniscus (i.e., cartilage and joint/synovium). For the meniscus itself (Fig. 6A), most studies performed histology and provided macroscopic image analysis (>100 out of 128 manuscripts). Likewise, most studies reported on integration/healing. However, beyond these measures, other outcomes were measured and reported on sporadically, with 11 out of 16 possible measures performed in less than a third of the studies analyzed. Notably, biochemical analysis, MR imaging, gene expression, and arthroscopy were all performed in less than 20% of all reviewed studies. Analysis of the median year of reporting for some outcome measures also provides insight suggesting the early elimination of some outcome methods and the late adoption of others. For example, assessment of vascularization had a median year of 1999, revealing a preference for this measure in earlier papers. In contrast, immunohistochemistry had a median year of 2011, revealing later popularity in implementing this outcome measure.

Most papers focused on the meniscus as expected, whereas others have extended this analysis to the surrounding cartilage, the synovium, and the functioning of the joint as a whole. This is in recognition of the reciprocal relationship between meniscus function and cartilage and joint health. For cartilage-related outcomes, 55 manuscripts provided some level of reporting, with macroscopic analysis and cartilage damage and wear assessment being most common (50 and 48 manuscripts, respectively). In general, cartilage-related outcome measures had a later median year (average 2006) than those related to the meniscus, indicating that cartilage assessment has only more recently become a priority in these studies (Fig. 6B). Similarly, joint-level and synovium-related outcomes were reported in 55 papers (with 35 of these coinciding with those that also provided

some level of cartilage assessment). In this domain, macroscopic analysis and joint inflammation were the most commonly reported metrics (39 and 27 manuscripts, respectively). As with cartilage, outcome measures in joint/synovium occurred at a later median year (average 2007) than did the meniscus (Fig. 6C).

Details on commonly reported outcome measures

Beyond simply noting whether a particular outcome was assessed, we also cataloged the type of assay that was performed for the most common outcomes reported. For the meniscus, the most common outcome measures utilized was histologic evaluation and macroscopic assessment (Fig. 6A). Of the studies that performed histologic analysis, 32% used a scoring system (Fig. 7A), though these were often custom scales. Most studies used hematoxylin and eosin staining to assess general tissue features, with considerable variability and less frequent use of other stains for individual matrix components (Fig. 7B). Vascular assessment was performed in nearly 1/3 of all studies, with histologic assessment of vascular networks or microangiography via arterial cannulation and dye injection being the most common methods for assessing this outcome (Fig. 7C). Mechanical testing was carried out in ~1/5 of all studies, with most assays focused on measurement of bulk tensile and compressive properties (Fig. 7D). Immunohistochemistry was performed in ~1/4 of all studies, with staining focused on types I and II collagen, two of the primary constituents of the meniscus (Fig. 7E). Biochemical analysis was performed infrequently (~1/10 of all studies), with assays for glycosaminoglycans and collagens occurring with nearly the same frequency (Fig. 7F).

Discussion

The past 35 years have witnessed a rapid development of translational research focused on meniscus repair and regeneration. This increased research activity is largely, in

FIG. 5. (A) Meniscus laterality (medial or lateral) across all studies. (B) Limb laterality (unilateral or bilateral) across all studies. (C) Histograms showing the percentage of studies reporting on specific study descriptors as a function of the animal model in which the study was performed. Darker hues indicate positive reporting on these descriptors for each category. Dashed line indicates the average reporting percentage across all species. Color images available online at www.liebertpub.com/tec

FIG. 6. Heat maps (*left*) depicting inclusion of specific outcome measures in each paper analyzed. Measures are sorted by frequency of inclusion, with the accompanying plot (*right*) indicating the total number of papers performing each analysis. Individual publications are ordered chronologically along the x-axis. Darker bars indicate inclusion of a given analysis, whereas lighter bars indicate absence of that analysis for a given study. Contrasting bars (red) show the median year of reporting for each outcome measure. (A) Meniscus-related outcome measures. (B) Cartilage-related outcome measures. (C) Joint/synovium-related outcome measures. Color images available online at www.liebertpub.com/tec

FIG. 7. Detailed analysis of outcome assays. (A) Plot of histology and macroscopic outcome measures, where the *darkest hue* indicates assessment, inclusion of images, and scoring; the *middle hue* indicates assessment and sample images; and the *lightest hue* indicates only assessment. (B–F) Plots detailing specific methods used for histological staining, vascular assessment, mechanical testing, immunohistochemistry, and biochemical analysis. The number of manuscripts including each outcome is reflected by the maximum y-axis value for each sub-panel, with the number of manuscripts employing a specific assay reflected in the categorical grouping. Note that many studies included more than one type of assessment within a category (i.e., two types of staining). Color images available online at www.liebertpub.com/tec

part, due to the common occurrence of injuries to the meniscus as well as the significant limitations in its treatment in current clinical practice. Despite this activity, the spectrum of *in vivo* models used, the wide range of methods employed, and the disparate outcomes assessed the limit of reasonable comparison between studies. In contrast, the field of cartilage repair has benefited from guidance documents published by regulatory agencies that seek to standardize study design and outcome measure reporting.¹⁰ Although these guidance documents are not always followed, that field has generally increased in the uniformity of study performance and reporting standards over time.⁹ In the field of meniscus translation, no such guidance documents exist and, consequently, there has been little uniformity in study execution and reporting. To address this lack of a meniscus guidance document and using the cartilage guidance documents as a framework, this systematic review assessed the current state of the field in large animal studies focused on meniscus repair and regeneration. Our goal was to identify trends in study design and outcome reporting in the published literature that could inform the development of guidance documents and be widely adopted by the field.

Growth of the field and trends in animal models and interventions

As clinical practice has evolved to recognize the fundamental role of the meniscus in maintaining long-term joint

health, research efforts and translational models have increasingly focused on this area. Since the early 1970s, when arthroscopic partial meniscectomy was first being routinely performed,¹³ considerable innovation has occurred. This includes the introduction of outside-in and inside-out suturing methods (early 1980s^{14,15}), meniscus allograft transplantation (late 1980s¹⁶), and all-inside repair devices (early 1990s¹⁷). These advances in clinical practice have been paralleled by increased utilization of animal models for meniscus repair. Our analysis shows a steady increase in publications over that time. Preclinical animal models are important for the generation of safety and efficacy data supporting these new devices as they progress through to regulatory clearance for human application.⁶

Beyond finding increasing activity, our analysis also showed interesting trends with respect to the species utilized. For instance, from the beginning of our analysis (1980), rabbit models were heavily utilized and have become even more prevalent through to the present day (Fig. 3). This likely reflects the ease of use of this species and utility in evaluating new approaches in a cost-effective manner. For larger, more expensive species, our data indicate that although dogs were the model system of choice in early studies, the field is shifting toward sheep models. For instance, meniscus repair studies using sheep showed a twofold increase in usage patterns over the past 5 years, whereas the rate of utilization of dogs decreased during that same period. These data suggest that rabbits are an entry

point for evaluation of new technologies, potentially due to their lower cost and smaller size. However, canine and ovine models are the most used of the larger animals, perhaps due to their structural similarity to humans and similar length scales of intervention. Canine models also have the added benefit of ability to control postoperative rehabilitation and validated clinical assays of function.⁶ Each species has its own strengths and weaknesses, and selection is based on the stage of translation and scientific question being asked. Ultimately, however, these technologies will need to be assessed in model systems that most closely approximate the human length scale before clinical translation.

Another interesting feature that emerged from this analysis was the type of intervention being studied as a function of time. From the very earliest stages, augmented repair has been the most prevalent of interventions assayed in animal models. Moreover, these studies have seen the largest increase, doubling in number over the past 10 years. This focus is consistent with the growing interest in the preservation of native meniscus structure to the greatest extent possible, as well as advances in delivery systems for cells, growth factors, and other agents to meniscus defects that might aid in repair.¹⁸ Examples of this include scaffolds that deliver native tissue cells or mesenchymal stem cells,^{19–25} scaffolds that deliver factors to recruit new vascular supply or promote matrix formation,^{26–28} scaffolds that deliver degradative enzymes to improve local tissue integration,²⁹ and acellular scaffolds.

Also of interest is the recent emergence of technologies to provide for partial replacement of the meniscus. These have increased to a greater extent than have complete replacement approaches. This may reflect again a shift away from full removal and toward preservation, as well as trends in industry, where two partial replacements that retain the meniscus periphery have made it to market.^{30–32} This partial replacement focus also reflects improvements in material fabrication, including 3D printed and/or woven meniscal substitutes that are meant to replace all or a part of native tissue after injury.^{33–37} As these technologies continue to develop, it will be interesting to determine whether and when the animal models start to reflect these new technologies.

Consensus on study design and details on study execution

In addition to capturing emerging trends in terms of the focus of the field, this data set also provides valuable information on how such studies are being conducted. For instance, across all species, the majority of studies focused on medial menisci. This may reflect precedence or might be secondary to the relative ease of the surgical approach relative to the lateral meniscus.^{38,39} Another interesting observation was that >40% of studies are designed in a bilateral fashion. In one sense, this makes economic sense and reduces the number of animals required for a given study. However, some studies report a shift in baseline properties of cartilage in contralateral shams after a meniscus procedure.⁴⁰ Additional studies on the time course of changes in contralateral limbs would be of value to the field, and an official recommendation (via clarification of best practices in a guidance document) would be helpful in future study design.

Several additional study descriptors, beyond meniscus and limb laterality, were captured in this systematic review.

Our data indicated that most studies described animal weight, though those that did often failed to denote animal age. This may be due to well-defined correlations between weight and age for some species. Indeed, 78 out of 108 studies describing weight and/or age of animals only reported one out of those two measures. In terms of sex, most studies reported this important category (>60% of cases) though porcine models tended to underreport. This is a particularly important finding given recent NIH initiatives to clarify sex as a biologic variable in animal studies.⁴¹ This inconsistency in annotation of a potential biologic variable represents another area for improvement via guidance documentation. In addition, the recent adoption of the Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines⁴² by many journals may improve reporting of these key parameters.

The metrics noted in the previous paragraph are ones that are applicable to all such animal studies. Those specific to meniscal defects included the region of meniscus in which the defect was made (reported by 96%), the defect geometry (reported by 89%), and the surgical approach (reported by 91%). These specific methods were all consistently described. One poorly reported metric in this area was the relative size of the defects (reported by only 21% of studies). Though defect size (reported by 69%) is important, the relative defect size should also be considered given the size variation in animal model sample populations. In studies including some form of implant (101 of 128 total manuscripts), implant composition was well documented (83%). However, the geometry of the implant was documented in only 56% of studies. This may be due to the presupposition that the implant will completely fill the defect; efforts should be made to clarify this issue.

Overall, in terms of study descriptors, our analysis shows that the field, as a whole, performs well in providing sufficient detail to allow other researchers to replicate approaches and methods of intervention. This is consistent with our recent analysis of the field of cartilage repair.⁹

Reporting on common (primary) outcome measures

Study descriptors were generally well addressed, whereas study outcome measures were much more variable in their reporting. We focus first on those measures that were present in >50% of the reviewed manuscripts, those deemed “primary” by the field by virtue of their utilization. For the meniscus itself, these primary outcomes included microscopic (histologic) and macroscopic evaluation of meniscus tissue, images of histology and macroscopic images of the tissue, and assessment of healing/integration. Given their high prevalence, it is clear that the research community views these meniscus-focused outcomes as essential. Interestingly, none of the outcome measures related to cartilage or joint/synovium were implemented by >50% of studies, despite the importance of the meniscus to both cartilage health and joint stability.

Another point we noted was that although macroscopic and microscopic evaluation are commonly performed, standardized scoring criteria are used only in a minority of studies (22% for macroscopic scoring and 32% for histologic scoring). The lack of a standardized scoring system poses the problem of reproducibility, generalizability, and interpretation. It is interesting to note that scoring for both

histology and macroscopic assessment became more prevalent later in the review period, suggesting that perhaps scoring systems that are now under development will emerge as the field standard. For consensus and regularization, it is essential that the meniscus community develops and adopts a scoring system comparable to the International Cartilage Repair Society (ICRS-II) score used for assessment of cartilage repair⁴³ for evaluation of this important outcome. In this same vein, although histology was commonly performed, there did not appear to be any consensus with respect to the staining protocols employed. Most studies used hematoxylin and eosin to identify general tissue structure and cellularity. This basic stain was often coupled with additional staining for proteoglycan and/or collagen, including Safranin-O, Toluidine Blue, and Mason's Trichrome. These latter, more specific stains were used indiscriminately, with little consensus between studies (Fig. 7B). Many papers (101 out of 115 performing histology) used only one or two histological stains, one of which was hematoxylin and eosin. Considerable improvement in inter-study comparability could be achieved if protocols for primary outcome measures were defined in a guidance document, and validated scoring systems were developed, comparable to the ICRS-II scoring system for articular cartilage repair.

Reporting on less common (secondary) outcome measures

Beyond these primary measures, there were additional outcomes that appeared somewhat frequently, but they were not universally applied. We defined those outcomes that were reported in 20–50% of studies as “secondary outcome” measures. These outcomes included assessment of meniscal vascularization, meniscus inflammation, cartilage histology, cartilage macroscopic analysis, cartilage damage/wear assessment, joint/synovium inflammation, and joint/synovium macroscopic analysis. These secondary outcome measures represent deeper investigation into the specific mechanisms of meniscal repair and/or a consideration of other joint structures that are either impacted by meniscus repair (cartilage/joint) or may reflect overall joint health (joint/synovium). As the goal of many meniscal treatments is to prevent degenerative changes in the knee (and of the cartilage surface), measures of cartilage damage, wear, and histology are vitally important. Likewise, measures of meniscus tissue and joint-level inflammation would similarly inform one as to whether a healing process was successful, or potentially identify why healing failed to occur. Indeed, improved reporting on these outcomes would accelerate our collective understanding of how meniscal repair could be improved.

Other secondary measures that arose from this analysis included mechanical testing and immunohistochemistry of the meniscus. Given that the mechanical properties of the meniscus are important for the restoration of normal joint loading to prevent downstream cartilage degeneration, this outcome should warrant more consistent assessment. Most studies that evaluated mechanics did so in tension^{44,45} and compression,^{46,47} with only a few considering the viscoelastic properties of the repaired tissue.^{33,48} Consensus on the minimal mechanical attributes to report, and the appropriate testing configurations to measure these properties, should be defined by the field. In selecting mechanical tests

to perform, consideration might be given to nondestructive tests, wherever possible, to enable sequential mechanical testing and histological analysis on the same sample to reduce animal numbers. In addition, assessment of whole joint, *in vivo* contact mechanics is essential for determining the joint-level function of an implant or repair, though this outcome is only reported in a few studies.^{49,50} Similarly, we found that IHC staining was occasionally carried out, with antibodies most often targeted to collagen types I and II to quantify content of the extracellular matrix (ECM) of the regenerative tissue. Although these are two of the most abundant extracellular constituents of the meniscus, the field should define the suite of ECM molecules that are the most important to characterizing tissue composition.

In addition to what has been mentioned earlier, there were other outcomes that were performed in fewer than 20% of studies. These included all other measures (Fig. 6), and they span the majority of potential cartilage and joint/synovium outcomes, including meniscal biochemistry, MR imaging, gene expression, and arthroscopy. Reasoning behind the lack of regular usage of these outcome measures, though unclear, may be related to resource availability (i.e., equipment and/or cost), poor interest, and/or lack of training. Field consensus on the importance of particular assays could help standardize practice and improve the reliability of potentially significant underutilized outcome metrics.

Conclusions

This systematic review highlights the importance of defining study design methodology as well as standardizing primary and secondary outcome measures to advance the field of meniscus translation. Each study design will, of course, ultimately be defined to test the specific outcomes in question, and the standardization of outcomes should not stand in the way of innovation. That said, in carrying out this analysis, we identified the study design elements and outcome measures that have implicitly become the “standard of care” in the absence of a formal guidance document. Outcomes to assess histological and macroscopic features and integration are widely accepted and performed, though these could be further codified by meniscus-specific objective scoring systems. Other outcomes, such as mechanical testing, are underrepresented in the published literature, but these data are vital for objective functional evaluation and the development of predictive models of successful interventions.⁵¹ Finally, annotation and evaluation of other joint structures (e.g., cartilage wear and synovial inflammation) and joint-level function (including gait and measures of pain) will be essential for showing efficacy in any new technology. Given that efforts are now underway to develop a meniscus guidance document, this systematic review should provide insight into the general trends that have defined the field to date, as well as identify areas for potential improvement. Publication of guidance documents for cartilage repair have standardized reporting in that field, and would greatly benefit the field of meniscus repair and regeneration as well.

Acknowledgments

This work was supported with funding from the New Investigator Grant from the Orthopedic Research and Education Foundation, the Department of Veterans Affairs (I01

RX000174), and the National Institutes of Health (R01 AR056624 and P30 AR050950).

Disclosure Statement

No competing financial interests exist.

References

- Lubowitz, J.H., and Poehling, G.G. Save the meniscus. *Arthroscopy* **27**, 301, 2011.
- Moulton, S.G., Bhatia, S., Civitarese, D.M., Frank, R.M., Dean, C.S., and LaPrade, R.F. Surgical techniques and outcomes of repairing meniscal radial tears: a systematic review. *Arthroscopy* **32**, 1919, 2016.
- Bedi, A., Kelly, N.H., Baad, M., Fox, A.J., Brophy, R.H., Warren, R.F., and Maher, S.A. Dynamic contact mechanics of the medial meniscus as a function of radial tear, repair, and partial meniscectomy. *J Bone Joint Surg Am* **92**, 1398, 2010.
- Arnoczky, S.P., and Warren, R.F. Microvasculature of the human meniscus. *Am J Sports Med* **10**, 90, 1982.
- Pereira, H., Frias, A.M., Oliveira, J.M., Espregueira-Mendes, J., and Reis, R.L. Tissue engineering and regenerative medicine strategies in meniscus lesions. *Arthroscopy* **27**, 1706, 2011.
- Arnoczky, S.P., Cook, J.L., Carter, T., and Turner, A.S. Translational models for studying meniscal repair and replacement: what they can and cannot tell us. *Tissue Eng Part B Rev* **16**, 31, 2010.
- Pfeifer, C.G., Kinsella, S.D., Milby, A.H., Fisher, M.B., Belkin, N.S., Mauck, R.L., and Carey, J.L. Development of a large animal model of osteochondritis dissecans of the knee: a pilot study. *Orthop J Sports Med* **3**, 2325967115 570019, 2015.
- Cook, J.L., Hung, C.T., Kuroki, K., Stoker, A.M., Cook, C.R., Pfeiffer, F.M., Sherman, S.L., and Stannard, J.P. Animal models of cartilage repair. *Bone Joint Res* **3**, 89, 2014.
- Pfeifer, C.G., Fisher, M.B., Carey, J.L., and Mauck, R.L. Impact of guidance documents on translational large animal studies of cartilage repair. *Sci Transl Med* **7**, 310re9, 2015.
- FDA. Guidance for Industry: Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage. Center for Biologics Evaluation and Research, 2011. www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm (accessed July 19, 2017).
- Moher, D., Liberati, A., Tetzlaff, J., and Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* **6**, e1000097, 2009.
- Heatley, F.W. The meniscus—can it be repaired? An experimental investigation in rabbits. *J Bone Joint Surg Br* **62**, 397, 1980.
- Ikeuchi, H. Meniscus surgery using the Watanabe arthroscope. *Orthop Clin North Am* **10**, 629, 1979.
- Clancy, W.G., and Graf, B.K. Arthroscopic meniscal repair. *Orthopedics* **6**, 1125, 1983.
- Warren, R.F. Arthroscopic meniscus repair. *Arthroscopy* **1**, 170, 1985.
- Milachowski, K.A., Weismeier, K., and Wirth, C.J. Homologous meniscus transplantation. Experimental and clinical results. *Int Orthop* **13**, 1, 1989.
- Albrecht-Olsen, P., Kristensen, G., and Tormala, P. Meniscus bucket-handle fixation with an absorbable Biofix tack: development of a new technique. *Knee Surg Sports Traumatol Arthrosc* **1**, 104, 1993.
- Mauck, R.L., and Burdick, J.A. From repair to regeneration: biomaterials to reprogram the meniscus wound microenvironment. *Ann Biomed Eng* **43**, 529, 2015.
- Zellner, J., Mueller, M., Berner, A., Dienstknecht, T., Kuja, R., Nerlich, M., Hennemann, B., Koller, M., Prantl, L., Angele, M., and Angele, P. Role of mesenchymal stem cells in tissue engineering of meniscus. *J Biomed Mater Res A* **94**, 1150, 2010.
- Pabbruwe, M.B., Kafienah, W., Tarlton, J.F., Mistry, S., Fox, D.J., and Hollander, A.P. Repair of meniscal cartilage white zone tears using a stem cell/collagen-scaffold implant. *Biomaterials* **31**, 2583, 2010.
- Desando, G., Cavallo, C., Sartoni, F., Martini, L., Parrilli, A., Veronesi, F., Fini, M., Giardino, R., Facchini, A., and Grigolo, B. Intra-articular delivery of adipose derived stromal cells attenuates osteoarthritis progression in an experimental rabbit model. *Arthritis Res Ther* **15**, R22, 2013.
- Kwak, H.S., Nam, J., Lee, J.H., Kim, H.J., and Yoo, J.J. Meniscal repair in vivo using human chondrocyte-seeded PLGA mesh scaffold pretreated with platelet-rich plasma. *J Tissue Eng Regen Med* **11**, 471, 2017.
- Qi, Y., Yang, Z., Ding, Q., Zhao, T., Huang, Z., and Feng, G. Targeted transplantation of iron oxide-labeled, adipose-derived mesenchymal stem cells in promoting meniscus regeneration following a rabbit massive meniscal defect. *Exp Ther Med* **11**, 458, 2016.
- Yu, H., Adesida, A.B., and Jomha, N.M. Meniscus repair using mesenchymal stem cells—a comprehensive review. *Stem Cell Res Ther* **6**, 86, 2015.
- Fisher, M.B., Henning, E.A., Soegaard, N., Bostrom, M., Esterhai, J.L., and Mauck, R.L. Engineering meniscus structure and function via multi-layered mesenchymal stem cell-seeded nanofibrous scaffolds. *J Biomech* **48**, 1412, 2015.
- Kopf, S., Birkenfeld, F., Becker, R., Petersen, W., Starke, C., Wruck, C.J., Tohidnezhad, M., Varoga, D., and Pufe, T. Local treatment of meniscal lesions with vascular endothelial growth factor. *J Bone Joint Surg Am* **92**, 2682, 2010.
- Steinert, A.F., Palmer, G.D., Capito, R., Hofstaetter, J.G., Pilapil, C., Ghivizzani, S.C., Spector, M., and Evans, C.H. Genetically enhanced engineering of meniscus tissue using ex vivo delivery of transforming growth factor-beta 1 complementary deoxyribonucleic acid. *Tissue Eng* **13**, 2227, 2007.
- Ishida, K., Kuroda, R., Miwa, M., Tabata, Y., Hokugo, A., Kawamoto, T., Sasaki, K., Doita, M., and Kurosaka, M. The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. *Tissue Eng* **13**, 1103, 2007.
- Qu, F., Lin, J.M., Esterhai, J.L., Fisher, M.B., and Mauck, R.L. Biomaterial-mediated delivery of degradative enzymes to improve meniscus integration and repair. *Acta Biomater* **9**, 6393, 2013.
- Rodkey, W.G., DeHaven, K.E., Montgomery, W.H., 3rd, Baker, C.L., Jr., Beck, C.L., Jr., Hormel, S.E., Steadman, J.R., Cole, B.J., and Briggs, K.K. Comparison of the collagen meniscus implant with partial meniscectomy. A prospective randomized trial. *J Bone Joint Surg Am* **90**, 1413, 2008.
- Bulgheroni, E., Grassi, A., Campagnolo, M., Bulgheroni, P., Mudhigere, A., and Gobbi, A. Comparative study of collagen versus synthetic-based meniscal scaffolds in treating meniscal deficiency in young active population. *Cartilage* **7**, 29, 2016.
- Verdonk, P., Beaufils, P., Bellemans, J., Djian, P., Heinrichs, E.L., Huyse, W., Laprell, H., Siebold, R., and Verdonk, R. Successful treatment of painful irreparable partial meniscal

- defects with a polyurethane scaffold: two-year safety and clinical outcomes. *Am J Sports Med* **40**, 844, 2012.
33. Lee, C.H., Rodeo, S.A., Fortier, L.A., Lu, C., Eriskien, C., and Mao, J.J. Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep. *Sci Transl Med* **6**, 266ra171, 2014.
 34. Patel, J.M., Merriam, A.R., Culp, B.M., Gatt, C.J., Jr., and Dunn, M.G. One-year outcomes of total meniscus reconstruction using a novel fiber-reinforced scaffold in an ovine model. *Am J Sports Med* **44**, 898, 2016.
 35. Puetzer, J.L., and Bonassar, L.J. High density type I collagen gels for tissue engineering of whole menisci. *Acta Biomater* **9**, 7787, 2013.
 36. Ballyns, J.J., and Bonassar, L.J. Dynamic compressive loading of image-guided tissue engineered meniscal constructs. *J Biomech* **44**, 509, 2011.
 37. Ballyns, J.J., Gleghorn, J.P., Niebrzydowski, V., Rawlinson, J.J., Potter, H.G., Maher, S.A., Wright, T.M., and Bonassar, L.J. Image-guided tissue engineering of anatomically shaped implants via MRI and micro-CT using injection molding. *Tissue Eng Part A* **14**, 1195, 2008.
 38. Janusz, M.J., Bendele, A.M., Brown, K.K., Taiwo, Y.O., Hsieh, L., and Heitmeyer, S.A. Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. *Osteoarthritis Cartilage* **10**, 785, 2002.
 39. Orth, P., and Madry, H. A low morbidity surgical approach to the sheep femoral trochlea. *BMC Musculoskelet Disord* **14**, 5, 2013.
 40. Cook, J.L., Fox, D.B., Malaviya, P., Tomlinson, J.L., Kuroki, K., Cook, C.R., and Kladakis, S. Long-term outcome for large meniscal defects treated with small intestinal submucosa in a dog model. *Am J Sports Med* **34**, 32, 2006.
 41. NIH. Consideration of Sex as a Biological Variable in NIH-funded Research. NIH, 2015. <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html> (accessed July 19, 2017).
 42. NC3Rs Reporting Guidelines Working Group. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *J Physiol* **588**, 2519, 2010.
 43. Mainil-Varlet, P., Van Damme, B., Nesic, D., Knutsen, G., Kandel, R., and Roberts, S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. *Am J Sports Med* **38**, 880, 2010.
 44. Cook, J.L., and Fox, D.B. A novel bioabsorbable conduit augments healing of avascular meniscal tears in a dog model. *Am J Sports Med* **35**, 1877, 2007.
 45. Abdel-Hamid, M., Hussein, M.R., Ahmad, A.F., and Elgezawi, E.M. Enhancement of the repair of meniscal wounds in the red-white zone (middle third) by the injection of bone marrow cells in canine animal model. *Int J Exp Pathol* **86**, 117, 2005.
 46. Esposito, A.R., Moda, M., Cattani, S.M., de Santana, G.M., Barbieri, J.A., Munhoz, M.M., Cardoso, T.P., Barbo, M.L., Russo, T., D'Amora, U., Gloria, A., Ambrosio, L., and Duek, E.A. PLDLA/PCL-T scaffold for meniscus tissue engineering. *Biores Open Access* **2**, 138, 2013.
 47. Kobayashi, M., Chang, Y.S., and Oka, M. A two year in vivo study of polyvinyl alcohol-hydrogel (PVA-H) artificial meniscus. *Biomaterials* **26**, 3243, 2005.
 48. Welch, J.A., Montgomery, R.D., Lenz, S.D., Plouhar, P., and Shelton, W.R. Evaluation of small-intestinal submucosa implants for repair of meniscal defects in dogs. *Am J Vet Res* **63**, 427, 2002.
 49. Brophy, R.H., Cottrell, J., Rodeo, S.A., Wright, T.M., Warren, R.F., and Maher, S.A. Implantation of a synthetic meniscal scaffold improves joint contact mechanics in a partial meniscectomy cadaver model. *J Biomed Mater Res A* **92**, 1154, 2010.
 50. von Lewinski, G., Stukenborg-Colsman, C., Ostermeier, S., and Hurschler, C. Experimental measurement of tibiofemoral contact area in a meniscectomized ovine model using a resistive pressure measuring sensor. *Ann Biomed Eng* **34**, 1607, 2006.
 51. Leatherman, E.R., Guo, H., Gilbert, S.L., Hutchinson, I.D., Maher, S.A., and Santner, T.J. Using a statistically calibrated biphasic finite element model of the human knee joint to identify robust designs for a meniscal substitute. *J Biomech Eng* **136**, 0710071, 2014.

Address correspondence to:

Miltiadis H. Zgonis, MD

McKay Orthopaedic Research Laboratory

Department of Orthopaedic Surgery

Perelman School of Medicine

University of Pennsylvania

110 Stemmler Hall

36th Street and Hamilton Walk

Philadelphia, PA 19104

E-mail: miltiadis.zgonis@uphs.upenn.edu

Received: February 19, 2017

Accepted: May 9, 2017

Online Publication Date: August 4, 2017