ligament injuries account for up to 50% of sporting injuries, with the majority being to capsular and extracapsular ligaments (such as the knee and ankle collateral ligaments). Injuries to these ligaments have traditionally been thought to heal in a straightforward manner; however, preclinical studies have shown that ligament healing occurs by the formation of a reparative scar, rather than via regeneration, which leaves a deficiency in mechanical properties at the completion of healing. This persistent tissue weakness, combined with any residual neuromuscular deficiency, may explain why a history of ligament injury is a strong risk factor for subsequent injury. Some patients (up to a third) also continue to experience significant symptoms even up to 3 years following capsular or extracapsular ligament injury, and injuries to these ligaments can contribute to the development of osteoarthritis.

To address the short- and long-term consequences of capsular and extracapsular ligament injuries, there is a need for simple interventions that facilitate early recovery (accelerate healing) and/or result in a better final outcome (augment healing). By accelerating tissue-level healing, the injured tissue may be less susceptible to reinjury during early rehabilitation and the individual may be able to return to function quicker. By augmenting tissue-level healing, the final product of the healing process may be enhanced such that the healed tissue more closely approximates that of the native tissue.

Cross-fiber massage (CFM) may be a method for accelerating and/or augmenting capsular and extracapsular ligament healing. CFM refers to the application of specifically directed forces transverse to the direction of the underlying collagen substructure in order to induce physiological and/or structural tissue changes.
It differs from other massage techniques in that there is little motion between the therapist’s contact and the patient’s skin. Instead, CFM involves moving the skin and subcutaneous tissues over deeper connective tissues to exert controlled mechanical forces on the latter. As the reparative cells (fibroblasts) responsible for producing collagen and forming a scar following ligament injury are mechanosensitive, it is theorized that CFM facilitates matrix production and the restoration of tissue-level mechanical properties.

An addition to the practice of CFM has been the use of rigid instruments, with the resultant technique referred to as instrument-assisted CFM (IACFM). IACFM appears to be effective in promoting tissue remodeling, with Davidson et al. and Gehlsen et al. having found increased fibroblast recruitment and activation in an animal model of Achilles tendon injury. Results of clinical pilot studies also suggest that IACFM reduces symptoms in individuals with carpal tunnel syndrome, patellar tendinopathy, and chronic ankle pain.

Based on the hypothesized mechanical mode of action of IACFM and preliminary evidence demonstrating its potential efficacy, the aim of this study was to examine the short- and long-term effects of IACFM on tissue-level healing of knee medial collateral ligament (MCL) injuries in an established animal model. The primary variable of interest was ligament mechanical properties, as the ultimate outcome of any healing process in a load-bearing tissue (such as a ligament) is the restoration of mechanical properties. The secondary variable of interest was ligament morphology, as this may explain differences in tissue mechanical properties.

**METHODS**

**Animals**

Fifty-eight 6-month-old, virgin, female Sprague-Dawley rats (body mass, 280-300 g) were purchased from Harlan Sprague-Dawley, Inc (Indianapolis, IN) and acclimated for a minimum of 7 days prior to experimentation. Animals had *ad libitum* access to standard rat chow and water at all times, and were housed 2 per standard size cage (length, 40 cm; width, 20 cm; height, 20 cm). All procedures were approved a priori by The Institutional Animal Care and Use Committee of Indiana University.

**Ligament Injury**

Fifty-one animals underwent surgery on entry to the study to create bilateral knee MCL injuries of their hindlimbs (injured animals). The remaining 7 animals served as age-matched, ligament-intact cage controls and were not operated on (control animals). Following a preoperative subcutaneous dose (0.05 mg/kg) of buprenorphine hydrochloride analgesia (Buprenex; Reckitt & Colman Pharmaceuticals Ltd, Richmond, VA), surgical anesthesia was achieved using a mixture of ketamine (60-80 mg/kg) (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (7.5 mg/kg) (Sedazine; Fort Dodge Animal Health), introduced intraperitoneally. Using a sterile technique, a 5-mm longitudinal incision was made over 1 knee’s medial joint line, and the MCL sharply transected at the joint line using a size-11 scalpel blade. This resulted in complete disruption of the MCL at its midsubstance and transverse to the underlying collagen fiber alignment. No ligament material was removed, and the ligament ends were juxtaposed but not sutured prior to closing of the skin incision with a single subcuticular absorbable suture. The procedure was repeated on the contralateral knee to create bilateral injuries. All animals demonstrated normal, symmetrical hindlimb use upon recovery from surgery and were allowed normal cage activity (without access to exercise wheels) for the duration of the study.

**Intervention**

IACFM was performed using a rigid tool fabricated from stainless steel (GT6; Graston Technique, TherapyCare Resources, Indianapolis, IN). The GT6 instrument was used because it is designed to apply force through its tip to small structures, such as finger collateral ligaments in humans (in the present study, rat knee-size ligaments) (FIGURE 1). IACFM was initiated 1 week postoperatively (postinjury) to allow the initial inflammatory response/phase of ligament healing to subside. This initial delay in the introduction of IACFM is consistent with its suggested clinical use following an acute injury. IACFM was administered with the animals under isoflurane anesthesia (3% at 1.5 L/min for initial knockdown in a plastic container, and 1.5% at 1.5 L/min via a face mask for maintenance of anesthesia). Approximately 250 to 300 g of instrument downward force was applied during treatment. This

**FIGURE 1.** Instrument-assisted cross-fiber massage (IACFM) intervention. (A) The rigid Graston Technique GT6 tool fabricated from stainless steel has a tapered tip, which permits treatment of small structures. IACFM of a (B) human finger, and (C) similar-size rodent knee joint medial collateral ligament using the GT6 tool. Arrows indicate the direction of movement/force application perpendicular to the collagen substructure of the ligament.
force is equivalent to that previously used to demonstrate benefits of IACFM on injured rat Achilles tendons, and was determined by using the massage instrument on a force plate, with kinesthetically similar pressure to that which would be used clinically to treat a ligament of comparable size at an equivalent tissue depth (e.g., collateral ligament of a human interphalangeal joint). Thirty-one injured animals were treated 3 times per week for 3 weeks (total treatments, 9), while the other 20 injured animals were treated 3 times per week for 10 weeks (total treatments, 30). The number of treatments in the latter animals is more than would typically be introduced in a clinical setting; however, these were implemented to maximize the potential of finding any long-term benefit of IACFM. IACFM was applied to the left MCL in injured animals for 1 minute per session (IACFM-treated). This treatment duration was based on the recommended clinical use of IACFM for the treatment of isolated tissue lesions and evidence from previous preclinical studies demonstrating the efficacy of short-duration IACFM interventions. The contralateral injured MCL in these animals served as an internal control and did not receive IACFM (nontreated). The 7 control animals were not treated with IACFM.

Assessment Time Points and Specimen Preparation

Animals were euthanized postinjury at either 4 weeks (all animals treated for 9 sessions [n = 31] and 2 control animals) or 12 weeks (all animals treated for 30 sessions [n = 20] and 5 control animals). Animals euthanized at 4 weeks had both hindlimbs harvested and prepared for mechanical testing (injured animals, n = 18), scanning electron microscopy (injured, n = 11; control animals, n = 2), or histological assessment (injured animals, n = 2). Animals euthanized at 12 weeks had both hindlimbs removed and prepared for mechanical testing (injured, n = 17; control animals, n = 4) or histological assessment (injured, n = 3; control animals, n = 1).

Mechanical Testing

Ligament mechanical properties were assessed as previously described. Hindlimbs destined for mechanical testing were initially stored at −80°C, with the knee tissues intact. Postmortem storage of ligaments by freezing does not influence their mechanical properties. On the day of mechanical testing, the hindlimbs were allowed to thaw to room temperature in phosphate-buffered saline (PBS). Femoral-MCL-tibia (FMT) complexes were prepared by dissecting clear extraneous tissue (including the joint capsule and adherent medial meniscus), while keeping the MCL and its insertion sites hydrated with PBS. The femoral and tibial insertions of the MCL were left intact, and the proximal tibia growth plate was removed to permit more space within the knee joint during testing. MCL thickness and width were measured optically at the knee joint line, and MCL area estimated using an elliptical geometry. Each FMT complex was placed in a customized testing jig, with the knee joint positioned in 70° flexion, for MCL testing. This position appears to load all ligament fibers simultaneously. The femoral and tibial portions were embedded in Wood’s low-melting-point metal (bismuth alloy LMA-117; Small Parts, Inc, Miami Lakes, FL) for fixation. The jig was coupled to an electromagnetic material testing device (TestBench 200 N ELF LM-1; EnduraTEC Systems Group, Bose Corp, Minnetonka, MN), equipped with a 50-N load cell (FIGURE 2A). This system possesses a force and displacement resolution of 0.01 N and 0.001 mm, respectively. A preload of 0.05 N was applied and the ligaments were preconditioned by cyclically loading at 1 Hz for 10 cycles to 1% strain to reduce the effect of deep freezing on low-load mechanical properties. The ligaments were unloaded and allowed to recover for 1.5 minutes, while being kept moist with PBS. Following tissue recovery, ligaments were again preloaded (0.05 N) and pulled to tensile failure in displacement control at a rate of 0.8 mm/s (~10%/s). Force and displacement data were collected at 100 Hz, and the mechanical properties of ultimate force (N), stiffness (N/mm), and energy to failure (mJ) obtained from the force-displacement curves (FIGURE 2B).

Scanning Electron Microscopy

Immediately after harvest, specimens for scanning electron microscopy were placed in a custom limb frame that held the knee positioned in 70° flexion. The MCL was exposed and drip fixed for 1 hour with 2.5% glutaraldehyde in 0.1 mol sodium cacodylate buffer (pH 7.4) (Electron Microscopy Services, Hatfield, PA). After drip fixation, the MCLs were removed using a microsurgical scalpel, with the femoral insertion marked by an angled

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FIGURE 2. Tensile mechanical testing of rat knee medial collateral ligament (MCL). (A) Representative image of the setup for testing. Femur-MCL-tibia (FMT) complexes were placed in fixtures that supported (“cupped”) the distal femur and proximal tibia to prevent bone slippage, and fixed in place using Wood’s low-melting-point metal. The knee joint was positioned in 70° of flexion for testing. (B) Representative force displacement curve for a rat knee MCL tensile mechanical test. Derived properties include ultimate force (peak on the curve on the y-axis), stiffness (slope of the linear portion of the curve), and energy absorbed prior to failure (area under the curve).
plane thin (4 μm) sections were cut using a rotary microtome (Reichert-Jung Model 2050; Reichert-Jung, Heidelberg, Germany), mounted onto microscope slides, and stained with Harris hematoxylin and eosin on a linear stainer (Shandon Linistain GLX; Thermo Electron Corp, Waltham, MA). Three sections per specimen were qualitatively assessed under light microscopy using a Nikon Optiphot 2 microscope (Nikon, Inc, Garden City, NY).

Statistical Analyses
Statistical analyses were performed using SPSS, Version 16.0 (SPSS Inc, Chicago, IL). All comparisons were 2-tailed, with a level of significance set at 0.05. Unpaired t tests were performed to assess time (4 versus 12 weeks postinjury) and group (injured versus control animals) effects on body mass. IACFM effects were principally determined using paired t tests to compare IACFM-treated and contralateral nontreated MCLs. Paired t test results were subsequently confirmed by calculating mean percent differences between IACFM-treated and nontreated MCLs [(IACFM-treated – IACFM-nontreated) ÷ nontreated × 100%], which were analyzed using single sample t tests with a population mean of 0%.

RESULTS

Animal Characteristics
There were no operative or postoperative complications. Animals assessed at 12 weeks postinjury were significantly heavier than those assessed at 4 weeks (mean ± SD, 291.4 ± 13.2 g versus 313.5 ± 22.6 g; P < .05). There were no differences in weight between injured and control animals (P = .76).

Ligament Macroscopic Morphology
All surgically induced ligament defects were bridged with scar tissue at the time...
ligaments did not differ significantly at either 4 (5.46 ± 1.01 mm² versus 5.16 ± 1.55 mm²; \( P = .45 \)) or 12 (3.80 ± 1.02 mm² versus 4.09 ± 0.79 mm²; \( P = .29 \)) weeks postinjury.

### Ligament Mechanical Properties
At 4 weeks postinjury, IACFM-treated ligaments could resist 6.4 N (95% confidence interval [CI], 1.6 to 11.2 N; \( P = .01 \)) greater force than contralateral nontreated ligaments (FIGURE 4A). This was reflected by IACFM-treated ligaments having 43.1% (95% CI, 8.2% to 78.0%; \( P = .02 \)) greater mean difference in tensile strength than nontreated ligaments. Similarly, IACFM-treated ligaments had 4.9 N/mm (95% CI, 2.4 to 7.4 N/mm; \( P = .001 \)) (FIGURE 4B) and 5.8 mJ (95% CI, 0.7 to 10.9 mJ; \( P < .05 \)) (FIGURE 4C) greater stiffness and energy to failure at 4 weeks postinjury than nontreated ligaments, respectively. This was reflected by IACFM-treated ligaments being 39.7% (95% CI, 15.9% to 63.5%; \( P < .01 \)) stiffer and being able to absorb 57.1% (95% CI, 3.4% to 110.9%; \( P = .04 \)) greater energy before failure than nontreated ligaments.

At 12 weeks postinjury, IACFM-treated ligaments had 2.6 N/mm (95% CI, 0.2 to 5.0 N/mm; \( P < .05 \)) greater stiffness than nontreated ligaments, resulting in the former being 15.4% (95% CI, 0.1%–30.7%; \( P < .05 \)) stiffer (FIGURE 5B). However, there were no differences at 12 weeks postinjury between IACFM-treated and nontreated ligaments in ultimate force (1.1 N; 95% CI, –2.6 to 4.7 N; \( P = .54 \)) (FIGURE 5A) or energy to failure (–0.6 mJ; 95% CI, –6.7 to 5.5 mJ; \( P = .84 \)) (FIGURE 5C). Mechanical properties of ligaments in injured animals at both 4 and 12 weeks postinjury remained inferior to intact, nontreated ligaments from control animals (\( P < .05 \)).

### Ligament Microscopic Morphology
Light microscopy of noninjured ligaments from control animals revealed a uniform appearance of tightly packed, well-aligned collagen fibrils with interspersed fibroblasts aligned parallel to
In contrast, ligaments from injured animals appeared to have scar morphology with extracellular matrix disorganization and hypercellularity, particularly at 4 weeks postinjury (FIGURE 6B-E). The scar region of IACFM-treated ligaments at 4 weeks postinjury also appeared to have greater cellularity, with collagen fiber bundles appearing to be orientated more along the longitudinal axis of the ligament than observed in contralateral nontreated ligaments (FIGURE 6B-C). At 12 weeks postinjury, there were limited histological differences between IACFM-treated and nontreated ligaments (FIGURE 6D-E).

Ligaments from injured, but not control, animals had granular tissue at low magnification (×25) on scanning electron microscopy and IACFM-treated ligaments appeared to have less surrounding granular tissue compared to nontreated ligaments, supporting the macroscopic observations (FIGURE 7). At higher scanning electron microscopy magnifications (>2500×6500), the scar region of IACFM-treated ligaments appeared to have improved collagen fiber bundle formation and orientation within the scar region compared to nontreated ligaments, supporting the light microscopy observations (FIGURE 8).

**DISCUSSION**

This study investigated the potential utility of manual therapy in the form of IACFM on ligament healing. Results indicate that IACFM-treated ligaments were 43% stronger, 40% stiffer, and able to absorb 57% more energy than contralateral, nontreated, injured ligaments at 4 weeks following injury. These mechanical differences may have resulted from favorable effects of IACFM on the organization of the underlying collagen substructure, as suggested by preliminary light microscopy and scanning electron microscopy analyses. The latter needs to be confirmed by way of more in-depth quantitative analyses in future studies. In contrast, there was
How IACFM facilitates the restoration of ligament tensile mechanical properties following injury was not investigated in detail in the current study, as the primary purpose was to provide proof-of-concept evidence for the utility of IACFM. However, preliminary light microscope and scanning electron microscopy assessments suggest that IACFM may enhance restoration of ligament biomechanical healing by optimizing the organization of the collagen substructure. Collagen (in particular, type 1 collagen) is the primary load-bearing molecule in ligament that endows tensile strength, and is ordered hierarchically into fibrils and fibers. The alignment and organization of newly formed collagen fibers in the direction of tensile loads during healing influences ligament mechanical properties. The qualitatively improved collagen fiber organization observed within the scar region of IACFM-treated ligaments in the current study is a possible explanation for why IACFM-treated ligaments had enhanced tensile mechanical properties. This will be the focus of future quantitative studies into IACFM effects on ligament morphology.

In addition to more detailed studies into IACFM effects on ligament morphology, studies are planned to explore potential molecular mechanisms by which IACFM generates its biomechanical effects. Our working hypothesis is that IACFM has an underlying effect on collagen, which may include effects on its synthesis, maturation, and/or cross-linking. To have such effects, IACFM must influence the fibroblastic cells responsible for producing collagen. This potential effect is supported by previous work that found that IACFM increases fibroblast recruitment and activation in a rodent Achilles tendon injury model. It is plausible that IACFM must influence the fibroblastic cells responsible for producing collagen. This potential effect is supported by previous work that found that IACFM increases fibroblast recruitment and activation in a rodent Achilles tendon injury model.

The findings of the current study are interesting in that a relatively simple and practical manual therapy technique was found to enhance early recovery of ligament biomechanical properties following acute injury. This may be clinically relevant, as there are currently limited established treatment options for mediating tissue-level ligament healing. It is clear from preclinical and clinical studies that surgery with or without immobilization is not indicated for most capsular and extracapsular ligament injuries. This holds true for both partial- and full-thickness ligament tears, with comparative studies showing conservative treatment and surgical repair producing similar outcomes irrespective of the extent of the initial ligament damage. Consequently, there is a need to establish interventions other than surgery for influencing ligament healing. Numerous preclinical studies have investigated the utility of novel interventions targeting ligament healing, including the use of gene therapies, growth factors, biological scaffolds, stem cell therapies, and biophysical modalities. While each of these directions has shown promise in influencing ligament healing, the techniques are far from being translated into the clinical realm and their eventual costs may prohibit wide use in mainstream clinical practice. In contrast, IACFM may have clinical utility, as it is currently readily available and practical from the sense that gains in ligament biomechanical properties were produced in the current study using a relatively limited number of short treatment sessions (9 total sessions of 1-minute duration each).

Minimal to no effect of IACFM on ligament healing when assessed at 12 weeks following injury, with the only difference between IACFM-treated and nontreated ligaments being 15% greater stiffness in the former. Overall, the findings of this study suggest that IACFM accelerates early tissue-level healing following ligament injury, but does little in terms of augmenting healing.

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axis. Integrins are a family of glycoprotein molecules that connect extracellularly with the extracellular matrix and intracellularly with the cytoskeleton and other cytoplasmic constituents. This creates a transmembrane axis that mechanically links the extracellular matrix with the cytoplasmic constituents of the cell to transmit external stimuli (such as those associated with IACFM) directly to the internal environment of the cell to alter gene expression and protein synthesis.

The current study provides supportive evidence for the potential clinical use of IACFM in the treatment of capsular and extracapsular ligament injuries; however, the findings need to be carefully interpreted in light of acknowledged limitations. First, the study was performed in an animal model, wherein the knee MCL was injured via surgical transection. This is a highly reproducible and established model for the preclinical testing of interventions for ligament injuries; however, the ability of the model to predict the clinical scenario wherein ligaments are injured via excessive tensile load has not been established. Second, the size of rodent tissues in relation to those of humans raises scaling issues in terms of intervention application and response. We addressed this issue by performing IACFM to rodent ligaments with the same tool and force as those used clinically to treat similar-size ligaments (finger collateral ligaments). Third, between-animal differences in activity levels may have influenced ligament-healing rates, as activity has previously been shown to mediate healing of isolated MCL injuries in rodents. We controlled for this possibility in the current study by establishing within-animal IACFM effects, wherein IACFM-treated ligaments were compared to contralateral nontreated ligaments that were presumably exposed to equivalent activity levels. Fourth, the current study did not consider IACFM effects on clinically measurable outcomes, such as recovery from symptoms such as pain. The restoration of mechanical properties is the ultimate tissue-level outcome of the ligament-healing process and is an important preclinical outcome; however, it is plausible that IACFM accelerates ligament biomechanical healing without influencing symptom recovery. Also, the establishment of IACFM benefits via the ex vivo testing of MCL tensile properties with the knee at 70° flexion may not represent the most clinically translatable outcome. While testing at 70° knee flexion is reported to load all fibers of the rat MCL simultaneously, it is plausible that alternative joint positions provide better tests of functionally important portions of the ligament. Similarly, as the MCL was isolated for mechanical testing, the contribution of other structures that contribute to the in vivo resistance of knee valgus forces were not assessed. It is possible that other passive and active restraints are able to compensate for injury to the MCL in the clinical setting, reducing the potential clinical effect size of IACFM during ligament healing.

CONCLUSION

In summary, this study suggests that IACFM may accelerate early tissue-level healing following acute capsular/extracapsular ligament injury but it has minimal to no effect in terms of augmenting the overall outcome of the ligament-healing process. This finding supports a theoretically sound argument for the use of IACFM after acute ligament injury; however, careful interpretation of this controlled laboratory study is warranted until its findings are confirmed by clinical studies.

KEY POINTS

**FINDINGS:** IACFM accelerated early tissue-level healing following acute capsular/extracapsular ligament injury but had minimal to no effect in terms of augmenting the overall outcome of the ligament-healing process.

**IMPLICATION:** IACFM is a relatively simple and practical therapy technique that may facilitate earlier return of ligament tissue-level biomechanical properties, enabling quicker return to function with less susceptibility to reinjury.

**CAUTION:** Careful interpretation of this controlled animal study is warranted until its findings are confirmed by clinical studies.

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