Original Research

Chondrogenic Effect of Intra-articular Hypertonic-Dextrose (Prolotherapy) in Severe Knee Osteoarthritis

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Abstract

Background: Dextrose injection is reported to improve knee osteoarthritis (KOA)−related clinical outcomes, but its effect on articular cartilage is unknown. A chondrogenic effect of dextrose injection has been proposed.
Objective: To assess biological and clinical effects of intra-articular hypertonic dextrose injections (prolotherapy) in painful KOA.
Design: Case series with blinded arthroscopic evaluation before and after treatment.
Setting: Physical medicine and day surgery practice.
Participants: Symptomatic KOA for at least 6 months, arthroscopy-confirmed medial compartment exposed subchondral bone, and temporary pain relief with intra-articular lidocaine injection.
Intervention: Four to 6 monthly 10-mL intra-articular injections with 12.5% dextrose.
Main Outcome Measures: Visual cartilage growth assessment of 9 standardized medial condyle zones in each of 6 participants by 3 arthroscopy readers masked to pre-/postinjection status (total 54 zones evaluated per reader); biopsy of a cartilage growth area posttreatment, evaluated using hematoxylin and eosin and Safranin-O stains, quantitative polarized light microscopy, and immunohistologic cartilage typing; self-reported knee specific quality of life using the Western Ontario McMaster University Osteoarthritis Index (WOMAC, 0-100 points).
Results: Six participants (1 female and 5 male) with median age of 71 years, WOMAC composite score of 57.5 points, and a 9-year pain duration received a median of 6 dextrose injections and follow-up arthroscopy at 7.75 months (range 4.5-9.5 months). In 19 of 54 zone comparisons, all 3 readers agreed that the posttreatment zone showed cartilage growth compared with the pretreatment zone. Biopsy specimens showed metabolically active cartilage with variable cellular organization, fiber parallelism, and cartilage typing patterns consistent with fibro- and hyaline-like cartilage. Compared with baseline status, the median WOMAC score improved 13 points ($P = .013$). Self-limited soreness after methylene blue instillation was noted.
Conclusions: Positive clinical and chondrogenic effects were seen after prolotherapy with hypertonic dextrose injection in participants with symptomatic grade IV KOA, suggesting disease-modifying effects and the need for confirmation in controlled studies. Minimally invasive arthroscopy (single-compartment, single-portal) enabled collection of robust intra-articular data.
Level of Evidence: IV

Introduction

The Agency for Healthcare Research and Quality and the Institute of Medicine have called for evaluation of new knee osteoarthritis (KOA) therapies [1,2] Hypertonic dextrose injection (prolotherapy) is a treatment for chronic musculoskeletal pain, including KOA [3]. Functional and symptomatic benefit from hypertonic dextrose injection in knee osteoarthritis has been reported in 3 randomized controlled trials (RCTs) and 3 open-label studies, with stability of benefit at 30-month follow-up [4-9]. Both in vivo (animal) and in vitro model data suggest cartilage-specific anabolic growth as a result of intra-articular dextrose injection [10]. A chondrogenic effect of intra-articular dextrose injection in human osteoarthritic knees has been hypothesized and assessed by radiograph and magnetic resonance imaging (MRI), but has not been clearly demonstrated [6,11].

Arthroscopy has been used for postprocedure "second-look" to evaluate the biological response of articular cartilage following stem cell injection and surgical procedures [12,13]. Direct visualization with arthroscopy and biopsy has the potential to detect subtle biological changes and may detect early cartilage change more accurately than MRI [14], enabling the robust screening of potential chondrogenic effects in disease modification studies. We therefore tested the hypothesis that, among participants with severe symptomatic KOA, intra-articular hypertonic dextrose injections will be associated with chondrogenesis and clinical improvement compared with baseline status, as assessed by masked arthroscopic video review before and after treatment, post-treatment biopsy, and a disease-specific outcome questionnaire, respectively.

Methods

The study protocol was approved by the Bioethics Committee of the National University of Argentina in Rosario, Argentina. Because of the self-funded and preliminary nature of this study, enrollment was limited to 6 participants.

Eligibility Criteria and Participant Recruitment

Inclusion criteria included knee pain for at least 6 months, clinically diagnosed KOA [15] a weight-bearing radiograph consistent with high-grade medial compartment cartilage loss (Kellgren-Lawrence Grading Scale level IV) (Figure 1), and confirmation of exposed subchondral bone by high-resolution knee ultrasonography [16]. Exclusion criteria included anticoagulation therapy, inflammatory or postinfectious knee arthritis, systemic inflammatory conditions, knee flexion of less than 100°, knee extension of less than 165°, any valgus, varus deviation of more than 20°, or less than 90% acute pain relief after intra-articular injection of 10 mL of 0.2% lidocaine.

Assessment

Age, pain duration, prior knee interventions, and body mass index (BMI) were recorded at study entry. Biological, clinical, and functional assessment occurred at a single follow-up time point after completion of treatment. Biological assessment included methylene blue stain for cartilage at arthroscopy, followed by a pre- and posttreatment zone-by-zone videography of the medial condyle and a posttreatment biopsy with histologic and immunohistologic evaluation, as described below. Clinical and functional assessment included knee range-of-motion measurement using a goniometer [17] disease-specific quality-of-life score (composite Western Ontario McMaster University Osteoarthritis [WOMAC, 0-100 points]) [18], and knee pain severity with walking (0-10 numerical rating scale [NRS]) [19].
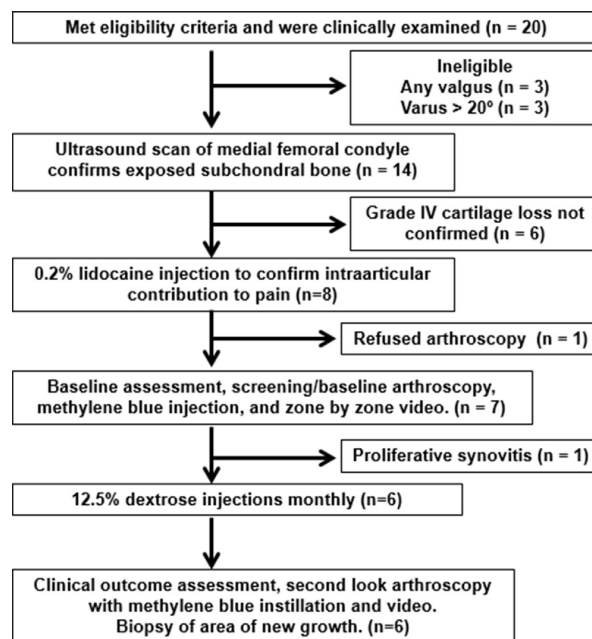

Figure 1. Enrollment of participants and completion of the study. Eligibility and exclusion criteria, grade IV change on ultrasound of the medial femoral condyle, and analgesia with lidocaine injection were required for candidacy. Methylene blue staining was used to visualize cartilage cells. Video recordings of the entire medial condyle were performed in a fixed sequence both before and after treatment. A biopsy sample was obtained from an area of visible growth during the second arthroscopy. Changes in pain, flexibility, cartilage status, histology, and immunohistology were followed.

Pretreatment Arthroscopy, Treatment, Posttreatment Arthroscopy, Biopsy, and Histology

A single orthopedic surgeon (L.A.P.) performed all arthroscopies in an outpatient hospital setting. Analgesia for arthroscopy consisted of intra-articular injection of 20 mL of 0.5% bupivicaine with epinephrine, 20 mL of 2% lidocaine with epinephrine, and 20 μg of fentanyl in 20 mL of sodium chloride. The procedure was minimally traumatic using 1 entry port; inspection was limited to the medial femoral condyle without flipping the scope to view the patellar surface. A 60-mL quantity of 0.14% methylene blue solution was instilled and left in place for 10 minutes; the knee was then flushed with 4 L of sterile water. A video of the entire medial compartment was then made with standardized zone-by-zone scope movement through each of 9 portions of the medial condyle (Figure 2a; A-I) [20]. Each of the 9 portions was labeled with the appropriate letter using video editor software (AVS Video Editor 6.1.2.211, On-line Media Technologies Ltd), and the video was then libraried.

Treatment consisted of sterile preparation with chlorhexidine gluconate, followed by intra-articular injection of 12.5% dextrose (5 mL of 25% dextrose, 5 mL of
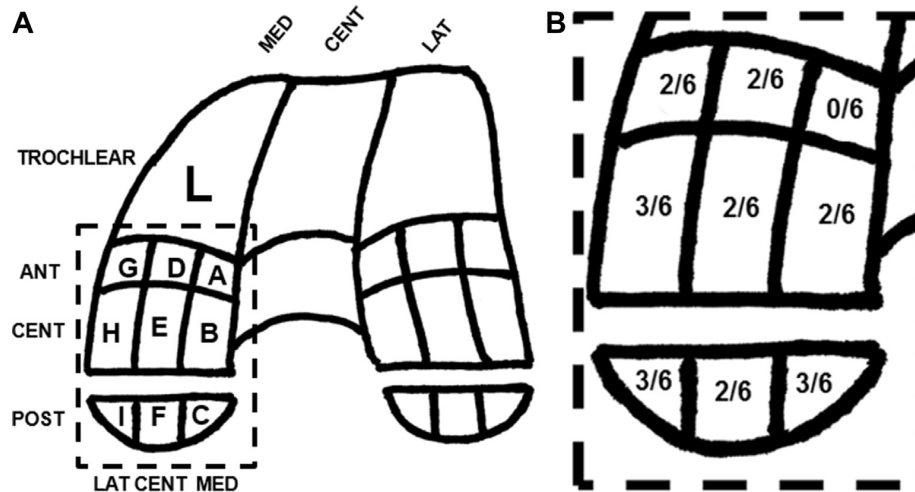
Figure 2. Areas of cartilage growth on the medial femoral condyle. Panel A shows the entire medial condylar surface of the left knee divided into 9 sections (A-I) per International Cartilage Research Society (ICRS) guidelines [28]. A cut-out area is shown. In Panel B, the magnified cutout, a fraction is seen in each of the sections. The denominator of each fraction is 6, the number of knees evaluated arthroscopically before and after treatment. The numerator is the number of knees that showed growth as agreed upon by all 3 arthroscopists.

normal saline solution) via a lateral approach to the supra-patellar pouch under ultrasound guidance [21]. Participants were asked to avoid taking glucosamine and chondroitin and to minimize weight bearing for 3 days after injection by using the support of the arms and opposite leg when rising from a chair, and to avoid running and squatting during the remainder of the study. Assistive devices such as canes or crutches were not required.

The original protocol called for 4 monthly intra-articular dextrose injections (baseline and 1, 2, and 3 months) followed by arthroscopy at 4 months after the baseline injection. However, after the first participant completed injection and follow-up arthroscopy, and while participants 2-6 were receiving injections, an unforeseen construction project closed the arthroscopy facility for several months. This delayed the acquisition of arthroscopy for participants 2-6. Because the investigators were concerned that weight-bearing ambulation for several months in the absence of monthly injections could eliminate evidence of a dextrose-related chondrogenic effect should one exist, we requested and received permission from the human subjects committee to increase the number of injection sessions from 4 to 6. Although this created a difference in the planned number of injections between participant 1 (4 injections) and participants 2-6 (6 injections), our intention was to obtain all arthroscopies within 3 months of the final injection.

Video recordings from the first arthroscopy were reviewed by the lead surgeon prior to the follow-up arthroscopy, who then repeated the method of the index procedure; a biopsy sample was obtained from an area of potential new growth, as defined by a new area of methylene blue dye uptake on the base of the

exposed subchondral bone. The same single portal was used to place the 11-gauge 10-cm Jamshi needle (Cardinal Health DJ4011X). A photograph of the biopsy site was taken pre- and postbiopsy. The biopsy was subjected to 1% Safranin-O and hematoxylin and eosin (H&E) staining (RG, Anatomopathology Consultation Clinic in Rosario, Argentina) according to standard histotechnologic methods [22]. Quantitative polarized light microscopy (QPLM) was performed (Department of Bioengineering at the University of California, San Diego) along with timed immunohistologic stain applications for type I and type II cartilage of the specimens and normal human cartilage controls [23].

Comparative Zone-by-Zone Readings of Libraried Arthroscopies

Three orthopedic surgeons (V.O.G., Y.U.K., and A.C.) who were otherwise uninvolved with the study and its participants, and with 14, 16, and 20 years of experience performing knee arthroscopies, respectively, volunteered to be outside reviewers. They performed comparative zone-by-zone readings of the arthroscopies and were masked to the date on which the arthroscopy was obtained. Computer randomization prepared by the statistician (A.L.C.) was used to assign the pretreatment arthroscopy to either "Arthroscopy A" or "Arthroscopy B"; then the same assignation was performed for the posttreatment arthroscopy, and both were loaded onto the timeline of the video editing program, saved in that randomized order, and reviewed independently by each reviewer. The reviewers were asked to view each zone A-I, moving the video timeline back and forth between Arthroscopy A and B, and to answer the following question: "Comparing arthroscopy

A with arthroscopy B, which zone has the appearance of additional cartilage growth, A, B, or N (neither)?" Reviewers completed a table with 54 responses (9 zones for each of 6 participants). Unblinding occurred after the arthroscopies were scored.

Analysis

The results of section-by-section arthroscopic video analysis of each of 9 sections of the medial condyle in all 6 participants by each surgeon-reader were summarized for display on a medial condyle map, and compared for inter-reader reliability using a Fleiss κ statistic [24]. Histologic findings were summarized using photographs in a per-participant manner. Nonarthroscopic data were analyzed using PASW 18 (Predictive Analytics Software 18.0.0; IBM). Descriptive statistics (median and interquartile range; or number/percent) described nonarthroscopic data at baseline and each follow-up time point. A paired-samples *t*-test was used to compare the WOMAC scores, 0-10 NRS pain scores, and knee flexion and extension measures at baseline to those collected before the second arthroscopy.

Results

Enrollment and follow-up occurred from February 2010 to June 2013. Twenty potential participants were referred to the study team (Figure 1). Of these, 8 met the initial eligibility criteria. One patient declined arthroscopy, and 1 patient was disqualified due to severe hypertrophic synovitis confirmed by multiple synovial proliferation folds. Thus, 6 participants were enrolled with 1 assessed knee each. Data from 2 right knees and 4 left knees were included in the analysis.

The study sample consisted of 5 men and 1 woman with a median age of 71 years, BMI of 26.25, and knee pain duration of 9.6 years (Table 1). A median initial composite WOMAC score of 57.5 and limitation of knee flexibility suggested moderate to severe baseline symptomatic KOA.

Ultrasound imaging showed a partially denuded medial femoral condyle as well as cortical irregularities (Figure 3, column 1). All participants showed baseline multicompartmental osteoarthritis on lateral and AP films (Figure 3, columns 2 and 3). Exposed subchondral bone was confirmed in each participant on initial arthroscopy.

Participant 1 received 4 injections and participants 2-6 received 6 injections prior to the follow-up arthroscopy at a median of 7.75 months (range 4.5-9.5 months).

Arthroscopic Zone-by-Zone Outcomes

In 19 of 54 zones evaluated (35%), all 3 readers agreed that the posttreatment zone showed cartilage growth compared with the pretreatment zone. In 35 of

Table 1
Baseline characteristics of participants (N = 6)

Female gender, n (%)	1 (16.7)
Age, y, median (IR)	71 (15)
Duration of knee pain, y, median (IR)	9.6 (10.8)
BMI, n (%)	
≤25	2 (33%)
26-30	3 (50%)
≥31	1 (17%)
Prior knee intervention, n (%)*	
Physical therapy	6 (100%)
Hyaluronic acid injection	1 (17%)
Corticosteroid injection	3 (50%)
Arthroscopic surgery	0 (0%)
WOMAC† median points (IR)	
Composite	57.5 (8)
Pain	57 (7)
Stiffness	57.5 (9)
Function	58 (8)
NRS pain, median (IR)	8.5 (3.25)
Flexibility	
Flexion range, median, (IR)	112.5 (22)
Extension deficit, median (IR)	7.5 (11)

IR = interquartile range; BMI = body mass index; WOMAC = Western Ontario McMaster University Osteoarthritis Index; NRS = numerical rating scale.
* Percentage does not sum to 100 due to participants' varied use of conventional therapies.
† 100-point WOMAC.

54 zones assessed, the 3 readers did not all agree, consistent with a low Fleiss κ value of interreader agreement of 0.007. Figure 2a shows the zones of the medial condyle for orientation, and Figure 2b shows the number of zones for which all 3 reviewers agreed on growth. For example, all reviewers rated zone I as showing more growth in 3 of the 6 participants. In addition, all participants showed areas of growth; specifically, all 3 reviewers agreed that at least 2 zones showed cartilage growth in each participant.

Arthroscopic Documentation of Biopsy Locations

To confirm that the biopsy sample was taken from an area of new growth, the pre- and postarthroscopy pictures are provided (Figure 4; columns 2 and 3), the area of biopsy is outlined by a red box (column 4), and the postbiopsy defect is shown. Although the biopsy for participant 3 cut across an area that may have included some previous cartilage in addition to new growth, all others were from exclusively new growth areas.

Basic Stains and Immunohistology for Cartilage Type

Figure 5 shows histological and immunohistologic findings for the medial condyle biopsy site. All Safranin-O–stained slides showed orange stain uptake indicating the presence of negatively charged molecules in the matrix, consistent with glycosaminoglycans, and
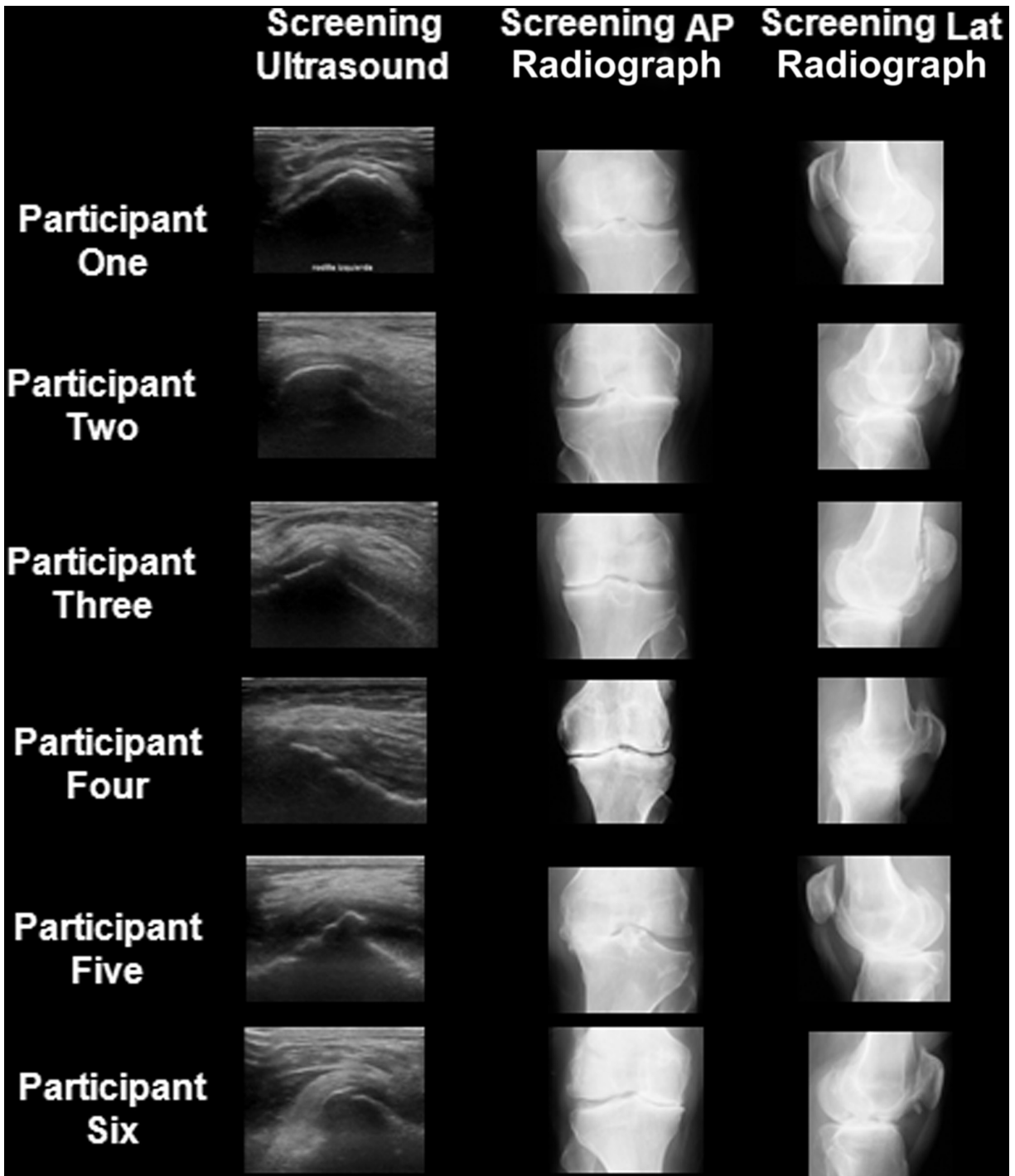
Figure 3. Baseline femoral condyle ultrasound and anterior-posterior (A-P) and lateral radiographs. The left-hand column is an ultrasound image of the medial femoral condyle showing at least focal full thickness loss of cartilage. A-P radiographs were taken in maximum extension with beam direction at joint height. Lateral compartments were consistent with multicompartmental involvement.

consistent with normal cartilage cell function. All H&E-stained slides, assessing the presence of organized tissue growth on formerly denuded bone, showed a

mixture of organized and disorganized tissue. QPLM assessment for fiber parallelism index showed areas of high fiber parallelism (orange or red areas), consistent
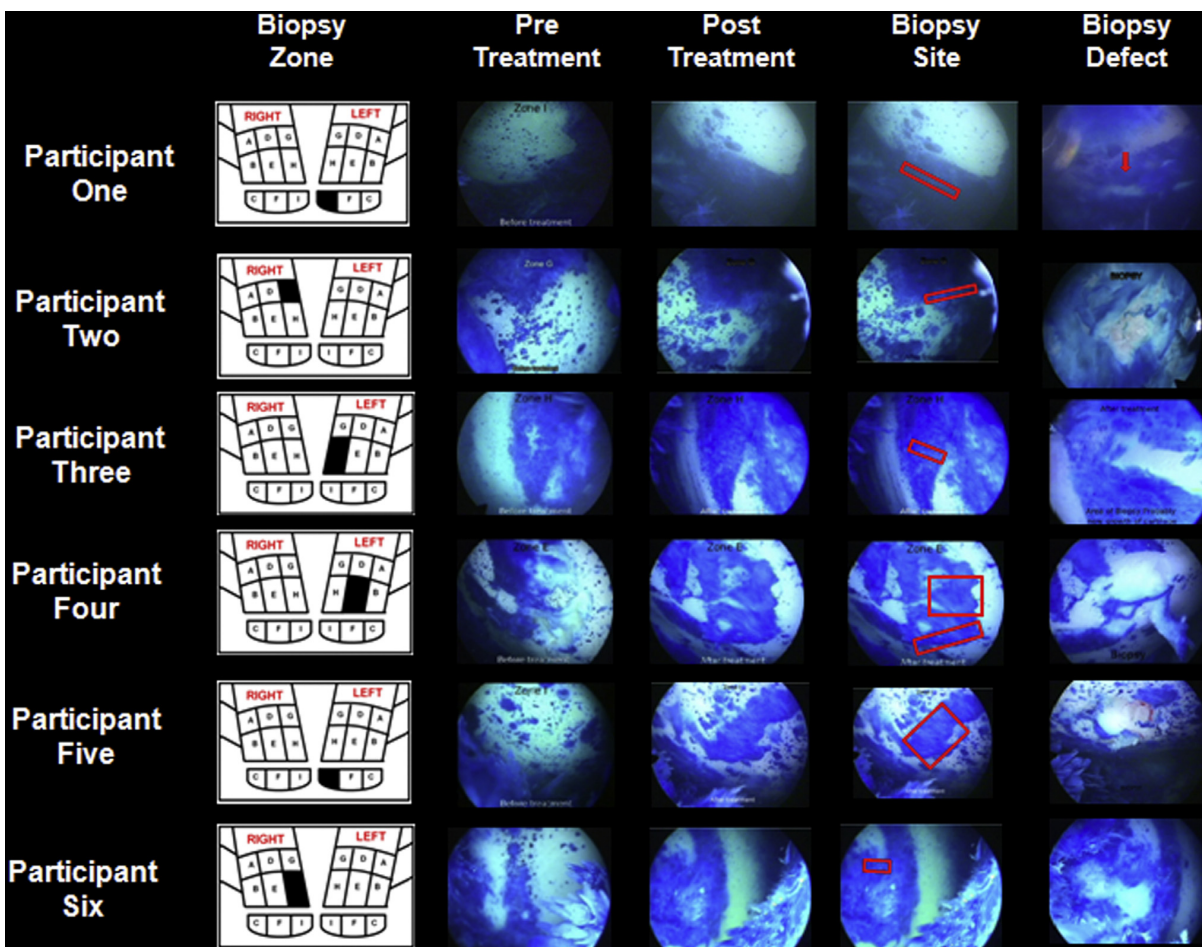
Figure 4. Arthroscopic confirmation of biopsy from an area of cartilage growth. In column 1, the darkened area for each subject indicates the section from which the biopsy was taken for that subject. In column 2, a still photograph of the area from which the biopsy was taken is shown from the first arthroscopy and at the time of the posttreatment arthroscopy (column 3). Column 4 shows the area of biopsy within the red box. Column 5 shows the biopsy defect.

with organized hyaline-like cartilage, in all but 1 participant (participant 5). Positive uptake for type I immunohistologic stain was noted in biopsy specimens from all participants, confirming that each specimen contained a fibrocartilage component. Positive uptake of type II immunohistologic stain, consistent with the presence of hyaline-like cartilage, was noted in all biopsy specimens except participant 5.

Clinical Outcomes

Median composite WOMAC scores improved from baseline to arthroscopic follow-up by 13 points ($P = .013$; Table 2). Median NRS-assessed pain severity decreased by 3.7 points ($P = .013$). Median knee flexion improved 7.5° ($P = .034$); median knee extension deficit improvement was not significant (2.5°; $P = .086$).

Side Effects and Adverse Events

There were no adverse events associated with the injection procedures or with arthroscopies. All participants noted self-limited mild-to-moderate, delayed-onset aching pain lasting hours to 3 weeks after arthroscopy. This was greater than that noted among nonstudy postarthroscopy patients not receiving methylene blue, and was thought to be related to a reaction to residual methylene blue after irrigation.

Discussion

This study assessed the hypotheses that intra-articular hypertonic dextrose injection is associated with chondrogenesis and that it provides clinical benefit compared to baseline status in participants with severe symptomatic KOA.
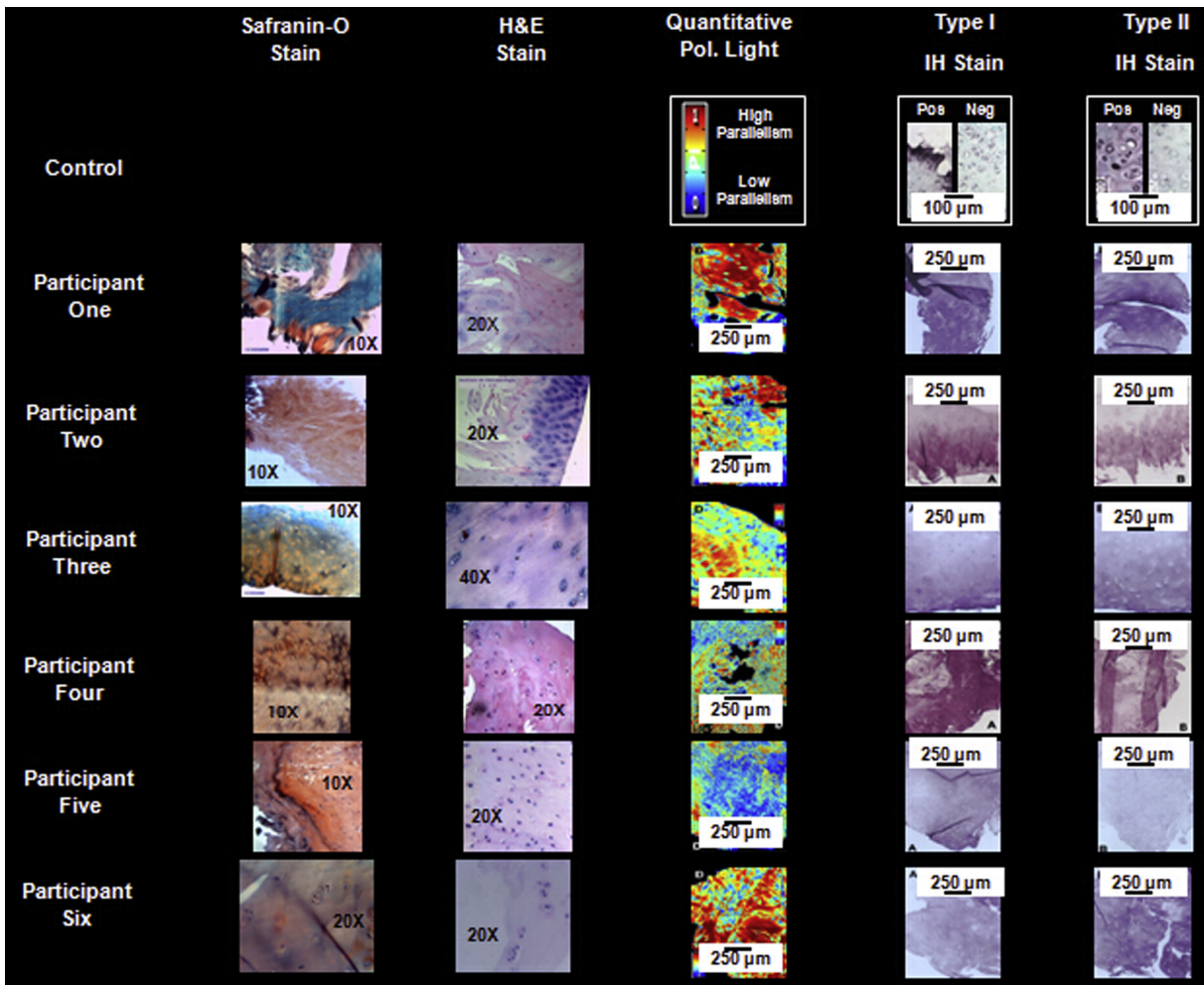
Figure 5. Safranin-O and H&E Stains, quantitative polarized light microscopy, and immunohistology for cartilage type of biopsy specimens. The normal positive uptake controls for immunohistologic stain for fibrocartilage and hyaline cartilage, respectively, were the perichondral (fibrous) region of nasal septal cartilage discarded at the time of routine nasal septal surgery and normal femoral condyle cartilage (cadaveric). An immunoglobulin-G (IgG) stain of the same normal femoral condyle cadaver cartilage served as the negative control, as IgG will not be taken up by normal cartilage.

The evidence favoring chondrogenesis includes agreement by all 3 reviewers of cartilage growth in 35% of possible evaluated zones and histological/immuno-histological presence of new cartilage in all 6 partici-pants, with a hyaline-like component in 5 of 6 biopsy samples from photographically confirmed areas of new methylene blue uptake. Mapping of growth zones con-firms that this growth occurred in both non—weight-bearing and weight-bearing areas. These outcomes were obtained postprocedure without the use of weight-reducing devices such as off-loading braces. Unloading of the knee remains the best care after cartilage repair procedures [25]; therefore, these data may underesti-mate the potential effect of the procedure in the presence of unloading. The improvement in clinical measures was statistically significant and clinically important, and consistent with 3 open-label studies and

3 RCTs of hypertonic dextrose prolotherapy injections for the knee OA [4-9].

These changes may result from the procedure tested; the small volume of methylene blue and subsequent saline lavage are not chondrogenic [26-28], and neither is expected to result in the observed clinical benefit. Although the cartilage growth was limited to a relatively small portion of the denuded surface, these are the first objective data to support the hypothesis that hyper-tonic dextrose injection may stimulate the growth of cartilage in the human knee.

Although this study suggests that cartilage growth and self-reported clinical improvement may be effects of hypertonic dextrose injection, we are not able to determine whether a single mechanism is responsible for either outcome, or whether the 2 outcomes are related. Several hypotheses for the mechanism of action

Table 2
Median baseline and change in WOMAC scores, NRS pain, and flexibility

Measure	Baseline Score (n = 6)	Improvement to Median 7.75 (Range 4.5-9.5 mo)* (n = 6)	P Value[†]
WOMAC composite score, median (IR)	57.5 (8.0)	−13 (22)	.013
WOMAC subscale scores, median (IR)			
Pain	57 (7.0)	−14 (21.0)	.010
Stiffness	57.5 (9.0)	−12.5 (23)	.017
Function	58 (8.0)	−13.5 (23)	.015
NRS (0-10) pain with walking, median (IR)	8.5 (3.25)	−3.7 (3.0)	.013
Flexion range, median (IR)	112.5 (22)	7.5 (13)	.034
Extension Deficit, median (IR)	7.5 (11)	−2.5 (7)	.086

WOMAC = Western Ontario McMaster University Osteoarthritis Index; NRS = numerical rating scale; IR = interquartile range.
  * Time until second arthroscopy. Values obtained in week before arthroscopy.
  [†] Significance (P value) is reported compared with baseline status.

of hypertonic dextrose injection have been advanced. The traditional view is that hypertonic dextrose initiates a brief inflammatory cascade stimulating native healing and subsequent tissue growth; clinical improvement then results from a restoration of tissue integrity [29]. Studies have reported increased cross-sectional area of MCL ligament in a rat model [30] and an increase in organized connective tissue width, thickening of collagen bundles, increase in energy absorption, and of load-before-rupture in a rabbit model [31,32] in response to hypertonic dextrose injection. Ultrasound data suggest that hypertonic dextrose injection is followed by tissue regeneration in ligamentous tissue [33,34]; however, analogous cartilage-specific data are lacking.

A direct pain-modulating effect has also been hypothesized. Recent clinical trial data suggest that hypertonic dextrose may decrease pain via a sensori-neural mechanism through direct exposure of dextrose to multiple intra-articular KOA pain generators, including the fat pad, synovium, and menisci. Two recent RCTs have suggested that sugar (dextrose) and a sugar alcohol (mannitol) have an analgesic effect in low back pain [35] and a capsaicin pain model [36], respectively, consistent with a potential sensori-neural mechanism of these agents.

An alternative view is that glucose has direct anabolic effects [37]. In vitro data on glucose-specific effects on chondrocytes demonstrate proliferative effects that vary according to such factors as oxygen tension, osmolarity, and the source of the chondrocyte (osteoarthritic or nonosteoarthritic knees) [38-43]. Synovial explants harvested from human donors and cultured in 0.45% dextrose demonstrated up to a 5-fold elevation of IGF-1 gene expression and secreted IGF-1 into the tissue media [44]. Park et al injected a solution that included 10% dextrose compared to normal saline into ACL-transection−induced osteoarthritic knee joints of New Zealand White rabbits and reported decreased erosion of articular cartilage overall compared to saline control, and minimal differences compared to normal cartilage that did not undergo ACL transection [10].

The current study is not able to identify the source of new cartilage. Progenitor cells within the synovial joint environment may contribute to endogenous cartilage repair [45-47]. Human synovium contains cells that, after culture expansion, display properties of mesenchymal stem cells [48]. Another potential source of the cartilage growth is cartilaginous aggregates within the exposed subchondral bone. Zhang et al documented the presence of cartilaginous deposit aggregates in the subchondral bone in areas of the human osteoarthritic knee with exposed bone [49].

Study Limitations

The primary limitations of this study are small sample size and absence of a control group. Potential conclusions are therefore modest. However, the cohort was thoroughly evaluated; cartilage growth among all participants suggests a modest but real chondrogenic response to hypertonic dextrose, and the WOMAC-assessed response is consistent with blinded and non-blinded studies of hypertonic dextrose injections for KO [6,7]. The low overall agreement rate among arthroscopy reviewers masked to date of arthroscopy limits slightly the confidence of our conclusions. Several aspects of the review process may account for uncertainty and subsequent lack of agreement; these include the presence of very subtle growth, the lack of published guidelines on visual assessment of cartilage on exposed subchondral bone, and review instructions that did not define exactly what constituted cartilage growth and were therefore open to interpretation by the reviewers.

Generalizability is limited by 3 factors. First, eligibility criteria included only the most severely affected knees; therefore, we are not able to address the potential effects of dextrose injection in patients with less severe KOA. Although prior studies have enrolled participants with K-L I-III KOA [4,7], we chose to include participants with KL IV and exposed subchondral bone, because prior studies suggested positive clinical effects from prolotherapy on all grades of KOA [4-9], and because detection of cartilage growth is more clear on a

denuded bone surface than on a cartilaginous surface. Second, the injection protocol varied slightly between 1 participant and the other 5 participants; however, both 4 and 6 injection sets fall within the clinically used number of injections. Third, a biopsy using a single entry port cannot obtain samples at the preferred angle of entry of 90°. Although this could affect precise assessment of tissue depth by layer, the use of QPLM allowed an assessment of hyaline-like tissue quality via fiber parallelism, and photographic confirmation of biopsy site confirmed that the biopsy location was in an area of new methylene blue uptake.

Conclusions

In this study, intra-articular hypertonic dextrose injections were associated with chondrogenesis in areas of exposed subchondral bone in participants with symptomatic grade IV osteoarthritic knees. Participants improved clinically in self-reported and objectively assessed functional outcomes consistent with previous randomized clinical trials. Minimally invasive, single-compartment, single-portal arthroscopy enabled collection of robust data from a small number of participants, and may provide an attractive, cost-effective means by which to evaluate potentially disease-modifying therapy.

References

1. Samson DJ, Grant MD, Ratko TA, Bonnell CJ, Ziegler KM, Aronson N. Treatment of primary and secondary osteoarthritis of the knee. Agency for Healthcare Research and Quality (Publication No. 07-E012): Evidence Report/Technology Assessment (Prepared by Blue Cross and Blue Shield Association Technology Evaluation Center Evidence-based Practice Center under Contract No. 290-02-0026). Rockville, MD 2007; 157.

2. Greenfield S, Sox H. Committee on Comparative Effectiveness Research Prioritization Board on Health Care Services. Initial National Priorities for Comparative Effectiveness Research. Institute of Medicine of the National Academies. 2009. Available at http://www.iom.edu/Reports/2009/ComparativeEffectivenessResearchPriorities.aspx. Accessed September 11, 2014.

3. Rabago D, Slattengren A, Zgierska A. Prolotherapy in primary care practice. Prim Care 2010;37:65-80.

4. Reeves KD, Hassanein K. Randomized prospective double-blind placebo-controlled study of dextrose prolotherapy for knee osteoarthritis with or without ACL laxity. Altern Ther Health Med 2000; 6:68-80.

5. Dumais R, Benoit C, Dumais A, et al. Effect of regenerative injection therapy on function and pain in patients with knee osteoarthritis: A randomized crossover study. Pain Med 2012;13:990-999.

6. Rabago D, Patterson JJ, Mundt M, et al. Dextrose prolotherapy for knee osteoarthritis: A randomized controlled trial. Ann Fam Med 2013;11:229-237.

7. Rabago D, Zgierska A, Fortney L, et al. Hypertonic dextrose injections (prolotherapy) for knee osteoarthritis: Results of a single-arm uncontrolled study with 1-year follow-up. J Altern Complement Med 2012;18:408-414.

8. Rabago D, Patterson JJ, Mundt M, et al. Dextrose and morrhuate sodium injections (prolotherapy) for knee osteoarthritis: A prospective open-label trial. J Altern Complement Med 2014;20:383-391.

9. Rabago D, Mundt M, Zgierska A, Grettie J. Hypertonic dextrose injection (prolotherapy) for knee osteoarthritis: Long term outcomes. Complement Ther Med 2015;23:388-395.

10. Park YS, Lim SW, Lee IH, Lee TJ, Kim JS, Han JS. Intra-articular injection of a nutritive mixture solution protects articular cartilage from osteoarthritic progression induced by anterior cruciate ligament transection in mature rabbits: A randomized controlled trial. Arthritis Res Ther 2007;9:R8.

11. Rabago D, Kijowski R, Woods M, et al. Association between disease-specific quality-of-life and magnetic resonance imaging outcomes in a clinical trial of prolotherapy for knee osteoarthritis. Arch Phys Med Rehabil 2013;94:2075-2082.

12. Koh YG, Jo SB, Kwon OR, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. Arthroscopy 2013;29:748-755.

13. Miao Y, Yu JK, Ao YF, Zheng ZZ, Gong Z, Leung KK. Diagnostic values of 3 methods for evaluating meniscal healing status after meniscal repair: Comparison among second-look arthroscopy, clinical assessment, and magnetic resonance imaging. Am J Sports Med 2011;39:735-742.

14. Guermazi A, Hayashi D, Roemer FW, Felson DT. Osteoarthritis: A review of strengths and weaknesses of different imaging options. Rheum Dis Clin North Am 2013;39:567-591.

15. Altman RD. Criteria for classification of clinical osteoarthritis. J Rheumatol Suppl 1991;27:10-12.

16. Martinoli C, Bianchi S. Knee. In: Bianchi S, Martinoli C, eds. Ultrasound of the Musculoskeletal System. New York: Springer; 2007; 727-729.

17. Peters PG, Herbenick MA, Anloague PA, Markert RJ, Rubino LJ. Knee range of motion: Reliability and agreement of 3 measurement methods. Am J Orthop 2011;40:E249-E252.

18. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: A health status instrument for measuring clinically important patient relevant outcomes in anti-rheumatic drug therapy in patients with osteoarthritis of the knee. J Rheumatol 1988;15:1833-1840.

19. Salaffi F, Stancati A, Silvestri CA, Ciapetti A, Grassi W. Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. Eur J Pain 2004;8: 283-291.

20. Brittberg M, Winalski CS. Evaluation of cartilage injuries and repair. J Bone Joint Surg Am 2003;85-A(Suppl 2):58-69.

21. Bum Park Y, Ah Choi W, Kim YK, Chul Lee S, Hae Lee J. Accuracy of blind versus ultrasound-guided suprapatellar bursal injection. J Clin Ultrasound 2012;40:20-25.

22. Prophet E, Mills B, Arrington J, Sobin L. Metodos Histotecnologicos: Versión en castellano editada y traducida por: Clara Heffes MD y Florabel G Mullick MD. Washington, DC: Armed Forces Institute of Pathology; 1995.

23. Raub CB, Hsu SC, Chan EF, et al. Microstructural remodeling of articular cartilage following defect repair by osteochondral autograft transfer. Osteoarthr Cartil 2013;21:860-868.

24. Fleiss JL. Measuring nominal scale agreement among many raters. Psychol Bull 1971;76:378-382.

25. Bardos T, Varncsodi J, Farkas B, et al. Pilot study of cartilage repair in the knee joint with multiply incised chondral allograft. Cartilage 2015;6:73-81.

26. Getgood A, McNamara I, Kili S, Bhullar T, Henson F. Reduced chondrocyte viability is associated with the use of surgical marker pen ink. Am J Sports Med 2011;39:1270-1274.

27. Reichenbach S, Rutjes WW, Nuesch E, Trelle S, Juni P. Joint lavage for osteoarthritis of the knee. Cochrane Database Syst Rev 2010;(5):CD007320.

28. Health-Quality-Ontario. Arthroscopic lavage and debridement for osteoarthritis of the knee: An evidence-based analysis. Ont Health Technol Assess Ser 2005;5:1-37.

29. Reeves KD, Topol GA, Fullerton BD. Evidence-based regenerative injection therapy (prolotherapy) in sports medicine. In: Seidelberg PH, Beutler PL, eds. The Sports Medicine Resource Manual. Philadelphia: Saunders (Elsevier); 2008; 611-619.

30. Jensen KT, Rabago D, Best TM, Patterson JJ, Vanderby R. Longer term response of knee ligaments to prolotherapy in a rat injury model. Am J Sports Med 2008;36:1347-1357.

31. Yoshii Y, Zhao C, Schmelzer JD, Low PA, An KN, Amadio PC. The effects of hypertonic dextrose injection on connective tissue and nerve conduction through the rabbit carpal tunnel. Arch Phys Med Rehabil 2009;90:333-339.

32. Yoshii Y, Zhao C, Schmelzer JD, Low PA, An KN, Amadio PC. Effects of multiple injections of hypertonic dextrose in the rabbit carpal tunnel: A potential model of carpal tunnel syndrome development. Hand (N Y) 2014;9:52-57.

33. Ryan M, Wong A, Taunton J. Favorable outcomes after sonographically guided intratendinous injection of hyperosmolar dextrose for chronic insertional and midportion Achilles tendinosis. Am J Roentgenol 2010;194:1047-1053.

34. Ryan M, Wong A, Rabago D, Lee K, Taunton J. Ultrasound-guided injections of hyperosmolar dextrose for overuse patellar tendinopathy: A pilot study. Br J Sports Med 2011;45: 972-977.

35. Maniquis-Smigel L, Reeves KD, Lyftogt J, Rabago D. Analgesic effect of caudal 5% dextrose in water in chronic low back pain [Abstr]. Arch Phys Med Rehabil 2015;96:e103.

36. Bertrand H, Kyriazis M, Reeves KD, Lyftogt J, Rabago D. Topical mannitol reduces capsaicin-induced pain: Results of a pilot level, double-blind randomized controlled trial. PM R 2015;7: 1111-1117.

37. Mobasheri A. Glucose: An energy currency and structural precursor in articular cartilage and bone with emerging roles as an extracellular signaling molecule and metabolic regulator. Front Endocrinol 2012;3:153.

38. Hong YH, Park CW, Kim HS, Won KC, Kim YW, Lee CK. Effects of hypoxia/ischemia on catabolic mediators of cartilage in a human chondrocyte, SW1353. Biochem Biophys Res Commun 2013;431: 478-483.

39. Oswald ES, Ahmed HS, Kramer SP, Bulinski JC, Ateshian GA, Hung CT. Effects of hypertonic (NaCl) two-dimensional and three-dimensional culture conditions on the properties of cartilage tissue engineered from an expanded mature bovine chondrocyte source. Tissue Eng Part C Methods 2011;17:1041-1049.

40. Bertram KL, Krawetz RJ. Osmolarity regulates chondrogenic differentiation potential of synovial fluid derived mesenchymal progenitor cells. Biochem Biophys Res Commun 2012;422: 455-461.

41. Shanfield S, Campbell P, Baumgarten M, Bloebaum R, Sarmiento A. Synovial fluid osmolality in osteoarthritis and rheumatoid arthritis. Clin Orthop Relat Res 1988;235:289-295.

42. Rosa SC, Rufino AT, Judas FM, Tenreiro CM, Lopes MC, Mendes AF. Role of glucose as a modulator of anabolic and catabolic gene expression in normal and osteoarthritic human chondrocytes. J Cell Biochem 2011;112:2813-2824.

43. Cigan AD, Nims RJ, Albro MB, et al. Insulin, ascorbate, and glucose have a much greater influence than transferrin and selenous acid on the in vitro growth of engineered cartilage in chondrogenic media. Tissue Eng Part A 2013;19:1941-1948.

44. D'Lima DD. Glucose concentration increases IGF expression from human synovial membrane, Technical Report, August 17. 2009. Available at http://www.aaomed.org/Scripps-Report-Glucose-effect-on-Synovial-tissue-IGF-expression. Accessed April 14, 2016.

45. Kurth TB, Dell'accio F, Crouch V, Augello A, Sharpe PT, De Bari C. Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo. Arthritis Rheum 2011;63:1289-1300.

46. Hunziker EB, Rosenberg LC. Repair of partial-thickness defects in articular cartilage: Cell recruitment from the synovial membrane. J Bone Joint Surg Am 1996;78:721-733.

47. Hunziker EB. Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. Osteoarthr Cartil 2001;9:22-32.

48. De Bari C, Dell'accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928-1942.

49. Zhang D, Johnson LJ, Hsu HP, Spector M. Cartilaginous deposits in subchondral bone in regions of exposed bone in osteoarthritis of the human knee: Histomorphometric study of PRG4 distribution in osteoarthritic cartilage. J Orthop Res 2007;25:873-883.

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