Biocompatibility of three-dimensional chondrocyte grafts in large tibial defects of rabbits

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Objective—To evaluate biocompatibility and effects of implantation of 3-dimensional chondrocyte-agarose autografts in tibial defects in rabbits and to compare in vitro and in vivo chondrocyte-agarose constructs with respect to cell viability, differentiation, and matrix production.

Animals—24 adult New Zealand White rabbits.

Procedure—Three-dimensional constructs with (grafted group) or without (control group) autogenous chondrocytes were implanted into tibial defects of rabbits and cultured in vitro. During an 8-week period, defects were evaluated radiographically, grossly, histologically, biochemically, and immunohistochemically. In vitro constructs were evaluated histologically, biochemically, and immunohistochemically.

Results—Tibial defects had significantly higher radiographic densitometry values at 4 and 6 weeks after implantation in grafted group rabbits, compared with control group rabbits. Number of observed centers of enchondral ossification was significantly greater in defects of grafted group rabbits, compared with control group rabbits. On day 14, glycosaminoglycan concentration was significantly higher in tibial defects of grafted group rabbits, compared to defects of control group rabbits or in vitro constructs. At weeks 2, 4, and 8, glycosaminoglycan concentrations were significantly lower in the in vitro control constructs, compared with other groups. Collagen type I was present in bone and bony callous in defects of grafted and control group rabbits. Collagen type II was identified in cartilaginous tissues of grafted and control group rabbits. Collagen type X was associated with hypertrophic chondrocytes. Only type II collagen was found in the in vitro chondrocyte constructs.

Conclusions and Clinical Relevance—Chondrocyte-agarose grafts are biocompatible in large tibial defects and appear to provide a cell source for augmenting enchondral ossification. (Am J Vet Res 2003; 64:12-20)

The mineralization of cartilage and subsequent bone deposition through the process of enchondral ossification is an important means of bone formation. This process occurs in epiphyseal growth, callous formation, and osteophyte production. Chondrocytes initiate the process of enchondral ossification by creating matrix molecules consisting of proteoglycans and collagen.1-3 Chondrocytes progress through an ordered series of phenotypic stages to provide for eventual mineralization of the extracellular matrix. Invasion by capillaries brings osteoid-producing cells that resorb the mineralized matrix and deposit bone.1-4 Osteoblasts are considered to be the primary bone producing cells; however, the ability of chondrocytes to directly deposit bone has been reported.5 On the basis of these data, the chondrocyte plays integral roles in the process of enchondral ossification.

Tissue engineering and transplantation of cartilage have been investigated for many years. Most of this research has focused on joint injury and the potential for articular cartilage resurfacing.6-15 Recently, a protocol for 3-dimensional (3-D) chondrocyte culture in agarose has been developed.8,9,16,17 The beneficial effects of 3-D culture, compared with monolayer culture, include maintenance of chondrocyte phenotype with production of cartilage-specific extracellular matrix prior to transplantation.8,9,16,17 This culture system may allow for biomechanical and immunologic protection of the tissue-engineered chondrocyte-substrate construct.8 The agarose medium used in this system also provides a practical method of manipulation and implantation.

Given the importance of chondrocytes for enchondral ossification, the transplantation of autogenous chondrocytes and their matrix molecules may provide a means to expedite and augment this process of bone formation. In addition, the potential exists for chondrocyte transplantation into physeal defects to maintain or restore the physiologic function of the growth plate. Chondrocytes have been grafted into articular cartilage defects, bone defects, subcutaneous tissues, and muscle.6,10,13,15,18 Agarose has been implanted into mammals for repair of articular cartilage defects, as a carrier for growth factors, as a carrier for pancreatic islets, and other in vivo applications.19-21 However, to our knowledge, there are no studies reporting the use of chondrocyte-agarose grafts in bone defects. Therefore, the purposes of the study presented here were to evaluate the biocompatibility and effects of implantation of 3-D chondrocyte-agarose autografts in large tibial defects in rabbits and to compare in vitro and in vivo chondrocyte-agarose constructs with respect to cell viability, differentiation, and matrix pro-
duction. Our hypothesis was that chondrocyte-laden 3-D grafts implanted into stable bone defects would result in enhanced matrix production and bone formation via endochondral ossification when compared with acellular implants.

Materials and Methods

Cartilage harvest—All procedures were approved by the University Animal Care and Use Committee. Twenty-four adult New Zealand White rabbits (mean and median body weight, 2.5 kg) were used for our study. Two weeks prior to graft implantation, each rabbit was premedicated (ketamine hydrochloride, 40 mg/kg; IM; diazepam, 5 mg/kg; IM) and anesthetized (isoflurane in O₂) for cartilage harvest. Full-thickness articular cartilage slices were aseptically harvested from the trochlear groove of 1 randomly assigned stiffe joint of each rabbit. The cartilage slices were placed in Hank’s balanced salt solution (HBSS) with penicillin and streptomycin sulfate (PS) at concentrations of 100 IU/mL and 100 µg/mL, respectively, and transported to a laminar flow hood. Chondrocytes were obtained by collagenase digestion, cultured in monolayer, and cultured in a 3-D agarose medium as previously described. Briefly, cartilage slices were incubated in 20 mL of HBSS-PS containing clostridial collagenase (0.5 mg/mL) at 37°C with continuous stirring for 7 to 9 hours. The digest solution was filtered through sterile gauze to remove remaining cartilage fragments. Chondrocytes in the digest solution were pelleted by centrifugation at 500 × g for 15 minutes. The cells were resuspended in 5 mL of RPMI 1640 medium containing PS and 10% fetal bovine serum. The cell suspension from each rabbit was plated in individual 25 cm² culture plates and incubated at 37°C with 5% CO₂ and 95% humidity. The medium was changed every 2 to 3 days.

By day 13 of monolayer culture, chondrocytes in all 25 cm² culture flasks had reached confluency. The cells were then used to create 3-D chondrocyte-agarose constructs as previously described. The medium was discarded and the flasks rinsed with HBSS-PS. Chondrocytes were detached by trypsinization (0.05%), counted, and assessed for viability by trypan blue exclusion. Cells from each rabbit were centrifuged (400 × g for 10 minutes) and resuspended in equal volumes of double-strength RPMI 1640-PS plus 20% fetal bovine serum and 2% low-melting agarose (gelling temperature, 25 ± 5°C) in Dulbecco’s PBS solution to produce a cell concentration of 10¹⁵ cells/mL. For each rabbit (n = 24), 1 mL of the cell-agarose suspension (graft) was added to 1 well of a 24-well tissue culture plate. One milliliter of agarose suspension containing no cells (control) was added to each well of a 24-well tissue culture plate. In this manner, 2 constructs were created for each rabbit: 1 construct contained cells, and the other did not. The 24-well plates were placed in a refrigerator at 5°C for 15 minutes to allow the construct to gel. One milliliter of liquid RPMI 1640-PS plus 10% fetal bovine serum was added to each well, and the plates were incubated overnight at 37°C in preparation for implantation as subsequently described. The constructs (n = 24) that were not implanted were left in the tissue culture plate and incubated at 37°C until collected. Liquid medium was changed every 3 days.

Graft implantation—On the day of graft implantation, each rabbit was again premedicated and anesthetized by use of the previously described protocol. The hind limb contralateral to cartilage harvest was aseptically prepared for surgery. A type II external skeletal fixator was pre-placed on the limb for stabilization. Three 0.062-in-diameter stainless steel Kirschner wires were placed into the proximal portion of the tibia from medial to lateral, perpendicular to the long axis of the bone. Three 0.062-in-diameter Kirschner wires were placed in the distal portion of the tibia in a similar manner. Kirschner wires were then temporarily stabilized by use of a stainless steel external skeletal fixator connecting bar and clamps placed on the lateral aspect of the limb.

After medial approach to the tibial diaphysis, a 1-cm-long ostectomy was created at the mid-diaphysis of the tibia by use of a pneumatic oscillating saw. The ostectomy site was standardized for each rabbit and centered over the point midway between transversely placed needles in the stiffe and hock joints. The ostectomy gap was then filled with 1 × 1 × 0.5 cm section of a 3-D construct with or without cells as previously determined by random assignment. After routine closure of the surgical wound, one 0.5-in-diameter rod of polymethylmethacrylate was used to connect all 6 external skeletal fixator pins on the medial and lateral aspects of the limb. Rabbits were radiographed, recovered, and treated with analgesics (buprenorphine hydrochloride, 0.02 to 0.05 mg/kg, IM) for a minimum of 24 hours.

Radiographic assessment—A craniocaudal radiographic view of the tibial defect site was obtained immediately post-operatively and at 1, 2, 4, 6, and 8 weeks after surgery. An aluminum step-wedge was included for each radiograph, and the same equipment and technique (50 kVp, 0.30 milliampere seconds) were used throughout the present study. Optical densitometry of the tibial defect site was assessed to provide an objective measure of radiographic density of the replacement tissue. The densitometer was calibrated prior to evaluation of each radiograph by use of the aluminum step-wedge included in each radiograph. Densitometry readings were obtained from 4 standardized sites within the tibial defect. Five readings were obtained at each site. The mean of the 5 values was calculated and used for analyses.

In addition, radiographs were evaluated subjectively by 1 investigator (NW) who was unaware of rabbit number or treatment group (ie, control vs grafted group). Each radiograph was scored on the basis of area of bone density in the tibial defect according to the following grading system: 0 = no visible area of bone density in the tibial defect; 1 = ≤ 25% of the tibial defect composed of radiographic bone density; 2 = 26 to 50% of the tibial defect composed of radiographic bone density; 3 = 51 to 75% of the tibial defect composed of radiographic bone density; and 4 = ≥75% of the tibial defect composed of radiographic bone density.

Each radiograph was also subjectively scored for bony bridging of the tibial defect by use of the following grading system: 0 = no bony bridging of the tibial defect; 1 = partial bony bridging of the tibial defect; and 2 = complete bony bridging of the tibial defect. A total subjective radiographic score was calculated by adding the 2 individual scores for each rabbit. The total subjective radiographic score was used for analyses.

Gross and histologic assessment—Four rabbits (2 grafted group and 2 control group) were euthanatized at 1, 2, and 4 weeks following graft implantation. Eleven rabbits (5 grafted group and 6 control group rabbits) were euthanatized 8 weeks following graft implantation by IV injection of an overdose of pentobarbital-phenytoin. Immediately after euthanasia, a portion of the operated tibia that included the defect site and 1 cm of unaffected bone proximal and distal to the site was collected from each rabbit and examined to assess gross appearance. The corresponding in vitro construct was collected at the time of euthanasia for each rabbit. A portion from each tibial defect site and construct was weighed, placed in 1 mL of distilled deionized water, and stored at −80°C for subsequent spectrophotometric assay. The remaining tissue from each tibial defect site and construct was placed in neutral-buffered 10% formalin for histologic processing.

After routine histologic processing, hematoxylin-eosin-stained sections were evaluated for tissue type; amount of fibrous tissue,
cartilage, and bone; and the morphology of tissues filling the defect. Toluidine blue-stained sections were evaluated for defect morphology and proteoglycan staining. All stained sections were evaluated via light microscopy by 1 investigator (JLC) who was unaware of rabbit number or treatment group. The presence (1) or absence (0) of a histologically evident center of endochondral ossification was determined for each in vivo construct. A histologically evident center of endochondral ossification was defined as a defect that contained cells of histologic phenotype consistent with chondrocytes of resting, proliferative, hypertrophic, and calcifying zones and associated matrix staining typical of that found in physeal tissues and bony callus.

Immunohistochemistry—Unstained sections were processed for immunohistochemical analysis of collagen types I, II, and X as previously described.²²²³ All staining was performed by use of a commercially available avidin-biotin-peroxidase kit.² Endogenous peroxidase quenching was performed by use of 3% hydrogen peroxide in water. For collagen type X, testicular hyaluronidase digestion for 30 minutes was followed by a serum block and application of the primary antibody (mouse anti-deer monoclonal type X collagen)²³ at a 1:800 dilution for 18 hours at 4°C. For collagen types I and II, a 30-minute pepsin digestion was performed, a serum block used, and the primary antibody (rabbit anti-bovine type I or II collagen) applied at an appropriate dilution for 18 hours at 4°C. Positive (rabbit epiphyseal plate) and negative (nonsense antibody) controls were included in the staining protocol for each batch. Hematoxylin counterstaining was performed on all sections. All stained sections were evaluated via light microscopy by 1 investigator (JLC) who was unaware of rabbit number or treatment group. The sections were subjectively evaluated for the presence and intensity of staining for the respective collagen type.

Glycosaminoglycan quantification—Frozen samples were thawed and digested in 1.5 mL of papain (0.5 mg/mL) in distilled deionized water at 65°C for 12 hours. A 100 μL aliquot of the digest solution was assayed for total glycosaminoglycan (GAG) concentration by addition of 2.5 mL of dimethylmethylene blue solution and spectrophotometric determination of absorbance at 525 nm.²⁴ Known concentrations of bovine trachea chondroitin sulphate A were used to construct a standard curve. Results were standardized to correct for differences in sample weight. Total GAG content was reported in μg/mL/g.

Statistical analysis—All analyses were performed by use of a computer software program.²⁵ Mean (± SEM) values were calculated for each group at each data collection period. For densitometry and subjective radiographic assessment, a Student t-test or Mann-Whitney rank sum test was performed to compare differences between groups, and a 1-way ANOVA was performed to determine differences within a group over time. A Pearson's product-moment correlation was performed to determine whether significant correlations existed between densitometry values and subjective scores. For comparison of evidence of endochondral ossification centers, a Mann-Whitney rank sum test was performed to determine differences between groups. For GAG concentrations, a 1-way repeated measures ANOVA was performed. Significance was set at P < 0.05 for all analyses.

Results

Cartilage harvest—All 24 rabbits survived the cartilage harvesting procedure. No clinically evident lameness was apparent after surgery. One rabbit had a minor wound complication (self mutilation causing a full-thickness wound on the dorsum of the paw of the operated limb) that was repaired under sedation. Viable cells were obtained from all 24 rabbits. After 13 days in monolayer culture, the number of viable cells ranged from 1 × 10⁵ to 1.3 × 10⁶ cells/rabbit. Cells from all 24 rabbits were available for 3-D culture.

In vivo and in vitro 3-D constructs—One rabbit died approximately 20 minutes after implantation as a result of an apparent anesthetic overdose. All other 23 rabbits survived for the appropriate duration of our study. Minor external skeletal fixator problems (chewing on or cracking of the methylmethacrylate columns) occurred and were repaired in 7 rabbits. Minor skin problems (trauma or self mutilation causing partial- to full-thickness wounds on the dorsum of the operated limbs) occurred in 8 rabbits. All 24 in vitro constructs were harvested at the appropriate time.

Radiographic assessment—Tibial defect densitometric values and subjective assessment indicated increasing bone density throughout the 8-week period (Fig 1 and 2). Densitometric values and subjective scores from grafted group rabbits were higher than control group rabbits at 2, 4, 6, and 8 weeks after implantation.

![Figure 1](image-url)
However, only densitometric values were significantly different between groups and only at 4 weeks ($P = 0.016$) and 6 weeks ($P = 0.006$) after implantation. Values and scores from grafted group rabbits were significantly ($P < 0.001$) different from time 0 (immediate postoperative defects) values and scores at an earlier time frame (week 4) than control group rabbits (week 6). A strong positive correlation ($r = 0.95$, $P = 0.003$) existed between densitometry values and subjective scores.

**Gross and histologic assessment**—Gross examination of the tibial defect site revealed marked differences in appearance between grafted and control group rabbits at the time of euthanasia at 1, 2, and 4 weeks. Tibial defects in the grafted group rabbits appeared to contain tissue composed of cartilage and bone. Tibial defects in the control group rabbits contained only fibrous tissue. Extensive periosteal reaction present in both groups of rabbits at the time of euthanasia (at 8 weeks) made gross determination of differences in defect tissue type impossible.

Histologic evaluation of tibial defects revealed a distinct difference in tissue type and morphology throughout the 8-week period. Tissue from grafted group rabbits had histologic characteristics of endochondral ossification centers as early as 1 week following implantation (Fig 3). Tibial defects from grafted group rabbits continued to have features of endochondral ossification throughout the 8-week period. Histologic features in many of these sections were characteristic of functional epiphyseal plates in that an orderly arrangement of defects into histologic zones of proliferation, maturation, hypertrophy, and calcification was observed (Fig 4). Tibial defects from control group rabbits primarily consisted of fibrous and inflammatory tissue until 8 weeks after implantation at which time trabecular bone predominated in the defect.

At 2 weeks after implantation, agarose could be identified within tibial defects of both groups of rabbits. The agarose was surrounded and infiltrated by varying amounts of inflammatory cells, including neutrophils, macrophages, and lymphocytes. Chondrocytes with histologic characteristics of viability were observed within the agarose of tibial defects of grafted group rabbits. Chondrocytes in the agarose of tibial defects of grafted group rabbits had a histologic appearance similar to the chondrocytes in the in vitro constructs. No chondrocytes were observed in the agarose of tibial defects of control group rabbits.

The most striking histologic differences between groups of rabbits were observed at 2 and 4 weeks after implantation (Fig 3). All tibial defects from grafted group rabbits ($n = 4$) had areas of chondroid differentiation and the presence of a center of endochondral ossification. Tibial defects from control group rabbits ($n = 4$) were primarily composed of fibrous tissue, remaining agarose, and inflammatory cells. Tibial defects of control group rabbits contained some areas of cartilage and bone production. However, none fulfilled the criteria required to establish a center of endochondral ossification. Histologic identification of endochondral ossification centers in tibial defects were found significantly more often in grafted group rabbits at week 2 ($P < 0.001$), week 4 ($P < 0.001$), and throughout the 8-week period as a whole ($P = 0.02$), compared with control group rabbits.

Consistent cell viability and appearance of chondrocytic differentiation throughout the 8-week period was observed in the in vitro constructs that contained cells. After 1 week of 3-D culture, pericellular and territorial matrix production was evident and continued throughout the 8-week period. Interterritorial matrix was present in constructs collected after 8 weeks of culture. Toluidine blue staining of proteoglycans increased in area and intensity throughout the 8-week period until near confluency by week 8.

![Figure 2](https://example.com/figure2.jpg)  
**Figure 2**—Craniocaudal radiographic views of tibial defects in grafted and control group rabbits. Notice the changes in the radiographic appearance of tibial defects during the 8-week study period. The radiographic views are from different rabbits that were most representative of the radiographic appearance of each group at each evaluation time point.

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Figure 3—Photomicrographs of sections of tibial defects from grafted and control group rabbits. Notice the differences and changes in the histologic appearance of the tissues between groups during the 8-week study period. These high magnification images highlight the major differences found between groups; all sections from grafted group rabbits at each time point had evidence of organized chondral tissue at the defect site. Tibial defects of control group rabbits had only fibrous tissue and bone with no evidence of organized chondral tissue or endochondral ossification. In the section obtained from a grafted group rabbit at 8 weeks, notice the zonal columnar arrangement of chondrocytes with transition to bone (left to right). Toluidine blue stain; bar = 100 µm.
Immunohistochemistry—Collagen types I, II, and X were identified by immunohistochemical staining of histologic sections of tibial defects as early as 1 week after implantation. Collagen type I was consistently present in areas identified histologically as bone and bony callous in tibial defects of grafted and control group rabbits. Collagen type II was also identified in tibial defects that contained cartilage of grafted and control group rabbits. However, the most intense collagen type II staining was observed in the proliferative and maturation zones of ossification centers. Type II collagen was present in hypertrophic zones and appeared to be undergoing degradation (Fig 5) as the tissue was replaced with osteoid. Interestingly, type I collagen staining was observed in tissues at the junction of hypertrophic cartilage and osteoid. Collagen type X was found only in the extracellular matrix associated with hypertrophic chondrocytes and, therefore, was only present in sections with centers of ossification (Fig 6).

Only type II collagen was identified by immunohistochemical staining in the in vitro constructs that contained chondrocytes. Type II collagen was identified as early as day 14 in 3-D culture and continued to be identifiable in all chondrocyte constructs throughout the remainder of the 8-week period. Collagen type II was identified within the pericellular and territorial matrix of chondrocytes in 3-D culture.
Glycosaminoglycan content—Glycosaminoglycan concentration was significantly higher (P = 0.014) in tibial defects of grafted group rabbits on day 14 than in all other groups. Glycosaminoglycan concentrations in tibial defects of grafted and control group rabbits and in the in vitro constructs that contained chondrocytes were all significantly higher than in the in vitro control constructs at weeks 2, 4, and 8 (Fig 7). No measurable GAG concentration was detected in any in vitro control constructs.

Discussion

Results of our study indicate that chondrocyte grafts in 3-D agarose medium are biocompatible when placed in tibial defects of rabbits. The 3-D constructs containing cells appear to provide a source of cells for augmenting early endochondral ossification. This augmentation may occur through direct participation in matrix formation, through induction of matrix production by host cells, and by providing a substrate for tissue conduction. Radiolabeling grafted cells could delineate the source of cells that participated in the process of bone formation in defects. However, centers of endochondral ossification were present in significantly more tibial defects of grafted group rabbits, compared with control group rabbits. This fact, in conjunction with the knowledge that in vitro constructs containing cells also had a histologic appearance of differentiated cartilage, lends credence to the theory that grafted cells survived after implantation and maintained differentiation to a degree that allowed for physiologic-like appearance and function. Agarose and alginate constructs containing cells or growth factors have seal-like appearance and function. Agarose and alginate grafts in 3-D agarose medium are biocompatible when implanted in tibial defects of rabbits. The 3-D constructs. Collagen type II is the predominant collagen from grafted group rabbits and in vitro cellular constructs. This pattern of ossification was present. These data suggest that graft-derived defects reached highest concentrations 2 weeks after implantation, then decreased to concentrations similar to those of tibial defects of control group rabbits and in vitro cellular constructs. This pattern of GAG production is likely related to differentiated chondrocyte stimulation via biomechanical forces and endogenous physiologic factors. These influences act as stimuli for proliferation, differentiation, and synthesis through cell signaling and mechanotransduction mechanisms. The histologic data corresponding to this time frame supports these conclusions. Later, when GAG concentrations were similar between in vivo groups, findings on histologic evaluation also revealed similarities in tissue type (eg, bone) that would account for the changes in GAG concentrations.

Findings on histologic evaluation supported the biocompatibility of 3-D agarose-chondrocyte grafts. A minimal inflammatory response was observed in association with the implants. This response is typical to what is expected in any bone-healing situation. No long-term detrimental effects of inflammatory or immunologic responses were evident. Toluidine blue staining of sections revealed marked differences among groups. In tibial defects of grafted group rabbits, evidence of earlier and more substantial endochondral ossification was present. These data suggest that grafted cells maintained viability and phenotype and contributed to cartilage-specific matrix production through the process of endochondral ossification. These data strongly suggest that cultured cells survived transplantation and participated in the endochondral ossification process.

In our study, data obtained by collagen immunohistochemistry support the assumptions of transplanted chondrocyte survival and maintenance of differentiation. Collagen type II was observed in tibial defects from grafted group rabbits and in vitro cellular constructs. Collagen type II is the predominant collagen present in articular and physeal cartilage. Because the chondrocytes used in our study were of articular cartilage origin, it is likely that they maintained the ability to synthesize type II collagen through monolayer culture, 3-D culture, and implantation into the bone defect. Chondrocytes maintained in vitro continued to possess the ability to produce type II collagen throughout the 8-week study period. Interestingly, these cells did not dedifferentiate to a phenotype capable of collagen type I production, nor was a phenotypic alteration allowing for type X collagen production observed. These findings are consistent with the culture environment created, the lack of biomechanical stimulation, and the absence of other physiologic stimuli that are present in vivo. Tibial defects from grafted group rabbits contained collagen type II and type X indicating phenotypic differences and a progression toward terminal differentiation of chondrocytes to a hypertrophic
phenotype (type X) during the process of endochondral ossification. Type I collagen was observed at the junction of cartilage and bone consistent with normal endochondral ossification as has been previously described.

In our study, chondrocyte-agarose constructs were placed in vivo 24 hours after 3-D culture. The effects of longer in vitro culture may have important in vivo implications with respect to temporal matrix production and should be investigated. Effects of 3-D culture with respect to viability, differentiation, and extracellular matrix production have been well documented and may play important roles for in vivo viability, biocompatibility, and tissue production.

Agarose implants were biocompatible and were a practical means of providing a differentiated cell source to a bone defect. Constructs were easy to handle and were able to be precisely sized to completely fill each defect. Constructs could be cut to fit any defect shape. Because of the ability to manipulate cell type and number, pre-implantation matrix production, and construct size and shape, chondrocyte-agarose constructs provide a potential method for tissue engineering of cartilage for physreal reconstruction and regenerate bone production in distraction osteogenesis.

The focus of our study was to determine the effects of 3-D chondrocyte grafts on large bone defects. Stable fibula defects as small as 3 mm in length reportedly will not reach bony union within 3 weeks in rabbits, and 2-cm-long defects consistently go to non-union. In our study, 1-cm-long stabilized fibula defects in rabbits were used in an attempt to address clinically relevant questions. Specifically, bone replacement, distraction osteogenesis, and treatment of physreal disorders are currently limited by biological, temporal, and anatomic factors. The ultimate treatment method for these disorders would appear to be recapitulation of the normal process of endochondral ossification. Clinically, articular chondrocytes can be readily harvested from non-weight-bearing areas of joints in an easy manner with minimal resultant morbidity. If chondrocytes can be harvested, cultured in agarose, and placed in a bone or physreal defect such as they optimize regenerate bone formation, then we believe an important clinical benefit can be realized. Results of our study provide an initial step in testing the feasibility of this technique. Provided that the results of our study can be verified by repeated investigation with further data analysis, 3-D chondrocyte grafts may have potential use in physreal reconstruction, distraction osteogenesis, cartilage resurfacing, and disorders of endochondral ossification by providing an early stimulus and substrate for osteoinduction, osteoconduction, and osteogenesis. Furthermore, the results of our study, with respect to in vitro constructs, demonstrate the usefulness of 3-D chondrocyte culture as a model for study of physiologic and pathologic responses of chondrocytes and cartilage.

References


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