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Mechanical characterization of matrix-induced autologous chondrocyte implantation (MACI®) grafts in an equine model at 53 weeks

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ABSTRACT

There has been much interest in using autologous chondrocytes in combination with scaffold materials to aid in cartilage repair. In the present study, a total of 27 animals were used to compare the performance of matrix-assisted chondrocyte implantation (MACI®) using a collagen sponge as a chondrocyte delivery vehicle, the sponge membrane alone, and empty controls. A total of three distinct types of mechanical analyses were performed on repaired cartilage harvested from horses after 53 weeks of implantation: (1) compressive behavior of samples to measure aggregate modulus (HA) and hydraulic permeability (k) in confined compression; (2) local and global shear modulus using confocal strain mapping; and (3) boundary friction coefficient using a custom-built tribometer. Cartilage defects receiving MACI® implants had equilibrium modulus values that were 70% of normal cartilage, and were not statistically different than normal tissue. Defects filled with Maix™ membrane alone or left empty were only 46% and 51–63% of control, respectively. The shear modulus of tissue from all groups of cartilage defects were between 4 and 10 times lower than control tissue, and range from 0.2 to 0.4 MPa. The average values of boundary mode friction coefficients of control tissue from all groups ranged from 0.42 to 0.52. This study represents an extensive characterization of the mechanical performance of the MACI® grafts implant in a large animal model at 53 weeks. Collectively, these data demonstrate a range of implant performance, revealing similar compressive and frictional properties to native tissue, with inferior shear properties.

1. Introduction:

Articular cartilage has limited ability for self-repair, and as such any defects leave the affected joint susceptible to osteoarthritis (Inerot et al., 1978). Both autologous chondrocyte implantation (ACI) and matrix induced autologous chondrocyte implantation (MACI®) have been shown to effectively repair full-thickness chondral defects as evidenced by histology and integration (Inerot et al., 1978). ACI involves only the delivery of cells to a cartilage defect. This technique has been used clinically for over two decades. Despite this extensive record, surgical challenges still persist including graft hypertrophy, the damaging of adjacent tissue by suturing, and graft delamination.

More recently, modified ACI techniques have been developed using a variety of materials to deliver transplanted chondrocytes (Brittberg, 2010). Specifically, matrix-induced autologous chondrocyte implantation (MACI®) involves the use of porcine-derived type I/type III collagen bi-layer membrane secured into cartilage defects using fibrin adhesive (Frenkel et al., 1997). MACI® grafts eliminate the need for periosteal harvest, help maintain chondrocyte viability and phenotype, and potentially allows for a more even distribution of cells in the defect (Willers et al., 2005).

Despite the widespread clinical use of such techniques, there is relatively little known about the mechanical properties of the tissue that results use of various cartilage repair techniques. Several studies

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report compressive properties obtained through indentation (Peterson et al., 2002; Henderson et al., 2007), unconfined (Laasanen et al., 2003), and confined compression tests (Strauss et al., 2005; Peretti et al., 2001). Confined compression testing on ACI repaired equine tissue showed an aggregate modulus that was only 12% of native tissue (Strauss et al., 2005). A MACI graft with a type II collagen membrane was shown to have an aggregate modulus that was 15% of native tissue (Lee et al., 2000). Stiffness tests in an ovine model showed that MACI® grafts ranged from 16% to 50% of native cartilage (Russlies et al., 2003; Jones et al., 2008). The duration of implanted tissue ranged from as short as a few weeks to as long as several years. A general trend shown in this data is that longer implant durations tend to perform better, indicating that mechanical properties of repaired cartilage may improve over time.

The current study was motivated by the lack of data on other critical mechanical properties of autologous chondrocyte grafts. For example, there are no published papers that study friction or shear properties of the repaired cartilage in long term large animal models. In this study, we performed an array of mechanical tests to more fully understand the functionality and mechanical behavior of matrix-induced autologous chondrocyte grafts. Therefore, the primary objective of this study was to characterize the compressive, shear, and friction properties of repaired articular cartilage after 53 weeks in an equine model.

2. Materials and methods

2.1. Chondrocyte isolation and expansion

Cartilage biopsies were obtained arthroscopically from the femoral trochlear ridge of 24 horses. Cartilage samples were enzymatically digested and expanded in vitro before seeding on sterile processed collagen type I/III membranes (ACI-Maix™; Genzyme Corporation). Cells were seeded at 0.5 million chondrocytes/cm^2 of collagen membrane, and covered with DMEM and incubated for 48 h before implantation. Additionally, a collagen type I/III membrane was cultured without chondrocytes, for use in control defects implanted with membrane alone.

2.2. Implantation surgery

A total of 27 skeletally mature horses (1.5–6 yr of age, 300–400 kg weight) free of lameness were used. Arthrotomy surgeries were performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Cornell University. Two full-thickness chondral defects (15 mm diameter) were created on the femoral trochlear ridge of one hind-limb of each horse, using a trephine and cannula. These lesions extended down to, but not through, the subchondral bone plate, and resulted in minimal bleeding or seepage of marrow into the defect. Specifically, defects were placed in both the proximal and distal region of the trochlea in either the right or left joint, with the contralateral joint left unoperated for control (Fig. 1). Animals were assigned to one of three cohorts based on treatment provided in each of the two defects: (1) MACI® graft in one defect and the carrier scaffold, consisting of the ACI-Maix (Matricel) membrane without the presence of cells in other defect ($n=12$); (2) MACI® graft and an empty defect ($n=12$); and (3) both defect sites left empty ($n=3$). The choice of operation on the right or left joint along as well as location within the trochlea was randomized, with all subsequent histological and mechanical analysis performed in a blinded fashion. After 53 weeks, the horses were euthanized and samples were immediately harvested for histological examination and mechanical assessment (Fig. 1).

2.3. Histology and immunohistochemistry

Samples were decalcified in hemotoxylin and eosin (H&E) or ethylenediaminetetraacetic acid (EDTA) and sectioned at 6 μm, as previously described (Nixon et al., 2011). Sections were stained with toluidine blue for histochemical reaction to cartilage GAG moieties. Serial sections were used for positive and negative reactions for collagen type II immunohistochemistry (Nixon et al., 2011).

2.4. Confined compression

A total of 108 full thickness cylindrical plugs (3 mm diameter) were harvested from the defect region using a biopsy punch perpendicular to the articular surface. The plugs were thawed in a bath of phosphate buffer saline (PBS) containing protease inhibitors. This procedure was repeated for samples harvested from the proximal and distal region of the trochlea in the control joint. Prior to testing, sample heights were measured using a caliper. Samples were placed in a 3 mm confining chamber, covered with a porous plug and PBS with protease inhibitors, and mounted in a Bose EnduraTEC ELF 3200 for stress–relaxation testing. A series of 5% steps in compressive strain were imposed on each sample up to a total of 40% strain. For each step, the resultant load was measured for 10 min using a Honeywell 50 lb load cell at a frequency of 1 Hz. The stress–relaxation curves were fit to a poroelastic model and analyzed using custom MATLAB code to calculate aggregate modulus (Ha) and hydraulic permeability (k) (Gleghorn et al., 2007; Chang et al., 2001).

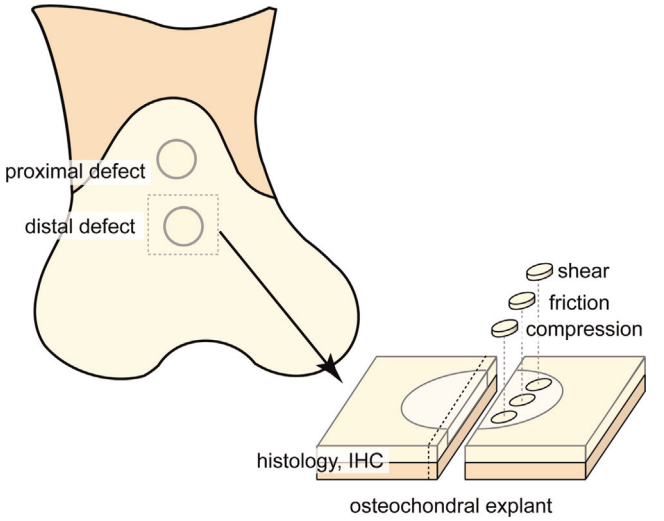

Fig. 1. Two 15 mm defects are placed into the trochlea of the right or left limb of each horse and either left empty, or filled with MAIX membrane or MACI graft. Osteochondral blocks were extracted after sacrifice with sections taken for histology containing both repair and local tissue and 3 mm plugs taken for each of shear, friction, and compression testing.

2.5. Friction testing

A total of 84 full thickness cylindrical plugs (3 mm diameter) were tested in a custom tribometer to measure boundary friction coefficients as previously described (Bonnevie et al., 2014; Gleghorn et al., 2007; Gleghorn et al., 2010; Galley et al., 2011). Samples were linearly reciprocated against glass at a speed of 0.1 mm/s under 40% strain while bathed in PBS, conditions known to induce boundary mode lubrication. During sliding, both normal and shear forces were collected to determine the friction coefficient. The friction coefficient was calculated by averaging shear force divided by normal force, for both directions of sliding.

2.6. Shear confocal strain mapping

A total of 84 full thickness cylindrical plugs (3 mm diameter) were prepared for confocal strain mapping as described previously (Griffin et al., 2014; Buckley et al., 2008, 2010, 2012; Silverberg et al., 2013). Samples were bisected longitudinally into hemi-cylinders, exposed to 7 μg/mL 5-dichlorotriazinyl–aminofluorescein (5-DTAF) for 2 h to uniformly stain the extracellular matrix, and rinsed in PBS for 30 min. Briefly, samples were glued to a tissue deformation imaging stage (TDIS) and compressed to 10% strain. The TDIS was mounted on an inverted Zeiss LSM 510 5 Live confocal microscope and imaged using a 488 nm laser. A line perpendicular to the articular surface was photobleached using the laser at full intensity. Sinusoidal shear displacements were placed on the articular surface by the TDIS at a frequency of 0.1 Hz and amplitude of 16 μm, and the resultant forces were measured with a load cell. Simultaneously, images of the sample deforming were collected at 10 frames per second. Using custom MATLAB code, the intensity minima corresponding to the location of the photobleached line was tracked, and the local strains

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