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Comparison of meniscal fibrochondrocyte and synoviocyte bioscaffolds toward meniscal tissue engineering in the dog

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ABSTRACT

Tissue engineering is a promising field of study toward curing the meniscal deficient stifle; however the ideal cell type for this task is not known. We describe here the extraction of synoviocytes and meniscal fibrochondrocytes from arthroscopic debris from six dogs, which were cultured as tensioned bioscaffolds to synthesize meniscal-like fibrocartilage sheets. Despite the diseased status of the original tissues, synoviocytes and meniscal fibrochondrocytes had high viability at the time of removal from the joint. Glycosaminoglycan and collagen content of bioscaffolds did not differ. Meniscal fibrochondrocyte bioscaffolds contained more type II collagen, but collagen deposition was disorganized, with only 30–40% of cells viable. The collagen of synoviocyte bioscaffolds was organized into sheets and bands and 80–90% of cells were viable. Autologous, diseased meniscal fibrochondrocytes and synoviocytes are plausible cell sources for future meniscal tissue engineering research, however cell viability of meniscal fibrochondrocytes in the tensioned bioscaffolds was low.

1. Introduction

The canine stifle menisci are c-shaped fibrocartilages which provide major weight-bearing functions, including proprioception (Zimny, 1988; Zimny et al., 1988) joint lubrication, (Clark et al., 1999) shock absorption, (Voloshin and Wosk, 1983) relief of femoral–tibial incongruity (Mow, 1992) load transmission, (Ahmed, 1983) and joint stability (Levy et al., 1982; Voloshin and Wosk, 1983). Meniscal extracellular matrix is composed primarily of type I collagen organized into circumferential bands bound by radial tie fibers (Fithian et al., 1990; Kambic and McDevitt, 2005). The menisci also contain type II collagen, located primarily in the axial region and around radial tie fibers (Eyre and Wu, 1983; Kambic and McDevitt, 2005) and glycosaminoglycans (GAG) (Adams and Ho, 1987; Nakano et al., 1997; Stephan et al., 1998), including aggrecan (Valiyaveettil et al., 2005).

This functionally critical fibrocartilage has limited healing capabilities; in particular, the avascular, axial white–white zone does not heal spontaneously. (Arnoczky and Warren, 1983; Kobayashi et al., 2004). Therefore, avascular meniscal injuries are treated with partial meniscectomy, to provide short term relief from clinical signs such as painful joint locking and popping. Unfortunately, partial meniscectomy does not replace the critical weight bearing functions of the meniscus and hastens the development of secondary arthritis (Berjon et al., 1991; Connor et al., 2009; Cox et al., 1975) which is increased proportionally to the amount of meniscal tissue resected (Berjon et al., 1991).

Tissue engineering through production of living replacement tissue may be one method for curing canine meniscal deficiency. Unfortunately, the ideal cell source and in vitro biomechanical and biological conditions for creating such a fibrocartilage implant have not been determined. The in vitro biomechanical environment may be particularly critical to the meniscal tissue engineering effort: application of biomechanical stimulation in vitro has a profound effect on extracellular matrix (ECM) formation due to the principles of mechanotransduction (Lavagnino and Arnoczky, 2005; Mauck et al., 2002). Type I collagen tends to form in tissues influenced by tensile forces, and GAG and type II collagen tend to form in tissues subjected to compressive forces (AufderHeide and Athanasiou, 2004; Benjamin and Ralphs, 1998; Kambic and McDevitt, 2005). To that end, the type I collagen of the abaxial 2/3 of the meniscus converts compressive weight bearing forces into tensile hoop strains, while the GAG and type II collagen of the axial meniscus sustain primarily compressive forces (Fithian et al., 1990; Mow et al., 1989). Thus successful tissue engineering of the meniscus will likely require a combination of tensile and compressive forces to induce functional ECM.

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Synovium is a non-weight bearing tissue lining the joint capsule, which produces hyaluronic acid and lubricin (Lee et al., 2010). Synovium contains loosely organized types I, III, and VI collagen, glycosaminogycans, fibronectin, and vitronectin (Ando et al., 2007, 2008; Okada et al., 1990; Price et al., 1996). Despite the dramatic differences in matrix architecture and function, synovium has been extensively investigated for meniscal tissue engineering and has been concluded to hold tremendous promise for this purpose (Fox and Warnock, 2011).

Autologous, osteoarthritic-joint origin synovium has been investigated as a cell source for fibrocartilage tissue engineering in dogs because of its ease of harvest, and in vitro (Warnock et al., 2011, 2012) and in vivo (Tienen et al., 2006) ability to synthesize fibrocartilage ECM. Additionally, canine synovium gathered during standard arthroscopic partial synovectomy can be used to synthesize collagenous tensioned bioscaffolds (Warnock et al., 2013). Use of normal synovium as a cell source would require violation of a healthy, unaffected joint, which is clinically undesirable. Healthy animal meniscal fibrochondrocytes have been used in meniscal tissue engineering research (Hoben et al., 2007; Tan et al., 2010); however, they also represent a poor cell source option for clinical application, requiring injury or sacrifice of a patient's unaffected meniscus. Axial meniscal tissue removed during partial meniscectomy is a logical source of autologous cells for reforming axial meniscal fibrocartilage in vitro. However, the synthetic capability and cell viability of meniscal fibrochondrocytes from diseased, excised meniscal tissue are unknown.

The long term goal of this research is to identify a viable and synthetically active cell source available for autologous meniscal tissue engineering purposes. Thus, the first objective of this study was to determine the viability and cell yield of autologous meniscal fibrochondrocytes obtained from arthroscopic meniscectomy debris in dogs, with the hypothesis that arthroscopic meniscectomy debris would yield fewer cells, with lower viability than arthroscopic synovectomy debris.

Surgically implanted collagenous sheets help regenerate the meniscus if the lesion extends to the vascular red zone (Cook et al., 1999, 2006a, 2006b) but not in white zone avascular lesions (Welch et al., 2002). Conceivably, tissue engineering a surgical implant consisting of a collagenous sheet of living tissue, which contains the ECM components of the axial meniscus, and can remodel and adapt to the intra-articular environment, could be helpful in guiding regeneration of lost axial meniscal tissue. Thus, toward engineering such an implant, the second study objective was to synthesize living, collagenous sheets. The collagen and glycosaminoglycan (GAG) content of meniscal fibrochondrocyte hyperconfluent monolayer cell sheets ('CS') was compared to tensioned meniscal fibrochondrocyte bioscaffold sheets, to determine the effect of long term culture with tension. The hypothesis was that with culture under tension as tensioned meniscal fibrochondrocyte bioscaffolds ('TMB'), meniscal fibrochdondrocytes would have higher GAG and collagen content relative to the meniscal CS, as has been determined in synoviocytes (Warnock et al., 2013). To compare the effect of cell type on collagenous sheet synthesis, cell viability and ECM formation were compared between meniscal fibrochondrocytes versus synoviocytes cultured as tensioned bioscaffolds (tensioned synoviocyte bioscaffold, or 'TSB'). We hypothesized that meniscal-like ECM content and cell viability of TMB would be greater versus TSB.

2. Materials and methods

2.1. Tissue harvest

With informed owner consent and Institutional Animal Care and Use Committee permission, synovial villi and meniscetomized debris were obtained from six dogs. Dogs received arthroscopy and TPLO for naturally occurring, chronic, non-contact cruciate ligament tears and medial meniscal bucket handle tears. Synovial villi which blocked the view of the cruciate ligaments and medial and lateral femoral condyles were arthroscopically resected as clinically required using a tissue shaver (Stryker, San Jose, CA) with a 3.5 mm aggressive shaver blade run at 1800 rpm, and were retained for culture as previously described (Warnock et al., 2012). All dogs had non-displaced, avascular medial meniscal bucket handle tears of the caudal body and horn. Torn tissue was removed by displacing the 'bucket handle' of meniscal tissue cranially with a hooked-tip arthroscopic probe (Arthrex, Naples FL); then the axial portion of the 'handle' at the meniscal body was released with a disposable arthroscopic push knife (Smith & Nephew, Inc., Andover MA); then the portion at the caudal meniscotibial ligament was released using an arthroscopic punch (Arthrex, Naples FL). The freed tissue fragments were removed using arthroscopic tissue graspers (Arthrex, Naples FL). Harvested synovial villi and meniscectomized tissue fragments were immediately placed in 50 ml polypropylene tubes containing 40 ml of Dulbeccos' Modified Eagle's Media (DMEM) with 10% fetal bovine serum (FBS), warmed to 37 °C and transported to the laboratory. Tubes containing synovial villi were centrifuged at 313 *g* and then media was decanted. Meniscal tissue was additionally sterily minced into 2–3 mm × 2–3 mm pieces using #10 Bard-Parker blade. Tissue fragments were transferred by pipette and/or sterile forceps into a digestion solution as will be described later.

2.2. Cell culture

All tissues were completely digested with sterile type 1A clostridial collagenase, 10 mg/ml, in RPMI 1640 solution (Invitrogen) at 37 °C. Prior to transfer to culture flasks, a 20 μl sample of tissue digest was analyzed with the trypan blue exclusion assay to determine harvest viability and cell counts. Cells were cultured in monolayer at 37.8 °C, 5% CO2, 95% humidity with daily media changes consisting of DMEM supplemented with 17.7% FBS and other additives ('SDMEM'; see Appendix). At the fourth passage (Han et al., 2010), cells were allowed to become hyperconfluent cell sheets, defined as cells overlapping each other in greater than 100% confluency. Upon spontaneous contraction off the flask floors, hyperconfluent cell sheets were removed from the flask in preparation for bioscaffold synthesis.

One meniscal fibrochondrocyte CS from three of the six dogs were harvested upon reaching hyperconfluence. One meniscal fibrochondrocyte CS was also retained from the fourth dog for real-time reverse transcriptase PCR. To determine the effect of long term culture with tension, the double stranded DNA (dsDNA), GAG, and collagen quantity of CS were compared to that of tensioned meniscal fibrochondrocyte bioscaffolds (TMB).

To compare the fibrochondrogenic potential of meniscal fibrochondrocytes versus synoviocytes, tensioned bioscaffolds were made from each cell type (TSB and TMB), using a previously described technique (Warnock et al., 2013). Briefly, hyperconfluent cell sheets were wrapped over 2.0 cm diameter, 22 ga cerclage wire hoops in three layers, with approximately 0.5 N of tension to avoid tearing. The TSB were placed in six-well plates in 9.0 ml of the supplemented DMEM described earlier, with the free end of the cell sheet facing down to prevent loosening.

All TSB and TMB were harvested for analysis after a total of 30 days in culture (Ando et al., 2008; Tan et al., 2010).

2.3. Tissue analyses

Tissue analyses examined the presence of ECM that is responsible for meniscal form and function, including type I collagen (Kambic and McDevitt, 2005), type II collagen (Kambic and McDevitt, 2005), α-smooth muscle actin (ASM) (Kambic et al., 2000; Spector,