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Adult equine bone marrow stromal cells produce a cartilage-like ECM mechanically superior to animal-matched adult chondrocytes

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ABSTRACT

Our objective was to evaluate the age-dependent mechanical phenotype of bone marrow stromal cell- (BMSC-) and chondrocyte-produced cartilage-like neo-tissue and to elucidate the matrix-associated mechanisms which generate this phenotype. Cells from both immature (2–4 month-old foals) and skeletally-mature (2–5 year-old adults) mixed-breed horses were isolated from animal-matched bone marrow and cartilage tissue, encapsulated in self-assembling-peptide hydrogels, and cultured with and without TGF-β1 supplementation. BMSCs and chondrocytes from both donor ages were encapsulated with high viability. BMSCs from both ages produced neo-tissue with higher mechanical stiffness than that produced by either young or adult chondrocytes. Young, but not adult, chondrocytes proliferated in response to TGF-β1 while BMSCs from both age groups proliferated with TGF-β1. Young chondrocytes stimulated by TGF-β1 accumulated ECM with 10-fold higher sulfated-glycosaminoglycan content than adult chondrocytes and 2–3-fold higher than BMSCs of either age. The opposite trend was observed for hydroxyproline content, with BMSCs accumulating 2–3-fold more than chondrocytes, independent of age. Size-exclusion chromatography of extracted proteoglycans showed that an aggrecan-like peak was the predominant sulfated proteoglycan for all cell types. Direct measurement of aggrecan core protein length and chondroitin sulfate chain length by single molecule atomic force microscopy imaging revealed that, independent of age, BMSCs produced longer core protein and longer chondroitin sulfate chains, and fewer short core protein molecules than chondrocytes, suggesting that the BMSC-produced aggrecan has a phenotype more characteristic of young tissue than chondrocyte-produced aggrecan. Aggrecan ultrastructure, ECM composition, and cellular proliferation combine to suggest a mechanism by which BMSCs produce a superior cartilage-like neo-tissue than either young or adult chondrocytes.

1. Introduction

Because of their capacity to undergo chondrogenesis (Barry et al., 2001; Johnstone et al., 1998; Pittenger et al., 1999), bone marrow derived stromal cells (BMSCs) have been the focus of numerous studies with the ultimate goal of repairing cartilage tissue damaged through disease or injury (Connelly et al., 2008; Kisiday et al., 2008; Mauck et al., 2006). Recent reports have suggested a robust chondrogenic and tissue forming capacity for BMSCs that is sustained with aging (Connelly et al., 2008; Im et al., 2006; Jiang et al., 2008; Scharstuhl et al., 2007), in contrast with primary chondrocytes which have decreased matrix synthesis and tissue repair potential with age (Barbero et al., 2004; Bolton et al., 1999; Plaas and Sandy, 1984; Tran-Khanh et al., 2005). This age-related behavior is particularly important given the potential advantages of using autologous tissue for cartilage repair (Chen and Tuan, 2008; Noth et al., 2008) making BMSCs an attractive candidate cell source.

Several recent studies have focused on encapsulation of BMSCs in 3D hydrogel culture with TGF-β1 or TGF-β3 stimulation to induce chondrogenesis and compared the differentiated cell phenotype with that of primary chondrocytes (Connelly et al., 2008; Erickson et al., 2009; Mauck et al., 2006). While these studies showed that chondrocytes produce a more cartilage-like and mechanically-functional extracellular matrix (ECM) than BMSCs, they all used skeletally-immature bovine tissue as the source for both cell types. Given that the relative chondrogenic potential of chondrocytes vs.

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BMSCs changes with age, evaluation of chondrocyte- and BMSC-seeded hydrogels at multiple times during development and aging is important.

To achieve cartilage repair, a successful cell-based strategy will be required to recapitulate the fine structure of the native cartilage ECM in order to produce a mechanically-functional tissue. Aggrecan, a large aggregating proteoglycan, is the primary cartilage ECM molecule that provides the compressive stiffness and load distribution functions of the tissue (Dudhia, 2005). Given the extensive changes in aggrecan biosynthesis (Kimura et al., 1981; Mitchell and Hardingham, 1982), processing (Buckwalter et al., 1994; Roughley and White, 1980), aggregation (Bolton et al., 1999) and degradation (Dudhia, 2005) with age, it will likely be important to evaluate the quality of aggrecan produced by any cell type used in a cartilage repair therapy. Numerous techniques exist for the study of aggrecan including chromatography (Hascall et al., 1994) and Western analysis (Patwari et al., 2000), which assess size distribution and cleavage products in an entire population of molecules, and imaging techniques such as electron microscopy (Buckwalter and Rosenberg, 1982) and atomic force microscopy (AFM; Ng et al., 2003), which allow for detailed measurements of individual molecules.

In this study, we hypothesized that adult BMSCs could produce mechanically-functional cartilage-like neo-tissue comparable to that of primary chondrocytes derived from animal-matched donors. Furthermore we hypothesized that neo-tissue quality for BMSC vs. chondrocyte cell sources would depend on the age of the animal donor. To test these hypotheses, equine bone marrow and cartilage tissue were both harvested from immature foal and skeletally-mature young-adult horses. BMSCs and chondrocytes were isolated and encapsulated in a self-assembling peptide hydrogel that has been shown to enhance TGF-β1 stimulated chondrogenesis of BMSCs and promote accumulation of an aggrecan and type II collagen rich neo-ECM (Kisiday et al., 2008; Kopesky et al., 2010). These peptides are being developed for use in cardiovascular (Davis et al., 2006; Hsieh et al., 2006), liver (Semino et al., 2003), and cartilage (Kisiday et al., 2002) repair, and have been successfully used in animal studies without inducing inflammation or immune response (Davis et al., 2006, Hsieh et al., 2006), making them candidate *in vivo* tissue engineering scaffolds.

Using dynamic compression testing, we measured the neo-tissue mechanical phenotype produced by BMSCs and chondrocytes from both young and adult animal sources after 21 days of culture. To understand the mechanisms which generate this mechanical phenotype, we quantitatively measured cellular content and ECM synthesis and accumulation. To further assess the quality of the ECM, proteoglycans were extracted and characterized by size-exclusion chromatography to examine the size distribution of proteoglycan monomers. Proteoglycan extracts were also purified and imaged by single molecule atomic force microscopy to enable detailed ultrastructural studies of individual aggrecan molecules.

2. Results

2.1. Cell viability

Both BMSCs and chondrocytes from foal and adult donors survived seeding in peptide hydrogels and were >70% viable one day post-encapsulation in the presence of TGF-β1 (Fig. 1). Similar viability was observed at day 1 in TGF-β1-free controls; however, by day 21, viability in TGF-β1-free controls decreased to 40%–50% for both cell types and both donor ages (not shown), consistent with previous studies (Mouw et al., 2007).

2.2. Mechanical properties

Both frequency and culture condition were significant main effects on dynamic stiffness (Fig. 2, $p<0.001$), and post-hoc pairwise comparisons on each main effect revealed significant differences between individual frequencies and between different culture

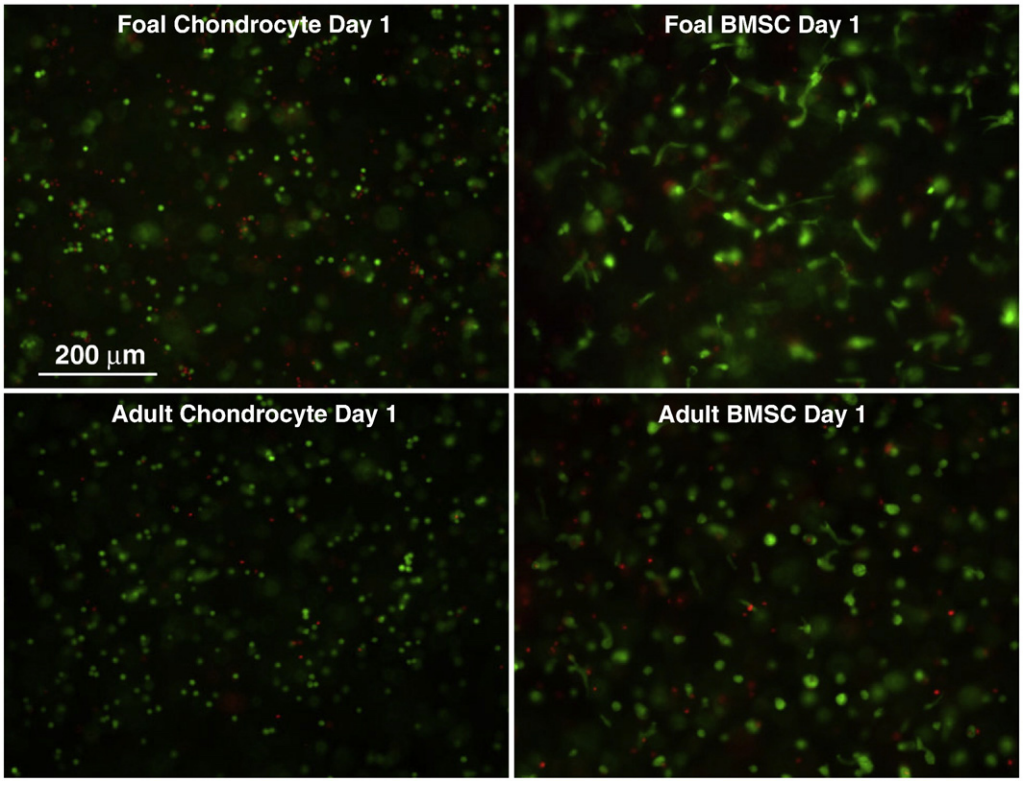

Fig. 1. Cell viability. Live (green) and dead (red) staining of self-assembling peptide hydrogels cultured with TGF-β1 at day 1.