INTRODUCTION

The menisci are fibrocartilaginous semilunar structures that function to transmit load across the knee [1]. These dense structures are populated by meniscal fibrochondrocytes (MFCs) that maintain the extracellular matrix (ECM) [2]. Damage to the menisci disrupts the structural integrity of the tissue, impairing its function and leading to cartilage damage [3]. The menisci are differentially vascularized, with a vascular periphery and an avascular inner region [4]. Tears in the outer periphery show limited repair, while those in the avascular inner regions fail to heal. This observation has led to the belief that vascularity is essential for healing, and consequently, surgical techniques have been designed to improve vascular access [4]. More recently, it has been observed in integration studies in explant cultures that different zones of the meniscus have differing healing potential, even in the absence of a blood supply [5]. It is generally accepted that endogenous repair is driven by multipotential cells within the tissue that migrate to injury sites, proliferate, and deposit new matrix. To determine if there exists a regional variation in this cellular component of repair, this study examined the multipotentiality of MFCs isolated from different regions of the meniscus: the outer (vascular), inner (avascular), and horn (mixed) regions. We hypothesized that MFCs from these regions possess different multi-lineage differentiation potential, and that regional variations in cell plasticity may modulate local healing capacity.

METHODS

MFCs were isolated from calves (3-6 month old, 3-5 donors/replicate, 4 replicates), by sectioning the meniscus into an inner (I), outer (O), and horn (H) zone, dicing, and plating the pieces in 10cm tissue culture plates in a basal medium consisting of high glucose DMEM containing 1XPSF and 10%FBS. Cells were allowed to grow out for 1-2 weeks, after which they were trypsinized, replated, and expanded through p2. Human mesenchymal stem cells (MSCs, p3-4) were used as a positive control for differentiation. For all studies, media were changed twice weekly for 21 days. To test for adipogenesis and osteogenesis, cells were plated at 20,000 cells/cm², and maintained in lineage specific differentiation media. Osteogenic medium (OS) consisted of DMEM supplemented with 10% FBS, 1X PSF, 10 nM dexamethasone, 10 mM β-glycerophosphate, 50 µg/mL ascorbate 2-phosphate and 10 mM 1,25 dihydroxyvitamin D3. Adipogenic medium (AS) consisted of DMEM supplemented with 10% FBS, 1X PSF, 1 µM dexamethasone, 1 µg/mL insulin and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX). Control (CT) cultures were maintained in basal medium (describe above). Cell number was determined using the MTT assay (Molecular Probes). OS samples were stained with Alizarin Red (2%, Rowley Biochemicals, Danvers, MA) for mineralized matrix. AS samples were stained with Oil Red O (ORO) to visualize lipid droplets, and ORO content was quantified by elution with isopropanol and absorbance measurements [6]. For both lineages, RNA was extracted with phenol-chloroform, reverse transcribed, and analyzed for adipogenic (fatty acid bind protein, FABP) and osteogenic (alkaline phosphatase, ALP) expression with real-time PCR [7]. For chondrogenesis, cells of each zone were formed into pellets containing 250,000 cells in 96-well polypropylene plates. Pellets were maintained in a chondrogenic medium consisting of hgdMEMM supplemented with 1X PSF, 0.1 µM dexamethasone, 50 µg/mL ascorbate 2-phosphate, 40 µg/mL L-proline, 100 µg/mL sodium pyruvate, 1X ITS+ with (CM+) or without (CM-) TGF-β3 (10 ng/mL). On day 21, several pellets from each MFC population were fixed, embedded, sectioned, and stained with H&E, Picosirius Red (PSR, collagen), or Alcian Blue (AB, proteoglycan). Bulk sGAG content (per pellet) was determined (6-8 pellet/replicate) after papain digest using the DMMB dye-binding assay [8]. RNA was extracted from monolayers and pellets (12-16 pellet/replicate), and cartilage ECM expression (aggrecan, Type I and Type II collagen) normalized to GAPDH using real-time PCR. Comparisons between groups were performed using t-tests.

RESULTS

Primary cells derived from different meniscal regions had a different morphology; inner cells were rounded while those from the outer zone were more spindle shaped. Culture in pellets in CM+ led to pronounced matrix production, with real-time PCR showing a marked induction of aggrecan (2-20 fold) and type II collagen (8-180 fold) gene expression in all MFC populations. In all pellets, abundant ECM deposition was observed, particularly in the periphery of MFC pellets (Figure 1). sGAG content increased with CM+ for each group (p<0.001, n=4), with no differences between groups on day 21 (I: 54±8, O: 66±5, H: 57±7, MSC: 61±3, µg/pellet). When cultured in AS conditions, MFCs increased expression of FABP by 10-100 fold (undetectable in CT). Oil Red O staining and quantification showed increases in lipid accumulation (Figure 2), with significantly more ORO bound in outer and horn MFC cultures (p<0.05 vs. inner, n=15). Cell number increased with AS treatment of inner MFCs (p<0.05), with no change in outer and horn MFCs (p=9). In OS, all cultures increased in cell number compared to CT (p<0.04, n=6-9). OS treatment resulted in an increase in ALP expression (treated versus control) in both inner and outer MFCs, and a small decrease in the horns (fold difference, I: 1.9, O: 3.5, H: 0.8, n=3). Furthermore, differing osteogenic potential was observed via staining of mineralized matrix, with the most deposition occurring in outer MFCs (Figure 3).

CONCLUSIONS

The results of this study demonstrate that meniscal fibrochondrocytes from all regions possess a multi-lineage differentiation potential. Of the three regions, cells from the outer zone were observed to differentiate along all three lineages, while those from the horn and inner regions failed to show convincing osteogenic potential. These findings suggest that the cells of the outer meniscus have a greater phenotypic range, and may be one reason that increased healing occurs in this region. The limitations observed in horn and inner region suggest that delivery of cells possessing a more complete differentiation spectrum, such as MSCs, may be necessary to enhance the repair of avascular meniscus defects.

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REFERENCES