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Applied osmotic loading for promoting development of engineered cartilage

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Abstract

This study investigated the potential use of static osmotic loading as a cartilage tissue engineering strategy for growing clinically relevant grafts from either synovium-derived stem cells (SDSCs) or chondrocytes. Bovine SDSCs and chondrocytes were individually encapsulated in 2% w/v agarose and divided into chondrogenic media of osmolarities 300 (hypotonic), 330 (isotonic), and 400 (hypertonic, physiologic) mOsM for up to 7 weeks. The application of hypertonic media to constructs comprised of SDSCs or chondrocytes led to increased mechanical properties as compared to hypotonic (300 mOsM) or isotonic (330 mOsM) media ($p < 0.05$). Constant exposure of SDSC-seeded constructs to 400 mOsM media from day 0 to day 49 yielded a Young's modulus of 513 ± 89 kPa and GAG content of $7.39 \pm 0.52\%$ ww on day 49, well within the range of values of native, immature bovine cartilage. Primary chondrocyte-seeded constructs achieved almost as high a Young's modulus, reaching 487 ± 187 kPa and $6.77 \pm 0.54\%$ ww (GAG) for the 400 mOsM condition (day 42). These findings suggest hypertonic loading as a straightforward strategy for 3D cultivation with significant benefits for cartilage tissue engineering strategies. In an effort to understand potential mechanisms responsible for the observed response, cell volume measurements in response to varying osmotic conditions were evaluated in relation to the Boyle–van't Hoff (BVH) law. Results confirmed that chondrocytes behave as perfect osmometers; however SDSCs deviated from the BVH relation.

Keywords

Chondrocytes; Synovium-derived stem cells; Tissue engineering; Cartilage; Static osmotic loading

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Conflict of interest statement

The authors certify that there is no conflict of interest related to the work presented in this manuscript.

Author contributions

All authors contributed to the conception and design of the study, collection and analysis of data, drafting and revising the manuscript, and gave final approval of this submitted work.

1. Introduction

The avascular nature of cartilage and the harsh loading environment intrinsic to the joint lead to a poor intrinsic healing capacity after joint injury, motivating the development of cell-based therapies for repair. We previously cultured juvenile bovine chondrocytes in 3D agarose hydrogels that achieved physiologic cartilage tissue properties (Bian et al., 2009; Lima et al., 2007; Lima et al., 2008; Mauck et al., 2003). Recently, the clinical potential of mesenchymal stem cells (MSCs) has motivated efforts toward their optimization for tissue engineering applications (Buxton et al., 2011; Gong et al., 2010; Huang et al., 2010; Lima et al., 2007; Moutos et al., 2010; Ronziere et al., 2010). Synovium-derived stem cells (SDSCs) have the potential for chondrogenic differentiation and aid in cartilage repair after damage in vivo (De Bari et al., 2001; Pei et al., 2008b). It has been established that cartilage and synovium develop from a common precursor pool (Archer et al., 2003).

SDSCs maintain their chondrogenic capacity regardless of donor age or disease condition (De Bari et al., 2001; Nagase et al., 2008) and have potential for tissue-engineering applications aimed at cartilage repair or regeneration (Bilgen et al., 2007; Han et al., 2010; Lima et al., 2007; Pei et al., 2008a; Pei et al., 2008b). We previously cultured SDSCs in a 3D hydrogel which achieved properties approaching those of native juvenile bovine cartilage. We also found that supplementation of 2D cultures with a growth factor cocktail was essential to SDSCs' production of cartilage-like tissue when encapsulated in 3D (Francioli et al., 2007; Sampat et al., 2011).

Our success in utilizing biomimetic chemical factors for priming SDSCs to grow functionally relevant cartilage grafts prompted us to manipulate another factor endemic to in situ cartilage, namely altered extracellular osmolarity, as an additional growth strategy (Sampat et al., 2011). The in situ osmotic environment of chondrocytes varies with proteoglycan content and tissue deformation (Urban, 1994; Urban et al., 1993). In response to osmotic loading, chondrocytes exhibit passive volume changes consistent with the Boyle-van't Hoff (BVH) relationship, which suggests that the chondrocyte behaves as a perfect osmometer. Unlike chondrocytes, the adherence of SDSCs to the BVH relationship has not been studied. SDSCs express the same aquaporin 1 water channels as chondrocytes so they may behave similarly (Mobasheri et al., 2010).

The typical in vivo osmolarity of human articular cartilage ranges from 350–450 mOsM depending on the zone (Koo et al., 2010; Urban et al., 1993). The loss of proteoglycans during osteoarthritis reduces the osmotic environment of cartilage by as much as 150 mOsM. Similarly, human synovium averages 400 mOsM (Shanfield et al., 1988). Relative to native osmolarity, standard chondrogenic culture media is hypotonic and typically 330 mOsM.

The transient response of chondrocytes to osmotic loading has been studied (Bush and Hall, 2001; Chao et al., 2006; Palmer et al., 2001); results have quantified osmolarity-induced effects on the biological response of chondrocytes in 2D and 3D culture (Koo et al., 2010; Oswald et al., 2011; Urban et al., 1993). Theoretical predictions of in situ chondrocyte osmolarity indicates that extracellular osmolarity does not vary significantly with physiologic loading (Oswald et al., 2008). Therefore, we assessed the properties of cells in 3D tissue engineered constructs, as a function of baseline osmolarity during long-term culture.

Our two-pronged experimental approach first assessed the effects of static osmotic loading on the mechanical and biochemical properties of 3D tissue-engineered constructs prepared from SDSCs and chondrocytes, and second, identified potential mechanisms responsible for the osmotic responses observed by evaluating cell volume changes in relation to the BVH

law. We utilized culture techniques, specific to each cell type, previously demonstrated to create functionally relevant tissue. We tested the hypothesis that application of a hypertonic, more physiologic environment (created by adding NaCl and KCl to 3D culture media) improves the biochemical composition and mechanical properties of the constructs, compared to hypotonic (300 mOsM) or isotonic media (330 mOsM).

2. Methods

2.1. Static osmotic loading

Articular cartilage and synovium were harvested from juvenile bovine knee joints (2–4 weeks old) and digested using collagenase type IV (Worthington) (Fig. 1). Following digestion, primary chondrocytes were encapsulated in 2% w/v agarose (Sigma-Aldrich) at a concentration of 30×10^6 cells/ml (Fig. 1) (Bian et al., 2009; Lima et al., 2007). SDSCs were seeded at a density of 1.76×10^3 cells/cm² and expanded for 2 passages with media containing alpha-minimum essential medium (α MEM), 10% fetal bovine serum (FBS), antibiotics (100 U/ml penicillin, 100 μ g/ml streptomycin, and amphotericin B), and a growth factor cocktail of 1 ng/ml TGF- β 1, 10 ng/ml PDGF- β β , and 5 ng/ml bFGF-2 (Barbero et al., 2003; Sampat et al., 2011). A negative isolation procedure was performed following the first passage (Pei et al., 2008a; Sampat et al., 2011). After passaging, SDSCs were encapsulated in 2% w/v agarose at a concentration of 60×10^6 cells/ml (Fig. 1) (Sampat et al., 2011). The gels were maintained individually in serum-free chondrogenic media (Dulbecco's modified Eagle's medium (DMEM), 1% ITS+Premix, 50 μ g/ml L-proline, 0.1 μ M dexamethasone, 0.9 mM sodium pyruvate, and antibiotics) plus 50 μ g/ml ascorbate and 10 ng/ml TGF- β 3 at an osmolarity of 330 mOsM overnight and then discs were cored to final dimensions (4 mm diameter, 2.34 mm thickness). Constructs were divided into chondrogenic media of osmolarities 300, 330, and 400 mOsM plus 50 μ g/ml ascorbate and 10 ng/ml TGF- β 3.

Medium of 300 mOsM was made by diluting DMEM (337 mOsM) to 300 mOsM with deionized water. Next, hyperosmotic media was prepared at 400 mOsM by the addition of NaCl and KCl at a ratio of 20.7 mol NaCl to 1 mol KCl, the same concentration as in DMEM. Standard chondrogenic media of 330 mOsM was kept as the isotonic control condition. TGF- β 3 was removed at day 21 for SDSCs and day 14 for chondrocytes, as previously established.

Mechanical testing was performed and biochemical composition was assessed for DNA, glycosaminoglycan (GAG), and collagen content. Time points for analyses occurred at days 0, 21, 35 and 49 for SDSC constructs and days 0, 14, 28, and 42 for chondrocyte constructs, based on removal of TGF- β 3. The equilibrium Young's modulus (E_Y) was determined under unconfined compression at 10% strain. The dynamic modulus (G^*) was determined by applying an additional $\pm 1\%$ strain at 1 Hz. The DNA content was determined using the PicoGreen kit (Invitrogen Co.). The collagen and GAG content were determined using the orthohydroxyproline and dimethylmethylene blue assays, respectively (Barbero et al., 2003; Stegmann and Stalder, 1967).

Samples for histological analysis were stained with Picrosirius Red and Alcian Blue to visualize collagen and GAG distribution, respectively. Immunohistochemical staining for type II and VI collagen (Abcam), cartilage oligomeric matrix protein (COMP) (Abcam), and transient receptor potential vanilloid 4 (TRPV4) (Aviva Systems Biology) was performed. Statistical analysis was performed with Statistica software using a two-way ANOVA with $\alpha < 0.05$ and Tukey's HSD *post hoc* test, with statistical significance achieved when $p < 0.05$. The two factors analyzed in the ANOVA were Factor 1 = osmotic media condition and Factor 2 = time. Factor 1 had three levels while Factor 2 had four levels. All data are presented as mean $\pm$ standard deviation.

2.2. Multi-differentiation potential of SDSCs

Following passage 1, SDSCs were plated at 50 cells/cm² and cultured in α MEM with 10% FBS and antibiotics, for 7 days. For adipogenic differentiation, the medium was replaced with DMEM with 10% FBS and antibiotics, plus 10⁻⁶ M dexamethasone, 0.5 mM isobutyl-1-methyl xanthine, 100 μ M indomethacin, and 10 μ g/ml insulin, for 14 days. Following differentiation, cells were fixed in 10% formalin and stained with Oil Red-O. For osteogenic differentiation, the medium was replaced with DMEM with 10% FBS and antibiotics, plus 10⁻⁷ M dexamethasone, 0.2 mM ascorbic acid, and 10 mM β -glycerolphosphate for an additional 14 days, followed by fixing with ethanol and staining with Alizarin Red (pH 4.2) (Pei et al., 2008b). As a negative control, SDSCs were expanded in serum-containing media before staining with Oil Red-O and Alizarin Red.

2.3. Cell volume measurements

In an effort to identify differences in the intrinsic osmotic behavior of chondrocytes and SDSCs, the volume response of isolated cells to variation in applied osmotic conditions was monitored. At passage 2, cells were trypsinized and allowed to recover for at least 3 h in FBS media. Cells were then independently suspended for 30 min in DMEM of the following osmolarities: 200, 300, 330, 400*, 500*, and 600* mOsM (*created by the addition of NaCl and KCl), at 4.0×10^4 cells/ml before measurements were taken at room temperature (~22 °C). Only passive volume response was evaluated in this study. Cell size was determined using the Beckman–Coulter Multisizer™ 4 particle counter at each osmolarity ($n = 3$ replicates from same initial cell population/osmotic condition; ~200,000 cells/replicate). The iso-osmotic condition, 330 mOsM, was established by standard culture media. Cell volumes (μ m³) were determined from measurements of cell diameter (μ m).

To determine the applicability of the BVH relation to both cell types, the following equations, taken from Katkov (2011), were utilized to calculate the osmotically inactive volume, ν_b , and plot the true BVH line (Katkov, 2011). The average normalized volume at the given osmolarity was found using:

$$\bar{\nu}_i = \frac{\sum_{j=1}^{S_i} \frac{V_{ij}}{V_{iso,j}}}{N_i} \quad (1)$$

where $V_{iso,j}$ is the iso-osmotic volume obtained at a particular j th replicate. The osmotically inactive volume was found using:

$$\nu_b = \frac{\sum_{i=1}^N [(x_i - 1)(x_i - \bar{\nu}_i)]}{\sum_{i=1}^N (x_i - 1)^2} \quad (2)$$

where x_i is the i th normalized inverse osmolarity and $\bar{\nu}_i$ is the average normalized cell volume at a given i th osmolarity. Each value of aniso-osmotic measurement was normalized to the individual iso-volume obtained for each replicate j ($j = 1, 2, 3$). The values obtained from Eqs. (1) and (2) were entered into the following equation:

$$\nu_{BVH} = (1 - \nu_b)x + \nu_b \quad (3)$$

from which the true BVH line was plotted. Deviation of the experimental data from the true BVH line was used to assess whether the cells behaved as ideal osmometers (i.e. within 5%) as determined using the following equation:

$$\alpha = \frac{\text{Abs}(\sum_{i=1}^N \{\bar{v}_i - [(1-\nu_b)x + \nu_b]\})}{\sum_{i=1}^N \bar{v}_i} \geq 5\%. \quad (4)$$

In cases where $\alpha \geq 5\%$, cellular behavior was not that of an ideal osmometer.

3. Results

3.1. Multi-differentiation potential of SDSCs in 2D

We tested the multi-differentiation potential of SDSCs by culturing in both adipogenic and osteogenic media. Fig. 2B and E shows evidence of lipid vacuoles and calcified nodules, confirming the mesenchymal potential of SDSCs. As expected, no adipogenic and osteogenic phenotype were detectable in the negative controls (Fig. 2C and F).

3.2. Effects of static osmotic loading in 3D

We revisited chondrogenic differentiation of SDSCs and chondrocytes, to test the hypothesis that hypertonic loading during culture enhances tissue properties. Representative gross morphology of the constructs revealed the greatest volume and tissue opacity for the 400 mOsM group for both SDSCs (Fig. 3) and chondrocytes, as compared to the 300 mOsM group ($p < 0.05$ for volume difference). At day 49, the SDSC constructs had the following average dimensions: 300 mOsM: diameter = 3.85 ± 0.07 mm, thickness = 2.49 ± 0.02 mm, 330 mOsM: diameter = 3.84 ± 0.06 mm, thickness = 2.52 ± 0.12 mm, and 400 mOsM: diameter = 4.40 ± 0.13 mm, thickness = 3.02 ± 0.08 mm (Fig. 3). At day 42, the 400 mOsM chondrocyte group had a larger average thickness than the other conditions ($p < 0.05$). The chondrocyte constructs had the following average dimensions: 300 mOsM: diameter = 4.12 ± 0.03 mm, thickness = 2.49 ± 0.03 mm, 330 mOsM: diameter = 4.01 ± 0.10 mm, thickness = 2.49 ± 0.02 mm, and 400 mOsM: diameter = 4.13 ± 0.06 mm, thickness = 2.58 ± 0.07 mm.

With the application of static osmotic loading, constructs seeded with either primary chondrocytes or passaged SDSCs responded most favorably to culturing under the most physiologic osmolarity ($p < 0.05$). With 400 mOsM static exposure, SDSC constructs exhibited significant increases in Young's modulus (Fig. 4A), reaching 513 ± 89 kPa on day 49, well within native values of immature bovine cartilage (400–1000 kPa). At day 49, the 400 mOsM group of SDSC constructs exhibited a 15-fold higher Young's modulus when normalized to the isotonic condition. Chondrocyte constructs followed a similar trend; the hypertonic condition led to the most favorable mechanical properties, reaching 487 ± 187 kPa (day 42). This value was significantly higher than the other two conditions ($p < 0.05$) (Fig. 4E), and was within native levels. The overall trends for equilibrium modulus were paralleled in dynamic modulus ($G^* 1$ Hz) values, although these were not within native levels (Fig. 4B and F). The results of each study were repeated and consistent.

The increased stiffness in response to hypertonic loading was accompanied by corresponding increases in GAG (Fig. 4C and G) and DNA content (Fig. 4D and H) for both studies. GAG content for both physiologically loaded SDSCs and chondrocytes were within native levels, ranging from 7–8% as a function of tissue wet weight (Fig. 4C and G). At day 49, a significant ~6-fold higher GAG (% ww) was observed in the 400 mOsM SDSC constructs ($7.39 \pm 0.52\%$ ww) as compared to the 300 mOsM constructs ($1.28 \pm 0.13\%$ ww) ($p < 0.05$). Chondrocyte constructs exposed to hypertonic conditions had significantly higher levels of GAG ($6.77 \pm 0.54\%$ ww) than the other conditions at day 42 ($p < 0.05$).

Collagen levels in SDSC constructs failed to reach native levels, unlike the mechanical properties and GAG content. In the 400 mOsM SDSC constructs at day 49, the collagen level was $1.32 \pm 0.27\%$ ww and was not significantly different from the 300 and 330 mOsM groups (β S1). The collagen content in the 400 mOsM chondrocyte constructs at day 42 was $1.53 \pm 0.57\%$ ww and was similar across groups (Table S1).

Because of the volume (and weight; see below) differences caused by culture in hypertonic medium, the absolute amount of collagen (per construct at day 49) for SDSCs was the highest in the 400 mOsM constructs ($297 \pm 63 \mu\text{g}$), with significantly less collagen in the 300 ($119 \pm 26 \mu\text{g}$) and 330 ($131 \pm 34 \mu\text{g}$) mOsM constructs ($p < 0.0005$). In contrast, the absolute collagen (per construct) for chondrocyte constructs at day 42 was more similar amongst the three treatments, with the 400 mOsM having only slightly higher content ($282 \pm 104 \mu\text{g}$) than the 300 ($233 \pm 22 \mu\text{g}$) ($p = 0.58$) and 330 ($273 \pm 47 \mu\text{g}$) ($p = 0.98$) mOsM groups. In addition to having the highest absolute collagen content, the hypertonic-loaded SDSC constructs also exhibited linearly increasing (as a function of time) ($R^2 > 0.99$) total volume and wet weight when these values were normalized to day 0 values at each time point (data not shown). The isotonic and hypotonic conditions did not lead to temporal variation of volume and wet weight.

Histological staining for GAG and collagen distribution indicated stronger peripheral staining for both 300 and 330 mOsM SDSC conditions. Staining of the hypertonic media condition showed a different distribution pattern with stronger central GAG and collagen staining (Fig. 5A and B). In chondrocyte constructs at day 42, all three media conditions showed similar uniform GAG and collagen distribution (Fig. 5C and D).

Immunohistochemical staining indicated the presence of COMP (Fig. 6A and E), type II (Fig. 6B and F) and type VI collagen (Fig. 6C and G), and TRPV4 (Fig. 6D and H) in both the SDSC and chondrocyte constructs that were cultured in 400 mOsM hypertonic media for the duration of the study (Fig. 6).

3.3. Boyle–van't Hoff relation

SDSCs exhibited a larger average cell diameter ($14.39 \pm 0.12 \mu\text{m}$) than chondrocytes ($13.25 \pm 0.02 \mu\text{m}$) at the iso-osmotic condition of 330 mOsM ($p = 0.0001$). The normalized cell volumes for both SDSCs and chondrocytes (iso-osmotic: 330 mOsM) were independently plotted against the inverse osmolarity (normalized to 330 mOsM) to evaluate agreement with the true BVH line for each cell type (Eqs. 1–3) (Fig. 7A and B). We employed Eq. 4 to assess deviation from the true line and found that chondrocytes deviated by 4% from the true BVH line, while SDSCs deviated by 8%. According to the customary 5% deviation criterion, therefore, it was determined that chondrocytes behave as ideal osmometers and SDSCs do not. According to the ideal BVH line, the normalized inactive osmotic volume (v_b) was determined to be 58% of the chondrocyte cell volume (Fig. 7B).

4. Discussion

In the current study, we investigated the potential of utilizing static osmotic loading as a 3D culturing technique for cartilage tissue engineering. The osmolarity of hypertonic medium (400 mOsM) was chosen to mimic the environment of native cartilage, while the osmolarity of hypotonic medium (300 mOsM) was chosen to mimic an osteoarthritic environment. The osmolarity of standard chondrogenic medium (330 mOsM) was used as the isotonic control. Our results support the hypothesis, that SDSCs and chondrocytes cultured in individually encapsulated agarose hydrogels are improved in cartilaginous properties by exposure to a hypertonic physiological osmolarity, compared to a hypotonic or isotonic environment.

In addition to the measured effects of extracellular osmolarity on tissue growth, we also found that, within 5–7 weeks of culture, SDSC constructs achieved a Young's modulus representative of native immature bovine cartilage, with corresponding GAG content also within native values (Fig. 4A–D) (Bian et al., 2009; Lima et al., 2007; Mauck et al., 2002). The equilibrium modulus, GAG content, and collagen levels achieved for SDSCs cultured in 400 mOsm media are higher than previously obtained after 56 days (E_Y : 267 ± 96 kPa, GAG: $5.19 \pm 1.45\%$ ww, collagen: $0.51 \pm 0.03\%$ ww) (Sampat et al., 2011). The mechanical and biochemical data suggests that the magnitude of the osmotic response may depend on cell type; nonetheless, growth of 3D constructs in hypertonic media enhances their chondrogenesis significantly ($p < 0.05$). Additionally, the results indicate that the 300 mOsm media may have a detrimental effect on the SDSCs as demonstrated by the DNA, GAG, and mechanical properties (Fig. 4A–D), although this same effect was not seen for the chondrocytes (Fig. 4E–H).

SDSCs and chondrocytes produce similar proteins, including COMP, link proteins, and sulfated GAGs (Pei et al., 2008b). The ECM formed by SDSC constructs that were cultured in hypertonic media was determined by immunohistochemistry to contain COMP (Fig. 6A) and types II (Fig. 6B) and VI collagen (Fig. 6C). This evidence supports the notion that a hypertonic environment promotes SDSC chondrogenesis and production of cartilaginous ECM.

Studies in multiple species have shown that static osmotic loading of chondrocytes during 2D passaging influences cell proliferation rate and subsequent properties achieved in 3D culture. Culturing human articular chondrocytes in 2D in serum-free media at a hypotonic osmolarity was found to increase cellular proliferation rate (Koo et al., 2010). Recent work from our lab indicates that hypertonic loading of mature chondrocytes in 2D culture leads to improved mechanical properties in 3D (Oswald et al., 2011). The current strategy demonstrates that, regardless of cell type, the osmolarity of the media during 3D culture affects the construct properties of both juvenile SDSCs and chondrocytes similarly.

To date, most studies have focused on evaluating aggrecan promoter activity and gene expression, and sulfate incorporation, in response to osmotic loading (Hung et al., 2003; Palmer et al., 2001; Urban et al., 1993). In this study, we explored the impact of hypertonic conditions created through the addition of the physiologic ionic osmolytes, NaCl and KCl, on construct properties (Urban et al., 1993). According to the literature, it is not definitive whether ionic and nonionic osmolytes lead to similar metabolic activity in chondrocytes. Preliminary work in our lab indicates a detrimental effect of hypertonic media created through a nonionic osmolyte, sucrose, on the culture of engineered constructs. With respect to ionic osmolytes, results may depend upon which solutes are used to increase osmolarity. The results of this study appear inconsistent with the above-mentioned work with mature chondrocytes, which indicated that hypertonic culture (prepared with NaCl) decreased cell number and per-cell GAG content in 3D cell constructs, despite the benefit of increased osmolarity shown in 2D cell culture (Oswald et al., 2011). However, these studies may not be inconsistent; differences may be due to variances in cell maturity and osmolytes used. The osmotic effects observed here should be considered to be specific to the application of hypertonic NaCl/KCl conditions on the two cell types used.

It should be noted that the 330 mOsm media was prepared from undiluted DMEM, whereas the 400 mOsm condition was made from diluted 300 mOsm hypotonic media. Therefore, the concentration of DMEM components and sodium bicarbonate were higher in the 330 mOsm media. The differences observed may be not only a response to the osmolarity but also to different concentrations of original media components. For this study, however, it was important to compare our experimental groups to our normal culture conditions used in

the lab. In a separate study to assess the impact of the higher DMEM component concentrations, two separate isotonic medias were evaluated, one in which the 330 mOsM media was made from the diluted 300 mOsM media with the addition of NaCl and KCl solutes and the other being standard undiluted 330 mOsM media. While the standard 330 mOsM media produced higher properties than the modified 330 mOsM media, both formulations were inferior to the 400 mOsM media condition, further corroborating the results of the present study.

The mechanisms that may be involved in the response of both SDSCs and chondrocytes to long-term culture in varying osmolarities remain to be elucidated. Based on previous studies that have evaluated the morphological response of cells to short-term changes in osmolarity, the $\text{Na}^+ - \text{K}^+ - \text{Cl}_2^-$ ion co-transporter (NKCC) and the TRPV4 ion channel are potential mechanisms to consider (Kerrigan et al., 2006; Lewis et al., 2011; Phan et al., 2009). TRPV4 is involved in the regulatory volume decrease response and is present in both chondrocytes and SDSCs (Fig. 6D and H). Recently, activation of TRPV4 was used to grow engineered cartilage with improved tensile properties (Eleswarapu and Athanasiou, 2013).

SDSCs and chondrocytes may be differentially responding to the osmotic environment and therefore it is unknown whether the same mechanisms are responsible for sensing and translating changes in osmolarity for both cell types. The SDSC constructs exposed to hypertonic media exhibited increased volume, wet weight, stiffness, GAG (%ww), and absolute collagen content. However, the volume, wet weight, and absolute content of collagen stayed relatively similar for all groups in the chondrocyte study. For chondrocytes, the increased tissue stiffness seen with the physiologic osmolarity could be attributed to the increased GAG content (Fig. 4G) whereas the increased GAG and collagen could both play a role for SDSCs. The decreased collagen production by the SDSCs may be due to a potentially mixed cell population despite the negative selection procedure or passaging (Edwards, 1994; Hallbeck, 2007; Smith, 2011; Vandenabeele et al., 2003).

To further support the claim that different mechanisms may be responsible for the results seen in this study, previously MSCs were isolated from umbilical cord blood due to their resistance to extended hypotonic lysis. MSCs were determined to have a higher ineffective cell volume (~60%) relative to other mammalian cell types (~30%) (Parekkadan et al., 2007). The normalized cell volume results in this study confirm that chondrocytes behave as ideal osmometers. In contrast, SDSCs do not behave as perfect osmometers along the entire range tested, and therefore do not satisfy the BVH law. The SDSC normalized cell volume measurements deviated from linear behavior at extreme hypotonic (200 mOsM) and hypertonic (600 mOsM) conditions. Possible causes for this disparate cellular behavior warrant further study.

Our findings establish the potential of SDSCs as a clinically relevant cell source for regenerative strategies for articular cartilage repair. Hypertonic loading is a straightforward 3D cultivation technique for both SDSCs and chondrocytes with significant benefits for cartilage tissue engineering strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jbiomech.2013.07.043>.

Fig. 1.

Schematic of the experimental groups. After 3D encapsulation in agarose, SDSC- and chondrocyte-seeded constructs were cultured with serum-free chondrogenic media of osmolarities 300, 330, and 400 mOsM. TGF-β3 was removed at Day 14 for chondrocytes and Day 21 for SDSCs, which were previously demonstrated to create functionally relevant tissue.

Fig. 2.

Multi-differentiation potential of juvenile bovine SDSCs in 2D monolayer culture. (A) and (B): Adipogenesis of SDSCs. After 7 days of expansion in serum-containing media, SDSCs were incubated in adipogenesis medium for an additional 14 days before staining with Oil Red-O. Lipid vacuoles are visible in (B). (C): SDSCs were expanded in serum-containing media before staining with Oil Red-O as a negative control. (D) and (E): Osteogenesis of SDSCs. After 7 days of expansion in serum-containing media, SDSCs were incubated in osteogenic media for an additional 14 days before staining with Alizarin Red. Calcified nodules are visible in (E). (F): SDSCs were expanded in serum-containing media before staining with Alizarin Red as a negative control. Bars: 50 μ m. (A) and (D): Stereoscopic images; (B), (C), (E), and (F): 20 $\times$ magnification.

Fig. 3.

Representative gross morphology of SDSC-seeded constructs at day 49. The 300 mOsM group (left) had final average dimensions of diameter = 3.85 ± 0.07 mm, thickness = 2.49 ± 0.02 mm, the 330 mOsM group (middle) had final average dimensions of diameter = 3.84 ± 0.06 mm, thickness = 2.52 ± 0.12 mm, and the 400 mOsM group (right) had final average dimensions of diameter = 4.40 ± 0.13 mm, thickness = 3.02 ± 0.08 mm.

Fig. 4.

(A) Equilibrium Young's modulus (E_Y), (B) Dynamic modulus (G^*), (C) glycosaminoglycan (GAG) (% ww), and (D) DNA content (μ g) for SDSC-seeded constructs cultured over 7-week study period. (E) Equilibrium Young's modulus (E_Y), (F) Dynamic modulus (G^*), (G) glycosaminoglycan (GAG) (% ww), and (H) DNA content (μ g) for primary chondrocyte-seeded constructs cultured over a 6-week period. Solid line indicates release time point (* $p < 0.05$). The results of each study were subsequently repeated and consistent. (For clearer interpretation of the hatching patterns in this figure legend, the reader is referred to the web version of this article for the high quality image.)

Fig. 5. Representative GAG and collagen staining (A) Alcian Blue staining (GAG) and (B) Picrosirius Red staining (collagen) for constructs seeded with SDSCs at days 0 and 49. (C) Alcian Blue staining (GAG) and (D) Picrosirius Red staining (collagen) for constructs seeded with primary chondrocytes at days 0 and 42.

Fig. 6.

Representative immunohistochemical staining for (A) COMP (green), (B) type II collagen (green), (C) type VI collagen (green), and (D) TRPV4 (green) for SDSC constructs and (E) COMP (green), (F) type II collagen (green), (G) type VI collagen (green), and (H) TRPV4 (green) for chondrocyte constructs cultured in 400 mOsM media at final time point. (I) Negative control image. Cell nuclei (A–I) stained with TOTO-3 (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 7.

Boyle–van’t Hoff (BVH) plots of average normalized data for (A) chondrocytes (4% deviation from BVH line) and (B) SDSCs (8% deviation from BVH line). True Boyle–van’t Hoff line (red) ($v_{BVH} = (1 - v_b)x + v_b$) plotted to determine inactive cell volume (v_b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)