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Short communication

Dynamic nanomechanics of individual bone marrow stromal cells and cell-matrix composites during chondrogenic differentiation

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ABSTRACT

Dynamic nanomechanical properties of bovine bone marrow stromal cells (BMSCs) and their newly synthesized cartilage-like matrices were studied at nanometer scale deformation amplitudes. The increase in their dynamic modulus, $|E^*|$ (e.g., 2.4 ± 0.4 kPa at 1 Hz to 9.7 ± 0.2 kPa at 316 Hz at day 21, mean ± SEM), and phase angle, δ, (e.g., $15 \pm 2°$ at 1 Hz to $74 \pm 1°$ at 316 Hz at day 21) with increasing frequency were attributed to the fluid flow induced poroelasticity, governed by both the newly synthesized matrix and the intracellular structures. The absence of culture duration dependence suggested that chondrogenesis of BMSCs had not yet resulted in the formation of a well-organized matrix with a hierarchical structure similar to cartilage. BMSC-matrix composites demonstrated different poro-viscoelastic frequency-dependent mechanical behavior and energy dissipation compared to chondrocyte-matrix composites due to differences in matrix molecular constituents, structure and cell properties. This study provides important insights into the design of optimal protocols for tissue-engineered cartilage products using chondrocytes and BMSCs.

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1. Introduction

During the past decade, various nanomechanical approaches have been used to understand the mechanical integrity of individual primary chondrocytes and chondrocytes associated with pericellular matrices, including compression (Koay et al., 2008), indentation (Darling et al., 2010; Darling et al., 2006; Sanchez-Adams et al., 2013; Wilusz et al., 2013), shear (Ofek et al., 2010) and tension (Trickey et al., 2004). These studies laid the background on how biomechanical signals play a major role in chondrocyte gene expression and biosynthesis. Besides chondrocytes, bone marrow stromal cells (BMSCs) were recently shown to be a promising alternative cell source for cartilage tissue repair (Mauck et al., 2006). When seeded within a variety of tissue engineering scaffolds and subjected to chondrogenic factors, BMSCs can undergo chondrogenesis within a few days, and produce PCMs mainly composed of types II and VI collagen, aggrecan and other macromolecules found in articular cartilage (Kopesky et al., 2010a). Aggrecan molecules synthesized in vitro by BMSCs harvested from immature and adult equines have significantly longer glycosaminoglycan (GAG) chains and higher nano-compressive stiffness than aggrecan synthesized by chondrocytes (Kopesky et al., 2010a; Lee et al., 2010b).

As previous studies have focused on the biochemical composition and elastic-like tissue-level mechanics of the BMSC-synthesized neocartilage matrices (Connelly et al., 2007; Kopesky et al., 2010b), little is known about the dynamic nanomechanical properties of individual cells. Similar to chondrocyte-associated matrix, the BMSC-associated matrix provides important functions in cell signaling and mechanotransduction (Millward-Sadler et al., 2000; Potier et al., 2010) in response to both static and dynamic loadings. Knowledge of the dynamic nanomechanical properties of the BMSC-matrix composites will provide a critical measure of the potential success of BMSC-based cartilage tissue engineering (Han et al., 2011b). Here, we adapted the atomic force microscopy (AFM)-based dynamic oscillatory nanoindentation (Han et al., 2011a) to assess the dynamic mechanical properties of individual BMSC-matrix composites. The dependence

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on dynamic frequency and culture duration was investigated to study the poro-viscoelastic properties of this composite, and its culture time-dependent evolution. The results were compared to our previous study of chondrocyte-matrix composites (Lee et al., 2010a) to highlight the differences in engineered products from these two cell sources.

2. Materials and methods

2.1. Cell culture and isolation

Bovine BMSCs were isolated and seeded into 2% alginate hydrogel beads at 20 × 10^6 cells/mL density, similar to the culture of chondrocytes (Ng et al., 2007). The beads were placed in chondrogenic culture medium containing high-glucose DMEM supplemented with 1% Insulin-Transferrin-Selenium (Sigma-Aldrich, St. Louis, MO), 100 nM dexamethasone (Sigma-Aldrich) and 10 ng/mL recombinant human transforming growth factor-β1 (R&D System, Minneapolis, MN) (Connelly et al., 2008). Culture medium was replaced every other day. Groups of BMSCs with their neo-matrices were released from the alginate beads on days 7, 14, and 21 and maintained in high-glucose DMEM for subsequent measurements.

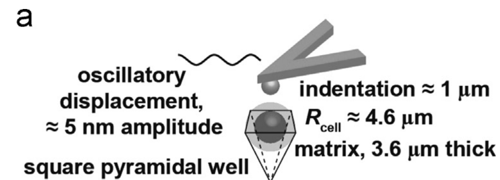
2.2. AFM-based dynamic oscillatory test

Aided by an optical microscope attached to an AFM, individual BMSCs or BMSC-matrix composites were positioned within microfabricated inverted pyramidal silicon wells (Fig. 1a) (Ng et al., 2007). Following previous procedures (Lee et al., 2010a), a Multimode Nanoscope IV AFM with a PicoForce piezo (BrukerNano, Santa Barbara, CA) and an electronic wave generator (Rockland 5100, Victoria, British Columbia, Canada) were used to perform dynamic oscillatory indentation on individual cells using a ~5 nm dynamic oscillation amplitude superimposed on a ~1 μm static indentation depth at dynamic frequencies, f (1–316 Hz), through spherical silica colloidal probe tips ($R_{\mathrm{tip}} \approx 2.5$ μm, #SS06N, Bang Labs; tipless cantilever, NP-020, $k \approx 0.06$ N/m, BrukerNano). At each time point, we tested $n \geq 10$ cells from $N=3$ animals; the data were pooled since we did not detect significant differences across animals ($p > 0.05$ through one-way ANOVA).

The resulting dynamic indentation force, F^*, displacement amplitude, D^* and phase angle of the force to the displacement, δ, were calculated for each test using a previously developed calibration algorithm (Han et al., 2011a). The dynamic indentation modulus, $|E^*|$, was calculated using a Taylor expansion of the elastic Hertz model (Mahaffy et al., 2004),

$$F^* = 2\frac{|E^*|}{(1-\nu^2)}R^{1/2}D_0^{1/2}D^*,$$

where D_0 is the static indentation depth, ν is the Poisson's ratio, and R is the reduced contact radius (i.e., $1/R=[(1/R_{\mathrm{tip}})+(1/R_{\mathrm{cell\text{-}matrix\ composite}})]$). In addition, to quantify the mechanical properties of individual cells, dynamic nanomechanical properties of freshly isolated BMSCs and knee cartilage chondrocytes of the same-aged bovine calves were measured on day 0, prior to seeding into an alginate gel, and therefore, prior to the development of cell-associated matrices. For all the tests, $\nu \approx 0.4$ measured on chondrocytes (Freeman et al., 1994) was used. For the PCM, though it was reported to be $\nu \approx 0.04$ (Alexopoulos et al., 2005), the effects of ν on $|E^*|$ (≈ 15% over $\nu=0.04$ to 0.4) do not affect our major conclusions.



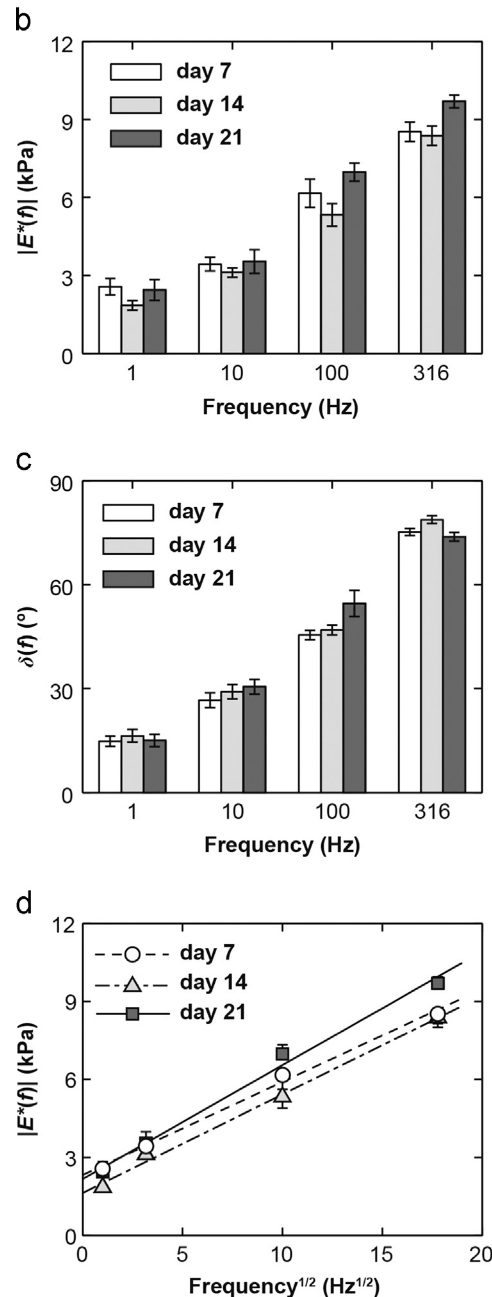


Fig. 1. (a) Schematic of AFM-based dynamic oscillatory nanoindentation on BMSC-matrix composites in a pyramidal silicon well using a spherical tip ($R_{\mathrm{tip}} \approx 2.5$ μm) in PBS. (b) Dynamic complex modulus $|E^*|$ and (c) phase angle, δ, of BMSC-matrix composites as a function of frequency, f, at 7, 14 and 21 days of culture. (d) Least squares linear regression of $|E^*|$ versus $f^{1/2}$ for BMSC-matrix composites at all three culture days, where dashed lines were the regression fits, $R^2 \geq 0.81$ ($n \geq 10$ cells, mean ± SEM for (b-d)).

2.3. Statistical tests

For individual BMSCs, chondrocytes, and BMSC-matrix composites, the effects of dynamic frequency on $|E^*|$ and δ were tested through one-way ANOVA, followed by Tukey-Kramer post-hoc tests.

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