Dynamic Compressive Loading Enhances Cartilage Matrix Synthesis and Distribution and Suppresses Hypertrophy in hMSC-Laden Hyaluronic Acid Hydrogels

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INTRODUCTION. Recent studies have demonstrated that dynamic compression enhances the expression of chondrogenic markers and cartilage matrix synthesis by mesenchymal stem cells (MSCs) encapsulated in various hydrogels, including agarose [1], alginate [2] and fibrin [3]. Another study showed that mechanical loading improves cartilage matrix distribution in agarose gels, leading to improved mechanical properties [4]. We previously identified hyaluronic acid (HA) hydrogels as stable 3D environments that are conducive to the chondrogenesis of MSCs in the presence of growth factors [5]. However, neocartilage in MSC-laden HA gels was inferior to that of native tissue and MSCs eventually developed into a hypertrophic phenotype under chondrogenic induction, resulting in calcification of the ECM after ectopic transplantation [6]. Mechanical signals can stimulate matrix production and organization in hydrogels and these same signals may regulate growth plate and articular cartilage chondrocyte hypertrophy via the IHH-PTHrP (India hedgehog, Parathyroid hormone-related protein) pathway [7; 8]. Therefore the objectives of this study were to evaluate the effect of dynamic compressive loading on (i) chondrogenesis and cartilage matrix production and distribution of human MSCs encapsulated in HA gels and (ii) the effect of dynamic compressive loading on hypertrophic differentiation of human MSCs and mineralization of MSC-seed HA gels using an in vitro MSC hypertrophy model [9].

MATERIALS AND METHODS. Methacyrlated HA (MeHA) was synthesized as previously reported [10]. Passage 3 human MSCs (Lonza) were photoencapsulated in 1.5% MeHA hydrogel disks (20 million cells/ml, Ø5mm, 2.5mm thickness) and cultured in chondrogenic media supplemented with TGF-β3 (10 ng/ml) for 70 days. In the MSC hypertrophy study, media was switched to hypertrophy induction or control media (+/-T3: with/without 1nM triiodothyronine (T3) and 10mM β-glycerophosphate (β-gly)) after 14 days of culture in chondrogenic media [9]. Dynamic compressive loading (5% tare, 10% peak to peak) was applied from day 3 to the end of culture period. Young’s moduli (E) and dynamic moduli (G*) of samples were calculated from static and dynamic (1Hz) unconfined compression testing. GAG and collagen content was determined via DMMB and hydroxyproline assay, respectively. Real-time PCR was performed using Taqman primers and probes specific for GAPDH (housekeeping gene) and other genes of interest. Samples for histological analysis were fixed in formalin and embedded in paraffin. The histological sections were stained for targets of interest using the Vectastain ABC kit and the DAB substrate kit for peroxidase (Vector Labs). Calcium content was quantified using a commercial kit (BioVision). Statistical comparisons were performed with Tukey’s HSD post hoc analyses with α=0.05.

RESULTS. Dynamically loaded (DL) constructs exhibited significant increases in Young’s modulus, dynamic modulus, GAG and collagen compared to the free swelling (FS) group after 70 days (Figure 1). Dynamic loading also improved the spatial distribution of chondroitin sulfate and type II collagen in HA hydrogels (Figure 2). When compared to the FS group, the expression of major hypertrophic markers (type X collagen, MMP13 and alkaline phosphatase (ALP)) were suppressed by DL in the presence (+T3) or absence (-T3) of hypertrophic inducing factors (T3, β-gly) (Figure 3).

DISCUSSION. In this study we successfully demonstrated that dynamic compressive loading enhances the biosynthesis of cartilage specific ECM by human MSCs in HA hydrogels, leading to superior mechanical stiffness compared to FS controls. Besides the elevated GAG and collagen contents, the improved mechanical properties in the DL constructs are also likely due to more uniform matrix distribution in the scaffold promoted by the loading. Furthermore, DL suppressed the hypertrophy of MSCs and decreased calcification in the engineered cartilage, possibly via its regulation in the IHH-PTHrP pathway. Future work is needed to elucidate the specific mechanism of mechanical regulation of MSC hypertrophy.

SIGNIFICANCE. The clinical implication of this finding is that joint loading, or in vitro loading in bioreactors for delayed implantation, may enhance the maturation of engineered cartilage and inhibit hypertrophic differentiation of MSCs, resulting in superior outcomes compared to immobilization of joint.


Figure 1. Young’s modulus (A), dynamic modulus (Hz) (B), GAG (C) and collagen (D) content of free swelling (FS) and dynamically loaded (DL) gels after 30 and 70 days. *p<0.05 vs. FS group at the same time.

Figure 2. Immunohistochemical staining for chondroitin sulfate and type II collagen on day 70, @4x: bar=1mm, @20x: bar=200µm.

Figure 3. Expression of hypertrophic markers in fold changes on day 30, *p<0.05 vs. FS+T3/-T3 group (n=4).

Figure 4. Calcium content (A) and Von Kossa staining (B) of the HA hydrogels on day 30, *p<0.05 vs. FS+T3 group (n=4); bar=500µm.

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