Variations in Media Formulation Impact ECM Synthesis and Retention in NP Cell-laden HA Hydrogels

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Introduction

Degeneration of the intervertebral disc (IVD) is a major cause of back pain, and tissue engineering has emerged as a promising method for the generation of replacement structures. Various growth factors and media formulations have been used to fabricate tissue engineered constructs using both progenitor and native tissue cells.[1] However, the media formulations that best produce engineered constructs during in vitro culture and maintain this state upon in vivo implantation remain to be determined. In particular, TGF-β3 has been shown to induce differentiation of MSCs, as is indicated by significant increases in chondrogenic gene expression and extracellular matrix production.[2,3] Dexamethasone, a synthetic glucocorticoid, is widely used to stimulate chondrogenesis and maintain the integrity of the cartilaginous matrix in chondrogenic cell lines.[4,5] In addition, ascorbate has been shown to play a role in collagen hydroxylation, and its absence impairs collagen secretion and assembly.[6] In the present study, starting from a chemically defined, serum free medium, we assessed the impact of three molecules (TGF-β3, ascorbate, and dexamethasone) on proteoglycan and collagen deposition by nucleus pulposus (NP) cells in a 3D hyaluronic acid (HA) hydrogel culture system, and further examined the persistence of these properties after implantation.

Methods

NP cells were isolated from adult bovine caudal discs and encapsulated in a 1% w/vol MeHA solution at a density of 20 million cells/ml.[7] Constructs (diameter: 4 mm, thickness: 2.25 mm) were cultured for 8 weeks in one of five media conditions: chemically defined media (CDM)[8,9] with/without 10 ng/ml TGF-β3 (+TGF-β3/−TGF-β3), CDM with TGF but lacking ascorbate (−Ac), CDM with TGF but lacking dexamethasone (−Dexa), or a basal, serum containing media (BM) supplemented with ascorbate (BM+Ac). For in vitro analysis, cell viability of constructs was assessed using the Live/Dead staining kit and unconfined compression tests were performed to determine construct mechanical properties as a function of different media formulations. For each construct, a constant 2g load was applied and creep displacement was monitored until equilibrium was attained (~300 s). Then, a stress relaxation test was carried out via a single compressive deformation to 10% strain at a rate of 0.05% s^{-1} followed by 20 min of relaxation to equilibrium, at which point stress and strain values were used to calculate equilibrium modulus. Sulfated glycosaminoglycan (s-GAG) and collagen content were assessed as in.[7] Sections were also stained with Alcian blue and picrosirius red to visualize proteoglycans and collagen, respectively. Based on in vitro study results, four formulations (+TGF-β3, −TGF-β3, −Ac, and −Dexa) were implanted into the rat subcutaneous space after 6 weeks of pre-culture. Biochemical and histological assessment of matrix deposition was performed 5 weeks after implantation (11 weeks).

Results

In the in vitro study, Live/Dead staining showed a similar number of live cells in all formulations except for BM+Ac, where the number of live cells was lower (Figure 1A). Mechanical properties of +TGF-β3, −Vc and −Dexa constructs were higher than all other constructs at 4 and 8 weeks, with no differences observed between the +TGF-β3 and −Dexa groups (Figure 1B). GAG content increased over time in all groups except for −TGF-β3 constructs, with the +TGF-β3 and −Dexa constructs reaching significantly higher levels than the −Ac constructs at 8 weeks (Figure 1C). Collagen content of the +TGF-β3 and -Dexa constructs were significantly higher than -Ac constructs at both 4 and 8 weeks (Figure 1D). In the in vivo study, after subQ implantation, proteoglycan staining decreased slightly for all groups (Figure 1E), while collagen staining increased markedly (Figure 1F), with similar trends observed via quantification of construct content (Figure 1C-D).

Discussion

This study explored the impact of different media formulations on the maturation of NP cell-laden HA hydrogels, both in vitro and after implantation into the rat subcutaneous space. Our findings demonstrate that inclusion of TGF-β3 is most essential for establishing disc-like mechanical properties and ECM content.

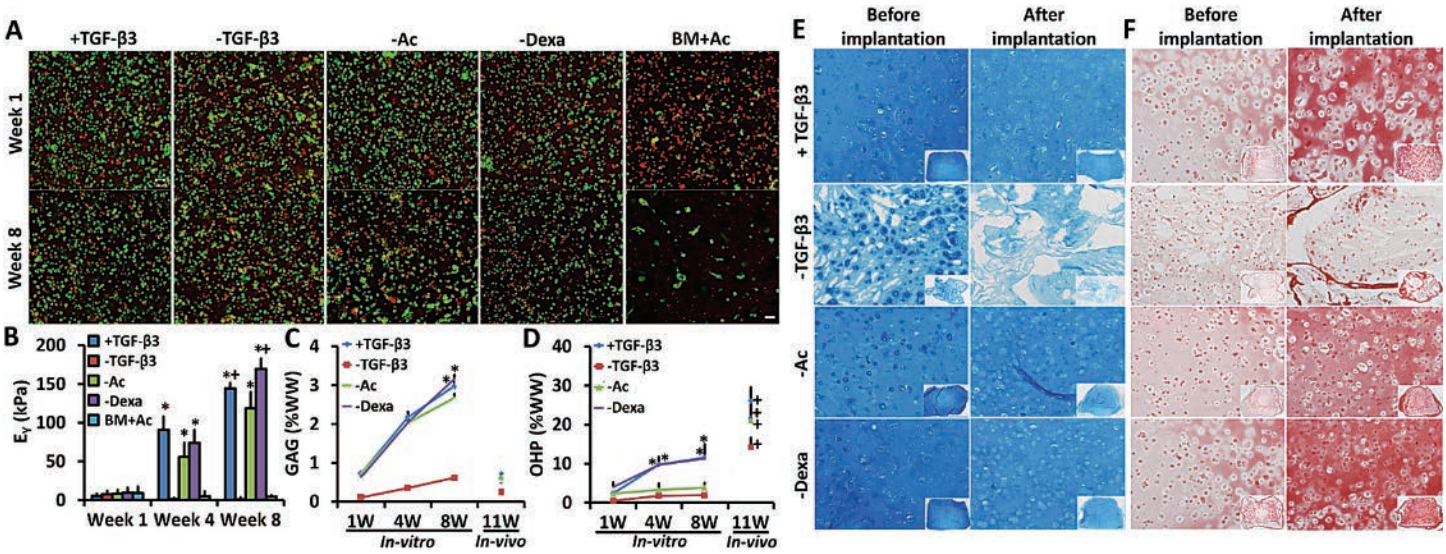

Figure 1. (A) Representative images of Live/Dead staining of NP cell-laden HA gels cultured in five different media formulations (Bar = 50 μm). (B) Equilibrium compressive modulus (n = 4~5, *:p < 0.05 vs. −TGF-β3, + :p < 0.05 vs. −Ac). (C) GAG content (n = 4 ~ 5, *:p < 0.05 vs. −Ac). (D) Collagen (OHP) content (n = 4 ~ 5, *:p < 0.05 vs. –Ac, + :p < 0.05 vs. 8 weeks). E: Alcian Blue staining of proteoglycans. F: Picrosirious Red staining of collagens.

Notably, removal of dexamethasone had no impact on construct properties, while removal of ascorbate resulted in lower mechanical properties and collagen content, consistent with the established role of this molecule in collagen crosslinking. Furthermore, we noted, for all media formulations, a decrease in proteoglycan content and marked increase in collagen deposition after subcutaneous implantation.

Significance

Our findings suggest that chemically defined media can be refined to promote NP cell-based construct maturation, but that transfer to the in vivo space alters construct composition and function, and so must be considered in any translational application.

Acknowledgements

This work was supported by the Department of Veterans' Affairs (I01 RX001321 and IK2 RX001476) and the Penn Center for Musculoskeletal Disorders (P30 AR050950).

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