Trajectory-based Tissue Engineering for Cartilage Repair: A Methodology to Better Predict In-Vivo Success

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Introduction

Given the limitations of current surgical approaches to treat articular cartilage injuries, tissue engineering (TE) approaches have been aggressively pursued over the past two decades, and recently, biochemical and biomechanical properties on the order of the native tissue have been achieved.[1-5] However, in-vitro and in-vivo data suggest that increased tissue maturity may limit the ability of engineered constructs to remodel and integrate with surrounding cartilage, although results are variable.[2,6-8] Thus, "static" measures of construct maturity (e.g. compressive modulus) upon implantation may not be the best indicators of in-vivo success, which likely requires implanted TE constructs to mature, remodel, and integrate with the host over time to achieve optimal results. In order to better predict in-vivo outcomes, it is hypothesized that time-dependent increases in construct maturation in-vitro prior to implantation (i.e. positive rates) may provide a better predictor of in-vivo success. The goal of this "trajectory-based" tissue engineering (TB-TE) approach is to maximize maturation rates before implantation. To explore this hypothesis, the current objective is to quantify and model the time course of maturation of TE constructs during in-vitro culture.

Methods

Bovine mesenchymal stem cells were isolated and cultured, as previously described.[1-2] Cells were encapsulated within methacrylated hyaluronic acid (HA) (1% w/v) at a seeding density of 20 or 60 million cells/ml (20M and 60M groups, respectively). Following polymerization via UV light, cylindrical constructs (4 mm diameter) were formed and cultured in chemically-defined media containing TGF-β3 on an orbital shaker for up to 9 weeks. At weekly intervals, stress relaxation testing (10% compressive strain, 1000s hold) and cyclic testing (1% amplitude, 1 Hz) were performed to determine equilibrium modulus and dynamic modulus, respectively. Glycosaminoglycan (GAG) content was measured by the DMMB assay, and its distribution determined by Alcian blue staining of histological sections.[5-6] Biomechanical and biochemical data were plotted versus time and fit individually with a sigmoidal curve ($y=C1*e^{(C2*e^{(C3*x)})}$). Using the determined parameters (C1, C2, and C3), the 1st derivative of the function was calculated. For statistical analysis, mechanical and biochemical data were compared between 20M and 60M groups at each time point using unpaired t-tests. Significance was set at $p<0.007$ following a Bonferroni correction. Experimental significance for the 1st derivative was defined as a difference greater than 20% of the peak value for the 60M group. Data were also normalized by their respective peak value to compare relative changes in mechanical and biochemical properties using unpaired t-tests ($p<0.002$).

Results

The equilibrium modulus data for both groups followed a sigmoidal shape as a function of time, with an initial lag phase for the first 3 weeks, followed by a linear region with increased slope, before plateauing at 7 weeks (Fig. 1A). Following a curve-fit of the modulus data, the plot of the 1st derivative had a parabolic appearance peaking around 5 weeks (Fig. 1B). Similar shapes were obtained for the dynamic modulus and GAG content. Comparing the values for the 20M and 60M groups (Fig. 2), at early time points (3 & 5 weeks), the equilibrium and dynamic modulus were similar between groups ($p>0.007$); yet, the 1st derivative of these parameters at 5 weeks were 233% and 87% higher for the 60M group, respectively (experimentally significant). By 9 weeks, the equilibrium and dynamic modulus of the 60M group was 193% and 60% higher, respectively ($p<0.007$); however, the rates of these parameters were similar between groups and near zero. To examine the relative changes in mechanics and biochemistry, values were normalized to their respective peak values (Fig. 3A). For the 60M group, GAG content was approximately 50% of the peak value at 3 weeks. In comparison, the relative values for moduli were only ~10% of their peak values at 3 weeks ($p<0.002$ vs. GAG). These values remained significantly lower than the GAG content up

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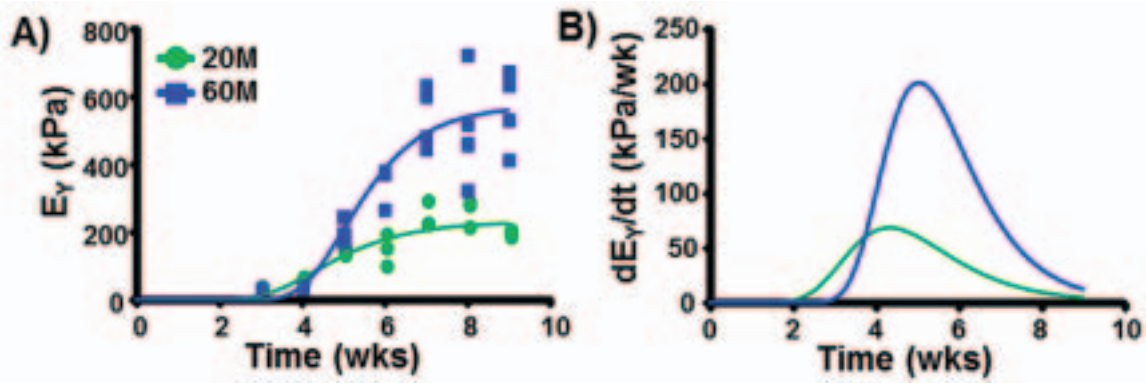

Figure 1. Maturation of MSC-laden HA constructs. **(A)** Equilibrium modulus as a function of time for 20M and 60M groups and respective model fits (solid lines). **(B)** First derivative of equilibrium modulus with respect to time.

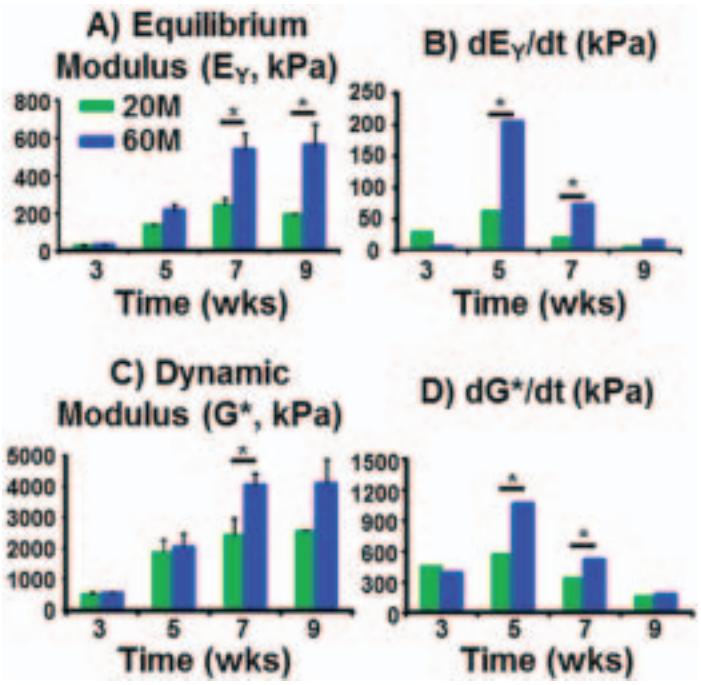

Figure 2. Comparison of 20M and 60M groups in terms of equilibrium modulus **(A)** and dynamic modulus **(C)** as well as their respective first derivatives **(B,D)**. (*p<0.0125 between groups, +difference greater than 20% of peak value for 60M group).

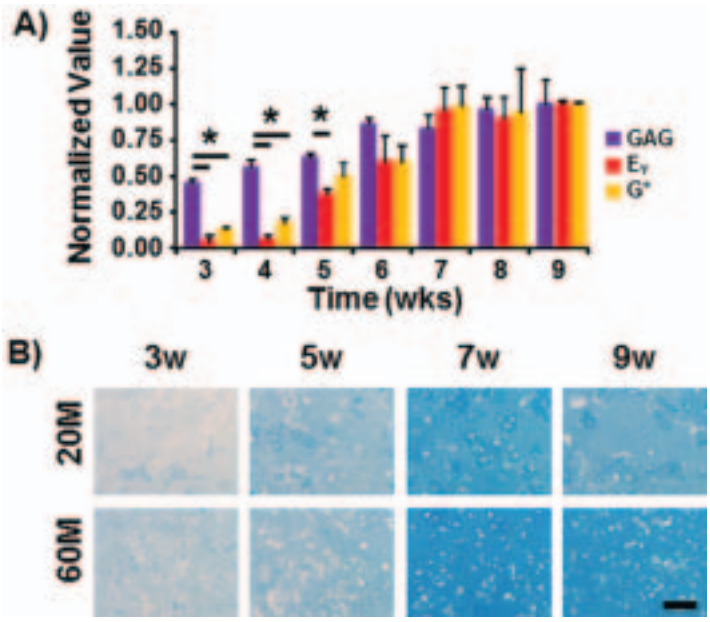

Figure 3. Relative increases in biochemical and biomechanical properties during maturation. **(A)** GAG, equilibrium modulus, and dynamic modulus normalized to their respective peak values (*p<0.002). **(B)** Histological staining for proteoglycans throughout maturation.

to the 5 week time point ($p<0.002$), before reaching similar relative values ($p>0.002$). Similar results were obtained for the 20M group. Histological staining for GAGs reflected the quantitative data, with notable changes in staining from 3 to 7 weeks and less drastic changes thereafter. Additionally, the 20M and 60M groups stained similarly at 5 weeks, but the 60M group had substantially more intense staining by 9 weeks.

Discussion

In this study, we performed an investigation into a TB-TE approach by quantifying and modeling the maturation of MSC-laden HA hydrogels during in-vitro culture. The constructs featured non-linear maturation profiles, with the peak rates occurring at 5 weeks. Furthermore, substantial differences in the rate of maturation could be determined for constructs of different cellular composition (changing seeding density). Thus, this system will allow us to test the overarching hypothesis of TB-TE within an in-vivo setting, which is that maximizing maturation rates, not states, prior to implantation will provide a better predictor of in-vivo success. Future work will seek to further increase maturation rates in-vitro through the use of chemical and mechanical stimuli.Additional analyses such as gene expression and more specific analysis of the matrix will be performed and correlated to mechanical data to identify other indicators of construct maturation. In summary, the current work provides a quantitative framework that will allow for the assessment of how constructs of differing maturation states and rates are able to appropriately remodel and integrate in-vivo, in order to optimize TE constructs to effectively restore function of injured cartilage.

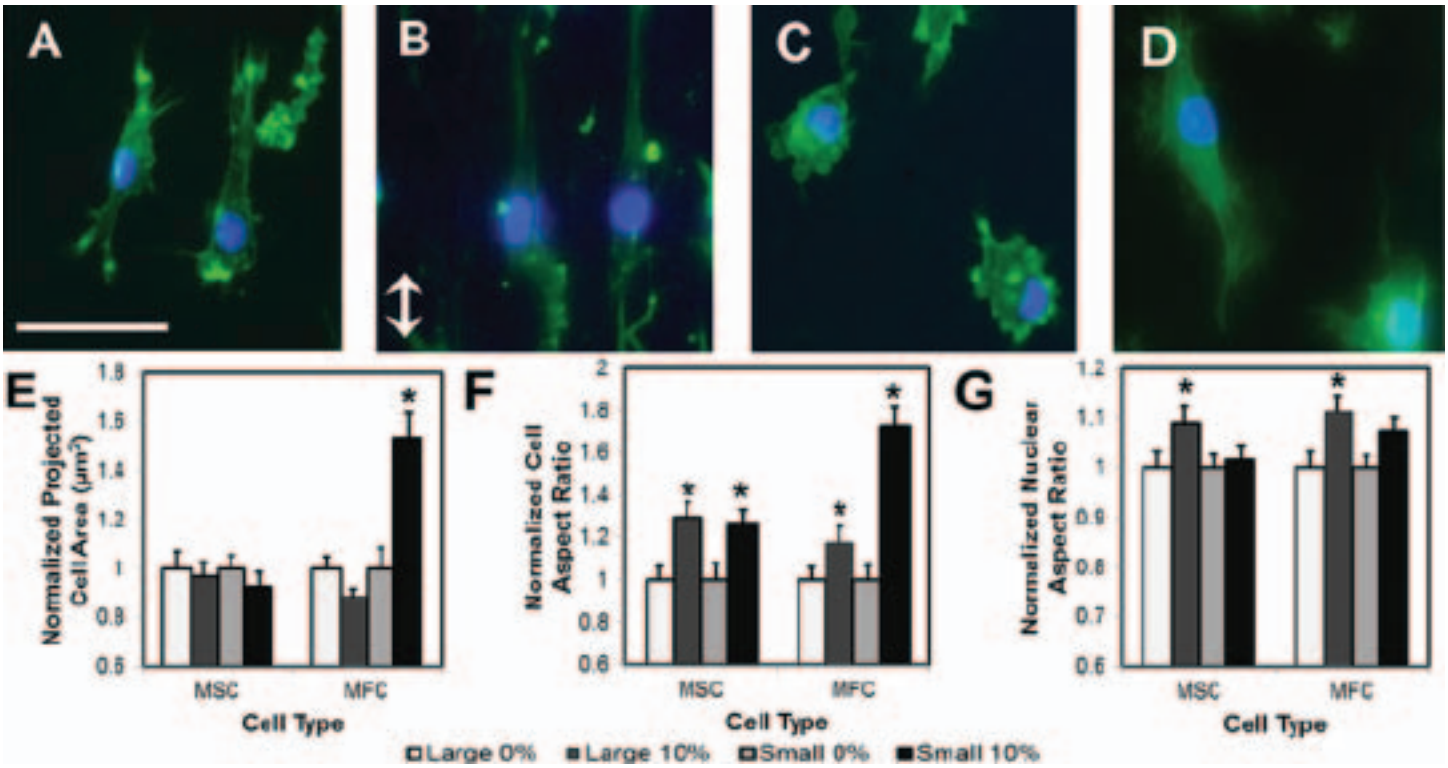

Figure 3. MFCs on large **(A,B)** or small **(C,D)** fiber scaffolds after 0% and 10% strain. Projected cell area **(E)**, CAR **(F)** and NAR **(G)** as a function of cell type and normalized to 0% strain. Data represents mean ± SEM. Scale bar = 50 μm. Arrow indicates fiber direction/direction of stretch.

Significance

This study provides an objective methodology to allow appropriate selection of TE constructs to maximize their in-vivo potential. Successful validation of this approach will allow better prediction of outcomes following implantation, thus enhancing their therapeutic potential.

Acknowledgements

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