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Trajectory-Based Tissue Engineering for Cartilage Repair: Impact of Maturation State and Rate on Integration Potential

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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. Critical biochemical and biomechanical properties on the order of native tissue have been achieved in a variety of TE contexts.[1-5] However, several in-vitro and in-vivo studies indicate that increased tissue maturity may limit the ability of engineered constructs to remodel and integrate with surrounding cartilage, although results from individual studies are highly variable.[1,6-8] We recently introduced the concept of "trajectory-based" tissue engineering (TB-TE), which is based on the general hypothesis 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 rather than "static" measures of construct maturation state.[9] As a first step toward evaluating this concept, we hypothesized that time-dependent increases in the biochemical and biomechanical properties of TE constructs (a metric of growth) would correlate with their ability to integrate to cartilage. To test this hypothesis, the current objective was to determine and model the time course of maturation of TE constructs during in-vitro culture and to assess their ability to integrate to cartilage at various points during maturation.

Methods

Bovine mesenchymal stem cells (MSCs) were isolated and cultured, as previously described.[1] Cells were encapsulated within methacrylated hyaluronic acid (HA) (1% w/v) at a seeding density of 60 million cells/mL. Cylindrical constructs (4 mm diameter) were formed via UV polymerization and cultured in chemically-defined media containing TGF-β3 for up to 17 weeks. Stress relaxation testing (10% compressive strain, 1000s hold) and cyclic testing (1% amplitude, 1 Hz) were performed at weekly intervals (n=4-5/timepoint) to determine equilibrium and dynamic modulus, respectively.[1,2] Collagen and glycosaminoglycan (GAG) content were quantified via the ortho-hydroxyproline and 1,9-dimethylmethylene blue assays.[1,2] Biochemical and biomechanical 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. To determine integration capacity, juvenile bovine cartilage explants (8 mm diameter) were obtained, trimmed, and cored (4 mm diameter) as previously described.[1] TE constructs at 1, 2, 3, 4, 5, 6, 8, and 11 weeks of culture were press-fit into the cartilage rings and cultured in chemically-defined media containing TGF-β3 for 6 weeks. Cartilage cores were also placed back into the cartilage rings as a control. At 3 and 6 weeks, integration testing was performed (n=6) as previously described[1,6,7] using a materials testing machine and indenting with a cylindrical flat ended indenter (4 mm diameter) until failure. The peak force was divided by the area of integration to determine the integration strength, which was then normalized with respect to the cartilage control. Histological (n=2) assessments were performed to visualize GAG at the interface (Alcian Blue stain). Statistical Analysis: Pearson correlation coefficients were calculated between the biochemical and biomechanical data or their 1st derivatives and integration strength, with statistical significance set at $p<0.05$.

Results

The equilibrium modulus of the MSC-seeded HA constructs followed a sigmoidal growth trajectory over time, with an initial lag phase for the first 3 weeks, followed by a linear region with increased slope, before slowing down by 7 weeks (Figure 1A). The 1st derivative of the modulus was parabolic over time, peaking at ~5 weeks (Figure 1B). Similar findings were obtained for the dynamic modulus as well as the GAG and collagen content (data not shown). In terms of integration, TE constructs implanted after 4-6 weeks of pre-culture reached the highest values for integration strength at both 3 and 6 weeks. No significant correlation was found between equilibrium modulus or dynamic modulus of the constructs at implantation and the resulting integration strength at 3 weeks (R^2=0.01 and 0.01, respectively, $p>0.05$) (Figure 1C, Table 1).

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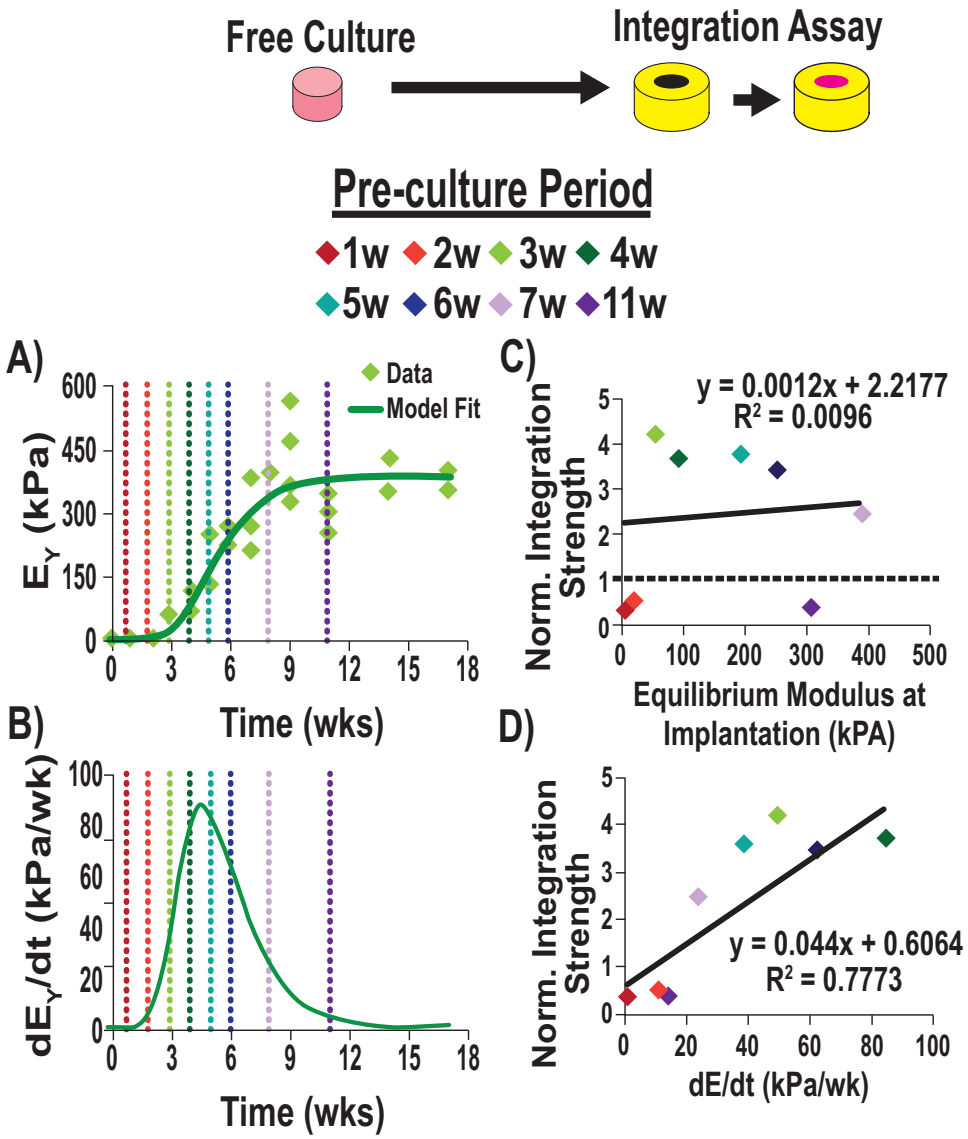

Figure 1. Correlation of maturation with integration capacity. Equilibrium modulus (A) and its first derivative (B). Dashed lines represent duration of pre-culture before initiating integration assay. Correlations between integration strength and equilibrium modulus (C) and its first derivative (D) of constructs at time of implantation.

Table 1. Correlations between integration strength and biochemical and biomechanical properties of constructs at the time of implantation and their first derivatives (*p<0.05).

	Normalized Integration Strength			
	3w		6w	
Values	**p-value**	**R^2**	**p-value**	**R^2**
Equilibrium Modulas	0.817	0.01	0.432	0.11
Dynamic Modulas	0.861	0.01	0.406	0.12
GAG Content	0.837	0.01	0.457	0.10
Collagen Content	0.794	0.01	0.186	0.27
1st Derivative				
Equilibrium Modulas	0.004*	0.78	0.013*	0.67
Dynamic Modulas	0.002*	0.82	0.004*	0.78
GAG Content	0.010*	0.70	0.002*	0.73
Collagen Content	0.027*	0.59	0.222	0.24

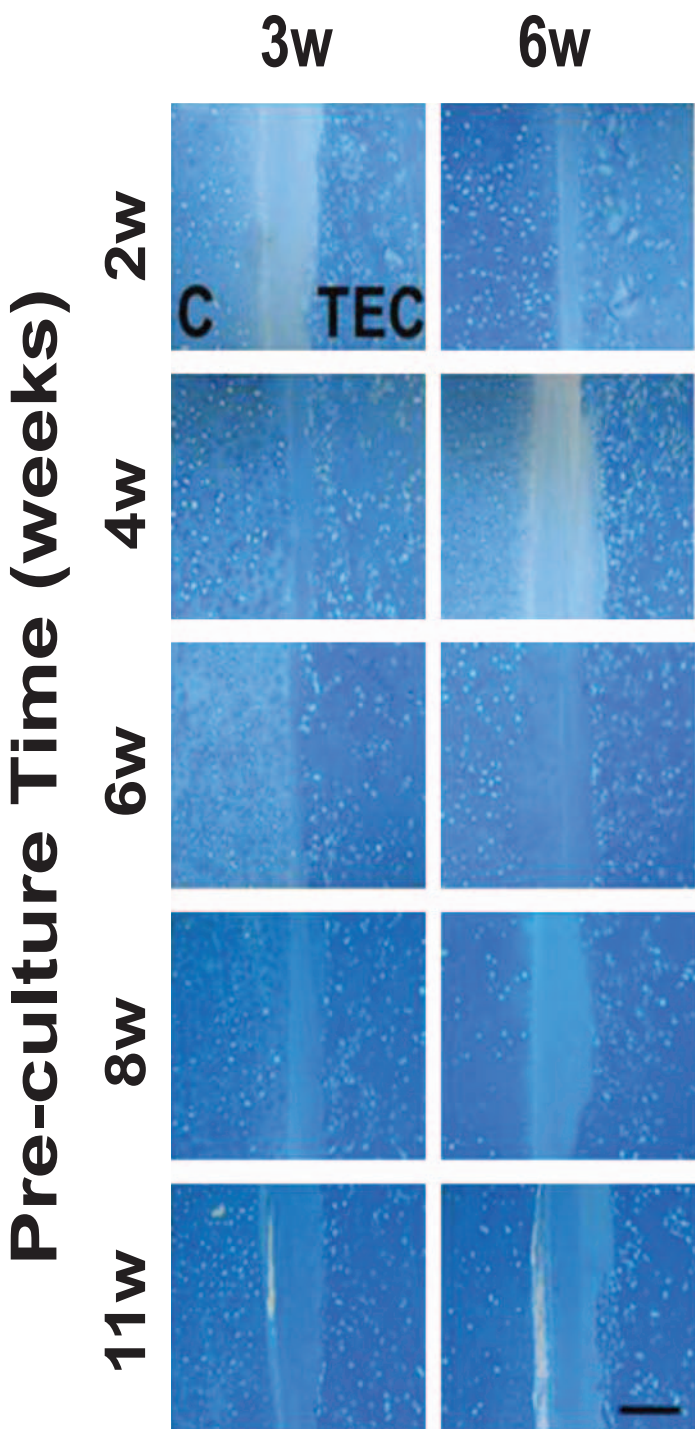

Figure 2. Histological staining for proteoglycans after integration for 3and 6 weeks (C-TEC= cartilage-tissue engineered construct). TECs were cultured for varying durations of pre-culture prior to implantation (scale bar = 200μm).

Similar findings were obtained for the GAG and collagen content (R^2=0.01 and 0.01, respectively, p>0.05, Table 1). However, a clear correlation was achieved between the first derivative of all biochemical and biomechanical measures and integration strength (R^2=0.59-0.86, p<0.05) and R^2 values ranging from 0.67 to 0.83 for their 1st derivatives (p<0.05, Table 1). These data were confirmed via histological assessment (Figure 2). The greatest integration occurred with constructs that had been pre-cultured for 4-6 weeks, with dark and homogenous staining across the interface, while earlier or later pre-culture periods showed incomplete integration with diffuse staining.

Discussion

In this study, we modeled the maturation of MSC-laden HA hydrogels during in-vitro culture and examined the importance of time-dependent parameters on the ability of these constructs to integrate to cartilage. In support of our hypothesis, the integration strength of constructs to cartilage was linearly correlated to the change in biochemical and biomechanical properties as a function of time (its rate), but not the static levels of these properties. Previous studies have attempted to correlate construct maturation to its ability to integrate to cartilage both in-vitro and in-vivo, with conflicting results.[1,6-8] The current data suggest that a TB-TE approach may be able to resolve these differences by highlighting the importance of time-dependent maturation rates, rather than static measures of maturation, allowing determination of an optimal period for in-vivo implantation. Ongoing and future work will extend these findings to the investigate maturation states and rates at the time of implantation to in-vivo outcomes using a large animal model of cartilage repair.

Significance

This study provides an objective methodology by which to appropriately select 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.

Acknowledgments

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