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Maximizing cartilage formation and integration via a trajectory-based tissue engineering approach

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ABSTRACT

Given the limitations of current surgical approaches to treat articular cartilage injuries, tissue engineering (TE) approaches have been aggressively pursued. Despite reproduction of key mechanical attributes of native tissue, the ability of TE cartilage constructs to integrate with native tissue must also be optimized for clinical success. In this paper, we propose a "trajectory-based" tissue engineering (TB-TE) approach, based on the hypothesis that time-dependent increases in construct maturation in-vitro prior to implantation (i.e. positive rates) may provide a reliable predictor of in-vivo success. As an example TE system, we utilized hyaluronic acid hydrogels laden with mesenchymal stem cells. We first modeled the maturation of these constructs in-vitro to capture time-dependent changes. We then performed a sensitivity analysis of the model to optimize the timing and amount of data collection. Finally, we showed that integration to cartilage in-vitro is not correlated to the maturation state of TE constructs, but rather their maturation rate, providing a proof-of-concept for the use of TB-TE to enhance treatment outcomes following cartilage injury. This new approach challenges the traditional TE paradigm of matching only native state parameters of maturity and emphasizes the importance of also establishing an in-vitro trajectory in constructs in order to improve the chance of in-vivo success.

1. Introduction

Given the limitations of current surgical approaches to treat articular cartilage injuries [1–5], tissue engineering (TE) approaches have been pursued extensively over the past two decades. Using a variety of scaffolding materials, cell types, and culture conditions, engineered tissues with biochemical (e.g. glycosaminoglycan (GAG) content) and biomechanical properties (e.g. compressive modulus) on the order of the native tissue have been achieved with extended in-vitro culture durations [6–13] (Fig. 1A). Despite this progress, the ability of these TE cartilage constructs to integrate with native tissue must also be optimized for successful clinical therapies to be realized. Indeed, functional integration may be just as important (if not more) than functional properties of the construct itself [14]. Failure to integrate results in marked stress concentrations at the implant boundaries, predisposing both the construct (and the surrounding native tissue) to further degenerative processes [15,16].

The cartilage tissue engineering community has not yet come to a consensus on the best means by which to integrate an engineered cartilage construct with the native tissue [6,17–20]. The prevailing notion is that as TE cartilage constructs mature, their ability to integrate into the native tissue is diminished (Fig. 1B). Indeed, one clinical cartilage repair strategy, osteochondral allograft transplantation (or OATs), involves the transfer of a cylinder of cartilage and bone from a non-load-bearing region to a cartilage defect site [4]. This immediately restores load transfer capacity [21], but is plagued by poor integration at the cartilage margins [22,23]. Such findings suggest that there may exist a "trade-off" between functional maturation (to provide load transmission) and integration (to evenly distribute stress across the repaired cartilage surface) (Fig. 1B). Indeed, in one of the earliest papers to examine in-vitro integration of engineered constructs to native cartilage, Obradovic et al. reported that immature constructs (5 days of culture) integrated with native cartilage to a much greater extent than mature constructs (5 weeks of culture) [6]. However, other studies

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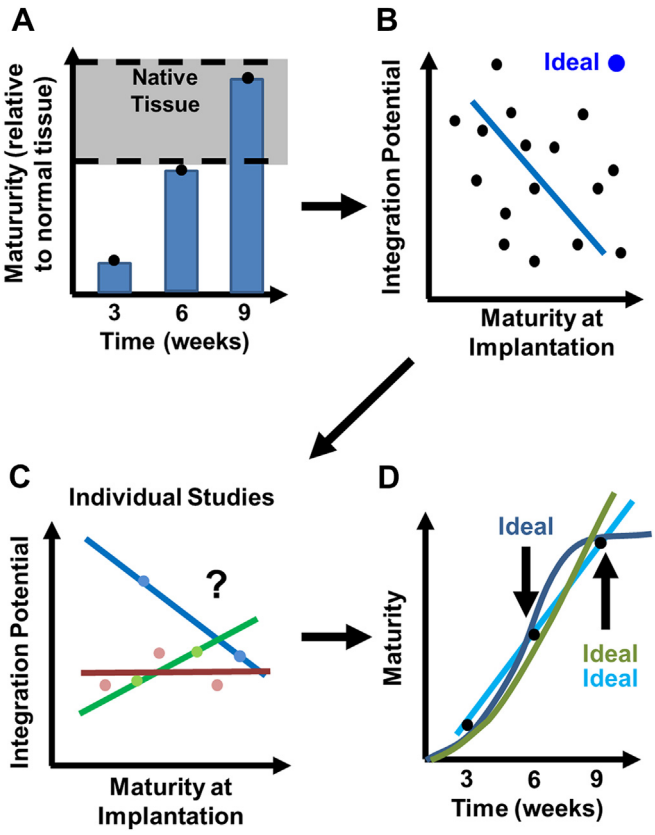

Fig. 1. Schematic illustration of the question of construct state versus trajectory. Current practice in cartilage TE allows for the formation of constructs with some properties matching native tissue (A). While there is a general negative correlation between construct maturity and its ability to integrate with native tissue (B), individual studies are less clear regarding this trend and are limited by few data points (C). One important factor in correlating construct maturity and integration potential might be its "trajectory" or time-dependent properties, however, the shape of maturation for these constructs has yet to be elucidated, which could influence the ideal time for implantation (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

revealed a more complicated situation [20,24,25]. For example, using similar TE constructs, Hunter et al. saw little to no difference in the integration potential between immature and mature constructs pre-cultured for the same period [17]. In our laboratory, constructs made from hyaluronic acid (HA) hydrogels seeded with mesenchymal stem cells (MSCs) that had been pre-cultured for 4 weeks integrated better than constructs that were formed immediately within a cartilage defect [18]. Similarly, Miot et al. recently examined the role of maturation state at the time of implantation in the goat model [19]. Autologous chondrocytes were harvested and cultured in-vitro within hydroxyapatite/hyaluronic acid sponges for two days, two weeks, or six weeks prior to implantation into an osteochondral defects. Interestingly, the constructs cultured for two weeks showed superior results in terms of histological scoring at 8 months compared to those cultured for shorter or longer period of time. These studies depict a more complex relationship between construct maturity and integration potential (Fig. 1C).

In light of these conflicting data, we posited that "static" measures of construct maturity (e.g. compressive modulus) alone may not be the best indicator of in-vivo success. An ideal TE construct will be required to mature, remodel, and integrate with the host over time. Since the growth state that best promotes such activities need not be (and likely is not) the most mature state, less mature but rapidly developing constructs may need to be selected for implantation, thereby eliminating the "trade-off" between maturity and integration potential (blue dot (in web version) in Fig. 1B).

To formalize this concept, we propose the general notion of "trajectory-based" tissue engineering (TB-TE). This is based on a 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 than static measures of construct maturation. Under this hypothesis, the shape of construct maturation (i.e. its trajectory) becomes critically important for determining the ideal time for implantation. Such an approach requires almost real-time assessment of construct maturation to identify the correct shape of the growth trajectory. However, given this limited sampling frequency in traditional tissue engineering endeavors, several general shapes are possible, which leaves the ideal time for implantation an open question (Fig. 1D).

To overcome this limitation and to validate the TB-TE concept, our first objective was to rigorously assess and model how engineered cartilage constructs mature over time in-vitro using a well-defined culture platform. After determining the general shape of maturation, and its perturbation by changing input parameters (such as cell seeding density and mixing environment), the second objective was to model this response, as well as to perform a sensitivity analysis to determine how often and how much data should be collected to accurately describe the shape of the maturation. Finally, to test this concept in a scenario relevant to cartilage repair, the third objective was to correlate biochemical and biomechanical properties of the engineered cartilage constructs as a function of the derived TB-TE parameters and to their time-dependent variation in integration potential using an in-vitro integration assay. Our findings provide quantitative selection criteria, based on a TB-TE approach, to improve cartilage repair.

2. Materials and methods

2.1. Experimental design and analyses-study 1

2.1.1. MeHA macromer synthesis

Methacrylated HA (MeHA) was synthesized by reacting methacrylic anhydride (Sigma, St Louis, MO) and 74 kDa HA (Lifecore, Chaska, MN) as previously described [7,18,26]. Using 1H NMR characterization, the final MeHA product was determined to be 20–25% methacrylated. HA macromer was then lyophilized and stored at −20 °C. One day prior to construct formation, the MeHA macromer was sterilized by exposure to a biocidal UV lamp for 15 min and dissolved in sterile PBS at a concentration of 1% (mass/volume) with 0.05% Irgacure-2959 photoinitiator (2-methyl-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone; Ciba–Geigy, Tarrytown, NY).

2.1.2. Mesenchymal stem cell isolation and construct formation

Juvenile bovine hind limbs (3–6 months old) were obtained (Research 87, Boylston, MA). For each study replicate, two donors were used. MSCs from the femoral bone marrow were isolated as previously described [7,18,27] and expanded through passage 2 in basal medium consisting of DMEM with 10% fetal bovine serum and 1% penicillin–streptomycin–fungizone (PSF) (Invitrogen, Carlsbad, CA).

For the first experiment, cells were encapsulated within methacrylated HA (1% w/v). Following polymerization via UV light, cylindrical constructs (4 mm diameter) were formed and cultured in chemically-defined media (1 mL per construct) containing TGF-β3 (10 ng/mL) for up to 9 weeks in free-swelling conditions [7,18,27]. To assess repeatability, this experiment was replicated three separate times with different donor sources at a seeding density of 60 million cells/mL. To examine the impact of cell density, one replicate study included groups with constructs containing MSCs at a density of 20 or 60 million/ml (20 M or 60 M, respectively). To examine the impact of environment, one replicate study included a group that underwent orbital shaking (1 Hz) during in-vitro culture.

2.1.3. Biomechanical analysis

The mechanical properties of free-swelling constructs (n = 4–5/group/time point) were assessed via uniaxial unconfined compression as previously described [7,18,27]. Constructs were first equilibrated under creep (0.02N tare load) for 300 s. Then, a stress relaxation test was performed by applying 10% strain at a strain rate of 0.05%/s followed by a 1000 s relaxation phase. Equilibrium modulus was calculated from equilibrium load and sample cross-sectional geometry. After the stress relaxation test, a 1% sinusoidal strain was applied at 1 Hz. The dynamic modulus was