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A high throughput mechanical screening device for cartilage tissue engineering

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ABSTRACT

Articular cartilage enables efficient and near-frictionless load transmission, but suffers from poor inherent healing capacity. As such, cartilage tissue engineering strategies have focused on mimicking both compositional and mechanical properties of native tissue in order to provide effective repair materials for the treatment of damaged or degenerated joint surfaces. However, given the large number design parameters available (e.g. cell sources, scaffold designs, and growth factors), it is difficult to conduct combinatorial experiments of engineered cartilage. This is particularly exacerbated when mechanical properties are a primary outcome, given the long time required for testing of individual samples. High throughput screening is utilized widely in the pharmaceutical industry to rapidly and cost-effectively assess the effects of thousands of compounds for therapeutic discovery. Here we adapted this approach to develop a high throughput mechanical screening (HTMS) system capable of measuring the mechanical properties of up to 48 materials simultaneously. The HTMS device was validated by testing various biomaterials and engineered cartilage constructs and by comparing the HTMS results to those derived from conventional single sample compression tests. Further evaluation showed that the HTMS system was capable of distinguishing and identifying 'hits', or factors that influence the degree of tissue maturation. Future iterations of this device will focus on reducing data variability, increasing force sensitivity and range, as well as scaling-up to even larger (96-well) formats. This HTMS device provides a novel tool for cartilage tissue engineering, freeing experimental design from the limitations of mechanical testing throughput.

1. Introduction

Cartilage tissue engineering has made marked progress, with numerous studies arriving at methods for the production of mechanically functional cartilage, based on either native chondrocytes (Kelly et al., 2006; Novotny et al., 2006; Lima et al., 2007; Byers et al., 2008; Bian et al., 2010; Cheng et al., 2011; Ng et al., 2011) or mesenchymal stem cells (MSCs) grown as three dimensional (3D) constructs (Mauck et al., 2006; Huang et al., 2010; Moutos and Guilak, 2010; Thorpe et al., 2010; Erickson et al., 2012). However, the degrees of freedom present in any experimental design can make even the simplest of tissue engineering studies difficult to execute, where an investigator can vary materials (Mouw et al., 2005; Chung et al., 2009; Chung and Burdick, 2009; Hwang et al., 2011), cell number (Mauck et al., 2003; Weinand et al., 2009), growth factor doses and combinations (Blunk et al., 2002; Gooch et al., 2002; Appel et al., 2009; Johnstone et al., 2013), and the mechanical loading environment (Ng et al., 2009; Thorpe et al., 2010). Moreover, complexity in experimental design leads to difficulties in capturing outcome parameters in a cost- and time-efficient manner. This need for increased throughput in assessing outcomes is not unique to tissue engineering. Indeed, high throughput screening (HTS) methods emerged very early in the pharmaceutical industry (Drews, 2000), where such methods were essential for screening large chemical libraries for biologic activity relevant to disease.

The underlying premise of HTS is that if a suitable assay can be developed that is (1) sufficiently sensitive to measure a relevant cellular response, (2) of a low cost per sample, (3) easy to automate, and (4) reproducible, then one can expedite drug

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discovery. While most HTS assays are performed in monolayer culture, recent studies have begun to implement assays in 3D constructs as well. For example, 3D multi-cellular spheroids have been used to screen for tumor suppressive agents (Kunz-Schughart, 2004). A few studies have applied HTS principles towards applications in bone and cartilage biology and regeneration. For instance, HTS-based assays focused on MSC osteogenesis in monolayer (Brey et al., 2011) and chondrogenesis in micro-scaled pellet cultures (Huang et al., 2008) have been used to screen small molecule libraries in a 384-well format. The potential of such HTS approaches is perhaps best illustrated by a recent study, employing an image-based HTS method that identified molecules that promoted the formation of chondrogenic MSC nodules. Identified molecules were subsequently shown to protect cartilage from degeneration in a small animal model of joint instability (Johnson et al., 2012).

While most HTS assays focus on molecular events, functional outcomes are equally important for musculoskeletal tissues (Vandenburgh, 2010). This is particularly relevant for cartilage, as the properties of the engineered tissue will dictate function in the load-bearing joint environment (Ateshian and Hung, 2005). Thus, it would be ideal if HTS approaches could be modified to include mechanical measures. However, traditional one-at-a-time assessment of mechanical properties can be prohibitively time consuming, where a typical stress relaxation test can take several hours per sample (Mauck et al., 2000; Soltz and Ateshian, 2000). In even relatively simple experimental designs (Erickson et al., 2012), involving just two different seeding densities, four different material formulations, one growth factor at a single dose (and a growth factor-free control), and five samples per group, over 80 h of testing is required at each time point. Given the continued development of novel materials and new factors influencing cartilage growth, and the requirement that each of these inputs be carefully evaluated in a combinatorial context, throughput in mechanical analysis has become a significant barrier to further advances. As such, development of a high throughput mechanical screening (HTMS) system would represent a valuable tool to advance cartilage tissue engineering.

Towards this end, several mechanical testing systems have been introduced that enable multi-sample evaluation. For instance, the Myoforce Analysis Device was developed to monitor bioartificial muscles to identify compounds that alter contractile strength (Vandenburgh, 2010). The MATE system incorporated real-time measures of load during dynamic stimulation of engineered cartilage, using a six-sample actuating system (Lujan et al., 2011). Still more recently, a 12-sample tissue stimulator was developed that recorded load from each sample via individual force sensitive resistors (Salvetti et al., 2012). These devices illustrate how real-time and multi-sample mechanical analysis can be incorporated into tissue culture systems. While promising, throughput in these devices is restricted to a relatively small sample capacity, and expansion to higher throughput formats might be limited by sensor technology (Lujan et al., 2011; Salvetti et al., 2012). Additional development is needed to make such devices compatible with HTS of chemical libraries.

To address this, we developed a novel high throughput mechanical screening (HTMS) device that can assess mechanical properties of biomaterials and engineered cartilage in a 48-well format. Our system utilizes a custom force sensitive resistor (FSR) array to measure instantaneous and time-dependent mechanical response of up to 48 samples simultaneously. The increased capacity of this device provides a platform to evaluate properties in complex, combinatorial studies for the screening and optimization of engineered cartilage. The objective of this study was to design, optimize, and validate this system, and to screen mechanical properties of multiple materials and engineered cartilage in several experimental configurations.

2. Materials and methods

2.1. HTMS device: Components

The HTMS device was designed to interface with mechanical testing systems utilized in most orthopaedic and bioengineering laboratories. A schematic is shown in Fig. 1. The device housing consists of an aluminum base plate and two parallel side plates onto which linear bearings (Maintenance-Free Ball Bearing Carriages and Guide Rails, McMaster-Carr, GA) are affixed to align and maintain smooth vertical displacement of the platen. The sensor platen was integrated via two plates: an upper plate to which it is directly attached, and a bottom plate fixed to the upper plate. A custom force sensitive resistor (FSR) array was mounted via adhesive backing to this plate (Custom 48 Matrix FSR Sensor Array, Sensitronics, WA). To control vertical displacement of the sensor platen, an Instron (Model 5848, Instron, MA) was connected via an adaptor to the sensor platen (Fig. 1A and D).

Opposing the sensor surface, a well plate assembly was designed to accommodate standard 48-well plates (BD Falcon, Multiwell Cell Culture Plate, #75875, NJ) and an indenter array and hole plate to align with each sensor on the FSR array

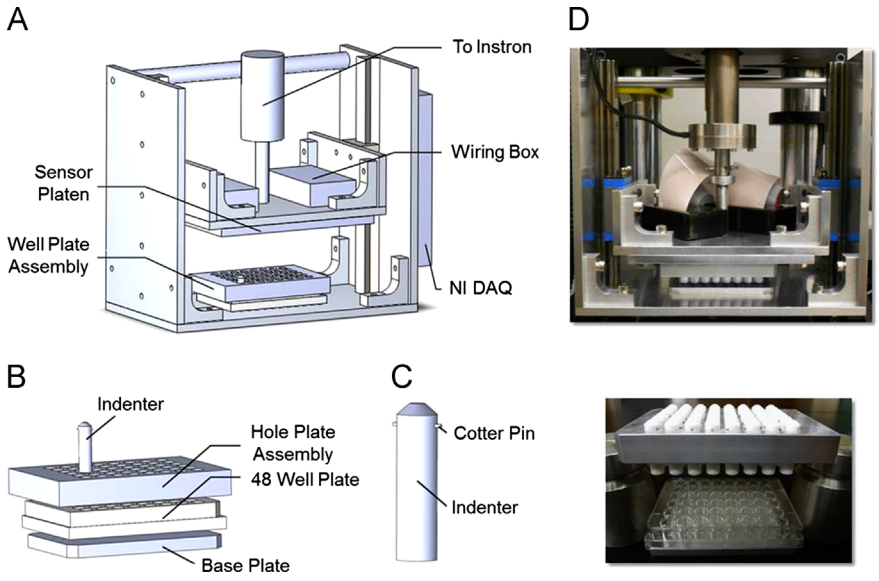

Fig. 1. Schematic of HTMS device. (A) The system includes an aluminum housing frame, sensor platen controlled via Instron displacement, and ((B)–(C)) well plate assembly designed for a standard 48-well culture dish with indenter platens. (D) Fully assembled HTMS device on Instron platform and complete 48 sample array of indenters in well plate assembly.

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