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Collagen abundance in mechanically stimulated osteoblast cultures using near infrared microscopy

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ABSTRACT

Collagen abundance in osteoblast cell cultures was determined using near infrared microscopy with chemical imaging (NIR-CI) with and without mechanical stimulation of the the cells. MC3T3-E1 mouse osteoblast cells seeded on a polycarbonate substrate were mechanically stimulated using static loads of 13.5 N, 27 N and 40 N applied to the substrates during 2, 4, 6 and 8 days of incubation. Results show that the cells increased their collagen production with 13.5 N and 27 N loads when compared to the control sample with a 27 N load resulting in a noteworthy increase (109%) in collagen production. The 40 N load on the other hand, resulted in an initial decrease in the collagen expression in the extracellular matrix, possibly as a result of cell death or inhibition of the protein secretion process followed by an increase in collagen after cell recovery and proliferation. Qualitative confirmation of these results was performed using confocal microscopy.

1. Introduction

Progress in surgical techniques, organ preservation, and improved methods to decrease graft rejection, including advances in histocompatibility testing and immune tolerance have contributed to successful outcomes in tissue engineering (Niklason and Langer, 2001). Nevertheless, these techniques have certain drawbacks concomitant with the use of implantable foreign body materials such as new lesions, infection at the host body/tissue interface, fracture, and migration of the implants over time. In addition, the use of allografts has to pass the stringent test of biocompatibility to avoid biological changes due to abnormal interaction with the host tissue at its implant location (Gourishankar and Halloran, 2002; Ingber et al., 2006). Tissue substitutes are being engineered and their generation involves the use of matrices containing specific cellular and growth factors which provide the necessary support for the proliferation, biological maintenance, cell function and signaling, along with defining tissue architecture through the synthesis and appropriate functioning of the extracellular matrix (ECM) (Boudreau et al., 1995; Martins-Green, 2000; Stevens et al., 2008). Mechanical signals can be utilized to stimulate cells and influence cellular function at multiple levels in the process of tissue engineering (Orr et al., 2006) possibly including the manipulation of collagen architecture in the ECM. Collagen is a key ingredient in skeletal tissue engineering scaffolds due to the fact that the mechanical properties of the ECM are largely a function of the collagen fiber alignment; its interaction with the substrate material also regulates the behavior of cells that migrate to the site of the ECM remodeling (Laurencin et al., 1999). Mechanical stimulus of cells and tissue results in an increase in the concentration of various constituents of the ECM including collagen and other proteins (Kim et al., 2002; Ignatius et al., 2005; Tang et al., 2006; Gilbert et al., 2007). Although, both quantitative and qualitative measurements of collagen in cell cultures have been described (Koshihara and Honda, 1994; Jones et al., 1999), a non-invasive, quantitative measure of the increase in collagen in mechanically stimulated cell cultures, has not been reported previously in the literature. This procedure is vital in the quality control and optimal in vitro production of engineered tissue for implantation. The motivation of this study is to understand the rate of collagen production in cell cultures and concomitantly the localization of collagen in the ECM. With the goal of producing tissue constructs engineered with a specific orientation of the collagen fiber architecture, further understanding of the application of mechanical stimuli to possibly increase and direct vesicu lar transport to the regions where collagen can be preferentially deposited is necessary. As a first step, the present study is significant in that unlike other analytical chemical methods, it employs a

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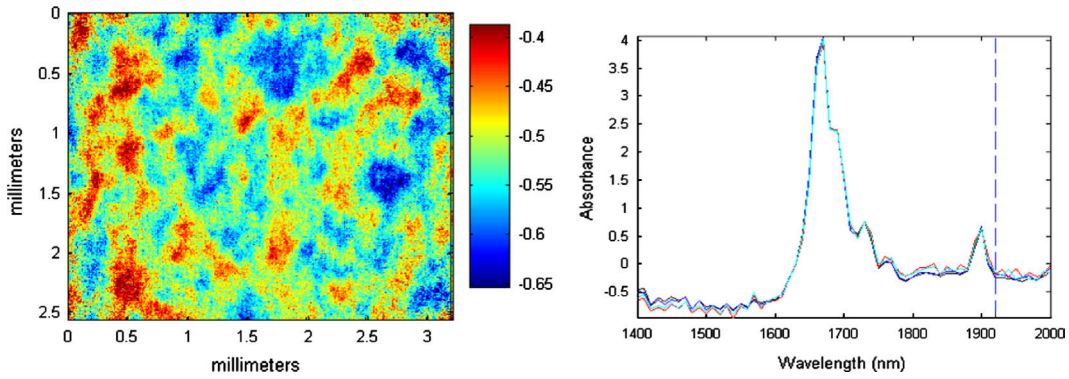

Fig. 1. Chemical image (left) and characteristic absorbance spectra of polycarbonate (right) obtained from the Near-Infrared Microscope. Note high intensity absorbance peaks at wavelengths corresponding to 1680 nm, 1730 nm and 1900 nm respectively.

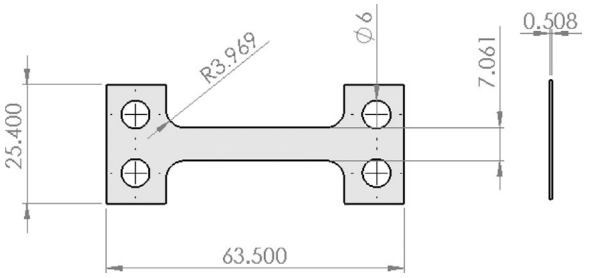

Fig. 2. Polycarbonate substrate test sample on which cells were seeded and the 13.5 N, 27 N and 40 N mechanical loads were applied. All dimensions are in mm.

remote-sensing, nondestructive method to determine collagen abundance in in vitro mouse osteoblast cell cultures using near-infrared (NIR) microscopy following the application of an appropriate mechanical stimulus. This will enable real time in vitro experimentation that will quantify collagen abundance and localization in a specific region of cell culture.

2. Materials and methodology

2.1. Cell culture

MC3T3-E1 mouse osteoblast cells (CRL-2593™), obtained from ATCC (Manassas, Virginia) were grown in 25 cm² plastic culture flasks (Corning, New York) and incubated at 37 °C until confluency. At approximately 100% confluence, cells were washed three times with Phosphate Buffer Saline solution (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2HPO_4) and harvested using 0.25% trypsin–0.53 mM EDTA (Gibco, Gaithersburg, Maryland) at 37 °C for 5 min. Cells were then pelleted by low speed centrifugation (3,500 rpm) for 5 min and subcultured at 1:3 ratio, and medium was renewed every 2 or 3 days. The growth medium utilized was Alpha Minimum Essential Medium with ribonucleosides, deoxyribonucleosides, 2 mM L-glutamine and 1 mM sodium pyruvate and 10% fetal bovine serum (FBS) without ascorbic acid.

2.2. Near infrared microscopy-chemical imaging (NIR-CI)

After culturing the cells for a specific time period, they were washed three times with PBS and rapidly observed in the NIR microscope. Near infrared hyperspectral images were acquired using a MALVERNSyNIRgi Near-Infrared Chemical Imaging System (Olney, MD). Spectra were collected with the system's focal plane array detector that has 256 × 320 pixel elements, with a total collection time of about 2 min. Images were acquired using a 10 μm per pixel objective in transmission mode over an area of approximately 3.2 × 2.6 mm. Spectra were obtained with one scan using a spectral range of 1200–2400 nm and a low-pass Fourier filter was applied to smooth high frequency noise without distorting the main spectral features. PIXIS® CI software from Malvern Instruments was used for data acquisition. The background image (without sample) was first obtained followed by the dark image (without any illumination of the microscope). Finally, the sample image was captured. The logarithm, $\log_{10}$ (1/R), was first applied to the data cube to convert the spectra to absorbance units. Bad pixels (Burger, 2009) were removed and replaced by the average value of the intensities of the surrounding pixels.

Recently NIR was used with biological samples to detect collagen/elastin in mouse aortas (Luypaert et al., 2007). In the present study, collagen Type I fascicles from rat tail tendons were used to detect the protein and identify the specific wavelengths at which collagen responds to the excitation of the infrared lamp. Using the wavelength spectrum obtained from the collagen fascicles, mouse osteoblast cell culture samples incubated for 7, 14 and 21 days on polycarbonate substrates were analyzed without any mechanical stimulus. This experimental set was developed with the aim of observing the interaction between the ECM and the polycarbonate substrate on which the cells were seeded and to evaluate the detection level of the NIR microscope when the collagen was expressed by the cells. The optical and chemical properties of polycarbonate were first evaluated with respect to the NIR which was then used to analyze the biological samples. Although polycarbonate has three absorbance bands that restrict the wavelength range for collagen detection (see Fig. 1), cells were still cultured on this sheet substrate in an effort to avoid interference. Based on the data acquired in these preliminary tests,

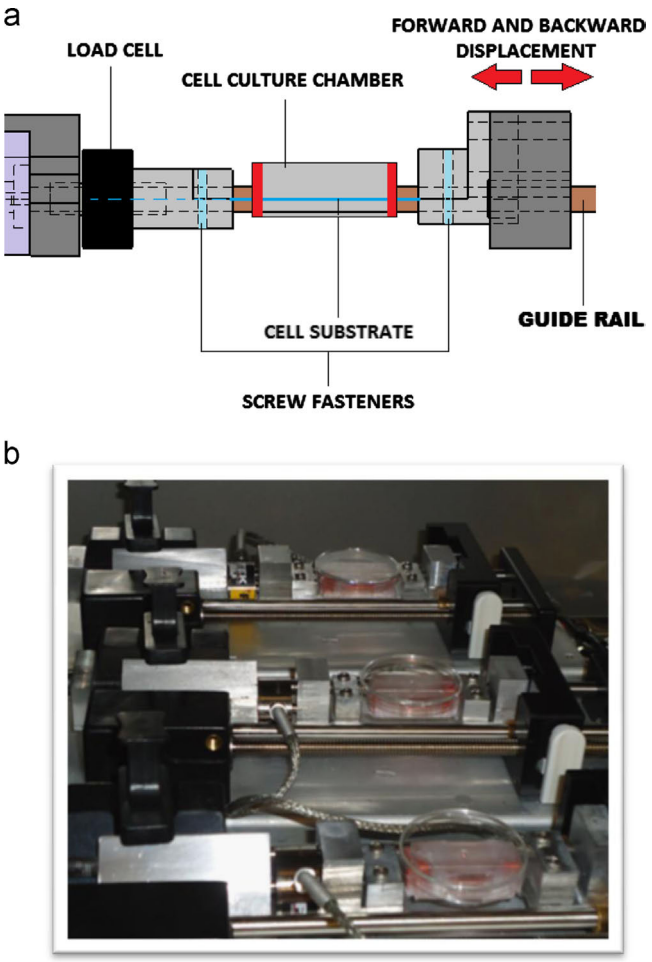

Fig. 3. (a) Schematic diagram of chamber/mechanical device system. (b) Actual loading devices to enable multiple testing which were simultaneously placed inside an incubator.