Acta Biomaterialia 7 (2011) 3294–3301

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

# Dynamic mechanical stimulations induce anisotropy and improve the tensile properties of engineered tissues produced without exogenous scaffolding

Robert Gauvin [1], Rémi Parenteau-Bareil, Danielle Larouche, Hugo Marcoux, Francis Bisson, Adrien Bonnet, François A. Auger, Stéphane Bolduc, Lucie Germain *

*Centre LOEX de l'Université Laval, Génie tissulaire et régénération : LOEX - Centre de recherche FRSQ du Centre hospitalier affilié universitaire de Québec, and Départements de Chirurgie et d'Ophtalmologie, Faculté de Médecine, Université Laval, Québec, QC, Canada*

ARTICLE INFO

*Article history:*
Received 1 February 2011
Received in revised form 10 May 2011
Accepted 25 May 2011
Available online 30 May 2011

*Keywords:*
Bioreactor
Connective tissue
Extracellular matrix
Mechanical properties
Tissue engineering

ABSTRACT

Mechanical strength and the production of extracellular matrix (ECM) are essential characteristics for engineered tissues designed to repair and replace connective tissues that are subject to stress and strain. In this study, dynamic mechanical stimulation (DMS) was investigated as a method to improve the mechanical properties of engineered tissues produced without the use of an exogenous scaffold, referred to as the self-assembly approach. This method, based exclusively on the use of human cells without any exogenous scaffolding, allows for the production of a tissue sheet comprised of cells and ECM components synthesized by dermal fibroblasts in vitro. A bioreactor chamber was designed to apply cyclic strain to engineered tissues in order to determine if dynamic culture had an impact on their mechanical properties and ECM organization. Fibroblasts were cultured in the presence of ascorbic acid for 35 days to promote ECM production and allow the formation of a tissue sheet. This sheet was grown on a custom-built anchoring system allowing for easy manipulation and fixation of the tissue in the bioreactor. Following the 35 day period, tissues were maintained for 3 days in static culture (SC), or subjected either to a static mechanical stimulation of 10% strain, or a dynamic DMS with a duty cycle of 10% uniaxial cyclic strain at 1 Hz. ECM was characterized by histology, immunofluorescence labeling and Western blotting. Both static and dynamic mechanical stimulation induced the alignment of assessed cytoskeletal proteins and ECM components parallel to the axis of applied strain and increased the ECM content of the tissues compared to SC. Measurement of the tensile mechanical properties revealed that mechanical stimulation significantly increases both the ultimate tensile strength and tensile modulus of the engineered tissues when compared to the non-stimulated control. Moreover, we demonstrated that cyclic strain significantly increases these parameters when compared to a static-loading stimulation and that mechanical stimulation contributes to the establishment of anisotropy in the structural and mechanical properties of self-assembled tissue sheets.



## 1. Introduction

Most connective tissues in the body – skin, blood vessels, heart valves, tendons, ligaments, etc. possess anisotropic viscoelastic mechanical properties and specific structural organization [1]. The reproduction of these structural features in engineered tissue is a tremendous challenge and it would be highly advantageous to optimize the performance of these tissues for potential clinical applications. Tissue mechanics depend on the individual cell features and extracellular matrix (ECM) properties, as well as on the complex organization and interactions occurring between cell types and their surrounding three-dimensional environment [2]. Numerous strategies have been designed to enhance the mechanical properties of engineered tissues, including the addition of growth factors to the culture media and the use of bioreactors and mechanical stimulations to induce tissue growth and remodeling [3]. The use of bioreactors to apply mechanical stimuli to engineered tissues has proven to be efficient in different studies, largely in the field of cardiovascular tissue engineering where tissues are subject to continuous stresses and strains of different amplitudes. These systems are ranging from uniaxial to biaxial bioreactors allowing for the application of stress and strain [4–6], through perfusion bioreactors which allow for a combination of stress, strain and pulsatile shear stress on the engineered tissues [7–9]. These bioreactors were used to mechanically stimulate tissue-engineered blood vessels (TEBVs) [10–13] as well as tissue-

* Corresponding author. Tel.: +1 (418) 682 7511x1696; fax: +1 (418) 990 8248.
*E-mail address:* lucie.germain@chg.ulaval.ca (L. Germain).
[1] Current address: Harvard-MIT Division of Health Sciences and Technology and Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA.

1742-7061/$ - see front matter © 2011 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2011.05.034

engineered heart valves [6,8,9,14]. Most studies have been performed using smooth muscle cells (SMCs) within scaffolds and have attempted to reproduce a physiological-like environment in order to enhance the functionality of engineered tissues. Dynamic stimulations have proven to be efficient at improving the mechanical properties of TEBVs produced using cell-seeded polymers [10,11] or using cells trapped within a collagen ECM [12,13,15]. More specifically, cyclic mechanical strain enhances the function and development of engineered tissues via an increased production of collagen and elastin [16–18].

The importance of mechanical loading in the physiology of connective tissues led to the use of in vitro mechanical preconditioning in order to enhance ECM production and to strengthen engineered tissues. Understanding the interactions between cells, ECM and mechanical stimulation, and how they influence tissue growth and organization, is of the utmost importance for the design of load-bearing engineered tissues. Previous studies performed in our laboratory have shown that uniaxial static loading could increase the tensile strength of a tissue sheet engineered in vitro [19]. Static tension developed in this tissue improves the tensile properties and induces cellular and ECM alignment towards the axis of stimulation, demonstrating the adaptation of fibroblasts in response to mechanical loading. Therefore this stimulus, described as a static mechanical stimulation (SMS) and based on cellular response, results in cell reorientation and collagen matrix remodeling.

The present study evaluated the potential of dynamic mechanical stimulation (DMS) as a method to improve the structure and mechanical properties of engineered tissues produced using the self-assembly approach. We report that mechanical stimulation increased significantly both the ultimate tensile strength (UTS) and tensile modulus of engineered tissues. Moreover, we demonstrated that cyclic strain increased these parameters significantly, and that both SMS and DMS contributed to establishing anisotropy in the structural and mechanical properties of self-assembled tissue sheets.

## 2. Materials and methods

This study was approved by the Centre Hospitalier Affilié Universitaire de Québec institutional review committee for the protection of human subjects. Tissues were obtained after informed consent was given.

### 2.1. Cell isolation and culture

Dermal fibroblasts (DFs) were isolated from a human skin biopsy as previously described [20]. Briefly, the dermis was separated from the epidermis by incubation in thermolysin (Sigma, Oakville, ON, Canada). DFs were enzymatically dissociated from the dermis using collagenase H (Roche, Indianapolis, IN, USA), centrifuged, plated in tissue culture flasks and cultured in Dulbecco-Vogt modified Eagle's medium (DMEM; Invitrogen, Burlington, ON, Canada) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and antibiotics (penicillin, 100 U $ml^{-1}$, Sigma, Oakville, ON, Canada; gentamycin, 25 μg $ml^{-1}$, Schering, Pointe-Claire, QC, Canada).

All cell types were maintained at 37 °C in a humidified incubator containing 8% $CO_2$ and culture medium was changed three times per week. Cells were used at passage 4 for tissue sheet production.

### 2.2. Engineered tissue sheet fabrication

Engineered tissues were produced using the tissue-engineering method previously described, i.e. the self-assembly approach [19]. DFs were seeded at a density of $1 \times 10^4$ cells $cm^{-2}$ in a 86 × 128 mm gelatin-coated tissue culture plate (NalgeNunc International, Napierville, IL, USA) containing a custom-built tissue-anchoring device made of a filter paper frame (Whatman International, Maidstone, UK) and stainless steel springs (McMaster-Carr, Aurora, OH, USA), similar to an anchoring method known as the "spiral-bound" gripping technique developed and previously reported by Engelmayr et al. [21,22], which allows for easy manipulation and fixation of the tissue on the bioreactor (Fig. 1A). DFs were cultured in DMEM supplemented with 10% FBS and antibiotics. Sodium L-ascorbate (50 μg $ml^{-1}$, Sigma, Oakville, ON, Canada) was added to the culture medium to stimulate ECM synthesis. DFs were cultured 35 days until they proliferated over the tissue-anchoring device and their neosynthesized ECM proteins had self-assembled into an adherent living tissue sheet. The tissue-anchoring device, around which the tissue sheet was grown, later served as a fixture point for the tissue in the bioreactor chamber.

### 2.3. Mechanical stimulations

In order to allow for the mechanical stimulation of the tissue, the lateral sides of the tissue-anchoring device were carefully cut using a sterile scalpel blade (BD AcuteCare, Franklin Lakes, NJ, USA) (Fig. 1B) and the tissue-anchoring device was inserted into stainless steel mounting brackets (Fig. 1C). Two different methods were used to stimulate the tissues. The SMS condition consisted in maintaining the tissue at a fixed length corresponding to 10% strain, using a rigid plastic frame (not shown). The original length of the tissue was determined as the reference length used for calculation. The DMS condition consisted in using a vertical mechanical tester (Instron Electropuls, Instron Corporation, Norwood, MA, USA), to which fixtures were adapted to link the tissue to the actuator, which served as a straining device for the application of cyclic stimulations. The DMS was set to 10% strain at a frequency of 1 Hz based on initial tissue length. Both stimulations were applied over a 72 h period. A third condition where the tissue was maintained in static culture (SC) without stimulation was used a negative control. Once mounted into the stainless steel brackets, tissue from both SMS and DMS were transferred into a custom-built bioreactor chamber consisting of a modified 225 $cm^2$ tissue culture flask (BD Biosciences, Mississauga, ON, Canada), which maintained the tissues in culture media at 37 °C during the stimulation period (Fig. 1D and E). This bioreactor chamber was designed to house a single tissue sheet and to be compatible with the mechanical tester. Culture medium in the bioreactor chamber was similar to medium used during tissue sheet fabrication and was buffered using HEPES (MP Biomedicals, Solon, OH, USA). Note that the bioreactor chamber was completely covered with aluminum foil during the stimulation period to avoid any phototoxicity attributable to the interaction between ambient fluorescent light and HEPES-buffered media [23–25].

### 2.4. Macroscopic aspect and morphology of the tissues

Macroscopic observations of the tissues following the stimulation period were imaged following the 72 h stimulation period using a digital camera (Canon, Mississauga, ON, Canada). Morphology of the tissue was observed using a Nikon Eclipse TS100 microscope coupled to a digital camera (Nikon, Mississauga, ON, Canada).

### 2.5. Histology

Following the stimulation period, biopsies of the central region of each tissue type were fixed overnight in Histochoice (Amresco, Solon, OH) and embedded in paraffin. Five μm thick cross-sections