Acta Biomaterialia 8 (2012) 4020–4030

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Crosslinking strategies facilitate tunable structural properties of fibrin microthreads

Jonathan M. Grasman [a,c], Raymond L. Page [a,c,d], Tanja Dominko [b,c,d], George D. Pins [a,c,*]

[a] Biomedical Engineering Department, Worcester Polytechnic Institute, Worcester, MA 01609, USA
[b] Biology and Biotechnology Department, Worcester Polytechnic Institute, Worcester, MA 01609, USA
[c] Bioengineering Institute, Worcester Polytechnic Institute, Worcester, MA 01609, USA
[d] CellThera Inc., Southbridge, MA, USA

ARTICLE INFO

Article history:
Received 23 March 2012
Received in revised form 11 July 2012
Accepted 16 July 2012
Available online 21 July 2012

Keywords:
Tissue engineering
Muscle regeneration
Fibrin
Microthreads
Crosslinking

ABSTRACT

A significant challenge in the design of biomimetic scaffolds is combining morphologic, mechanical, and biochemical cues into a single construct to promote tissue regeneration. In this study, we analyzed the effects of different crosslinking conditions on fibrin biopolymer microthreads to create morphologic scaffolds with tunable mechanical properties that are designed for directional cell guidance. Fibrin microthreads were crosslinked using carbodiimides in either acidic or neutral buffer, and the mechanical, structural, and biochemical responses of the microthreads were investigated. Crosslinking in the presence of acidic buffer (EDCa) created microthreads that had significantly higher tensile strengths and moduli than all other microthreads, and failed at lower strains than all other microthreads. Microthreads crosslinked in neutral buffer (EDCn) were also significantly stronger and stiffer than uncrosslinked threads and were comparable to contracting muscle in stiffness. Swelling ratios of crosslinked microthreads were significantly different from each other and uncrosslinked controls, suggesting a difference in the internal organization and compaction of the microthreads. Using an *in vitro* degradation assay, we observed that EDCn microthreads degraded within 24 h, six times slower than uncrosslinked control threads, but EDCa microthreads did not show any significant indication of degradation within the 7-day assay period. Microthreads with higher stiffnesses supported significantly increased attachment of C2C12 cells, as well as increases in cell proliferation without a decrease in cell viability. Taken together, these data demonstrate the ability to create microthreads with tunable mechanical and structural properties that differentially direct cellular functions. Ultimately, we anticipate that we can strategically exploit these properties to promote site-specific tissue regeneration.

1. Introduction

Large muscle defects profoundly impact patients' quality of life. In the military, over 50% of all wounds incurred on the battlefield affect the musculoskeletal system, leading to over 1,000 amputations and significant losses in musculoskeletal function [1,2]. Additionally, 35–55% of all sports injuries involve muscle damage [3,4]. Many traumatic military and civilian injuries to skeletal muscle, such as compartment syndrome, are characterized by volumetric muscle loss (VML), where a large amount of tissue, including the basement membrane architecture, is destroyed and functional impairment occurs [5,6]. While skeletal muscle has an innate repair mechanism, this mechanism is unable to compensate for large-scale injuries, resulting in the formation of massive amounts of scar tissue to fill the void left by the destroyed or lost muscle. Although scar tissue can be moderately successful in the conduction of uniaxial force, it is unable to contract and function like skeletal muscle, resulting in a permanent loss of function. The current gold standard for the repair of large-scale injuries is an autologous tissue transfer, where muscle is harvested from an uninjured site and is grafted to the injury site [7]. This process, which requires a team of highly skilled and specialized surgeons, results in limited restoration of lost muscle function. The success of autologous muscle grafting is also limited by complications such as infection, donor site morbidity, and failure of the grafted tissue due to necrosis [7,8]. As such, there is a clear need for an off-the-shelf scaffold that can direct endogenous skeletal muscle tissue formation within these large VML defect sites to facilitate functional tissue regeneration.

Scaffolds that direct skeletal muscle regeneration must facilitate alignment and organization of newly forming muscle fibers parallel to the uniaxial force conduction pathway [9,10]. To best promote *de novo* muscle regeneration in a large defect, the scaffold must also be biocompatible, support cellular ingrowth from the surrounding musculature, and ultimately degrade as new tissue

* Corresponding author at: Biomedical Engineering Department, Worcester Polytechnic Institute, Worcester, MA 01609, USA. Tel.: +1 508 831 6742; fax: +1 508 831 5541.
E-mail address: gpins@wpi.edu (G.D. Pins).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.07.018