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Microgrooved fibrillar collagen membranes as scaffolds for cell support and alignment

Robert B. Vernon[a,*], Michel D. Gooden[a], Stephanie L. Lara[b], Thomas N. Wight[a,b]

[a]Hope Heart Program, Benaroya Research Institute at Virginia Mason, 1201 Ninth Avenue, Seattle, WA 98101-2795, USA
[b]Department of Pathology, University of Washington, Seattle, WA 98195, USA

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Abstract

For several years, microgrooved substrates have been evaluated as a means to orient cells in engineered tissues. Recently, we fabricated thin (0.1–5.3 μm) planar and tubular collagen membranes (CMs) from air-dried hydrogels of native, fibrillar type I collagen (Vernon et al., Biomaterials 2004;26:1109–17). The CMs were strong, stable, and permeable and, hence, of potential use as scaffolds for tissue engineering. In the present study, planar CMs supported a robust attachment, spreading, and proliferation of human dermal fibroblasts (HDFs) and human umbilical artery smooth muscle cells (HUASMCs). Collagen hydrogels were air-dried onto microgrooved templates and subsequently removed in the form of grooved CMs with the potential to align cells. The grooved CMs were highly effective at inducing HDFs and HUASMCs to elongate and align, as revealed by scanning electron microscopy and by assays of f-actin and nuclear orientation. Alignment of cells was maintained at high cell densities. CMs with grooves of substantially different widths and depths were similarly effective in causing cell alignment; however, cells aligned poorly on CMs that had grooves less than 1 μm in depth. Grooved CMs with the capability to align cells might be of considerable use in the fabrication of tissue substitutes.

Keywords: Cell culture; Collagen; Fibroblast; Hydrogel; Membrane; Microgroove; Micropatterning; Scaffold; Smooth muscle cell; Surface topography

1. Introduction

A primary goal of regenerative medicine is the creation of material constructs to facilitate healing or to supplement or replace malfunctional tissues and organs. Typically, engineered tissue substitutes consist of a non-cellular, supportive "scaffold" that is either inoculated with cultured cells in vitro or cellularized after implantation in vivo. Although certain tissue substitutes (e.g., artificial cartilage) may function with non-oriented cells, optimal function for many tissue substitutes requires an alignment of cells along one or more axes. Axially aligned cells are necessary for simulated muscle (smooth, skeletal, cardiac) to contract effectively and for simulated tendons, ligaments, and blood vessels to resist applied tension. In the context of wound repair, axially directed cell migration within healing tissues (guided tissue regeneration) is desirable in many circumstances, e.g., for the regrowth of nerves [1], repair of tendons [2], and endothelialization of vascular grafts [3].

It has been known for many decades that cultured cells will orient in response to topographic cues provided by finely grooved planar surfaces (e.g. [4]) or filamentous materials [4,5]. More recently, the concept of topographic control of cell orientation has been integrated into the design of scaffolds for tissue engineering. For example, planar substrates with contact-printed patterns of extracellular matrix or cell adhesion proteins have been used to direct the

*Corresponding author. Tel.: +1-206-903-2027; fax: +1-206-903-2044.
E-mail address: rvernon@hopeheart.org (R.B. Vernon).

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outgrowth of neurons [6,7] and to orient vascular smooth muscle cells [8] and cardiomyocytes [9,10]. Sheets of axially aligned skeletal muscle cells have been formed on arrays of parallel, polymer microfibers [11]. Microgrooved surfaces have been used to align or guide the migration of fibroblasts [12–15], osteoblast-like cells [16], endothelial cells [3], glial cells [17,18], and neurite outgrowths [19].

Currently, the majority of scaffolds used to control cell orientation are made either partly or entirely of non-biological materials. As a notable exception, 3-dimensional hydrogels comprised of magnetically aligned type I collagen fibrils have been used to direct an axial migration of Schwann cells and neurite outgrowths in vitro [1] and to improve nerve regeneration in vivo [20]. Type I collagen is viewed by many as an ideal implantable material [21]. Collagen elicits minimal immune and foreign-body responses [22,23], promotes integration of healing tissues [24,25], and is non-toxic and biodegradable [26]. In the context of collagen as a biomaterial, we recently produced novel, self-supporting collagen membranes (CMs) with thicknesses of 0.1–5.3 μm by air-drying 3-dimensional hydrogels of native, fibrillar type I collagen on ring-shaped frames [27]. Our interest in CMs of micron-scale thickness is based on the organization of supportive extracellular matrices within most tissues and organs. Such supportive extracellular matrices typically consist of membranous investments, laminae, trabeculae, or septae with thicknesses of 10 μm or less. For example, the well-defined elastic laminae within the tunica media of the human aorta are approximately 2.5 μm thick [28]. The CMs we produced were strong, flexible and stable in the absence of chemical crosslinking. Such CMs, if incorporated into engineered tissues, could provide significant mechanical support while permitting high cell densities, facilitating diffusion, and offering minimal physical interference with cell function.

One property that the CMs lacked, however, was the capacity to align cells that were seeded onto their surfaces. We observed that collagen hydrogels conformed closely to contoured surfaces upon drying [27]; therefore, it seemed possible that CMs with linear microgrooves capable of inducing cell alignment might be produced by drying collagen hydrogels onto appropriately textured templates. Such a method was used in the present study, which reports on the fabrication of CMs with microgrooves of various configurations and describes the orientative responses of cells to them.

2. Materials and methods

2.1. Cell culture

Normal human dermal fibroblasts (HDFs) (Cambrex Bio Science, Walkersville, MD) were maintained in Dulbecco's-modified Eagle's medium (DMEM) (Gibco/Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS) and antibiotics. Normal human umbilical artery smooth muscle cells (HUASMCs) (Cambrex) were maintained in complete SmGM-2 medium (Cambrex) with antibiotics. HDFs and HUASMCs were used for experiments at passage 10 or earlier.

2.2. Grooved templates for CMs

Grooved substrates for cell culture can be "masters" with the grooves cut directly into the surface, or polymer replicas cast from grooved templates. Typically, grooves in masters and templates are cut by mask-and-etch techniques (e.g. [29]) or, alternatively, with scribes or controlled abrasion [3,18,30]. For the present study, templates were made from commercially available grooved materials—vinyl phonograph records (PRs), plane-ruled diffraction gratings (PRDGs) with 30, 75, or 150 grooves/mm (Jobin Yvon, Inc., Edison, NJ) and holographic diffraction gratings (HDGs) with 500 or 900 grooves/mm (Edmund Industrial Optics, Barrington, NJ) (Fig. 1). PRs, PRDGs, and HDGs are relatively inexpensive, manufactured to precise tolerances, and have linear grooves with dimensions appropriate for work with cells.

2.3. Collagen solution for CMs

One volume of native, rat-tail type I collagen (3–5 mg/ml) in 0.02 N acetic acid (BD Biosciences, Bedford, MA) was combined with 0.111 volume of $NaHCO_3$-saturated, 10X Medium 199 (Gibco). Subsequently, 1X DMEM,

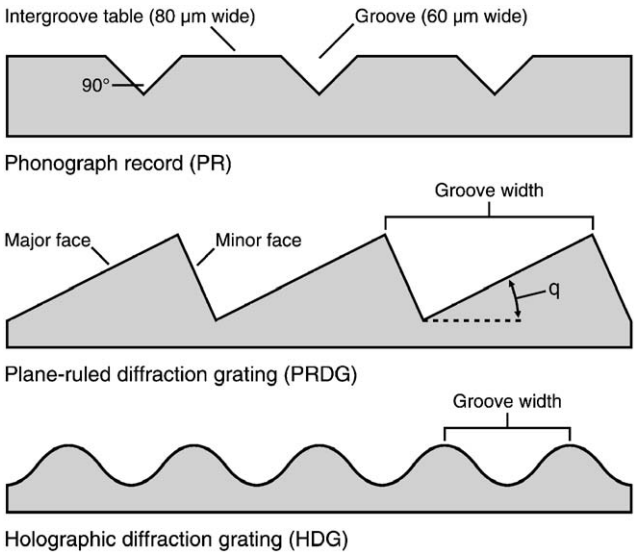

Fig. 1. Illustration of profiles of grooved templates viewed at right angles to the long axes of the grooves. All PRDGs used in this study have the same blaze angle "q" of 26.75°. Dimensions of the PRDG and HDG profiles are not in scale with the PR profile.