EXPERIMENTAL CELL RESEARCH 319 (2013) 2434–2439

Available online at www.sciencedirect.com

ScienceDirect

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

Review Article

Regulation of cell adhesion and migration by cell-derived matrices

*Matthew L. Kutys**, *Andrew D. Doyle*, *Kenneth M. Yamada*

Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial Research, United States

ARTICLE INFORMATION

Article Chronology:
Received 18 March 2013
Received in revised form
30 May 2013
Accepted 31 May 2013
Available online 7 June 2013

Keywords:
Cell-derived matrix (CDM)
Extracellular matrix
3D cell migration
Matrix adhesions
Fibrillar topography
Matrix elasticity

ABSTRACT

Three-dimensional *in vitro* extracellular matrix models provide a physiological alternative to regular two-dimensional cell culture, though they lack the full diversity of molecular composition and physical properties of whole-animal systems. Cell-derived matrices are extracellular matrices that are the product of matrix secretion and assembly by cells cultured at high density *in vitro*. After the removal of the cells that produced the matrix, an assembled matrix scaffold is left that closely mimics native stromal fiber organization and molecular content. Cell-derived matrices have been shown to impart *in vivo*-like responses to cells cultured in these matrices. In this review, we focus on mechanisms through which the distinct molecular and topographical composition of cell-derived matrices directs cellular behavior, specifically through regulation of cell-matrix adhesions and subsequent contributions to the process of cell migration.

Published by Elsevier Inc.

Contents

**Correspondence to:* National Institutes of Health, National Institute of Dental and Craniofacial Research, Building 30, Room 426, 30 Convent Dr MSC 4370, Bethesda, MD 20892-4370, United States. Fax: +1 301 402 0897.
E-mail addresses: kutysml@mail.nih.gov (M.L. Kutys), kyamada@mail.nih.gov (K.M. Yamada).

0014-4827/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.yexcr.2013.05.030

Introduction

The use of 3D *in vitro* models to bridge the gap between cell culture and *in vivo* systems permits greater understanding of the physical and chemical interactions between cells and the 3D extracellular matrix (ECM). Cell-derived matrices (CDMs) provide a more complex, physiological alternative to purified 3D matrix protein scaffolds, such as polymerized type I collagen or fibrin gels; CDMs consist of a heterogeneous mixture of proteins, proteoglycans and growth factors similar to the native stromal environment [1]. CDMs are naturally produced by the continued secretion and organization of ECM components into a 3D matrix when certain cell types are cultured at high density *in vitro*. Within several (3–10) days, a thick 3D matrix is generated that can be denuded of cells to yield a 3D ECM substrate [2]. Fibroblasts are responsible for *in vivo* stromal ECM deposition and maintenance and are commonly used for CDM generation, though 3D CDMs have been produced from endothelial, epithelial, stem cell, and cancer cell cultures [3–6].

Biochemical and biophysical characterization of CDMs

Fibroblast-generated CDM is a heterogeneous fibrous matrix, consisting primarily of a meshwork of linear fibronectin fibrils that can be oriented in parallel or more random in organization (Fig. 1, magenta fibronectin fibers). Additional matrix proteins such as collagen I and IV, perlecan, tenascin-C, hyaluronic acid, and heparan sulfate proteoglycans are present in lower abundance, as well as sequestered growth factors [3,7]. This diversity and spatial heterogeneity of CDM components mimic what is found in *in vivo* matrix, providing physiological properties not commonly found in traditional polymerized or synthetic 3D *in vitro* scaffolds. Because CDM is generated by the secretion and assembly of matrix fibers from layers of confluent cells *in vitro*, its topography consists of arrays of fibronectin fibers that are stacked to an approximate depth of 5–20 μm [5,8–10]. These fibers have an average inter-fiber spacing of approximately 1–2 μm [9], resulting in larger internal spaces in comparison to other 3D ECM models. This distinct matrix architecture provides an advantage for microscopy by minimizing light scatter through the matrix and additionally ensures relatively constant nutrient delivery and physical characteristics throughout the CDM model.

One concern raised about most 3D *in vitro* matrix models, whether comprised of a purified protein or CDM, is the physiological relevance of its matrix stiffness, particularly regions in close proximity to a rigid underlying glass or plastic surface (>1 GPa). This issue of local matrix properties is relevant biologically, since ECM stiffness is sensed by cells through cell-matrix adhesions and can alter intracellular signaling pathways, such as by Rho GTPases, leading to changes in migration, differentiation and proliferation [11–13]. Atomic force microscopy measurements of CDM have reported Young's modulus in the range of 200–600 Pa, which is comparable to reconstituted basement membrane (175 Pa), 3D collagen gels (concentration dependent, 15–1000 Pa), and dermal tissue explants (>300 Pa) [8,9,13,14]. Despite their relatively shallow depth and proximity to an artificial, rigid surface, CDMs are a physiologically-compliant matrix, allowing for flexibility and malleability similar to *in vivo* ECM. An additional physical parameter that has recently been identified as a regulator of cell migration and signaling is the elastic behavior of the matrix, *i.e.*, non-linear or linear elasticity. In contrast to non-linearly elastic 3D collagen matrices, CDMs are linearly elastic and do not undergo strain stiffening under increasing force, perhaps due to its distinct composition and crosslinking between fibers compared to meshwork collagen matrices. Using CDM, Petrie et al. demonstrated that fibroblasts have the ability to discern the elastic behavior of the ECM and accordingly switch modes of migration [14].

Besides possessing biochemical and physical characteristics that mimic *in vivo* matrices, CDM is by nature an *in vitro* system and thus more accessible to experimental manipulation. The composition of the medium used for culturing cells during CDM production can be adjusted to alter matrix composition, thickness, and topography. For example, the addition of ascorbic acid to culture media will increase the collagen content of the CDM and addition of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) will increase overall ECM production and matrix thickness [8,9]. Genetic ablation and RNA interference analyses of the cultured fibroblasts during CDM production can permit direct

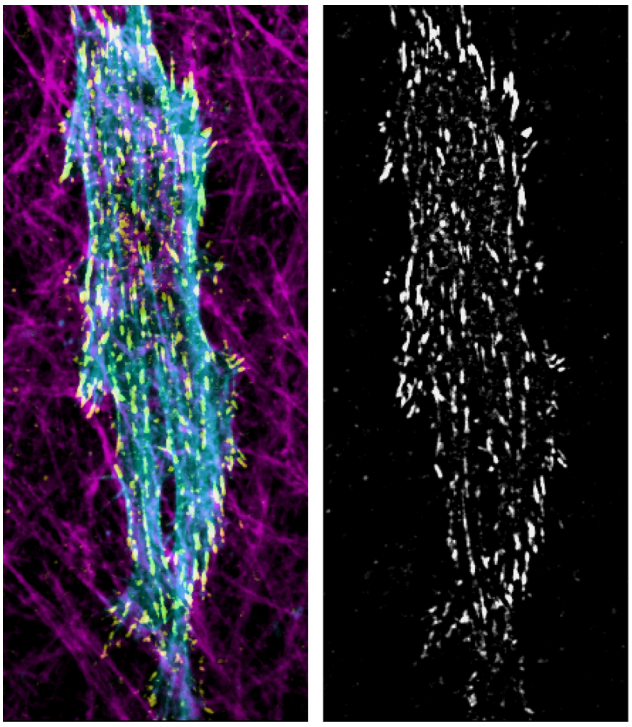

Fig. 1 – Fibroblast migration in 3D cell-derived matrix. Left: maximum projection confocal image of a human foreskin fibroblast (HFF) embedded within a matrix containing oriented CDM fibronectin fibrils (magenta, anti-fibronectin immunostaining). Cytoskeletal architecture visualized by staining for actin (blue, phalloidin) and paxillin-containing focal adhesions (yellow, anti-paxillin). Right: maximum projection image of focal adhesion profile of paxillin-containing adhesions during migration in CDM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)