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Effects of extracellular matrix analogues on primary human fibroblast behavior

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Abstract

In vitro cell culture is a vital research tool for cell biology, pharmacology, toxicology, protein production, systems biology and drug discovery. Traditional culturing methods on plastic surfaces do not accurately represent the in vivo environment, and a paradigm shift from two-dimensional to three-dimensional (3-D) experimental techniques is underway. To enable this change, a variety of natural, synthetic and semi-synthetic extracellular matrix (ECM) equivalents have been developed to provide an appropriate cellular microenvironment. We describe herein an investigation of the properties of four commercially available ECM equivalents on the growth and proliferation of primary human tracheal scar fibroblast behavior, both in 3-D and pseudo-3-D conditions. We also compare subcutaneous tissue growth of 3-D encapsulated fibroblasts in vivo in two of these materials, Matrigel™ and Extracel™. The latter shows increased cell proliferation and remodeling of the ECM equivalent. The results provide researchers with a rational basis for selection of a given ECM equivalent based on its biological performance in vitro and in vivo, as well as the practicality of the experimental protocols. Biomaterials that use a customizable glycosaminoglycan-based hydrogel appear to offer the most convenient and flexible system for conducting in vitro research that accurately translates to in vivo physiology needed for tissue engineering.

Keywords: 3-D culture; Cell proliferation; Bioresorbable hydrogel; Biomimetic substrate; Hyaluronan

1. Introduction

The practice of culturing cells in three dimensions has increased exponentially in the past decade, following numerous reports that classical two-dimensional (2-D) culturing conditions lead to aberrant cell behavior that may have limited relevance to in vivo conditions [1–4]. In organisms, a complex network of proteins and proteoglycans constitute the extracellular matrix (ECM) that surrounds every cell. Cell proliferation, migration, differentiation, angiogenesis and invasion are orchestrated by the ECM components and the signaling cascades in this three-dimensional (3-D) cellular microenvironment [5–7]. The ECM dictates the morphology and overall behavior of cells and, in turn, is constantly remodeled by matrix-specific enzymes produced by cells [8].

ECM scaffolds derived from natural sources meet many key requirements, such as biological recognition, presentation of receptor-binding ligands, cell-induced proteolytic degradation and remodeling [7]. One such material, PureCol™ (Vitrogen®), consisting of 99.9% pure type I collagen, is widely used in cell culture and tissue engineering, and as a coating material for medical devices [9–13]. A more complete suite of matricellular proteins and growth factors is provided by Matrigel™, an ECM preparation extracted from Engelbreth–Holm–Swarm (EHS) mouse sarcoma [14]. Matrigel™ has been successfully utilized for a variety of applications, such as cell growth and differentiation, angiogenesis and invasion assays, and promotes a natural

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cell morphology and behavior [14–18]. However, limited availability, batch-to-batch variability, pathogen transmission, immunogenicity, technical challenges in handling, and the inability to experimentally vary composition and compliance suggested the need for a more versatile ECM equivalent. Synthetic analogues of the natural ECMs were developed as 3-D scaffolds in an effort to accomplish in vivo-like environments in culture dishes, ex vivo tissue growth and engineering, and other scientific applications without posing health risks. One such material, PuraMatrix™, is a synthetic, self-assembling, peptide-based material that forms fibrous scaffolds and can be used for 3-D cell embedding or surface plating [19–23]. This non-animal-derived material is non-immunogenic and can be used for in vivo studies. Another ECM equivalent, Extracel™, consists of chemically modified hyaluronan (CMHA-S, also known as Carbylan™-S) and gelatin (Gtn-DTPH), which are co-cross-linked with polyethylene glycol diacrylate (PEGDA) [24,25]. This synthetic ECM (sECM) has been used successfully in numerous tissue engineering applications, has broad applicability in drug discovery [26], and is suitable for both 3-D and pseudo-3-D plating [27–34].

To date, no direct comparison of these four ECM equivalents with a primary human cell type has been described. Herein, we evaluate each of these materials for their efficiency as artificial scaffolds and convenience of use. Cell proliferation assays were designed to test the pseudo-3-D properties of these materials, while 3-D growth and cytotoxicity experiments were included to analyze the effect of the matrices on embedded cells. The results can be used to rank these sECM from a researcher's perspective, taking into consideration the biological performances of the matrices, as well as their preparation protocols and "user-friendliness". In an in vivo study, we also demonstrate that cells injected in vivo in Extracel™ show improved cell proliferation and hydrogel degradation relative to cells injected in Matrigel™.

2. Materials and methods

2.1. Preparation of gels and sponges

Matrigel™ (BD, Biosciences Discovery Labware, Bedford, MA) gels were made according to the manufacturer's protocol. Briefly, the material was thawed overnight on ice at 4 °C. Subsequently, the product was kept on ice and handled with cold pipettes. After casting, the material was allowed to gel for 10 min at room temperature in the hood before adding medium.

PuraMatrix™ (BD, Biosciences Discovery Labware) was prepared according to manufacturer's instructions. The peptide mix was bath-sonicated for 30 min prior to use. Sterile sucrose solution (10% w/v) was used to dilute the material twofold. The protocol instructions were followed to obtain gels. Briefly, at 5 min after casting, culture medium was gently added on the material to increase the pH of the matrix and promote gelation. The medium was changed three more times during the next 30 min.

PureCol™ (Inamed, Freemont, CA) was mixed with 10× phosphate-buffered saline (PBS) as recommended by the supplier and the pH of the solution was adjusted to 7.4 with 1 M NaOH. The prepared collagen solution was then filtered through a 0.45 μm syringe driven filter unit prior to casting, to ensure sterility.

Extracel™ hydrogels (Glycosan BioSystems, Inc., Salt Lake City, UT) were obtained by mixing 1% w/v CMHA-S solution (Glycosil) with 1% w/v Gtn-DTPH solution (Gelin-S) in a 1:1 volume ratio and cross-linking this mixture with 2% w/v PEGDA (mol. wt 3400) in a 4:1 volume ratio. All components were dissolved in DMEM/F12 + 10% newborn calf serum + 2 mM L-glutamine + penicillin/streptomycin (T31 fibroblast growth medium), and the pH of the CMHA-S and Gtn-DTPH solutions were adjusted to 7.5 with 0.1 M NaOH. All solutions were then filtered through a 0.45 μm syringe driven filter unit prior mixing to ensure sterility. The hydrogels were cast and allowed to gel in the hood at room temperature before adding medium.

2.2. Pseudo-3-D cell proliferation assay

T31 human tracheal scar fibroblasts were a generous gift from Dr. S.L. Thibeault, Department of Surgery, University of Wisconsin, Madison, WI). T31 fibroblasts [29,35] are sensitive, non-immortalized cells derived from primary culture and were selected as being representative primary cells. The T31 cells were evaluated under pseudo-3-D plating conditions in 96-well plates. Each material tested (50 μl well^{-1}) was used to coat one row per plate (a total of seven plates were used, one for each day of the assay). The hydrogels were allowed to gel and were seeded with 3.5×10^4 cells ml^{-1}. On the third day of culture, the medium was refreshed. Cell numbers were monitored each day by using the Cell-Titer 96 Aqueous One Solution Cell Proliferation assay (MTS assay) (Promega, Madison, WI). The A_{490} values, which are directly proportional to the number of viable cells, were plotted against the time course of the assay to yield the growth profile of the cells seeded on various sECMs.

2.3. 3-D cell culture

To determine the growth of T31 fibroblasts encapsulated in various gels, cells were entrapped in different materials at a final concentration of 10^5 cells ml^{-1} and 100 μl of cell + sECM mix was cast per well of a 24-well plate containing Corning Transwell permeable supports (inserts) with 8.0 μm membrane pore size (Corning Inc., Corning, NY). After gelation, cells were incubated with DMEM/F12 + 10% newborn calf serum + 2 mM L-glutamine + penicillin/streptomycin for 72 h and the MTS assay was used to estimate the number of viable cells in each material.

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