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An efficient rinsing procedure for 10 mm cell culture inserts prior to co-culture studies

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Abstract. A goal of our laboratory is to define putative intercellular communication mechanisms between myofibers and adipocytes. In pursuit of this objective we established a co-culture system incorporating commercially available microporous cell culture inserts. These cell culture inserts provide separation of cells bathed in a common media environment. Isolation and evaluation of the conditioned media from such systems allows detection of autocrine or paracrine agent production by either cell type. However, the process of rinsing cells plated on inserts prior to initiating co-culture may damage insert integrity and may dilute (added) defined treatment medium by introducing residual rinse medium. We have developed a method to rinse microporous membrane inserts before the addition of defined treatment medium. This rinsing technique decreases damage to inserts and reduces the volume of residual medium on all physical aspects of the insert.

Key words: Co-culture, Cell culture inserts, Rinsing

1. Introduction

Postnatal skeletal muscle is composed primarily of muscle fibers (myofibers) [3, 4]. Although a much smaller contributor to muscle mass, adipoctyes differentiate and form lipid reservoirs [15] within the myofiber environment [10]. The relative proportion of myofibers to adipocytes defines the muscle composition of an animal [13]. Defining intercellular communication pathways between myofibers and adipocytes, which may regulate filling of muscle lipid depots, may allow manipulation of the final composition of muscle. Therefore, in an attempt to delineate these potential intercellular communication links, we have established a defined co-culture system using microporous cell culture technology.

Microporous cell culture inserts are one component of an in vitro environment that may be utilized to simulate in vivo conditions [2, 9, 11]. Microporous membrane inserts provide a physical barrier to maintain separation of cell types [1, 7, 9, 12], while allowing cells access to communal [1] or divergent [8] conditions. Commercial inserts with multiple pore sizes within the porous membrane are available that may be utilized to determine the regulation of counter current flow of regulatory molecules across the membrane [2, 12, 14]. However, cell culture inserts are easily damaged during manipulation, and rinse techniques may leave residual rinse or treatment media. For example, when changing medium from apical and basolateral surfaces of the cell culture inserts with sterile pipets, we repeatedly observed that considerable time was spent performing rinses to prevent damage to membrane integrity from inadvertent membrane contact with pipet tips. Moreover, complete removal of rinse medium from inserts with serological pipets, specifically the basolateral surface, is extremely difficult and leaves residual medium that may compromise data from intercellular communication studies. To help address these technical difficulties, we have established a protocol that offers more efficient control of experimental variables associated with handling, rinsing and use of microporous cell culture inserts.

2. Materials

A. Aseptic technique materials
   1. Laminar flow hood (model EG-4252).[1]
   2. Peristaltic pump assembly, 115 V, single speed pump drive and head, silicone tubing (model XX80 202 00).[2]
   3. Ring stand
   4. Tri-grip clamp or thermometer clamp
   5. 13.12 cm Halstead hemostats, curved
   6. Ethanol (95% and 70% prepared in lab)

B. Glass and plasticware
   1. Pasteur pipets, 14.5 cm (catalogue No. 14672-200).[3]
   2. Cell culture dish 60 × 15 mm (catalogue No. 3060).[4]
   3. Cell culture plates, 6 well, 35 mm dia. (Mark II, catalogue No. 3406).[4]