Maintaining intact mature lung tissue in culture using low melt agarose

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Abstract. The development of methods to culture intact viable tissue is integral to understanding mechanisms of disease. Mature lung tissue, in particular, is difficult to maintain for any extended period of time in culture due to collapse of airspaces and rapid tissue necrosis. In the present study, we report the methodology for culturing whole lung tissue in vitro using low-melt agarose to maintain lung airspaces open and viable. Perfused rat lungs were inflated in situ with a warm liquid low-melt agarose solution. Upon cooling, the agarose polymerizes maintaining the airspaces in an open state and allows thin sections of lung to be cut and cultured in a defined environment. Viability of the cultured lung tissue was assessed biochemically. Protein synthesis was maintained for 7 days in cultured lung tissue and was dependent upon the oxygen concentration in which the tissue was cultured. Cell proliferation was assessed immunohistochemically in parenchyma of lung slices cultured in the presence of 5-bromo-2'-deoxyuridine (BRDU). BRDU labeling indices for tissue cultured in 21% O₂ or greater showed an oxygen-dependent increase in cell proliferation after 3 days in culture followed by a return to baseline levels after 7 days. The data describe the maintenance of mature lung tissue in culture and indicate the usefulness of the in vitro lung slice model for examining the mechanistic basis of lung injury and subsequent remodeling.

Key words: 5-bromo-2'-deoxyuridine (BRDU), Fractional synthesis rate (FSR), Lung slice, Organ culture

1. Introduction

Culturing intact tissue provides a means to examine the mechanistic basis for many disease processes in systems where cell-cell and cell-matrix interactions are maintained similar to those found in vivo. In these studies, we describe a method for culturing mature rat lung in vitro using low-melt agarose to maintain the vasculature and the airspaces of the lung open and distended under physiological pressures. A major advantage of this in vitro model is maintenance of the structural integrity and biochemical function of lung tissue while in culture.

The maintenance of intact mature lung tissue in culture presents a challenge in the collapse of airspaces and rapid necrosis of tissue is observed [1]. To address this problem, Hackney et al. [2] described a method for culturing lung tissue using agar to maintain the airspaces in an open state. Subsequently, others have made adaptations and demonstrated the utility of the model in studying lung injury [3–7]. In our studies, we report the methodology for culturing intact lung tissue using low-melt agarose instilled into the airways and pulmonary vasculature. A major modification of existing models is that the pulmonary vasculature is also instilled with agarose. We report that tissue protein synthesis and cell proliferation are maintained in lung tissue up to 7 days in culture. In addition, proliferative responses in the present in vitro model are similar to in vivo models of hyperoxia exposure [8, 9] suggesting the utility of the in vitro model in studying mechanisms of lung injury and disease.

2. Materials

A. Animals

B. Surgical dissecting instruments
1. Curved Kelly artery forceps (3), No. D2686-1A.
2. Tubing clamp forceps (2), No. D2682-1A.
3. Mayo scissors (2), No. D2655-1A.
4. Weitlaner retractor (1), No. 16-5360.
5. Surgical thread, 4-0, No. 108-S.
6. Autoclave to sterilize.

C. Chemicals
3. KCl, No. P4504.
5. KH₂PO₄, No. P5379.
8. EDTA, No. ED2SC.
9. Penicillin and streptomycin, No. 15070-014.
10. MEM essential amino acids, No. 21135.
11. MEM nonessential amino acids, No. 11140.
13. Low-melt agarose, No. 15517-022.
15. Dulbecco’s modified Eagles’s medium F-12 (DMEM-F12), No. 11320-025.
16. Heparin sodium injection, 1000 units/ml.
17. Enthyl alcohol, No. 0000517.
18. Sodium pentobarbital.

D. Disposables and incidentals
1. Culture dishes, 12 well, No. 25815.
3. 50 ml Falcon conical tube, No. 2098.
4. Polyethylene tubing (No. 7431, inside diameter 0.045", outside diameter 0.062").
5. 10 cc syringes.
6. 18 gauge tubing adapters.
7. Disposable underpads, No. 56616-018.
8. Sterile 50 ml beakers, 1 per animal, No. 13910-143.

E. Equipment
1. Harvard rodent ventilator, Model 863.
2. McIlwain tissue chopper, No. 23 40 100-2.
4. Sterile tissue culture hood.
5. Alcohol or gas bunsen burner.
6. Tissue culture incubator, Model 3159.
7. Gas monitor: O₂ analyzer, Model 5524.

3. Procedure
A. Preparation of solutions
1. Perfusion buffer:
   10 mM Hepes, 142 mM NaCl, 4.7 mM KCl, 1.7 mM MgSO₄, 1.2 mM KH₂PO₄, 2.8 mM CaCl₂, 5.0 mM glucose, 0.5 mM EDTA, pH 7.4, supplemented with penicillin 100 U/ml, streptomycin 100 μg/ml, MEM essential and nonessential amino acid solutions and vitamins. For 2 rats, 500 ml of buffer is filter sterilized using sterile bottle top filter (0.22 μm).
2. 0.5% Agarose instillation buffer:
   For each rat, dissolve 0.15 g low-melt agarose in 30 ml of perfusion buffer in a 50 ml conical tube in a water bath at 65–70 °C. This typically takes 20–30 min with mixing every 5 min. The liquid agarose solution is placed into a water bath set at 40 °C for at least 30 min prior to infusion.

B. Preparation of foam biopsy pads
Cut circles 1.8 cm in diameter from square foam biopsy pads using a leather punch and autoclave in a beaker covered with aluminum foil. These circles fit into 12 well culture dishes and support the lung slices.

C. Preparation of lung slices
A male Sprague-Dawley rat (200–250 g) is anesthetized with an intraperitoneal injection of sodium pentobarbital (150 mg/kg body weight), placed in a supine position and the ventral surface swabbed with 70% ethyl alcohol. It is important that the animal is still breathing and the heart is pumping. Check the animal for pain reception by applying increasing pressure with your finger and thumb on one of the hind feet. A section of the fur is removed from the abdominal region all the way to the head of the animal exposing the underlying muscle. The trachea is dissected free of surrounding tissues taking care not to sever major blood vessels in the neck and an 18 gauge tubing adapter is inserted into a small incision in the anterior trachea and tied in place with sterile surgical thread pre-positioned below the incision. The adapter is connected to a mechanical ventilator and ventilation is begun at a rate of 60 breaths/min with a tidal volume of 8 ml/kg body weight. A median sternotomy incision is made and the thorax exposed with retractor. It is very important that the retractor does not damage the lung tissue. The left ventricle of the heart is injected with 0.4 ml Heparin and allowed to circulate for 30–60 seconds. Using a Kelly artery forcep, a 15–20 mm long piece of surgical thread is positioned under the pulmonary artery several millimeters distal to the right ventricle. A polyethylene tube is inserted into a small incision where the main pulmonary artery connects with the right ventricle and tied in place with sterile surgical thread pre-positioned below the incision.