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Biocompatibility of Poly-DL-lactic acid (PDLLA) for Lung Tissue Engineering

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ABSTRACT: This study explores the possibility of growing lung cells on poly-DL-lactic acid (PDLLA) scaffolds, with a view to in future engineer pulmonary tissue for human implantation. As a first step in this process, the ability of PDLLA to maintain the growth of lung epithelium is tested using a robust cell line. Poly-DL-lactic acid has been investigated in two forms, as planar discs and as 3-D foams, and it has been demonstrated that PDLLA is not only non-toxic to pneumocytes but it also actively supports their growth. The initial findings suggest that the material is an appropriate matrix for engineering of distal lung tissue.

KEY WORDS: poly-DL-lactic acid (PDLLA), lung tissue engineering, scaffolds, thermally induced phase separation, murine lung epithelium cell.

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INTRODUCTION

Poly-DL-lactic acid (PDLLA) is a well-known super-high-molecular-weight acid that has good biocompatibility and degrades following *in vivo* implantation [1]. It has been previously shown that PDLLA, alone or as a composite, supports the growth of osteoblasts, chondrocytes, and lung carcinoma cells [2–5]. These features, plus the intrinsic adequate elastic properties and flexibility of PDLLA, led us to study the suitability of this biodegradable polymer for lung tissue engineering.

Lung disease occurs in many forms and is widespread and debilitating. When lung function continues to decline and medical or conventional surgical treatment is not likely to yield further results, transplantation has become the final option. However, donor organ availability has declined in recent years and around 40% of patients die while on a waiting list for lung transplantation [6]. Thus, there is an urgent need for new approaches to the treatment of lung diseases, such as replacing damaged, poorly or non-functioning tissue.

The rapidly burgeoning field of tissue engineering is aimed at repairing, replacing, and/or regenerating tissue and, to achieve this, two main elements are required; cells and appropriate scaffolds that promote the growth and proliferation of the specific cell phenotype/s [7]. There are several different sources of cells for use in tissue engineering but, for clinical applications, their normality, abundance, and ease of manipulation are all requirements. Embryonic stem cells are known to be pluripotent, and are capable of both self-renewal and differentiation into a variety of cell lineages [8]. We have previously identified embryonic stem cells as a potential source of lung epithelium for tissue engineering and are currently scaling up their production *in vitro* for further experimentation [9].

This study was carried out to explore the possibility of growing lung cells on specific PDLLA scaffolds, with a view to future engineering pulmonary tissue for human implantation. As a first step in this process, for the first time here the ability of PDLLA to maintain the growth of lung epithelium was tested using a robust cell line.

EXPERIMENTAL

Both dense (non-porous) 2-D films and 3-D foam scaffolds of high porosity were considered. Poly-DL-lactic acid films were prepared by dissolving 0.15 g of the polymer homogeneously in 3 mL of dimethyl carbonate (DMC) at 50°C. This polymer–solvent mixture was applied

to circular glass coverslips (16 mm in diameter to fit into 24-well cell culture plates) previously cleaned with acetone and alcohol. The coverslips were left at room temperature in a fume hood for 48 h to remove the solvent and then immersed in distilled water for 24 h. The PDLA films were peeled intact from the coverslips and stuck to the base of the cell culture wells (24-well plates; Triple Red, UK) using a small amount of DMC. The PDLA foams were prepared by thermal induced phase separation (TIPS) processing, as described previously [5]. Briefly, PDLA with an inherent viscosity of 1.62 dL/g was used (Purasorb[®], Purac Biochem, Goerinchem, The Netherlands). The polymer was dissolved in DMC to give a polymer weight to solvent volume ratio of 5%. The mixture was stirred overnight to obtain a homogeneous polymer solution. The solution was transferred to a lyophilization flask, immersed in liquid nitrogen, and maintained at -196°C for 2 h. The frozen solution was then transferred into an ethylene glycol bath at -10°C and connected to a vacuum pump (10^{-2} Torr). The solvent was sublimed at -10°C for 48 h and then at 0°C for 48 h. The foam samples were subsequently completely dried at room temperature in a vacuum oven until reaching a constant weight, as determined by using an electronic balance.

A sample of foam was sputter coated with gold for 2 min and its structure examined by scanning electron microscopy (SEM) at an acceleration voltage of 10 kV. The foams were found to comprise a bimodal pore system, with the larger cylindrical pores having diameters in the range 100–150 μm and the smaller pores, a size of 15–40 μm , forming an interconnected network (Figure 1). This pore structure is typical of TIPS processed foams [5]. The tubular macropores are highly orientated as a result of the unidirectional cooling process. As discussed in previous studies [2–5], foams made by TIPS have a sufficient pore volume and pores of a given controlled size and orientation for cell proliferation as required in tissue engineering scaffolds.

The multiwell plates containing films or foams were sterilized prior to use by exposure to UV light for 1 h. The discs and foams were preconditioned for 9 days in basal HITES medium (Invitrogen, Paisley, UK), which comprises 50% Kaighn's modification of F-12 (F-12K), 50% Dulbecco's modified Eagle's medium (DMEM) (LGC, UK) with an added 10% antibiotic/antimycotic (A/A) solution (Invitrogen, UK). The medium was changed each day with a decreasing A/A concentration. Murine lung epithelium (MLE-12) cells (ATCC, Manassa, VA, USA) [10] were used in this study. After 9 days of preconditioning, triplicate sets of MLE-12 cells were seeded at a density of 2000 cells/cm² (a total of 4000 cells per well) on to the PDLA films in 24-well plates and

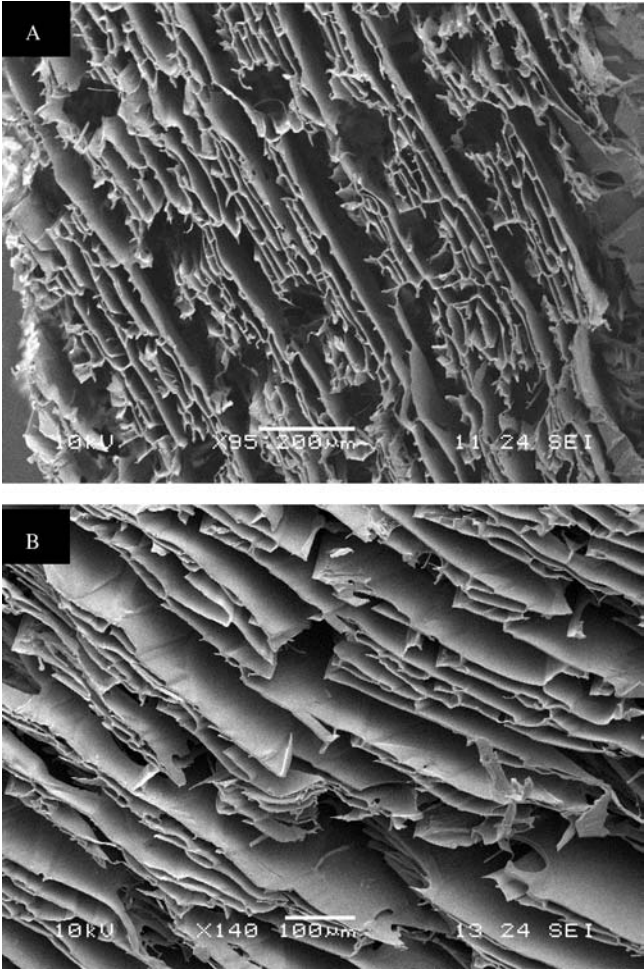


Figure 1. Scanning electron micrographs of a PDLLA foam scaffold cut in two different planes showing details of the oriented pore system typical of TIPS foams [5].

grown for periods of 1, 2, 4, 6, or 8 days, with the medium being changed each day. During the culture periods, cells were examined routinely under an Olympus IX70 inverted microscope. For a quantitative analysis of the number of living cells, the WST-1 assay (Roche, Welwyn Garden City, Herts, UK) [11] was carried out on triplicate sets of cultures grown on PDLLA discs and, as controls, culture plastic. The assay was only done on cells grown on discs as it was not possible to

elute all cells from the foams. Fifty microliters of the WST-1 solution was added to each well. Three hours later, 110 μ L of the medium was transferred to a 96-well plate and tested with a Dynes MRX multiwell plate reader. The test filter was at 450 nm and the reference filter at 650 nm. SEM was also carried out on the cells grown on PDLLA films following fixation in 4% formaldehyde, dehydration, and sputter coating with gold for 2 min. An acceleration voltage of 5 kV was used and the working distance was 32 mm.

The PDLLA foams were seeded with MLE-12 cells and cultured for 8 days in the medium that was changed every day. After 8 days, the foams were washed, fixed with 4% (v/v) formaldehyde for 1 h, and then cut into small pieces using a razor blade. The cells were visualized by staining with eosin nuclei. They were counterstained with DAPI (Vectashield; Vector Laboratories, Burlingame, California, USA) and examined under an Olympus BX-60 epifluorescence microscope. Images were captured with a Zeiss Axiocam digital camera and analyzed using KS-300 software (Imaging Associates, Thame, UK).

RESULTS AND DISCUSSION

During the 8 days of culture, cells were seen to colonize the films and foams gradually. Comparing the cells grown on PDLLA discs and the controls growing on plastic, no alteration in cell morphology or increase in the rate of cell death could be seen. It was not possible to follow the growth of the living cells into the foams but, although colonization could be seen, there appeared to be some slowness in penetrating into the pores compared with how quickly the cells spread onto the films. Examination of stained preparations revealed cell nuclei as fluorescent blue spots and confirmed observations made using inverted microscopy. Thus, it was clear that cells had attached to the discs and grown over the surface (Figure 2) and had penetrated into the pores of the foams (Figure 3). Although an even growth pattern could be seen on the films, the distribution and density of the cells within different pores in the foams was extremely variable. The results of the WST-1 assay showed that MLE-12 cell growth on PDLLA paralleled that on culture plastic with no significant differences (Figure 4). Growth peaked on day 6, when the cells reached confluence, and then declined.

The aim of this study was to evaluate the potential of PDLLA as a matrix for the growth of lung epithelium, specifically pneumocytes. The results showed that PDLLA not only maintained the cells but also caused a significant increase in their proliferation and, thus, confirm the

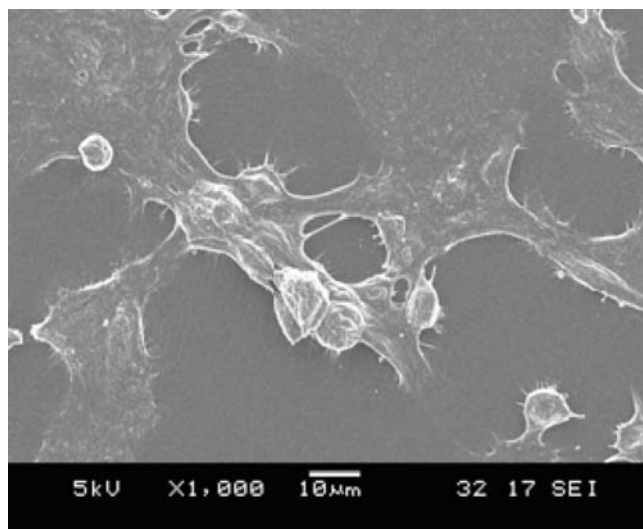


Figure 2. Scanning electron micrograph of MLE-12 cells spreading over and attaching to the surface of a PDLA disc.

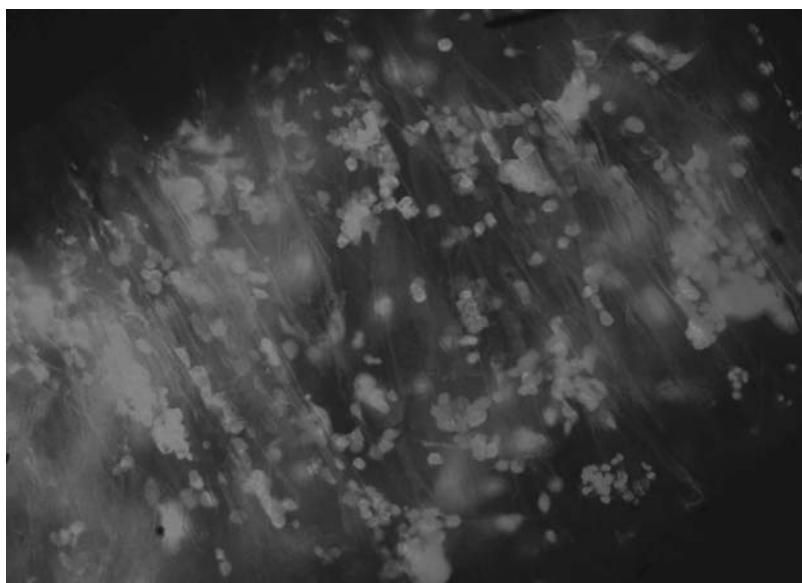


Figure 3. Fluorescence microscopy image of numerous MLE-12 cells growing over the surface of a PDLA foam and into the foam pores. (Original magnification $\times 100$).

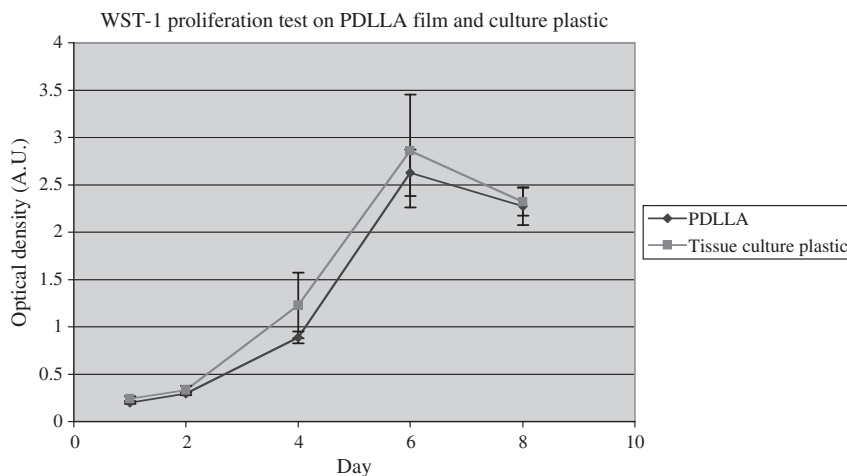


Figure 4. Results of the WST-1 assay. Similar rates of growth were seen for MLE-12 cells grown on PDLLA film and on cell culture plastic, with a peak being reached at day 6 when the cells achieved confluence.

suitability of this material for use as a scaffold in lung tissue engineering.

Poly-DL-lactic acid is being investigated with increasing success to produce scaffolds for tissue engineering. Most studies based on this polymer have been related to bone and cartilage tissues [2,3,5,12–16], with very limited previous work investigating the engineering or reconstruction of other tissues. Only the behavior of PDLLA in contact with fibroblasts [17] or as scaffold for peripheral nerve regeneration [18,19] has been given attention. In most previous studies, the surface of PDLLA has been modified to enhance cell adhesion and proliferation, either by combination with inorganic particles, such as hydroxyapatite [13] or bioactive glass [2–4], or by plasma treatment [13,20].

In the present study, we tested PDLLA in two forms, as dense 2-D films and as 3-D foams. Although *in vitro* work may include the use of flat surfaces of PDLLA, our overall goal is to engineer lung tissue. For this reason, we were particularly interested in determining whether pneumocytes can grow on PDLLA scaffolds configured in a 3-D porous structure that approximates that of the lung. However, we also wanted to evaluate whether pneumocyte proliferation is increased by the material PDLLA. This was not possible using only foams as the vastly increased surface area of the substrate could itself encourage

proliferation. Thus, both foams and flat films were used in this study and the combined results analyzed. Based on the positive results of this investigation, further quantitative work focussed on 3-D PDLA foam scaffolds with different engineered pore structures is planned.

CONCLUSIONS

For the first time, we have demonstrated that untreated PDLA not only has no toxic effect on pneumocytes but also it supports their growth. These initial findings suggest that the material is an appropriate matrix for engineering of distal lung tissue. The optimization of the 3-D pore structure of PDLA foams for this particular lung tissue engineering application is the focus of the current research.

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