COMPARISON OF NANOPOROUS SCAFFOLDS MANUFACTURED BY ELECTROSPINNING AND NANOFIBRILLAR COMPOSITE CONCEPT

S. T. C. Lin¹*, D. Bhattacharyya¹, S. Fakirov¹, B. G. Matthews², J. Cornish²
¹ Centre for Advanced Composite Materials, Department of Mechanical Engineering, The University of Auckland, New Zealand, ² Department of Medicine, The University of Auckland. * Corresponding author (slin122@aucklanduni.ac.nz)

Keywords: electrospinning, nanofibrillar composite (NFC) concept, poly(L-lactide) (PLLA), poly(ethylene terphthalate) (PET)

1 Introduction

Fibrillar networks have numerous applications in the biomedical field, which is evident from the extensive research that has been carried out to produce biomedical devices using a variety of manufacturing techniques. Electrospinning in particular has been used to produce devices for tissue engineering, drug release, wound dressing and enzyme immobilisation [1]. Other applications utilising fibrillar networks include filtration, protective clothing, reinforcement in composite materials and sensors [2]. The ability to perform tissue and organ transplantation is limited by the availability of suitable donor tissue and in many cases requires life-long use of immunosuppressant therapy. Manufacturing of biomaterials for tissue engineering is therefore an attractive prospect where many fabrication methods have been tested extensively [3]. This paper compares the morphological characteristics and ability to support cell attachment and growth of scaffolds produced using electrospinning and the nanofibrillar composite (NFC) concept. Two different materials were used for each manufacturing technique, producing four different scaffolds to study and compare.

2 Materials and Methods

2.1 Materials

Poly(L-lactide) (PLLA) 3051D from NatureWorks®, commercial grade poly(ethylene terphthalate) (PET), glycol modified poly(ethylene terphthalate) (PETG) GN119 copolyester from Eastar and poly(vinyl alcohol) (PVA) TC232 from Kuraray Poval were used to fabricate four different scaffolds. Solvents used for electrospun scaffolds consisted of dichloromethane (DCM), dimethylformamide (DMF) and trifluoroacetic acid (TFA). Distilled water was used for the production of the NFC scaffolds.

2.2 Electrospinning

PLLA and PET were used to fabricate nanoporous scaffolds using the electrospinning technique. A 0.1 g/mL PLLA solution was prepared by dissolving PLLA particles in a solvent mixture of 3:2 DCM/DMF (volume/volume). PET particles were dissolved in a solvent mixture of 1:1 DCM/TFA at a concentration of 0.2 g/mL. For both polymers a voltage of 10 kV and a feed rate of 1 mL/hour were used. Round glass cover slips were placed onto aluminium foil and used for collection of nanofibres. The target collector was positioned 100 mm from the needle tip. The electrospinning process was carried out at 23 ± 3°C with 40 ± 10% humidity. These parameters produced nano-sized fibres without the formation of beads and were selected based on studies carried out by Patra [4, 5].

2.3 Nanofibrillar Composite Concept

PLLA/PVA films were prepared by mixing dried PLLA and PVA pellets with a ratio of 1:4 (by weight). The melt blending of these thermodynamically immiscible components was carried out by extruding at 180°C. The extruded yarn was aligned and wound onto a stainless steel wire frame and immersed in a heated distilled water bath below 60°C for 4-5 days until the PVA component of the composite blend was removed. A thin film of PLLA was obtained after drying the aligned yarn with the PVA component removed, at room temperature.

To avoid PVA decomposing at higher temperatures, a lower melting copolymer of PET was used. Dried PETG and PVA were mixed with a ratio of 3:7 (by weight). The blend was extruded at 230°C to form a composite PETG/PVA yarn which was also wound onto a stainless steel wire frame in preparation for removing PVA with heated distilled water.
The NFC technique does not require the use of any toxic solvents in the scaffold production process and is one of the main advantages compared to the electrospinning technique.

2.4 Cell Culture

The electrospun scaffolds were sterilised by autoclaving at 120°C. NFC scaffolds were sterilised by soaking in 70% ethanol for at least 30 minutes. NFC scaffolds were also autoclaved, however, results from tests showed that cells favoured the scaffolds which were sterilised by ethanol at room temperature. Therefore, subsequent NFC scaffolds were sterilised by ethanol as they were more prone to structural disruption from heat and moisture when sterilised via autoclaving.

Sterilised scaffolds were placed into a 24-well plate and soaked in cell culture media; alpha-minimum essential medium with 5% fetal calf serum (αMEM + 5% FCS), overnight in an incubator at 37°C and 5% CO₂. After 24 hours of soaking, thinner samples were slightly transparent compared to thicker scaffolds. The medium was removed and the soaked scaffolds were then seeded with 0.4 mL mouse osteoblastic MC3T3-E1 cells at a density of 50,000 cells/mL.

Live-dead staining (Invitrogen) was performed after 7 days in culture to assess cell attachment and growth. Cells were washed twice with phosphate buffered saline (PBS) and stained with PBS containing 2 μM ethidium homodimer and 2 μM calceinAM for 15 minutes at 37°C. CalceinAM is converted to a green fluorescent product within live cells while ethidium enters cells with damaged membranes and produces a bright red fluorescence upon attachment to nucleic acids in dead cells [6]. Cells were viewed immediately after staining, on an inverted fluorescence microscope and images were taken.

2.5 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was carried out using the Philips XL 30S FEG SEM model to examine the morphology of the scaffold and how the cells interact with the scaffolds.

MC3T3-E1 cells grown on PLLA, PET and PETG nanofibres for seven days were also examined using SEM. To preserve the cell morphology, scaffolds with cells attached were washed with PBS twice and fixed with 2.5% glutaraldehyde in phosphate buffer for 24 - 72 hours. They were dehydrated in a series of 50, 70, 80, 95 and 100% ethanol washes before critical point drying, coating with platinum and observing under SEM.

3 Results

3.1 Scaffold Morphology Comparison

Electrospun scaffolds possess a highly porous, cross-plyed and fibrillar structure compared to the NFC scaffolds in which the fibres are interconnected due to branching. The average fibre diameter for electrospun PLLA is 125 ± 60 nm (Fig. 1) and for PET is 145 ± 100 nm (Fig. 2). The NFC technique produces a three-dimensional, interconnected, porous network after extraction of the PVA component with distilled water. The average diameter is 75 ± 50 nm (Fig. 3) and 70 ± 30 nm (Fig. 4) for PLLA and PETG, respectively.

3.2 Cell Morphology on Scaffolds

The scanning micrographs show that the cells have adhered well onto the scaffolds. The PLLA electrospun scaffolds in Fig. 5 illustrate a packed layer of cells completely covering the scaffold. The cells are one layer thick, which is typical of the type of cells used.

The PET scaffold (Fig. 6) is not as densely packed with cells. However, the cells have infiltrated and embedded themselves within the scaffold. In Fig. 7, it can be seen that the cells have migrated beneath the surface of the scaffold. This is promising as it is important for cells to penetrate through the scaffolds, forming tissue in the centre of the scaffold. When cells only adhere to the surface of the scaffold without migrating beneath the nanofibres towards the centre, the formation of a layer of tissue on the surface of a scaffold creates a blockage of nutrient exchange to the centre of the scaffold, resulting in a necrotic core [7].

The PETG scaffolds manufactured using the NFC concept, tend to follow the fibre direction (Fig. 8). The cells have also integrated into the fibres of the scaffold. However, the cells appeared to grow better on the electrospun material, as there were very few cells present on the PETG scaffolds manufactured using the NFC concept. The cells also do not look healthy (Fig. 9) compared to the cells grown on the electrospun scaffolds.
It is not clear at this preliminary stage of testing why the cell growth was better on the electrospun scaffolds than the NFC scaffolds. One possibility is the different structures of the nanofibres. Fig. 9 shows the individual fibres of the NFC scaffolds coagulate into clumps of solid mass which leads to a decrease of porosity of the scaffold. Thus, the transfer of nutrients to cells through the scaffold cannot be achieved successfully, which limits the successful growth of the MC3T3 cells. It is also possible that there is material contamination occurring during the extrusion process. Therefore, it is anticipated that the manufacturing process will be modified to avoid material contamination as much as possible. The coagulation of nanofibres associated with the NFC scaffolds will also need to be addressed, to increase the porosity of the NFC scaffolds. Further testing is required to improve the manufacturing of the NFC scaffolds to ensure they are suitable for cell growth. The advantage, if successful, will be the production of organic solvent-free scaffolds.

3.3 Cell Response

Fluorescent micrographs of live-dead stains conducted on Day 7 (Fig. 10) confirm that the PLLA electrospun scaffolds are covered with a dense layer of cells with fewer on the PET electrospun scaffolds. The corresponding image of dead cells is from the same field of view.
There are only a few dead cells on both the electrospun PLLA and PET scaffolds, which verify there is no obvious toxicity from the organic solvents that were used for preparing the electrospinning solutions.

Images of cell growth on PETG and PLLA scaffolds produced using the NFC concept after seven days in culture (Fig. 11), show that very few cells have grown on both the PETG and PLLA scaffolds, compared to the electrospun scaffolds. The cells tend to grow in clumps spread throughout the scaffold. There are more healthy patches of cells on the PLA scaffold produced via the NFC concept compared to the PETG scaffold.
4 Comparison of Manufacturing Process and Scaffolds

The main disadvantage of electrospinning compared to the NFC technique is that electrospinning involves the use of toxic organic solvents. This creates a major advantage for NFC scaffolds, as it can potentially be produced completely free of toxic solvents.

The electrospinning process is straightforward compared with the NFC technique which is more time consuming. Electrospinning is also a cleaner way of producing scaffolds, where materials are easier to keep sterile and uncontaminated, which is a problem for the NFC scaffolds as they are produced using large laboratory equipment that may have contaminated internal machine parts.

The NFC scaffold surfaces look disordered after being subjected to the cell culturing process, whereas for electrospun scaffolds, the structure of the nonwoven nanoporous fabric maintains its morphology. Maintaining the morphology of the scaffold through the entire manufacturing and cell culturing process is important and affects the results immensely. This is evident from the live/dead images where cells have completely covered the PLLA electrospun scaffolds after seven days compared to the NFC scaffolds where the cells seem to be clumped in patches. The MC3T3 cells also seem to either attach or grow better on the PLLA scaffolds manufactured using both electrospinning and the NFC technique compared to PET and PETG scaffolds.

Although from the initial results, cells tend to favour electrospun scaffolds over NFC scaffolds, the manufacturing process for the NFC scaffolds can definitely be improved and initial tests do look promising with some healthy looking cells present.

5 Conclusions

Electrospinning and the NFC concept have been used to fabricate nanofibrillar networks. An osteoblastic cell line has been successfully cultured on both the PLLA and PET electrospun scaffolds, where the cells have infiltrated into the PET structure. Further testing is required to confirm the success of the MC3T3 cells grown on the NFC scaffolds although the initial tests are promising where the fluorescent images do show patches of healthy cells.
References


