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Electrospun Bioresorbable Tissue Repair Scaffolds: From Laboratory to Clinic

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Abstract— The healing of soft tissue wounds and injury sites is a complex process requiring the participation of many different cells, tissues, proteins and tissue components in a co-ordinated manner. We describe the development of regenerative, electrospun, bioresorbable advanced material tissue scaffolds providing three dimensional (3D) structure for cells involved in the repair of soft tissue injuries. One product, EktoTherix™ provides a micron-scale 3D architecture to enhance the recruitment of reparative cells onto this temporary support and in this way the body's capacity to repair itself is utilised. EktoTherix and other electrospun tissue scaffolds have been translated from early stage laboratory work through manufacturing process development and clinical investigation.

Keywords— electrospinning; scaffold; tissue repair; medical device translation; bioresorbable polymer

I. Introduction

Over the past two decades there has been much research into the use of electrospun biomaterials for applications in regenerative medicine, drug delivery, and in vitro tissue modelling. Although a promising technique for the manufacture of a wide variety of functional biomaterials proposed for use in almost all clinical specialties, complex technical challenges and regulatory requirements must be satisfied in order to develop clinically-acceptable products using this process. Electrospinning uses high voltage to generate a large electric potential difference able to cause an electrically charged jet of polymer solution to be ejected from conducting needles towards a collector. Electrostatic repulsion, a rapid whipping motion of the jet, and solvent evaporation all contribute towards significant narrowing of the jet as it travels towards the collector. Very fine (10 nm to 10 µm diameter) fibres are formed as the jet dries, and these accumulate on the collector resulting in highly porous materials with very large surface areas.

Electrospinning offers a simple and convenient method for the manufacture of scaffolds that contain no biological material, using a range of cost-effective bioresorbable materials with a history of use in humans. Since 2007 Neotherix has been developing novel electrospun biomaterials, and has encountered and overcome a number of technical challenges in order to establish a reliable, scalable and commercially viable electrospinning process. The technical solutions generated have allowed the completion of, to the best of our knowledge, the first clinical trial of an electrospun regenerative device [1].

The use of scaffolds in tissue regeneration and repair has been the subject of significant research and development effort in recent years. Amongst other attributes, implanted scaffolds provide a three-dimensional (3D) porous framework for host cell attachment, proliferation and extracellular matrix deposition [2]. In this way they are able to support the body's own repair mechanisms, facilitating the repair, regeneration or replacement of diseased or damaged organs and tissues. The use of bioresorbable polymeric materials selected, designed and fabricated in such a way as to provide a temporary architecture allows the development of tissues that approximate the structure and properties of native tissues.

The clinical role and effectiveness of tissue scaffolds can be modified by the incorporation of bioactive agents to allow an enhanced range of features to be developed. For example, scaffolds designed to support the cells involved in the healing process can also act as a vehicle for the delivery of bioactive agents directly to a site of injury in a controlled manner, avoiding the need for systemic administration with its associated toxicity [3]. Manipulating the scaffold composition affords control over the release profile, either to limit release in order to avoid high concentrations of potentially cytotoxic substances or to deliver relative high doses of agents directly to affected cells and tissues by virtue of the scaffold's high surface area [4].

We have investigated the properties required of a porous bioresorbable scaffold for applications in soft tissue repair, the suitability of electrospinning as a process technology to realise the scaffold target design and characteristics and the feasibility of incorporating an agent to confer antimicrobial activity as an adjunct to the primary structural function of the scaffold.

II. Materials and Methods

Neotherix uses a custom-built electrospinning rig, designed to have a highly variable configuration to satisfy the requirements of biomaterial research, while having the capacity to act as an early stage manufacturing rig following release of a product onto the market. It can be set up in six different high voltage configurations: needles and target can be at positive, negative or zero potential (independently). Process settings can be independently varied including:

number of needles, potential difference, electric field polarity, collector diameter and collector rotation speed, needle to collector distance, and needle traversing rate (Fig. 1). It is capable of simultaneously delivering more than one type of polymer solution for electrospinning. Electrospun scaffold biomaterials were prepared using this rig according to methods described in several patent applications [5]. In the current preferred configuration, a sheet of 940 cm^2 can be produced from which scaffold units of sizes appropriate to the target clinical application can be cut.

Scaffolds have been characterised by a range of techniques including scanning electron microscopy (for example see Fig. 2), capillary flow porometry, inherent viscosity and residual solvent analysis. Cell populated scaffolds resulting from in vitro and in vivo experiments have been characterised by confocal microscopy, histology and analysis of resulting extracellular matrix composition. See, for example, Fig. 3 which shows fibroblast cells attached to a scaffold evaluated using the rat subcutaneous implant model.

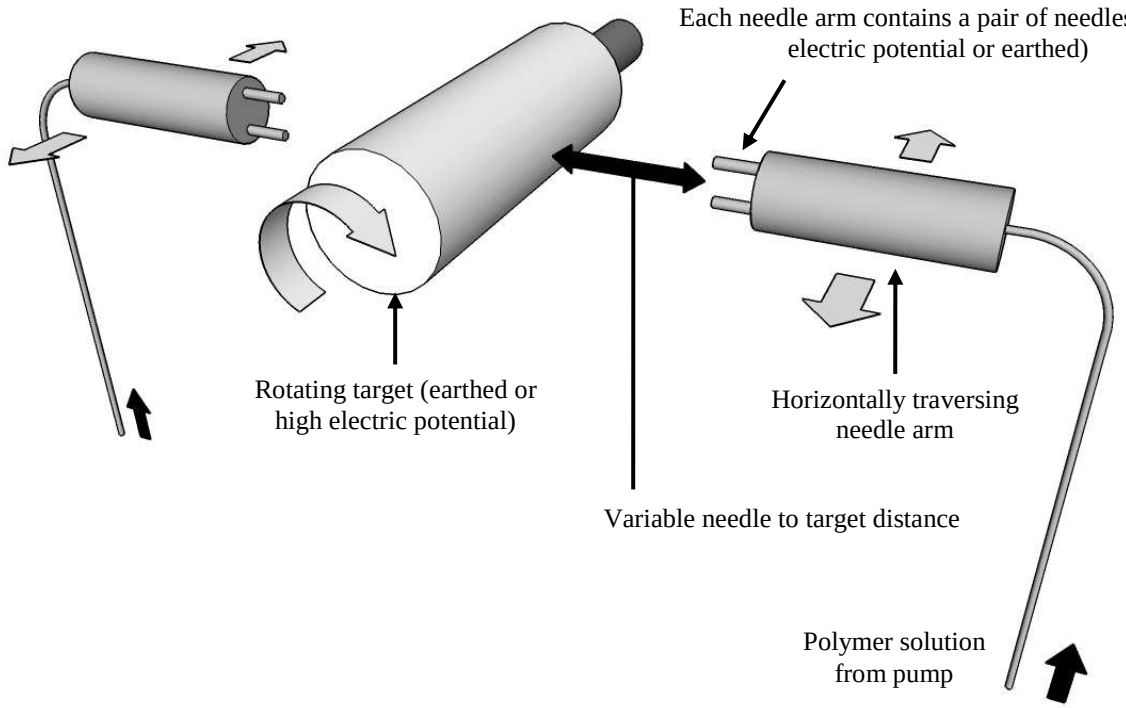


Fig. 1. Schematic of electrospinning rig.

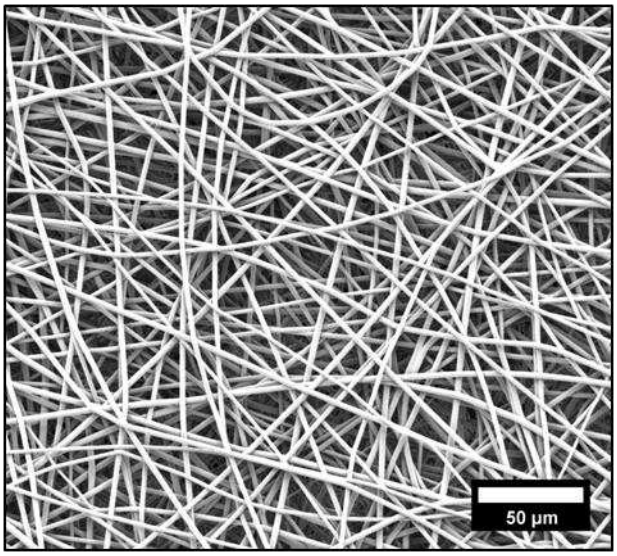


Fig. 2. Scanning electron micrograph of Neotherix' EktoTherix™ poly(glycolic acid) tissue repair scaffold (scale bar represents 50 µm).

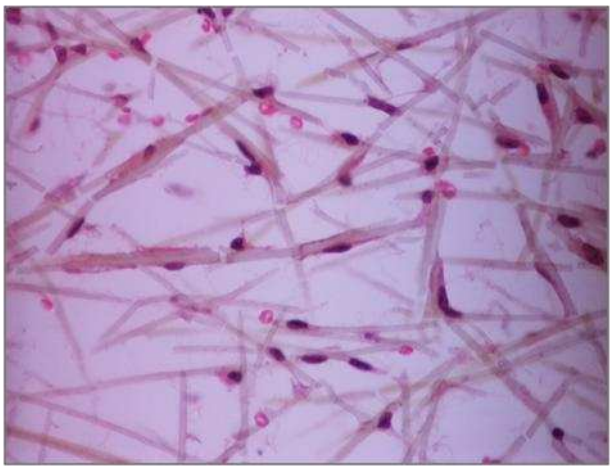
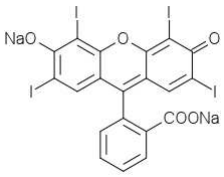
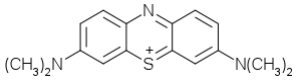
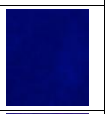
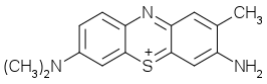


Fig. 3. Ability of scaffold to support growth of fibroblasts. Cells (stained by haematoxylin and eosin) are shown attached and spread over a partially degraded scaffold after 5 days implantation using the rat subcutaneous model (x 20 magnification).

Using this experimental and production set up, we have generated scaffolds targeted at the repair of acute and of chronic wounds, oral mucosal surgical sites (including infected or at risk of infection) and repair of fistula-in-ano. In each case the polymer was chosen on the basis of its rate of bioresorption (and thus residency time) so that this approximated the expected duration during which cells would need 3D support. Scaffolds electrospun from poly(glycolic acid; PGA), poly(lactic-co-glycolic) acid (PLGA) and polycaprolactone (PCL) were selected as being most suited to the various requirements of these different repair sites. In each case the solvent was hexafluoro-2-propanol (HFIP). Antimicrobial activity was achieved by incorporation of a photosensitiser (PS) at the polymer solution stage so that it is encapsulated within the resultant fibres when electrospun (see examples in Table 1). Subsequent release and activation using photodynamic therapy technology is expected to allow the translation of a scaffold with enhanced properties (e.g. antimicrobial activity) along the pathway from laboratory to clinic.

TABLE I. EXAMPLES OF SOME OF THE PHOTOSENSITISERS SUCCESSFULLY ENCAPSULATED WITHIN ELECTROSPUN FIBRES.

Photoactive Agent	Molecular Weight	Chemical Structure	Scaffold Image
Erythrosine B	879.9		
Methylene blue	319.9		
Toluidine blue O	305.8		

III. RESULTS AND DISCUSSION

Significant challenges exist in the development of any acellular bioresorbable tissue scaffold manufacturing technology [2,6,7]. Techniques that obtain scaffolds via the decellularisation of biological tissue (porcine, bovine, or human) [8,9,10] can result in tissues closely resembling the original tissues, and can have specific biological interactions with cells that are difficult to duplicate synthetically. However these materials require significant processing to remove almost all traces of material from the donor organism that may be pathogenic or trigger immune responses. There is also little control over the bioresorption rate of these materials and the processing can significantly affect the mechanical properties of the resulting matrix.

Synthetic scaffold materials offer greater potential for the tailoring of structural, chemical, and mechanical properties. A wide variety of fabrication methods exist, including phase separation, freeze-drying, high-pressure spinning, gas-assisted spinning, and force spinning. Although each of these techniques can have advantages when used for specific applications, none offer a broad range of advantages that would allow them to dominate electrospinning as a versatile method of scaffold manufacture.

Self-assembly has also been used to generate 3D structures for use as scaffolds [11,12], but this approach is limited to use of specific precursor materials, and can result in scaffolds with poor mechanical properties. The materials often have no established history of use in humans and can be expensive to manufacture. Micro-moulding and rapid prototyping technologies have also been directed towards the fabrication of scaffolds [13,14]. While such technologies can allow precise control over the 3D architecture, it is difficult to achieve features smaller than tens or hundreds of microns in a truly 3D scaffold (i.e. not a thin film).

A range of challenges were encountered in the development of our electrospun biomaterial scaffolds. These include biological (e.g. cell attachment and interaction; low or zero cytotoxicity; minimal local tissue inflammation on implantation), chemical (e.g. rate of hydrolysis and bioresorption), structural (e.g. sufficient porosity to allow transport of nutrients and appropriate pore size to allow ingress of cells), processability (e.g. ability to control the electrospinning operation to yield scaffolds of target specification in a reproducible manner) and scalability (e.g. production of uniform scaffolds in sufficient volume to support a clinical investigation or market introduction). Challenges have been addressed in a systematic manner to provide technical and design solutions. These include the optimisation of the 3D architecture for the cell types of interest, material selection, and selection of a suitable product sterilisation processes. Process related challenges include determining how to solubilise a difficult to dissolve polymer, the best means for encapsulation of active compounds, methods for stabilising problematic formulations, and how to manufacture bilayer scaffolds. Manufacturing challenges include the maintenance of process reliability and material quality whilst scaling up production volume, and the control of the manufacturing process to allow production of a sterile medical grade scaffold to international regulatory standards. Certification to ISO 13485 (Quality Management System (QMS) for the design and manufacture of Medical Devices as required for regulatory purposes) [15] was achieved in 2015 [16] and can be applied to all Neotherix scaffolds intended for human use.

The encapsulation of PS as exemplified in Table 1 into PGA electrospun fibres was found to have an attenuating effect on the resultant fibre diameter (Fig. 4). The causative mechanisms for this alteration may operate at the stage of fibre production (e.g. as a result of changes in solution viscosity) and are the subject of further investigation.

The successful translation of a novel biomaterial from laboratory to the clinic has been achieved on the completion of the First in Man (FIM) clinical investigation of EktoTherix for the repair of acute wounds [1]. A further scaffold product has now been approved to enter the FIM stage. This translational journey also demonstrates the potential of electrospinning as a

reliable, scalable and commercially viable process and the feasibility of application of the resulting scaffolds to a range of tissue repair targets. This versatility has been further demonstrated by the application of Neotherix' scaffolds to the generation of in vitro 3D tissue models, for example the modelling of bovine endometrium [17].

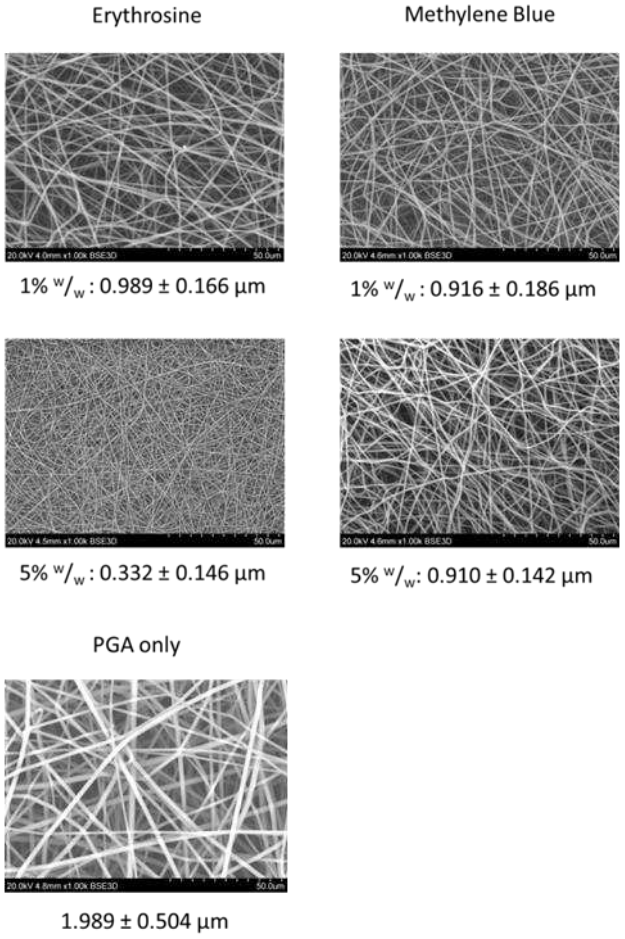


Fig. 4. Scanning electron micrographs of PGA scaffolds electrospun with PS (1 and 5%). Dimensions are mean fibre diameter measured by image analysis (images courtesy of Rina Haryani Binti Osman Basah, School of Dentistry, University of Leeds, UK).

IV. Conclusions

Neotherix has developed a reliable and scalable electrospinning process for the manufacture of safe and clinically-relevant implantable biomaterials. A resorbable tissue repair scaffold was successfully translated from laboratory to clinic using an ISO 13485 certified process, culminating in the first clinical trial of an electrospun regenerative device despite a challenging regulatory environment. Lessons learned in travelling this pathway are currently being applied to the development of other pipeline technologies, potentially leading to the translation of even more sophisticated electrospun biomaterials.

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