



## Analysis as a Micro Fibrous Graft for Components in Synthetic Biology

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### Abstract

A biocompatible and biodegradable poly (1,4-butylene succinate) microfibrillar tubular scaffold has been produced through the use of electrospinning. The scaffold's morphology was N-optimized to prevent cell infiltration through the graft's wall and to promote cell integration, adhesion, and growth as a micro-porous conduit with a small diameter. The scaffold's mechanical properties and morphology were examined and compared to those of native conduits. Scaffolds were then seeded with adult normal human dermal fibroblasts to test cytocompatibility in vitro. The hemolytic effect was assessed following incubation with whole blood that had been diluted. The graft is able to provide initial mechanical support and functionality thanks to the demonstrated degradation profile during colonization and subsequent replacement by host cells. Elastic modulus (less than 17.5 1.6 MPa), ultimate tensile stress (less than 3.95 0.17 MPa), strain to failure (less than 57 4.5%), and suture retention force (less than 2.65 0.32 N) were all within the physiological range for tubular conduits. There was no delamination of the scaffold's mechanical properties. This combination of properties may make it possible to use PBS as a biomaterial to create scaffolds that support host cell remodelling and provide structure and function over time.

**Keywords:** Poly (1,4-butylene succinate); Electrospinning; Biomaterials; Vascular grafts; Bile ducts; Tissue engineering

### Introduction

For tissue engineering, the search for novel scaffolding materials never ends. Cell growth should be directed toward the morphological, biological, and functional reconstruction of tissues by the three-dimensional scaffold [1]. It is necessary for tissue engineering because it creates a microenvironment similar to a physiological one that allows oxygen and nutrients to flow freely and transmits biochemical signals that affect the phenotype of cells and the formation of tissue [2]. The scaffolds, whether created artificially or naturally, are typically biocompatible, biodegradable, and capable of releasing drugs and growth factors as well as providing mechanical support [3]. The requirements of clinical applicability, such as ease of acquisition and long-term storage, ought to be taken into consideration when selecting a new biomaterial, whether natural or synthetic. Synthetic polymers can be combined with other polymers to meet any requirements that need to be met for the damaged tissue to function properly. They can also be controlled at the molecular level to control the rate of degradation and the modulation of cell behavior that mimics the ECM. Polyvinyl alcohol (PVA), polylactic acid (PLA), polyethylene oxide (PEO), polyglycolic acid (PGA), poly-caprolactone (PCL), polyethylene glycol (Stake), and polyurethane (PU) are all biodegradable polymers that are commonly found in engineered structures [4].

Rapid prototyping, particulate leaching, electrospinning (ES), and thermally induced phase separation are just a few of the many applications for which biodegradable polymeric materials with appropriate pore networks have been developed over the past few decades. ES is a versatile method that can be used in a variety of ways depending on the flow rate, applied voltage, polymer concentration, and air gap between the spinneret and the fabrication target. Continuous fiber-based fibrous scaffolds with diameters ranging from the nano to the microscale can be made using ES. By adjusting the collecting mandrel's rotation speed, the fibers obtained in this manner could even approximate the diameters and orientations of extracellular matrix components. The nanofibrous architecture greatly facilitates cell binding and other behavior-related activities [5].

A number of natural or synthetic polymers, such as collagen,

silk fibroin, chitosan, and alginate, or synthesized (such as PU, PCL, PLA, PGS, and so on) were successfully used in the electrospinning process to make porous scaffolds. Despite the fact that a number of electrospun conduits have been examined for various applications in tissue engineering, none are currently utilized for conduits with small diameters. Due to their susceptibility to inflammation, thrombosis, intimal hyperplasia, and compliance mismatch with the host vessels, current prosthetic grafts (ePTFE, Teflon®, or PET, Dacron®) fail to demonstrate long-term patency comparable to that of autologous grafts in settings with a diameter of less than six millimeters. In addition, tubular scaffolds aren't just used in the clinic for vascular grafts. By 2030, 50% of Americans will be affected by cardiovascular diseases, which are predicted to become more common worldwide [6]. An artificial bile duct, as opposed to choledochojejunostomy, which is the standard procedure for biliary reconstruction, could be used to maintain a physiological bile conduit. Regeneration of peripheral nerves, revascularization of the peripheral limbs, and arteriovenous fistulae for haemodialysis are additional requirements for tubular conduct substitutes. Because of these factors, a number of research groups have looked into alternatives to tubular scaffolds in recent years [7]. A tract that is no longer capable of transporting fluids in the correct manner should have these grafts inserted into it permanently [8]. Hemocompatibility, elasticity, leak prevention, compatibility with standard sutures, mechanical resistance, sterilizability, dependability, and the right length and diameter are all part of the design requirements. In addition, they must first function and provide mechanical support before it is anticipated that they will degrade and be replaced by cells. To get the best results for ECM synthesis and tissue regeneration, their properties and degradation profile could be controlled during

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manufacturing [9].

This examination centers around the utilization of poly(1,4-butylene succinate) (PBS) as an electrospun polymeric cylindrical platform for tissue designing applications. After succinic acid and butanediol have been polycondensed, PBS is a thermoplastic aliphatic polyester that is biodegradable and readily available on the market. PBS, which is currently available for purchase, is made up of monomers that are derived from chemicals. These monomers can come from fossil fuels or renewable sources. Butanediol (BDO) can be created from glucose utilizing an all-out biosynthetic strategy [10]. Succinic acid is produced by a number of microorganisms as a byproduct of anaerobic fermentation, making it an essential intermediate in the TCA cycle. As a result, industrial production of biobased PBS is possible.

PBS has a melting point of 120 °C, is insoluble in water, and has a glass transition temperature below 0 °C, making it a promising polymer for numerous biomedical applications. Additionally, it has been demonstrated to be biocompatible and very simple to process. Cicero and colleagues used PBS as a planar microfibrillar scaffold in a rat model to promote nerve regeneration and maintain nerve continuity. A high-resolution MRI examination 120 days after the implant revealed complete reabsorption, proving biodegradability. demonstrates that PBS is a promising material for applications in tissue engineering due to its support of cell adhesion and proliferation and its high level of processing control by developing and defining anisotropic planar scaffolds through the use of weft knitting. Due to its hydrophobicity and its manageability during the electrospinning process, PBS was selected as the polymeric ocular insert. The electrospun scaffold's surface was altered through plasma-induced chemical functionalization to enhance its biomimetic and mucoadhesive properties [11].

The mechanical properties can only exist if diisocyanates, which are used as chain extenders, are present. Without chain extenders, high molecular weight PBS is brittle and has a very short elongation at break, whereas isocyanates significantly lengthen it. PBS containing 1,6-diisocyanatohexane was our choice due to its ability to withstand and recover from short-term deformation, making it suitable for these dynamic applications. Small-diameter conduits were not examined in any of the studies that examined planar micro or nanofibrous scaffolds made from PBS by electrospinning. In this study, 2.6 mm-diameter conduits were electrospun with PBS. Vascular grafts, artificial bile ducts, revascularizing peripheral limbs, making arteriovenous fistulas for haemodialysis, and regenerating peripheral nerves are all potential applications. In vitro cytocompatibility testing was performed on adult normal human dermal fibroblasts, and the morphology and mechanical properties of the scaffold were thoroughly examined. In addition, as the host cells colonized and replaced the scaffold, its stability in physiological fluids like bile and plasma was sufficient to provide them with initial mechanical support and functionality [12].

## Materials and Methods

### Materials

The poly (1,4-butylene succinate) was provided by Aldrich Milan, Italy, along with Dulbecco's phosphate buffered saline (DPBS), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), and 1,6-diisocyanatohexane (Tm 120 °C) (PBS). Only the Tm for PBS is specified by this vendor.

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, l-glutamine, penicillin, streptomycin, and amphotericin were provided to the laboratory by Euroclone group (Milan, Italy).

The Istituto Zooprofilattico della Sicilia "A. Mirri" in Palermo, Italy, extracted porcine bile from pigs in accordance with European regulations regarding the use of animals in experiments.

Volunteers gave their informed consent for the collection of human blood, which was then isolated, at the University of Palermo in Palermo, Italy [13].

### Evaluation of Deterioration

There were three distinct buffer solutions used for the degradation test: After being cut, the electrospun grafts were lyophilized, washed four times in ultra-pure water, and weighed (w0). The weight loss was measured at scheduled intervals up to one month later. Human plasma, porcine bile, and sodium azide/PBS 0.02 M pH 7.4. The mean value minus the standard deviation of the recovered weight was used to represent these experiments' triplicate results. The data were plotted against t0, and the gold-coated samples were examined using SEM at the end [14].

### Tests of Suture and Uniaxial Retention

Tensile measurements were taken during mechanical testing of the fibrous scaffolds using a Bose TA Instruments ElectroForce Test Bench System. The dry scaffolds were cut into strips with a width of 5 mm and a length of 30 mm. These strips were cut in the circumferential or longitudinal directions. The thickness of each specimen was determined to be 400 µm with a digital caliper. The scaffold's firm retention at the initial length of 15 mm between the tensile system's robust metal clamps was guaranteed. After ten cycles of preconditioning to 15% strain at room temperature, the dry specimens in each direction were pulled at a crosshead speed of 10 mm/min until rupture. With the suspicion of incompressibility, load-relocation bends were utilized to compute pressure strain connections in view of current length and cross-sectional region [15].

As the maximum stress and strain prior to failure, ultimate tensile stress (UTS) and strain to failure (STF) were taken into consideration. The elastic modulus was determined by calculating the slope of the stress-strain curves in the elastic region. For tests on suture retention, rectangular specimens were clamped at the edge opposite the suture. Using the provided triangular needle (2-0), the thread was passed through the material and multiple knots were tied to create a loop. The specimen was 20 mm free length, had a width of 11 mm, and had a thickness of 0.2 mm. The suture bite was centered on the specimen's width and was 18 mm away from the clamp. The suture loop was initially pulled at 0.2 N.A. while the specimen was fixed. Once the suture wire was taut, a pulling rate of 1 mm/s was applied until the final specimen failed, which was characterized by suture pullout.

### In Vitro Exploration

For studies on in vitro cell culture, the electrospun grafts were cut into squares of 1.5 mm x 1.5 mm and sterilized with gas plasma. Then, they were transferred into a 48-well tissue culture polystyrene plate after being mounted on sterile CellCrown inserts (Scaffdex). For the purpose of culture seeding, approximately 30,000 cultured NHDF were suspended in complete DMEM. SEM and immunocytochemistry were used to examine cell morphology and proliferation, count cell nuclei, and observe the organization of the cytoskeleton. The samples were fixed for 15 minutes at room temperature in 4% formaldehyde in PBS after being washed with DPBS at each timepoint. After 24 and 72 hours of culture, the nuclei were counterstained with 4-6-diamidino-2-phenylindole (DAPI) for 10 minutes. After that, the samples were

mounted onto glass slides, washed three times with PBS for five minutes, and viewed through a Zeiss Axio vert.A1.

The image editing software ImageJ was used to count the number of DAPI-stained nuclei on the surface of the scaffold for the assessment of attachment and proliferation. Following the fluorescence analysis, the samples were dehydrated for ten minutes at varying ethanol concentrations (30%, 60%, and pure ethanol) for each wash. After being treated with hexamethyldisilazane (HMDS), the samples were dried in a flow hood. SEM was used to examine the gold-coated samples.

Due to intrinsic scaffold limitations like insufficient pore size or interconnectivity, obtaining highly bulk-cellularized scaffolds is a common obstacle in tissue engineering. The extent of cellular infiltration and tissue ingrowth into electrospun scaffolds is determined by their high porosity, pore interconnectivity, and large surface area to volume ratio. Moreover, they impact a scope of cell processes and are urgent for the dissemination of supplements, metabolites, and side-effects. Using ImageJ, we measured the area of thousands of pores to determine pore sizes. Gravimetry was used to calculate porosity, which is the difference between the predicted scaffold density and the polymer density. The results revealed a maximum pore size of 15.2  $\mu\text{m}$  and a porosity of 88.6  $\pm$  0.9 percent. In addition, the SEM analysis was used to determine whether the gas plasma and ethanol, which were used to sterilize the scaffolds for in vitro experiments, could cause harm. There were no morphological changes in the microstructure's revealed quality.

### Stability in Environments Similar to a Human Body

The debasement test was carried out using three distinct support responses in order to replicate the physiological environment of various human rounded channels. Bile is the common fluid that the bile ducts use to digest lipids. Water, salts, and bilirubin make up this fundamental arrangement. It was selected to test the PBS graft's degradation as a biliary reconstruction artificial duct.

Plasma is the portion of the extracellular fluid that enters the arteries without blood cells. In point of fact, it was utilized to assess the degradation of PBS as an alternative to artificial arteries. Finally, phosphate buffered saline was used to test the hydrolytic resistance of PBS scaffolds. After up to a month, there was no discernible difference in how these grafts degraded. The host remodelling mechanisms would likely be aided in gradually replacing the scaffold with native tissue if it maintained its mechanical integrity for an extended period of time.

### Conclusion

Using a tubular electrospun PBS scaffold, a tissue engineering application was described in this study. A controlled regular structure, long-lasting mechanical properties, and a slow rate of degradation characterize this aliphatic polyester. It can also be controlled in a way that is both reproducible and cost-effective, shipped, stored, and made in large quantities.

The advantage of the chosen processing method is that it lets you fine-tune the length, diameter, and even anisotropy ranges. As a result,

tubular scaffolds can be made with a wide range of properties to meet the needs of different applications. Metabolites and catabolites can also be exchanged in a physiological manner thanks to the electrospun fibers' tunable, interconnected pores and the material's permeability. During short-term mechanical conditioning, these grafts maintained their geometry and properties, according to morphological and mechanical characterization. The join can offer beginning mechanical help and usefulness on account of the exhibited debasement profile during colonization and ensuing substitution by host cells. Because of its non-hemolytic nature, surprising wrinkle obstruction, and simplicity of taking care of, it is additionally appropriate for implantation. PBS could be used as a biomaterial to create scaffolds that support host cell remodelling and provide structure and function over time thanks to this combination of properties.

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