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# Fabrication of bespoke nasal septal scaffolds

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ABSTRACT

An electrohydrodynamic printer designed and constructed in our laboratory was used to fabricate polycaprolactone nasal septal scaffolds. Three different polymer concentrations (15 wt.%, 18 wt.% and 23 wt.%) were used. The generated scaffolds, each with a unique architecture, were evaluated using PeakForce Quantitative Nanomechanical Mapping (QNM) Atomic Force Microscopy (AFM) to measure Young's modulus using the Dejaguin–Müller–Toporov (DMT) model. In addition, this allowed estimation of adhesion forces, deformation and dissipation energy of the scaffolds. The degree of crystallinity and purity of the polymer in the scaffolds were characterized by differential scanning calorimetry and Fourier transform infrared spectroscopy.



## 1. Introduction

From an engineering and biological point of view, tissue engineering holds many challenges which span beyond the strict definition of cells and scaffolds. Perhaps the most critical issue of all is to understand and to define the native tissue which is meant to be replaced. In fact, the function of the tissue must be completely understood, biologically and biomechanically, in order to replace it optimally. The mechanical characterization of biological tissue is, however, often complex due to testing conditions, the tissues' inherent anisotropy, and limited sample life-cycle. Furthermore, the mechanical signals regulating tissues must also be ascertained.

At a macroscopic level (mm range) the shape and composition of the scaffold will determine its cytotoxicity and the ability of the cells to penetrate its structure. At an intermediate level (100 μm), the pore size, orientation, interconnectivity and surface chemistry will determine cell differentiation and proliferation behaviour as well as the supply of nutrients and the removal of waste products. At a microscopic level (1 μm), the local surface texture and porosity will affect protein adsorption and cell adhesion [1]. Thus a thorough characterization of each level of the scaffold and a proper design are crucial for understanding its behaviour as a tissue engineering construct.

The specific cell signalling that is triggered by the surrounding environment which in this case is a scaffold will ultimately determine if the scaffold will eventually turn into an integrated tissue. Initially the cell types need to attach externally to the scaffold surface and then proceed to migrate within it [2]. This is only possible if the scaffold has cell adhesion sites dispersed within it throughout in the appropriate density to promote cell migration. After the scaffold is populated by cells, proliferation and differentiation need to take place to produce the replacement tissue. Integrins are responsible for anchoring onto the extracellular matrix while simultaneously eliciting cues for cell differentiation or proliferation along with cytokines and growth factors. The dynamic nature of the extracellular matrix makes it a challenge to replicate. Mechanotransduction pathways initiated by mechanical stimuli allow the cells to remodel the tissue. The proteins on the extracellular matrix facilitate cell attachment via the integrins. The cells can then exert traction forces on the extracellular matrix to stretch the extracellular matrix proteins to reveal binding sites on the protein structure [3–7]. Cellular mechanotransduction and cell signalling are vital issues that need to be fully understood and considered when designing artificial scaffolds in tissue engineering since it underpins cell differentiation, phenotype and proliferation [8,9].

Polycaprolactone (PCL) was chosen because it is a slowly degrading polymer (~2–3 years), elutes non-toxic by-products, and is biocompatible as evident through its wide array of FDA approved products (e.g., sutures and drug delivery) [10–12]. PCL degrades via hydrolysis of the ester linkages into water soluble hydroxycaproic acid monomers [10]. It is a semicrystalline polymer that degrades more slowly than many other biodegradable synthetics [11]. This slow degradation rate allows adequate time for the cells to regenerate native tissue, ideally

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**Table 1**
Physical properties of PCL solutions used in the experiments.

| Solution | Density ($kg\ m^{-3}$) | Viscosity (mPa s) | Surface tension ($mN\ m^{-1}$) |
|---|---|---|---|
| Dimethyl carbonate (wt.%) | 1.08 | 1.44 ± 0.2 | 30.5 ± 0.3 |
| PCL 15% | 1.20 | 1920.8 ± 8 | 40.3 ± 0.5 |
| PCL 18% | 1.45 | 2258 ± 6.25 | 42.5 ± 0.2 |
| PCL 23% | 1.90 | 8756 ± 161 | 65.3 ± 0.8 |

at the same rate as scaffold degradation. PCL also has a relatively low melting point of 60 °C allowing for easy manufacturing and scaffold fabrication [12,13].

Electrohydrodynamic printing is a flexible, versatile direct-write technique that enables the direct deposition of a variety of materials, eg. polymers [14,15], metals [16,17], ceramics [18], in a controlled fashion through the application of an external electric field to generate a micrometre sized jet. This jet is about two orders of magnitude smaller than the diameter orifice of the printing needle and thus permits high resolution patterning. In electrohydrodynamic printing liquid is pushed out via mechanical pumping and pulled out simultaneously by virtue of an applied electric field unlike in inkjet and flow based direct writing where liquid is only forced out of the orifice. In the absence of an electric field a hemispherical meniscus forms at the orifice of the needle due to the action of surface tension forces when the liquid comes in contact with the surrounding air. The bulk solution within the meniscus is electrically neutral but as the applied electric field strength increases, more ions arrive and the meniscal surface charge builds up. The build-up of charges results in columbic repulsion between mutually repulsive ionic charges which in turn introduce tangential electrostatic stresses on the hemispherical meniscus which compete with opposing surface tension forces that try to restore the original hemispherical shape. Once the electrostatic forces overcome the opposing surface tension forces, the meniscus distorts into a conical shape from which a thin jet emerges and this is used to print.

In recent years melt electrospinning has appeared as a competing additive manufacturing technology in comparison to solution based electrospinning [19]. The inherent versatility of solution based electrohydrodynamics is exhibited through the changes in concentration where at higher concentrations, higher geometric accuracy is attainable in the printed constructs. The solution based approach is not dependant on temperature and thus is capable of printing with multiple polymers simultaneously including temperature sensitive polymers. Additionally, melt electrospinning does not have the same flexibility as the solution based approach to electrospinning when it comes to varying the concentration of a polymer with a specific molecular weight. Structural changes brought about by crystallization of the polymer during the cooling down period in melt electrospinning after print depositing the melted polymer cannot be regulated which is in stark contrast to the solution based approach where deposition can take place at ambient temperatures [20,21,22].

Research described in [23–25] highlighted how distinct melt electrospun configurations could only be achieved through highly selective deposition on uniquely shaped conductive collectors like drums. The deposition and alignment of fibres on wire meshed collectors is established through focused electric fields along the contours and curves of the conductive wire meshed substrates. Work described in [26] points to how in the absence of such collectors it is difficult to maintain control when melt electrospinning poly (ε-caprolactone) scaffolds.

The mechanical properties of the scaffolds were studied at the nanoscale using Peak Force QNM AFM. The technique allows the user to gauge the force and distance at nanometre scale resolutions in numerous environmental conditions. The surface topography of hydrogels, cells and other biomaterials typically used in tissue engineering can be mapped out effectively. Through nanoscale probing, the technique provides information on the DMT modulus, deformation, adhesion and dissipation. The DMT model was applied in this study to quantitatively map the Young's modulus information.

Facial aesthetics and clear nasal pathways are determined by the cartilaginous craniofacial aspects of an individual. Autologous tissue is the preferred choice when attempting to reconstruct or repair nasal septal defects. Unfortunately septal cartilage from a donor is always in short supply which has led to tissue engineering to generate large quantities of autologous tissue. Using a small quantity of donor cartilage, it is possible to seed specially designed biodegradable scaffolds with the donor cells to create grafts of a definite geometry to suite the patient's reconstructive needs.

In this work we aim to outline a rational approach to scaffold fabrication of nasal septal plates. This involves in the first instance, an investigation of the link between concentration and scaffold mechanical properties. Concentration is the most dominant parameter and is the focal point since it directly impacts the mechanical properties of the scaffold. This in turn influences the way cells adhere and behave when introduced into the scaffold. The microfabrication system that was developed and optimized in our laboratory [27] is able to fabricate scaffolding supports of controlled geometry. It makes use of polymeric solutions that are electrohydrodynamically dispensed over a platform moving on motorized slides along three mutually independent orthogonal directions and following a specific and predetermined trajectory chosen by the operator. It is essential to note that an absolute scaffold design does not exist but each tissue requires a specific matrix design with defined material properties. The current challenge in tissue engineering is centred on producing neocartilage constructs with the optimal biomechanical and biochemical properties equivalent to those of native human nasal septal cartilage.

## 2. Materials and methods

### 2.1. Solution preparation

PCL (molecular weight 80 kDa) and dimethyl carbonate (DMC) were both purchased from Sigma-Aldrich. PCL pellets were dissolved in DMC to make solutions with three different concentrations of polymer 15%,

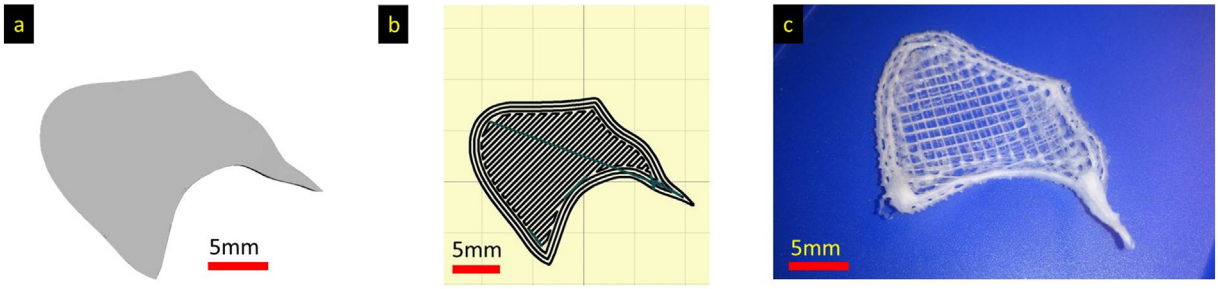


**Fig. 1.** The printing route from STL File to the final printed construct. a) The three-dimensional STL construct. b) Printing tool path for one layer. c) The actual printed construct.