Analysis and Comparison of Physical Characteristics of Electrospun Tissue Engineered Scaffolds for Temporomandibular Joint Repair

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Introduction

A major challenge in tissue engineering is the ability to facilitate the *in vivo* extracellular matrix (ECM) synthesis and organization with artificial tissue constructs that have sufficient biological and mechanical properties to function within the body. Therefore, the need for scaffold fabrication with properties mimicking those of the natural ECM is especially critical. For temporomandibular joint (TMJ) abnormalities, the latest challenge is to engineer suitable scaffolds for the repair of fibrocartilaginous tissue. The objective of this study is to explore the physical properties of tissues scaffolds fabricated by electrospinning so that the response of seeded fibrocondrocytes can be optimized for TMJ regrowth.

Materials and Methods

Polycaprolactone (PCL) (MW. 80,000; Sigma-Aldrich) was dissolved in chloroform. For each electrospinning run, polymer solution was placed into a plastic disposable syringe mounted on a syringe pump (KD-100; KD Scientific), which delivered the polymer solution at steady flow rates, whereas a positive high voltage (FC30; Glassman High Voltage) was applied to the metal syringe needle. A square aluminum sheet was used as the grounded target to collect the polymer fibers. Flow rate of polymer solution (10-30 mL/min), gap distance between the tip of syringe needle and the aluminum collector (20-30 cm), applied voltage (15-20 kV) and polymer solution (8-10% w/w) were varied to optimize the fabrication of the polymer scaffolds with reproducible surface properties. Digital images of fiber meshes were captured using an inverting microscope (Olympus) at 40X magnification. Image J was used to analyze the fiber diameters of electrospun scaffolds, taking 40 measurements from each trial scaffold. A multivariable input statistical analysis was performed on the measurements to determine the relationship between the electrospinning parameters and the resulting fiber diameters. For the evaluation of cellular biocompatibility of the electrospun polymer scaffolds, seeded cycle 7 mouse mesenchymal stem cells (MSC) on sterilized scaffolds were monitored every 24 hours for cell adhesion and viability through specific fluorescence staining.

Results and Discussion

12 distinct combinations of parameters (only five of them are shown in Fig. 1) are used to create measurable PCL fibers and three trial runs for each combination. The measured fiber diameter ranges from about 2 µm to 6 µm, with differing ranges of variability. Fluorescent imaging of stained MSC in culture show mostly green hues, indicating that they are still healthy and viable after a week. Statistical analysis indicated that gap distance, voltage, and flow rate have the highest correlation coefficients (Table 1). Gap distance, polymer concentration, and flow rate are all directly proportional whereas applied voltage is inversely proportional to fiber diameter. Of these parameters, gap distance and flow rate have the most significant correlation ($P<.05$). Analysis of fiber deviation showed that concentration and voltage have the highest coefficients. Voltage and flow rate are related to the deviation of fiber diameter in direct proportion, whereas polymer concentration and gap distance are inversely related. Additionally, polymer concentration has the most significant correlation to the deviation of fiber diameter ($P<.0001$).

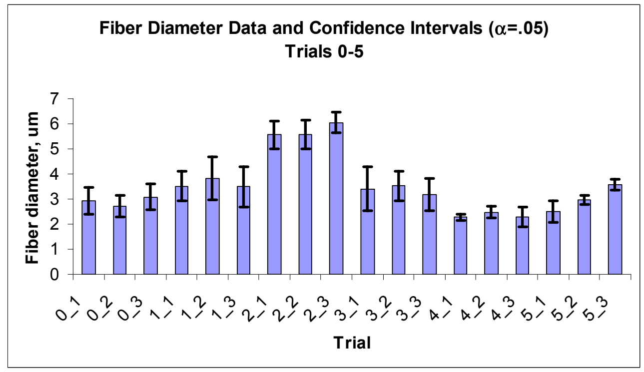

Figure 1. Combination of parameters for the creation of measurable polycaprolactone fiber. The first number (0-5) denotes the number of combinations and the second number the number of trial runs (1-3).

Table 1 Multivariable statistical analysis of the effect of electrospinning parameters on fiber diameter

	Fiber diameter		Fiber variation	
	Correlation Coefficient	P value	Correlation coefficient	P value
Polymer Concen-tration	.0345	.6803	-.09883	.0001
Gap Distance	.05696	.0011	-.04422	.2403
Applied Voltage	-.06584	.0861	.09243	.2921
Flow Rate	.06532	.0001	.0141	.2831

Conclusions

Fiber measurements and statistical analysis indicate that a consistent relationship exists between spinning parameters and fiber diameter, suggesting the possibility of manipulating the scaffold characteristics by altering spinning parameters. Cell studies suggest that the scaffold material may support cell adhesion and growth.

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