Tissue Engineering

Electrospinning Collagen Scaffolds

A variety of processing methods using both synthetic and natural polymers have been tried in attempts to fabricate scaffolds for the regeneration of tissue and organs.

The fibrillar collagens, types I, II, and III, are the most abundant natural polymers in the body and are found throughout the interstitium, where they impart structural integrity and strength to tissues. The extracellular matrix (ECM) they compose provides cells with the appropriate biological environment for embryologic development, organogenesis, cell growth, and wound repair. Collagen, then, is an obvious material choice for tissue engineering.

Other beneficial characteristics of collagen as a biomaterial are its high tensile strength, high water affinity, low antigenicity, resorbability, excellent cell compatibility, and ability to promote tissue regeneration (compared with synthetic materials), as well as its capacity for variable mechanical properties and degradation rates.

In tissue engineering applications, it is important to synchronize the collagen degradation rate, induced by fibrinolytic enzymes and phagocytosis, with the tissue regeneration or remodeling rate, in order to prevent catastrophic failures. Thus, control over the type and degree of crosslinking is a critical aspect of collagen biomaterials.

Type I collagen is found widely throughout the body and consists of three coiled subunits: two $\alpha 1$(I) and one $\alpha 2$(I) chains. These chains are wound in a right-handed, triple helix to form the collagen type I basic structural units, which are further packed together to form fibrils as small as 50 nm in diameter.

Type II collagen is also a triple helix that forms a homotrimer of three identical α chains [$\alpha 1$(II)]. These structural units pack together to form fibrils less than 80 nm in diameter. Type II collagen is found primarily in articular cartilage and intervertebral disks.

Type III is similar to type I collagen and usually occurs in a specific ratio with type I in tissues such as skin, muscle, and blood vessel walls. Its fibrils are formed of basic structural units composed of three $\alpha 1$(III) chains that pack together with varying diameters between 30 and 130 nm depending on the specific tissue and age.

The supermolecular structure (fibrils) of these three collagen types results from quarter-staggered packing of the collagen molecules side by side, with adjacent molecules offset by about 67 nm in the axial direction. This is caused by electrostatic interactions and gives the fibrillar collagens their distinctive banded structure at the ultrastructural level.

The process of electrospinning has been used to fashion a variety of synthetic and natural polymers into novel scaffolds for cell and tissue growth. A basic electrospinning system consists of a charge-injected polymer solution that is fed through a small nozzle or orifice opposite a grounded target some distance away.

The charge-injected solution is drawn toward the grounded target as a jet when the applied electric field strength overcomes the surface tension of the fluid. During jet travel, the solvent gradually evaporates, and a charged polymer fiber is formed and collected on the target. The charge on the fibers dissipates as the surrounding environment neutralizes it.

The resulting electrospun product collected on the grounded target is a nonwoven fibrous mat composed of fibers with diameters on the order of nanometers to microns. This fibrous mat can then serve as a tissue scaffold.

To date, electrospinning of fibrillar collagens has been completed with collagen types I (rat tail, bovine and human placenta), type II (chicken sternal cartilage), and type III (human placenta). These collagens were electrospun from solution in the solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) at concentrations of 0.04 to 0.16 g/mL to produce fibers of 80 nm to 5 μm in diameter. Details of the exact process have been presented in a recent article (*Biomacromolecules* 3: 232-38, 2002).

Scanning electron microscopy of electrospun type I fibrillar collagen scaffolds has revealed that the mats are composed of collagen fibers with diameters down to 100 nm with a uniform size distribution. This diameter is near the minimum diameter of 50 nm seen in native collagen type I fibers.

Transmission electron microscopy has shown that these fibers also exhibit the characteristic 67-nm banding pattern of native polymerized collagen. Evidence of this banding pattern, in addition to the very small fiber diameter, implies the creation of a truly biomimetic fiber and tissue scaffold.

Similar results were obtained with type II collagen, where scanning electron micrographs demonstrated that the electrospun scaffolds were composed of polymerized collagen fibers with a diameter of 110 nm. Again, this diameter is

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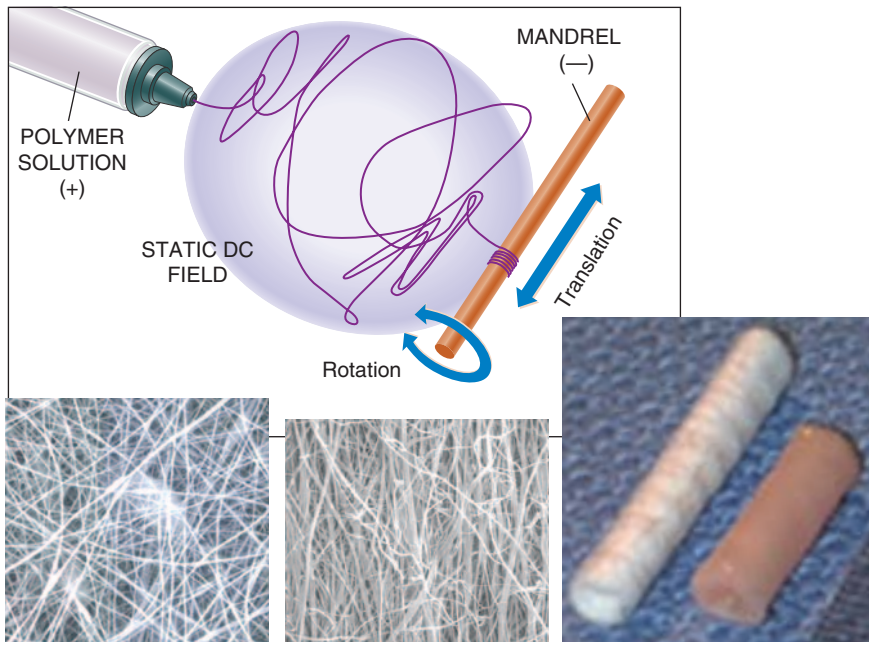

In electrospinning, a key component is the collection mandrel. Manipulating the rotational speed and translation of the mandrel allows the fabrication of random (*left*) or aligned (*middle*) collagen nanofiber scaffolds for use in applications such

near the 80-nm diameter of native type II fibrils. Finally, for type III collagen, electrospinning has produced fibers as small as 100 nm, which also falls in the physiologic range.

During scaffold fabrication by electrospinning, the fiber diameter produced depends on the type of collagen used and its concentration in solution. For example, collagen type I electrospun from concentrations of 30 to 100 mg/mL in HFP with all other parameters held constant produced fiber diameters of 100 nm to 5 μm, respectively, with a linear relationship between collagen concentration and scaffold fiber diameter.

This relationship of fiber diameter versus polymer concentration appears to be universal in the area of natural and synthetic polymer electrospinning. The wide range of possible fiber diameters introduces a large flexibility to the production of tissue scaffolds via electrospinning. In vitro and in vivo studies have shown that fiber size is crucial for proper interaction of the scaffold with cellular components (for adhesion, migration, proliferation, and differentiation).

Control over fiber orientation within the scaffold is another key factor to inducing proper interaction with the cellular environment and subsequent strength and function of the engineered tissue. In electrospinning, this control is achieved with rotational and translational motion of the mandrel during spinning.

In the figure, the aligned electrospun collagen type I scaffold (*middle*) was produced on a mandrel that was rotating 10 times faster than the mandrel that produced the random fiber scaffold (*left*). This combination of control over fiber diameter and orientation by simultaneous rotation and translation allows the process of electrospinning to manufacture any structure required to mimic the ECM of the tissue of interest.

There are other advantages of electrospinning as well. First, the thickness of the scaffolds and individual scaffold layers can be controlled by simply adjusting the electrospinning time to create scaffold thicknesses ranging from a single fiber diameter to several millimeters.

Second, scaffolds can be fabricated in virtually any shape, from simple tubes to complex, sculptured forms. Also, manufactured scaffolds are seamless and three dimensional, decreasing the potential for mechanical failure and facilitating cellular population and tissue remodeling of the scaffold. Finally, scaffolds can be tailored to a specific site and application by creating blended fibers composed of multiple collagen types (e.g., types I and III) or by co-electrospinning with additives such as growth factors.

Production of tissue-engineered forms such as blood vessels is an example of the use of the electrospinning process. For blood vessel scaffolds, collagen type I or a blend of collagen type I with a specific ratio of type III can be mixed in the same HFP solution and electrospun to form seamless, tubular scaffolds composed of collagen nanofibers.

The length and inner diameter of such a scaffold is dependent only on the dimensions of the mandrel used. In one variation, this scaffold can subsequently be seeded with smooth muscle cells to create a medial layer to be used alone as an implant or as the base-structure in engineering a multi-layered blood vessel construct (intima, media, and adventitia).

This basic example is just one of the many permutations possible using this novel scaffold fabrication method in the construction of complex tissue engineering products.

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