ELECTROSPINNING OF CHICKEN EGG WHITE LYSOZYME

Electrospinning is an emerging area of research whereby fibers with micro – to nanometer size are formed by passing polymer solution (or polymer melt) through a small aperture under the influence of an electrostatic field (5-30kV). The electrostatic repulsion overcomes the surface tension of the solution, causing the polymer to eject towards the grounded collector where the continuous fiber produces a free standing unwoven mesh network. Electrospun fibers are a very attractive potential matrix for variety of applications. They are easy to produce, have large surface-to-volume ratio with a lot of functional groups exposed and therefore easy for post-modification. Many natural and synthetic polymers have been electrospun resulting in materials with unique properties of interest in applied science, technology, and biomedical engineering.

The nanoscale size of the fibers is very important for the design and development of functional materials that mimic living matter. Biomaterials, also known as biocompatible materials, are meant to interact directly with the human bodies therefore they have to be non-toxic, not causing adverse immune or inflammatory reactions, and to have the necessary chemical and mechanical properties. For certain applications, it is very important for the materials to be biodegradable. Electrospun nanofibers from natural polymers, such as proteins, peptides, and DNA, are primary candidates for medical and biotechnological utilization.

Proteins are polymers composed of amino acid that have adopted a complex 3-dimensional structure which is crucial for their numerous biological functions. Several fibrous and globular proteins have been successfully utilized for fiber production, however most proteins are notoriously difficult to electrospin. For reasons which are not fully understood, electrospinning of proteins very rarely produces extended nanofiber morphologies. During each step of the electrospinning process, proteins undergo significant conformational changes before depositing

and assembling on the collector plate into a fibrous mat. The protein structure is ruled by numerous non-covalent interactions, such as van der Waals forces, hydrogen bonds, hydrophobic and hydrophilic interactions. Conformational adaptations are a result of re-arrangement and re-direction of the attractive and repulsive forces and each one of them is accompanied by the corresponding enthalpy and entropy changes. Spinnability is most often achieved by using mixtures of proteins and synthetic polymers, such as polyethylene oxide (PEO), polylactic acid (PLLA), and polyvinyl alcohol (PVA). The latter serve as "supporting polymers" enhancing the desired mechanical properties and helping to preserve the biological functions of the proteins. Despite of the recent advances in this area, the production of nanoscale fibers from proteins remains on empirical level.

Using of simple model proteins is one approach used to bring more inside of the process, the role of the electrospinning parameters, and the conformational changes that take place. Hen egg-white lysozyme (HEWL) is a convenient model protein due to its size, well known structure and biological activity. It is a relatively small single chain protein consisting of 129 amino acids with molecular mass of 14.3 KDa. One of the most extensively studied enzymes; HEWL was the first enzyme whose tertiary structure was elucidated by X-ray analysis. The single-chain molecule has Lys at the amino end and Leu at the carboxyl end as well as four disulfide bonds between the Cys residues. The first 40 residues form a right-hand wing where the peptide chain is coiled twice around the core of non-polar residues in a stable $\alpha-$ helix conformation. Amino acids 41-95 form a less rigid conformation containing a lot of polar residues. The $\alpha-$ helix structure accounts for about 40% of the content of the lysozyme. The rest of the chain wraps around the outside of the right-hand wing. The figure below shows the 3D structure of this enzyme.

HEWL was used in production of ultra-fine fibers by core-shell electrospinning where PVA was used as a shell and a lysozyme-gelatin mixture built the core structure. There are no reports for electrospinning of the lysozyme on its own. The main efforts of this research were directed toward the synthesis of nanofibers from the protein without a supporting polymer. From published data it was evident that the protein must undergo unfolding and subsequent formation of β- sheets before the fibers were deposited on the collector. Solvents, such as trifluoroethanol (TFE), hexafluoroisopropanol (HFP), as well as acidification and heating of the solution, work as denaturants to unfold the protein. Similar conditions also lead to formation of amyloid fibers. We have also investigated the possibility of amyloid formation and the structure and properties of the electrospun fibers resulting from the treated solution.