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The Controllable PVA-Chitosan Fiber Prepared by the Near-field Electro Spinning for Tissue Engineering

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Abstract: The cells in natural tissues and organs have diverse shapes and arrangements in structure. The tissue engineering scaffolds which have a specific extracellular matrix structure can be prepared by electro spun fibers having a diverse arrangement in structure and thus guide adherent cells grow, proliferate and divide into the regenerative tissue or organs which have specific cell morphology and orientation structure. This study is based on a Near-Field Electros Pinning (NFES) process and uses Polyvinyl Alcohol (PVA) mixed chitosan, a non-toxic, good hydrophobic and biocompatible mixed materials, to prepare a micro/nano-fiber with controllable arrangement used in tissue engineering. The purpose of this research is the realization of getting the fiber with controllable arrangement. In this study, laboratory equipment will be built which integrates a feeding system, a high voltage electric field control system, a on-line image acquisition system and a motion control system of the collection platform. It focuses on the process parameters of the micro/nano direct writing of this material. Meanwhile, verifying the controllability of the implementation of the near-field electrospinning process for preparing composite fiber using this experiment platform.

Keywords: Controllable, direct writing, electro spinning fibers, near-field, tissue engineering

INTRODUCTION

There is complex microstructure or a highly ordered structure in human tissue, such as nerves, smooth muscle vascular endothelial cells, skeletal muscle cells, etc. They are all having directionality in the growth of tissue. It plays a very important role in controlling cells' differentiation in accordance with the growth of a certain direction. If nanofiber scaffold has a similar structure with the extracellular matrix, it will be able to provide a suitable environment for the cells, affecting cell proliferation, differentiation, arrangement and migration. It has a guide role for the directional growth of cells and the repair of human tissue (Chew *et al*., 2008).

Currently, many researches have been done to use ordered fiber as the matrix of the cell. Yang *et al*. (2005) used L-polylactic acid (PLLA) to prepare the ordered fiber membrane scaffold and cultured neural stem cells (NSCs) as the seed cell tissue. He found that the extension of neural stem cells and the branch growth of axons are consistent with the direction of the PLLA fiber. Schnell *et al*. (2007) found that the ordered arrangement of electrospinning polycaprolactone (PCL) /collagen fiber membrane scaffold promotes directional growth of neurites and glial migration. It is conducive to the migration of nerve tip cells (Schwanncell) and the directional growth of axons. It can be used for in vivo nerve cell repairing. Xu *et al*. (2004) studied using ordered polylactide caprolactone copolymer fibrous scaffolds to culture human coronary artery Smooth Muscle Cells (SMCs). The research results show that SMCs adhere and migrate in axial direction along orderly nanofiber. The distribution of cytoskeletal protein in SMCs is parallel to the orientation of nano fiber. Compared with the ordinary disorderly fiber membrane, the adhesion and proliferation rate of cells in an orderly nanofiber scaffolds was significantly improved and showed a micro-structure similar to the blood vessels. Liao *et al*. (2008) compared the skeletal muscle cells cultured in the ordered Polyurethane (PU) fiber membrane and the disordered under the mechanical and electrical stimulation. The results showed that the tissue on the ordered PU fiber had more obvious elongation and its nucleus growth was better. Under the mechanical and electrical stimulation, skeletal myoblasts cultured the orderly fiber membrane better divided into the myotubes. Han *et al*. (2010) confirmed that t not only ordered fibers, but also its diameter and distribution density had implications on the neurite growth and breeding differentiation of the stem cells through experiments. Obviously, it has been fully validated that electrospun fibers can guide cells and promote their breeding differentiation.

Meanwhile, many scholars have done a lot of research to prepare the orderly arrangement of electro

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spinning. Yang *et al*. (2007) added a small amount of nano iron oxide particles into the polymer solution to prepare the magnetized polymer spinning solution. He prepared highly ordered polyvinyl alcohol nano-fiber membrane used additional magnetic field electro spinning. Li *et al*. (2003) used two parallel grounded conductive materials as the collecting substrate to prepare the ordered fiber membrane of Polyvinyl Pyrrolidone (PVP). Carnell *et al*. (2008) add the DC electric field control in the receiving means of the electro-spinning to prepare a polyglycolic acid (PGA), a highly ordered fiber membranes. Pan *et al*. (2006) the use of two separately connected to the positive and the negative voltage the spinneret is relatively discharged polymer solution, the runner as the receiving apparatus to obtain the orderly Polyvinylalcohol (PVA) fiber membrane. Hu *et al*. (2009) used a dial-collecting device to prepare a highly ordered PLLA fiber whose surface was coated with the chitosan solution. Daoheng *et al*. (2006) realized deposited fibers strictly controlled by the near-field electrospinning (NFES).

In summary, compared with the general porous scaffolds, the tissue engineering scaffolds prepared by the electro spinning nanofiber material can provide a higher permeability and cell adhesion capacity, so as to better promote cell growth (Sombatmankhong *et al*., 2007; Suwantong *et al*., 2007). It is more important is that the orientation of electro spinning fiber material not only can significantly enhance its adhesion ability of cell growth and proliferation, but also change its cell morphology and growth direction distinguished from the random arrangement of electro spun fibers, namely cell morphology will be stretched significantly along the direction of the fibers when cells grow on the orientated electro spun fibers and the growth of the cells has an obvious orientation along the direction of arrangement of the fibers.

Taking into account the composition of the natural tissue and organ cells, their diverse shapes and arrangements. The author try to get a various arranged structure of electro-spun fibers .Making the assembly form of the nanofibers in the extracellular matrix adapt to special features of the tissue so that guide adhering cell proliferate and divide into regenerating tissues or organs having a specific cell morphology and orientation structure. Based on the above idea, how to control the fiber deposition is crucial to ultimately achieve an ideal regenerative tissue scaffolds.

There is only NFES process which truly is able to control fiber strictly selected from many electro spinning process. However, this process is now mostly used in flexible electronics manufacturing and rarely involved in the field of micro/nano-structure manufacturing for tissue engineering. At the same time, due to the character of easy molding, most people choose PEO as the material of NFES. However, this material is not suitable for using in tissue engineering because it has low toxicity and is soluble in water. This study used the non-toxic, hydrophobic and biocompatible PVA-chitosan material in NFES experiment. Its purpose is achieving the controllable fiber in arrangement. The author built laboratory equipment which integrates a feeding system, a high voltage electric field control system, a on-line image acquisition system and a motion control system of the collection platform. It focuses on the process parameters of the micro/nano direct writing of this material. Meanwhile, verifying the controllability of the implementation of NFES process for preparing composite fiber using this experiment platform.

MATERIALS

Polyvinyl Alcohol (PVA) as a water-soluble synthetic polymer has biodegradability and biocompatibility. It is non-toxic to the human body and good biomedical materials. In view of the poor mechanical properties of pure chitosan fibers, its applications have been restricted as a medical fiber. Mixing PVA and chitosan to prepare electro spun fibers is expected to reach synergies in physiological effects and improved the mechanical properties of the fibers (Zhen *et al*., 2007) due to hydrogen bonding interaction between the two molecules and good compatibility.

In this study, chitosan and PVA were blended. Meanwhile, acetic acid and water were used as solvents. Firstiy, PVA (grades JP233, degree of polymerization 3500, alcoholysis degree of 88%, Kuraray Company of Japan, Ltd.) was dissolved in hot water with 8wt%.This solution was heated to boiling on a magnetic stirrer and stirred until it was completely dissolved. Secondly, chitosan (viscosity-average molecular weight $M\eta = 112\times105$, degree of deacetylation 82.5%, Zhejiang Golden-Shell Biochemical Co., Ltd.) was dissolved in the solvent of 10% acetic acid solution. Finally, PVA solution and chitosan solution were mixed with the volume ratio of 2:1 and stirred well.

EXPERIMENTAL PRINCIPLE

Electro spinning stretchs polymer solution by static electricity to prepare nanofibers. Electro spinning process can be divided into two stages: the first stage is the stable jet stage. The second stage is the spiral cleavage stage (Sun *et al*., 2007; Reneker *et al*., 2000; Shin *et al*., 2001). The distance between the nozzle and the collecting plate is reduced to 0.5~3 mm in NFES so that nano-fibers are collected in the stable jet stage of electro spinning to realize the controllability of the electro spinning process. NFES principle shown in Fig. 1a. Based on the principles of NFES, simultaneously with the formation of the near-field jet, the collection platform moves in accordance with the given direction.

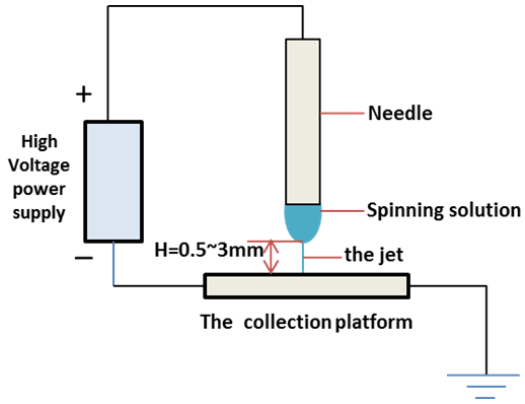


Fig. 1a: The near-field electro spinning principle

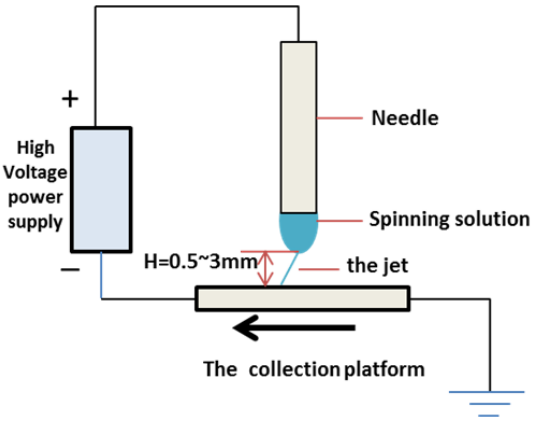


Fig. 1b: The near-field electro spinning process

The collection platform draws jet to deposit and ultimately collects the fibers with the arranged morphology which is same with the trajectory of the collection platform. NFES process shown in Fig. 1b.

Experimental device: The NFES device (Fig. 2) includes high-voltage DC power supply (DW-P503-1AC, Dongwen, Tianjin), syringe pump (TJ-3A/W0109-1B, Lange, United States), high-speed CCD industrial camera (CMLN-13S2M, PointGrey) and the collection platform. Positive and negative of the high-voltage DC power supply which can provide 0~ 50 KV voltages are respectively connected to the syringe needle and the collection platform. Syringe is promoted by the micro pump directly. The feeding speed is adjustable within a range of 1.07 μl/min ~10.7 mL/min. The collection platforms consists of three axis which is driven by three servo motors (A06B-0014-B203, Fanuc, Japan) whose maximum speed is up to 4000r/min and motion accuracy is up to the micro and nano level. The entire platform uses PAC RX3i controller as control system. The motion path can be programmed. The high-speed CCD camera will be used to monitor the entire spinning process. Using a self-developed online monitoring system to detect the Taylor cone

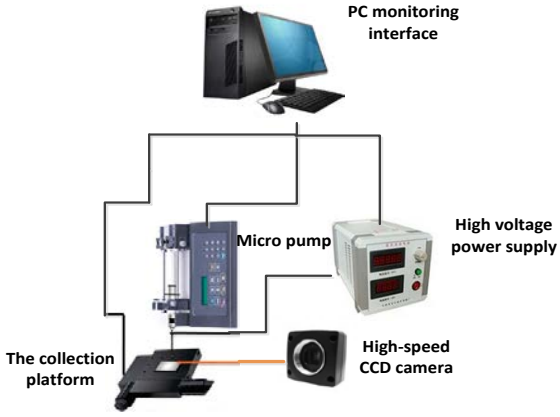


Fig. 2a: The schematic of NFES device

Fig. 2b: The real NFES device

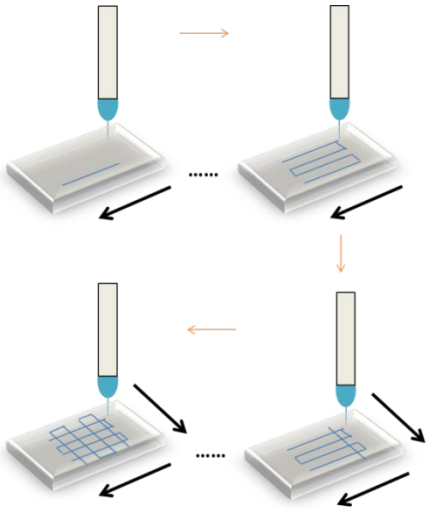


Fig. 3: The trajectory of the colletion platform

morphology to ensure a stable spinning. Voltage, feeding speed and the online monitoring system are integrated in the self-development PC interface. Based on the feedback of the online monitoring system, this equipment can achieve real-time remote control of the voltage, the feeding speed to ensure get the most stable of the process parameters.

Experiment process: In this experiment, a needle with 200 μm inner diameter was used and 3 mm away from

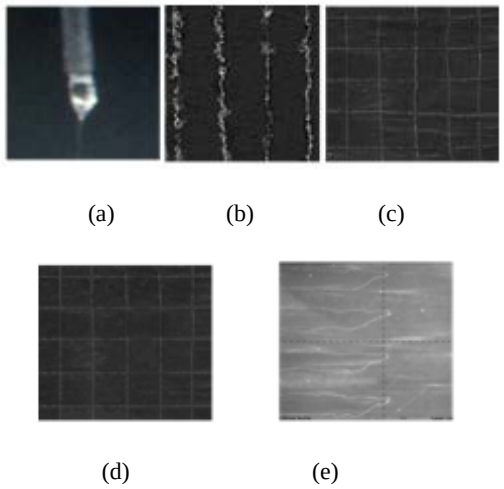
the collection platform. The voltage was set by 2 KV. The collection platform moved at a given trajectory during spinning process. The trajectory is shown in Fig. 3. During NFES process, the jet due to the drag of the collection platform deposited to a fiber arrangement which is consistent with the trajectory. In the case where the other variables were fixed, by changing the moving speed of collection platform and the pitch of moving track respectively, the author tested the controllability of the fiber deposition.

RESULTS AND DISCUSSION

The relationship between the velocity and the controllability of fibers: Figure 4a to d show the effect of the fiber deposition at different speeds of movement of the collection platform. The moving speeds of the collection platform, respectively are 5 cm/s, 10 cm/s and 15 cm/s. Figure 4b shows that, when setting the lower moving speed of the collection platform, the fluctuations in the linear direction of the deposited fiber are serious. The deposited fibers are spiral along the linear direction; Fig. 4c shows that, when the collection platform speed is increased to 10 cm/s, the fluctuations of the fiber are weakened, the stability is improved. Figure 4d shows that, when the speed is up to 15 cm/s, the effect of the deposited fiber is better. Its arrangement is consistent with the trajectory of the collection platform. The fluctuations along the linear direction are smallest. Figure 4e shows that, when moving the tuning points, the fluctuations along the trajectory are serious. The fibers cannot be deposited on the collection platform in accordance with the trajectory because the speed is slowing down. Be seen, when the speed is set at 15 cm/s, the fluctuation of the fiber is smallest. Meanwhile, it is very important that the speed of the moving platform must match with the trajectory.

The relationship between arrangement pitch and the controllability of fibers: The moving pitches of the collection platform were set to 400 μm and 200 μm and the speed of the collection platform is set to 15 cm/s in order to ensure get stable deposited fiber. Figure 5a and b respectively shows the measurement results of fiber spacing in different moving pitch of the collection platform: When the moving pitch of the collection platform is 400 μm, the fiber spacing is 387 μm; when the moving pitch of the collection platform is 200 μm, the fiber spacing is 387 μm. The second fiber spacing actually collected is 52.5% of the first collection. Simultaneously contrasting with the platform moving spacing, the error rates is less than 3.25%. It shows that using the self-built platform can control fibers strictly by NFES.

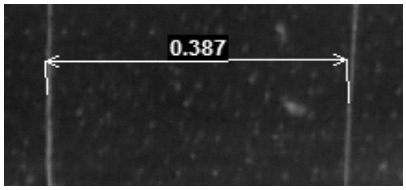
The experiment collected the electro spinning network cross structure consistents with the trajectory of the collection platform. It verified that taking advantage of this material as well as the experimental



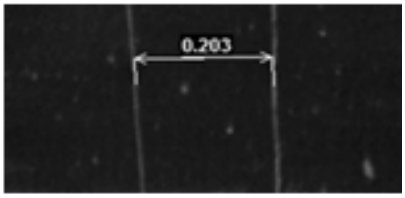
(a) (b) (c)

(d) (e)

Fig. 4: (a) The spinneret image acquisited from the high-speed CCD, (b) The fiber at the speed of the collection platform of 5 cm/s, (c) The fiber at the speed of the collection platform of 10 cm/s, (d) The fiber at the speed of the collection platform of 15 cm/s, (e) The fiber at the turning points



(a)



(b)

Fig. 5: (a) The moving pitch of the collection platform is 400 μm, the measurement of fiber spacing, (b) the moving pitch of the collection platform is 200 μm, the measurement of fiber spacing

platform can achieve controllable fibers by NFES process that people can take advantage of this material and this process to prepare tissue morphology according to demand. Meanwhile, comparing the moving pitch of the collection platform and the fiber spacing collected, the author verified that the control precise of fiber is very high.

CONCLUSION

Electro spun fibers can promote cell growth and cell adhesion ability due to its higher permeability and

is often used as scaffolds for tissue engineering. However, the shape and arrangement of the natural tissue and organ cells have diversity. Using electro spinning to prepare tissue engineering scaffolds with specific extracellular matrix structure which induce cells differentiate, multiply and eventually form the target tissues or organs. It has an immeasurable sense of building ideal scaffolds for tissue engineering and further promoting the tissue engineering applications.

This study is based on a NFES process and uses Polyvinyl Alcohol (PVA) mixed chitosan, a non-toxic, good hydrophobic and biocompatible mixed materials, to prepare a micro/nano-fiber with controllable arrangement used in tissue engineering. In this study, laboratory equipment will be built which integrates a feeding system, a high voltage electric field control system, a on-line image acquisition system and a motion control system of the collection platform. Through experiment, the deposition of PVA- chitosan fiber is most stable when the moving speed of the collection platform is 15 cm/s. The self - made equipment can control deposited fiber arrangement which provides for further constructing complex fibers' micro-morphology. Meanwhile, through the experimental detection, fibers can arrange at micron size and regulate on demand. It further validated the reliability of the process and equipment and ensure that the fibers can be control in high-precision.

This research provided a very good idea for the preparation and application of regenerative organ in the field of tissue engineering. The author used new materials and new process to prepare controllable electro spinning fibers and verified its controllability by experiments. It made use of electro spinning to build micro-structure of the target tissue becomes possible and further promote the development of tissue engineering applications.

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