ELECTROSPUN STARCH-POLYMER SCAFFOLDS FOR TISSUE ENGINEERING: A BRIEF REVIEW

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Abstract

A variety of biopolymers, such as starch compounded synthetic polymers or acetylated starch, have been proposed as promising candidates for tissue scaffolding. Electrospinning has become a unique technique for generating three dimensional matrices of those biopolymer matrix scaffolds. Finally, both starch blended biopolymer scaffolds and modified starch are capable of promoting cellular activities, which are essential in regenerative medicine.

1 Introduction

Tissue engineering, also commonly called regenerative medicine, is the application of knowledge from such interdisciplinary fields as medicine, biology, engineering as well as material science, which deals with fabricating therapeutic matrix scaffolds in order to be channeled with viable human cell systems or cell responsive biomolecules derived from such cells, with the aim of regenerating a new cellular matrix which has been destroyed by injury, disease, or congenital defects [1, 2]. Nevertheless, the physicochemical, as well as the mechanical and geometrical properties of the polymer-based scaffolds should fulfill all specific requirements in mimicking the native tissue, and eventually allow in-tissue growth [3, 4]. In this approach, a series of porous materials have been demonstrated to accommodate cells, produce three-dimensional cell composites, and potentially guide their growth by allowing specific individual cells to adhere to the surface of the scaffold, and maintaining the differentiated cell phenotypes. Both natural and synthetic materials have been generated as potential scaffolds for tissue engineering. The natural materials include collagen, silk protein, agarosa, alginate, chitosan, and starch in modified form. Meanwhile, synthetic polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(urethane) (PU), polyphosphoesters, poly(ε-caprolactone) (PCL) and its blended forms, for instance, gelatin compounded PCL and starch blended PCL have been employed as degradable constructs for various tissues and organs.

The electrospinning process has been known since the 1900's [5-6]. The process is a unique technique which creates electrified jets of polymer solutions and molten polymers contained in a capillary with a syringe tip and a collecting device by applying high voltage. To date, many types of polymers have been successfully electrospun into nanofibers with a diameter as small as 5 nm as reported in some literature [7-9]. The focus of the electrospinning process in regenerative medicine is on generating three dimensional matrix scaffolds which can ultimately be used to promote primary cell activities starting from cell attachment and proliferation, as well as complex processes such as differentiation which is completely advantageous, since the resultant fibers seemingly mimic the fibrillar native tissue structures.

But there are several necessary requirements for polymer-based scaffolds, including the use of non-toxic, biodegradable, biocompatible materials, which also do not cause an inflammatory response. Prior to the electrospinning process, material and system parameters have to be taken into account due to the fact that they can have an extreme influence on the deposited fibers compiled on the collecting screen, and are capable of acting as a mediator for regenerative processes within tissues.

This article provides a brief review, focusing on the use of polymer-based scaffolds made up of modified starch and its blended forms with one of polyester groups, PCL, and another synthetic polymer, PVA, by utilizing the electrospinning technique and considering several influencing parameters. Generally, several crucial points, for instance the electrospinning process of starch in a modified form and starch blended synthetic aliphatic polyester, PCL, and cell cultivation processes by means of seeding the specific cells on the surface of those matrix based scaffolds, will be succinctly highlighted in this article. In essence, this article is by no means a complete review of all literature associated with electrospun starch ester and starch blended polymer scaffolds, but it is based on the limited amount of tissue scaffolding research that points out the potential use of pristine or modified starch and its compounded forms with synthetic polymer-based scaffolds in tissue engineering. Hence, the authors would like to apologize to those who have been disregarded.

2 Tissue engineering

Tissue engineering in general was briefly introduced in the introductory part. It was mentioned there that tissue engineering consists of several fields of interdisciplinary studies; therefore, cell biologists, surgeons, and engineers could currently work within a team work. Artificial connective, epithelial, and neuronal tissue are being regenerated with a series of different biomaterials. The need for human 'spare parts' is still limited, with the stocks of a tissue or organ transplanted from a donor of the same species to the recipient with different genetic characteristics, widely known as allograft. Then, the enormous need for 'spare parts' comprises a driving force for research and requires various innovative techniques and creative solutions, for instance the development of artificial skin for severely burned patients, intensive research on the generation of cartilage and bone to heal articular joint diseases or injuries, as well as the construction of liver organoids for bridging comas, which are still currently being intensively investigated.

Tissue-engineered scaffoldings typically employ three dimensional extracellular matrices (ECMs) to mediate the formation of new natural tissues from isolated cells. The ECMs are manufactured not only to induce the specific cells of the damaged or injured tissue into an appropriate contact, but they also provide structural mechanical support until the host cells reform a stable and novel natural matrix and specific signaling pathways to guide the gene expression of cells in forming the tissue. One should bear in mind here to design artificial matrices for tissue engineering application is to mimic the functions of ECMs naturally found in the native tissue structure [10-11].

3 Electrospinning

Electrospinning, by which the filaments can be generated, has attracted significant interest in recent years as a simple and straightforward method [12]. However, in the typical electrospinning process (see Figure 1), based on a lot of scientific evidence, there are many influencing parameters which significantly affect the electrospinning process and the morphology of the resultant filaments. Such parameters are mainly categorized into two classes, namely, system and process parameters. The former includes solution concentration, polymer molecular weight, solution viscosity, solution conductivity, solution surface tension, and the latter one comprises applied voltage, nozzle-to collector distance (NCD), feed rate, temperature, humidity, as well as solvent volatility. Nonetheless, not all of those influencing factors are fundamental control parameters nor are they independent of each other. For instance, viscosity is a function of both solution concentration and polymer molecular weight (among other affecting factors) [13].

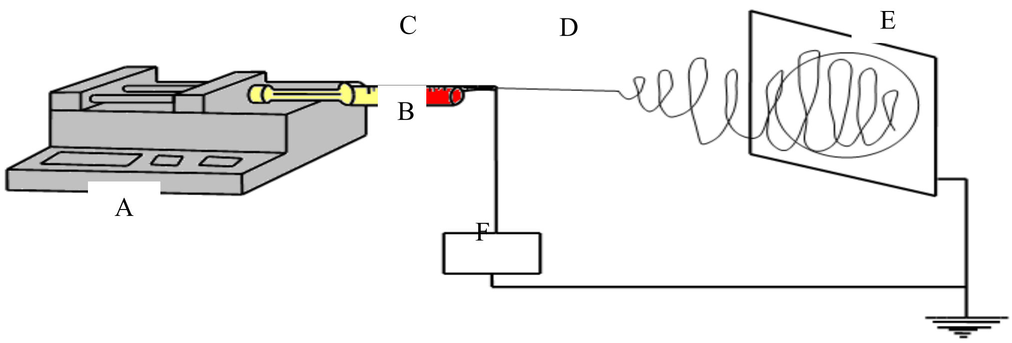

Figure 1 Schematic representation of the electrospinning apparatus: (A) syringe pump, (B) polymer solution in a capillary, (C) 20G-needle, (D) the resultant fibers, (E) a collector, and (F) high voltage.

3.1 The electrospinning of starch acetate

In fact, the electrospinning process by using a pristine starch polymer is still relatively new research. By contrast, cellulose acetate, one of the polysaccharides' families, was initially electrospun by Formhals in the 1930's; he demonstrated his first patent of electrospinning of cellulose acetate utilizing acetone as a solvent [14]. Recently, other polysaccharides such as arginin, dextrin, hyaluronic acid, as well as starch acetate have been able to be spun. As a matter of fact, many reports of numerous studies have been observed as dealing with polysaccharides and their derivatives for the manufacturing of electrospun nanofibers that could be potentially employed in regenerative medicine or broadly known as tissue scaffolding [15].

Nowadays, Reddy N *et al.*[16] have exhibited electroprocessing of starch acetate. They prepared numerous DS of starch acetate, namely 1.1, 2.3, and 2.8. Formic acid (90 % v/v) acted as a solvent. The fibers were then dried at an extremely high temperature about 105^{o}C, with the aim of completely evaporating the remainder of the formic acid.

3.2 The electrospinning process of starch blended biodegradable polymer scaffolds

Unlike starch acetate, which has so far been poorly investigated in terms of the production of fibers, starch compounded with several synthetic polymers, PCL, polyvinyl alcohol (PVA), has been observed by several authors. In this article, the authors focus purely on electrospinning of SPCL (starch-PCL), SPVA (starch-PVA) as well as SAPCL (starch acetate PCL). SPCL consisting of 30% starch and 70% PCL was prepared, thereafter electrospun. It was carried out at a high voltage and working distance, namely in the range of 25-30 kV and 20-25 cm. The feed rate was about 1 mL/h. [17-18]. Another report which emerged, Adomaviciute *et al.*[19] performed the electrospinning process of another synthetic polymer scaffold, PVA, compounded with one of the derivatives of starch, to manufacture three dimensional (3-D) matrices. They set up three main solutions, mainly PVA/Cationic Starch (CS) and PVA/hCS (homogenized Corn Starch) in water, and PVA/hCS in an ethanol/water mixture, and afterward conducted electroprocessing using a single roller electrospinning-"Nanospider™" (Elmarco, Czech Republic).

During the process, NCD was kept at a distance of 11 and 14 cm, the high applied voltage was varied ranging between 40 – 70 Kv and other influencing parameters such as temperature and humidity were significantly sustained at about 20 ± 2^{o} C and 42± 2 %, respectively. They finally showed that the filaments of PVA/CS were not created during electrospinning since CS itself has branchy polyelectrolytical swellable macromolecules. More essentially, the use of ethanol in the polymer solution has improved electroprocessing due to the fact that it is straightforward to evaporate. On the other hand, the solvent must totally evaporate before highly charged jets are deposited on the collecting device.

Then, Nugroho in 2009 [20] observed electrostatic spinning of starch acetate blended PCL and PCL. The prominent feature which resulted from this work was in a form of beaded fibers, as depicted in Figure 2 below.

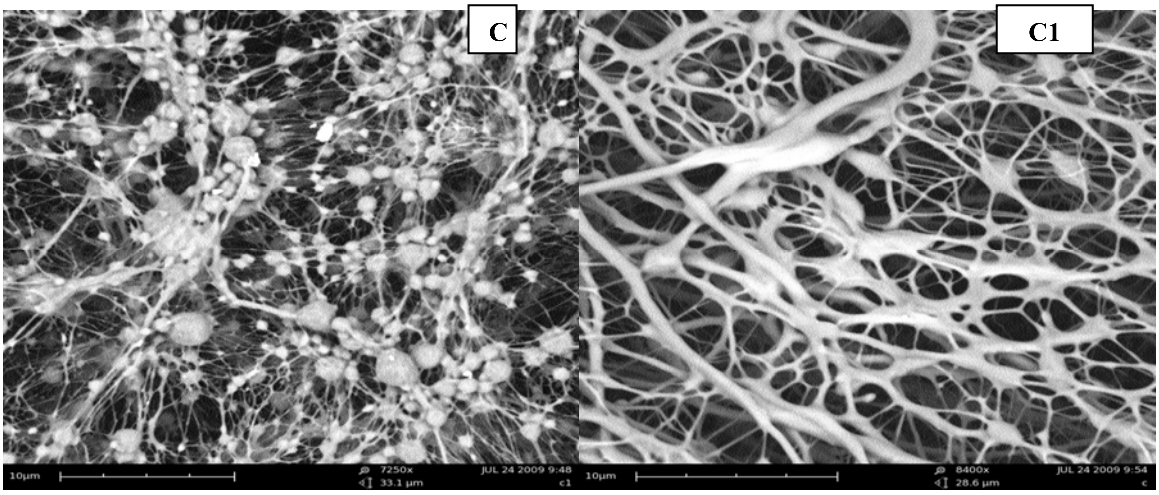

Figure 2 The resultant fibers of starch acetate blended PCL (C), and PCL (C1), respectively. The process was carried out at a high voltage, with the power supply ranging between 20-40 kV. The flow rate was in the range of 0.5-1 mL/h During electroprocessing, the humidity was extraordinarily high, generally the electrospinning process of starch acetate blended PCL and PCL is conducted in the range of 30-40 %. In addition, the electrospinning process was set up in the laboratory of the Department of Nonwovens, while the acetylated starch was produced in the laboratory of the Department of Chemistry, Technical University of Liberec.

4 Polymer scaffold prepared by electrospun starch acetate

Reddy *et al*. also reported that mouse fibroblasts (NIH 3T3) cultivated on the surface of starch acetate scaffold. The cells were well-adhered over the matrix scaffold surface. Furthermore, the desired degradation of the fibers in the body can be achieved by controlling the acetylation of the starch. As a conclusion, they have shown that starch acetate nonwoven mats are potential templates for mouse fibroblasts to perform cellular activities which are crucial in regenerative medicine. Thus, starch acetate could be a suitable polymer scaffold in tissue engineering.

5 Scaffolds prepared by nonwoven mats of starch blended synthetic biodegradable polymers

Da Silva MA *et al*. compared the test of glycosaminoglycan (GAG) quantification among two distinct matrix scaffolds, PCL and SPCL. Statistically, GAG production was slightly the same among the two types of 3D matrices, SPCL was a little bit higher than that of PCL. SPCL here is a blend form of corn starch and PCL. In this work, it has recently been demonstrated that it maintains adhesion, proliferation, as well as differentiation of bovine articular chondrocytes (BACs) in SPCL fibrous mats. It is known from previous chapters that a suitable scaffold must fulfill several pre-requisites needed in regenerative medicine. Two of those are that it must have good surface properties to allow cell adhesion and transport of essential nutrients to growing cells. SPCL is probably more hydrophilic and preferable for cell growth than PCL. Results also indicate that SPCL is capable of sustaining ECM production more effectively.

Generally, in tissue scaffolding, the cell-surface interactions are potentially governed by a series of several factors such as chemical properties of polymer, hydrophilicity/hydrophobicity, roughness, and rigidity. Furthermore, cell-matrix scaffold interactions are a very sophisticated phenomenon; it is not quite clear which property, hydrophilic or hydrophobic will be favorable for cell attachment, proliferation and other complex cellular activities [21]. Nugroho in 2009 then investigated the above phenomenon by cultivating chondrocytes cells on 3D fibrous mats manufactured by electrospinning, namely starch acetate blended PCL, after that compared with PCL. This work was carried out at the Institute of Clinical and Experimental Medicine, the Academy of Sciences of the Czech Republic. Figure 3 shows the first step of cellular activities in tissue engineering - cell attachment. From those images, chondrocytes communicated by making spheroid-like structures, on the other hand, the cell-cell interactions were much stronger than the cell-matrix interactions which are crucial in tissue scaffolding.

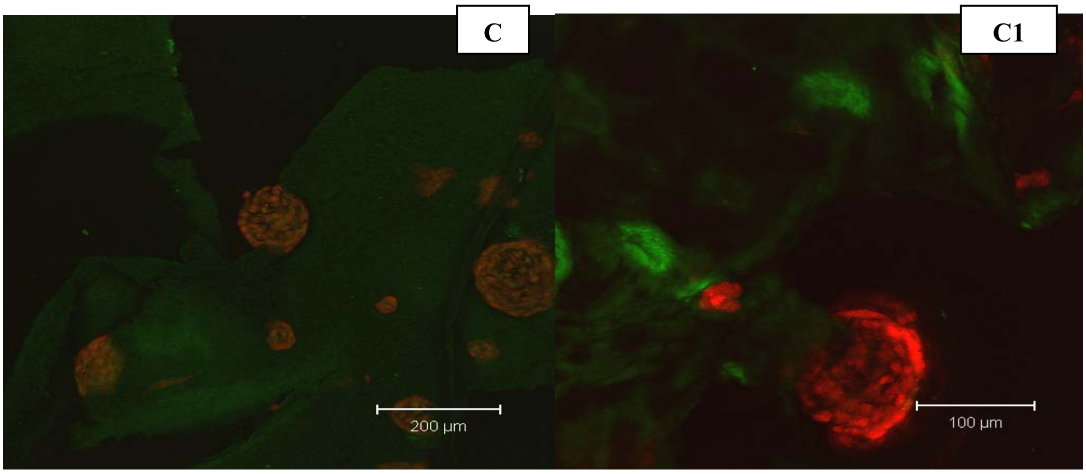

Figure 3 The cells strongly preferred interacting amongst themselves than spreading onto polymer-based scaffolds made up of starch acetate blended PCL (C) and PCL (C1), respectively.

The hydrophobic character conveyed by starch acetate blended PCL and PCL, respectively, was not sufficient to promote the first step after exposure of the fiber meshes in a biological environment. Perhaps, both polymer scaffolds are too hydrophobic to allow tissue-in growth. Surprisingly, the MTT test results exhibited a very distinct standpoint with microscope images as shown in Figure 4.

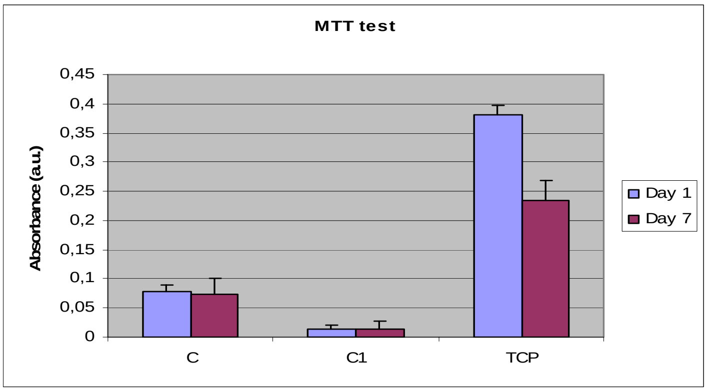

Figure 4 The results of MTT assay. Starch acetate blended PCL (C) exhibited higher cell viability than PCL (C1). Both of these results were much less than TCP.

The cell viability on C was higher than C1, even though this behavior could not obviously emerge in the exhibited pictures (see Fig.3). A control scaffold, Tissue Control Polystyrene (TCP), held a crucial role as compared to both starch acetate blended PCL and PCL scaffolds. In essence, the assay of MTT is needed to perceive the proliferation rate of the cells cultured on the surface of scaffolds and probably sustains the images captured by a confocal microscope.

6 Conclusion

The three dimensional fiber meshes made by the electrospinning process are becoming increasingly essential in tissue engineering. Numerous biomaterials, mainly either synthetic polymers such as PCL, PVA or a natural polymer, starch for instance, and a combination thereof have been positively offered as matrix scaffolds to temporarily replace ECM, in other words, to provide a supportive and preferable environment for tissue-in growth. More importantly, both starch acetate and starch blended synthetic polymer (SPCL) are capable of being potential templates for mouse fibroblasts to perform cellular activities, preferable for cell growth, capable of sustaining ECM production more effectively. Additionally, starch acetate compounded PCL could be potentially utilized to replace PCL as a promising candidate for the scaffolds in tissue engineering since the result of MTT assay given did significantly alter among those biodegradable matrix scaffolds, but other challenging efforts will obviously be required to improve their characters to be similar to the widely used control scaffold, TCP.

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ELEKTROSTATICKÉ ZVLÁKŇOVÁNÍ TKÁŇOVÝCH NOSIČŮ SLOŽENÝCH Z POLYMERŮ S PŘÍDAVKEM ŠKROBU: PŘEHLEDOVÝ ČLÁNEK

Různé biopolymery, například takové, které obsahují škrob nebo acetylovaný škrob, jsou slibnými materiály pro tkáňové inženýrství. Elektrostatické zvlákňování je jedinečnou technologií pro výrobu trojrozměrných biopolymerních tkáňových nosičů. Tkáňové nosiče obsahující směs polymeru a modifikovaného škrobu podporují buněčnou aktivitu, která je důležitá pro aplikaci v regenerativní medicíně.

ELEKTROSTATISCHES SPINNEN VON GEWEBETRÄGERN, DIE AUS POLYMEREN MIT EINER ZUGABE AN STÄRKE ZUSAMMENGESETZT SIND

Verschiedene Biopolymere, zum Beispiel solche, die Stärke oder Azetylstärke enthalten, sind vielversprechende Materialien fürs Gewebegerüst. Elektrostatisches Spinnen ist eine einzigartige Technologie für die Produktion dreidimensionaler Bioplymergewebeträger. Die Gewebeträger enthalten eine Mischung aus einem Polymer und modifizierter Stärke und unterstützen somit die Zellenaktivität, welche für die Anwendung in der regenerativen Medizin wichtig ist.

ELEKTROSTATYCZNE ZWŁÓKNIANIE NOŚNIKÓW TKANKOWYCH SKŁADAJĄCYCH SIĘ Z POLIMERÓW Z DODATKIEM SKROBII: ARTYKUŁ POGLĄDOWY

Różne biopolimery, przykładowo zawierające skrobię lub acetylowaną skrobię, są obiecującymi materiałami w inżynierii tkankowej. Elektrostatyczne zwłóknianie to wyjątkowa technologia produkcji trójwymiarowych biopolimerowych nośników tkankowych. Nośniki tkankowe zawierające mieszankę polimeru i skrobii zmodyfikowanej wspomagają czynność komórek, która jest ważna do stosowania w medycynie regeneracyjnej.