EECE 5002 Senior Design Report

Group 25: Jacob Long
Expo webpage: https://jrl52jc.weebly.com/

Tissue Engineering: Electrospinning Nanofiber Polymer Scaffolds for Dermal Wound Healing

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Senior Design

1. Abstract and Introduction

A) Abstract

The research project was initially to construct a nanofiber scaffold for wound healing using electrospinning, but upon closure of our lab due to the virus the project changed - instead, the project culminated in a review paper on the qualities of such a scaffold as outlined by available literature. The fabrication methods, bio-mimicking characteristics, and the cell-interacting features of the scaffolds are explored.

B) Introduction

The purpose of our project was to improve on designs of electrospun nanofiber scaffolds for wound healing applications. The goals were to learn the “electrospinning” technique for tissue engineering, the wound healing applications of tissue engineering, and how to perform an effective literature search.

The potential of treatment of chronic wounds by means of tissue engineering has grown substantially over the last 15 years, especially given that other current treatment options are relatively ineffective and impractical. One method of tissue engineering which has been explored extensively in this realm is biodegradable nanofiber scaffold construction. We set out to design, fabricate, and test a scaffold suitable for wound healing. We also postulated that natural additives (yarrow and goldenrod) and epidermal growth factor could increase the healing ability of these mats.)While additives were an initial component of our design plan, we never were able to implement this idea due to obstructions in the progress of our project.)

2. Theory of Operation, Progression of Project

The properties of the scaffold must be just right in tissue engineering. The content of the scaffold must be biocompatible - that is, cells must recognize the scaffold and react to it in the desired way. To the cells, the scaffold needs to seem like the extracellular matrix (ECM), a natural thin-fibrous structure that connects the cells to each other. A scaffold that effectively mimics the ECM can be generated by a process called electrospinning, which is displayed in figure 1. To generate a scaffold in this fashion, a high voltage is applied to the solution at the emitter (thin-gauge nozzle), which then projects downward towards the grounded collector in the form of a thin fiber. The solvent evaporates upon formation of the polymer "jet" towards the collector. The collected fiber forms a nanofiber scaffold (mat).

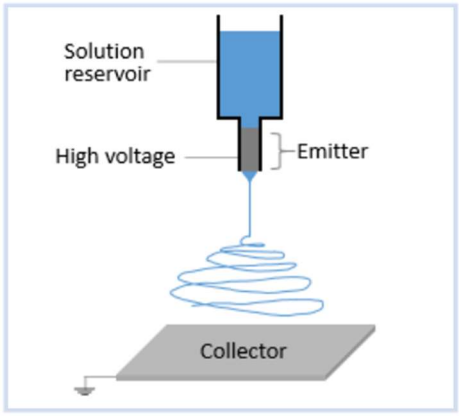


Figure 1: Typical electrospinning setup. A high voltage is applied to the solution at the emitter, which is projected to the collector as a "jet". The solvent evaporates and the polymer is collected as a fiber.
Image source: Nanoscience.com

The type of polymer used is crucial to the scaffold design. In general, there are two kinds of polymers used in tissue engineering - natural and synthetic. Natural polymers tend to be more biocompatible, but synthetic polymers are much more practical and possess superior structural properties. Initially we chose to use chitosan for our scaffold because of its well-established wound healing properties. However, primarily due to insufficient literature search, our protocol was ineffective and no matter how we adjusted the parameters we were unable to generate a nanofiber scaffold at all. The solution simply sprayed onto the collector (figure 2), rather than stretching into a fiber.

After this failure we improved our literature search process and found that there was evidence that a solution made from chitosan and polycaprolactone (PCL) could be electrospun effectively. However, we still failed to electrospin this combination, and after some protocol adjustments and safety considerations our advisor recommended that we modify our approach again.

A feature central to tissue engineering with nanofiber scaffolds is surface modification - that is, making the scaffold surface more suitable for cell migration and proliferation. Polymers can be grafted to other polymers (forming “copolymers”) more suited for prudent cell interactions. In the first week of March, just before closure of the University, this is what we intended to have guide our further progress.

3. Status and Results

A) Status

After successfully electrospinning PCL scaffolds, we began to investigate the biocompatibility of surface-modified PCL scaffolds in literature, but very soon after that the university closed. Our advisor then recommended that we write a review paper involving the ability of nanofiber scaffolds to mimic the ECM and the potential of surface modification. This was to help us improve our ability to perform effective literature search and learn further about this application of tissue engineering.

B) Results

Both the results of our efforts in the laboratory and the review paper are included in this report. Laboratory results: Chitosan and PCL/Chitosan solutions of all concentrations either sprayed or dripped onto the collector. PCL nanofibers were the only nanofibers that were successfully electrospun. One sprayed chitosan solution and the successfully electrospun PCL nanofibers are displayed in the figures below.

Review paper: The paper is included in its entirety, beginning on page 18.

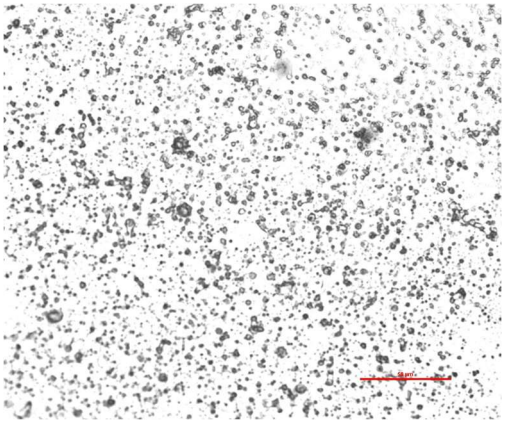


Figure 2: Attempt to electrospin Chitosan. Trial 12/10/2019 (scale 100μm)

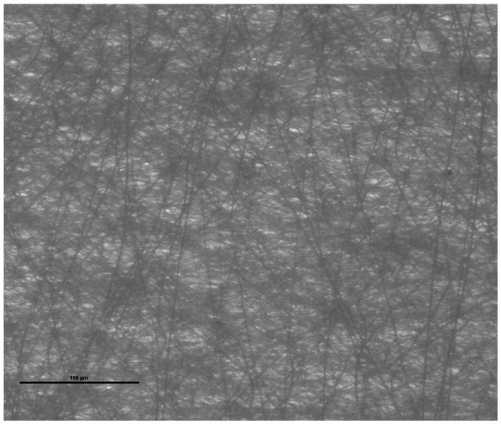


Figure 3: Electrospun PCL nanofibers. 02/27/2020 (scale 100μm)

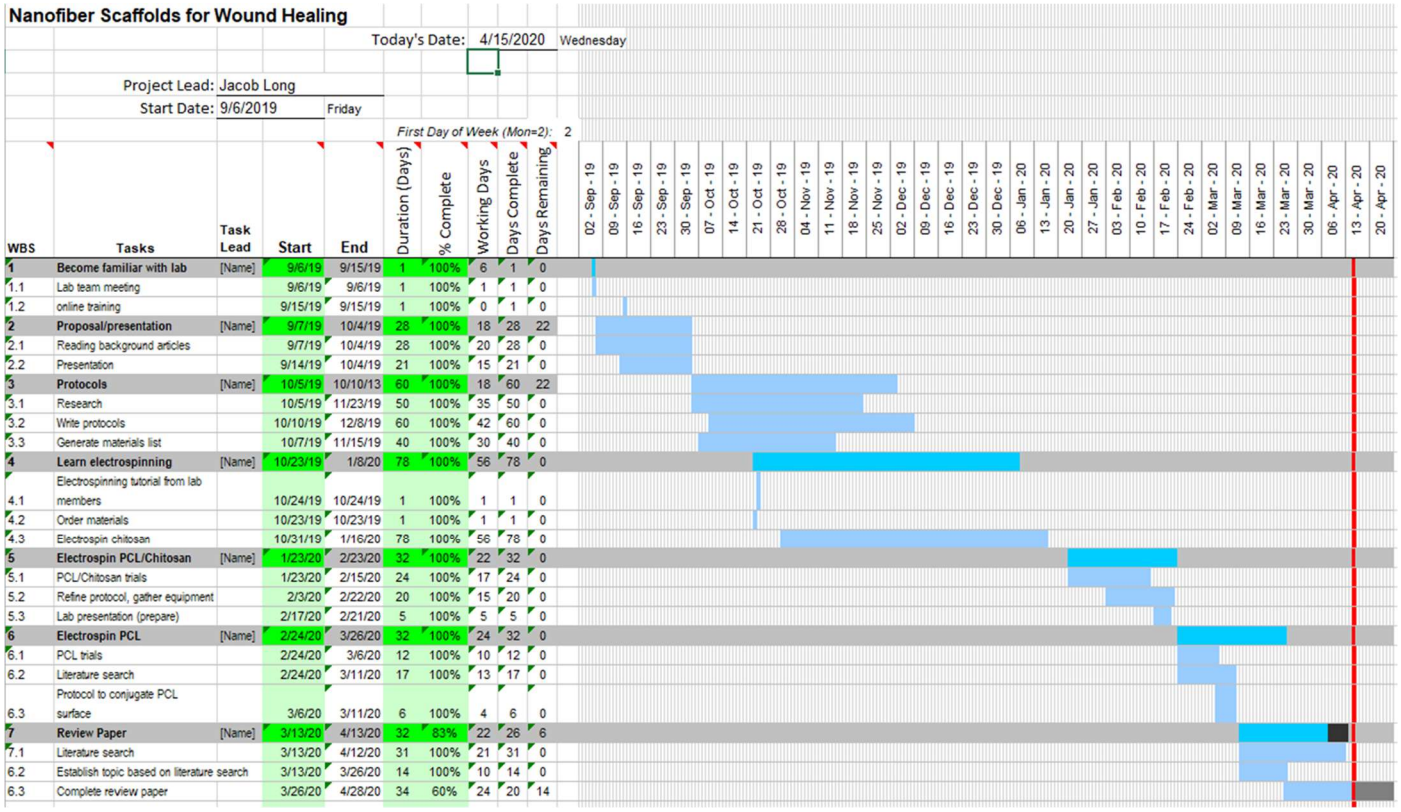
4. Future Actions Required

If the university had remained open, we would have been able to fabricate modified PCL scaffolds for wound healing applications. We could design several scaffolds with different characteristics and modifications, and then compare cell migration and proliferation on modified and unmodified PCL scaffolds (in vitro). If that experiment were very successful we could, with time permitting, test the scaffolds' wound healing abilities in vivo using laboratory mice (likely at another facility).

The eventual natural outgrowth of this project would be the publishing of a paper outlining our procedure, results, and findings.

5. GANTT Chart

Due to page constraints, the GANTT Chart is rather difficult to read in this report, even when rotated ninety degrees on the page and maximized in size. Please view the GANTT chart in full display on my Expo website: https://jrl52jc.weebly.com/gantt-chart.html



Nanofiber Scaffolds for Wound Healing

Today's Date: 4/15/2020 Wednesday

Project Lead: Jacob Long

Start Date: 9/6/2019 Friday

First Day of Week (Mon=2): 2

WBS	Tasks	Task Lead	Start	End	Duration (Days)	% Complete	Working Days	Days Complete	Days Remaining
1	**Become familiar with lab**	[Name]	9/6/19	9/15/19	1	100%	6	1	0
1.1	Lab team meeting		9/6/19	9/6/19	1	100%	1	1	0
1.2	online training		9/15/19	9/15/19	1	100%	0	1	0
2	**Proposal/presentation**	[Name]	9/7/19	10/4/19	28	100%	18	28	22
2.1	Reading background articles		9/7/19	10/4/19	28	100%	20	28	0
2.2	Presentation		9/14/19	10/4/19	21	100%	15	21	0
3	**Protocols**	[Name]	10/5/19	10/10/13	60	100%	18	60	22
3.1	Research		10/5/19	11/23/19	50	100%	35	50	0
3.2	Write protocols		10/10/19	12/8/19	60	100%	42	60	0
3.3	Generate materials list		10/7/19	11/15/19	40	100%	30	40	0
4	**Learn electrospinning**	[Name]	10/23/19	1/8/20	78	100%	56	78	0
4.1	Electrospinning tutorial from lab members		10/24/19	10/24/19	1	100%	1	1	0
4.2	Order materials		10/23/19	10/23/19	1	100%	1	1	0
4.3	Electrospin chitosan		10/31/19	1/16/20	78	100%	56	78	0
5	**Electrospin PCL/Chitosan**	[Name]	1/23/20	2/23/20	32	100%	22	32	0
5.1	PCL/Chitosan trials		1/23/20	2/15/20	24	100%	17	24	0
5.2	Refine protocol, gather equipment		2/3/20	2/22/20	20	100%	15	20	0
5.3	Lab presentation (prepare)		2/17/20	2/21/20	5	100%	5	5	0
6	**Electrospin PCL**	[Name]	2/24/20	3/26/20	32	100%	24	32	0
6.1	PCL trials		2/24/20	3/6/20	12	100%	10	12	0
6.2	Literature search		2/24/20	3/11/20	17	100%	13	17	0
6.3	Protocol to conjugate PCL surface		3/6/20	3/11/20	6	100%	4	6	0
7	**Review Paper**	[Name]	3/13/20	4/13/20	32	83%	22	26	6
7.1	Literature search		3/13/20	4/12/20	31	100%	21	31	0
6.2	Establish topic based on literature search		3/13/20	3/26/20	14	100%	10	14	0
6.3	Complete review paper		3/26/20	4/28/20	34	60%	24	20	14

02 - Sep - 19 | 09 - Sep - 19 | 16 - Sep - 19 | 23 - Sep - 19 | 30 - Sep - 19 | 07 - Oct - 19 | 14 - Oct - 19 | 21 - Oct - 19 | 28 - Oct - 19 | 04 - Nov - 19 | 11 - Nov - 19 | 18 - Nov - 19 | 25 - Nov - 19 | 02 - Dec - 19 | 09 - Dec - 19 | 16 - Dec - 19 | 23 - Dec - 19 | 30 - Dec - 19 | 06 - Jan - 20 | 13 - Jan - 20 | 20 - Jan - 20 | 27 - Jan - 20 | 03 - Feb - 20 | 10 - Feb - 20 | 17 - Feb - 20 | 24 - Feb - 20 | 02 - Mar - 20 | 09 - Mar - 20 | 16 - Mar - 20 | 23 - Mar - 20 | 30 - Mar - 20 | 06 - Apr - 20 | 13 - Apr - 20 | 20 - Apr - 20

6. Budget

All materials used in our experimental work were supplied by the Integrative Biosensing Laboratory (PI: Dr. Esfandiari) which contained most of the necessary equipment and experimental materials. Table 1 displays the costs of the materials purchased specifically for our project.

Product	**Company**	**Price**	**SKU**
Chitosan	Sigma-Aldrich	$65.20 (50g)	448877-50G
Acetic Acid	Sigma-Aldrich	$28.50 (100mL)	A6283
Trifluoroacetic Acid	Sigma-Aldrich	$44.50 (100mL)	8082600101
		$138.20 TOTAL	

Table 1: Purchased materials

7. Improvements and Conclusion

A) Improvements

Each step in this project revealed to us faults in our work, which I found very enlightening. There are several facets of the project which could have been improved, most of which centered around our unsatisfactory initial literature search.

In the world of research, a comprehensive familiarity with the topics of interest is absolutely essential. The quality of your endeavor is a direct representation of the degree to which you understand the problems, methodology, potential solutions, and sub-processes that make up the science at hand. This understanding emerges out of an extensive review of the relevant available scientific literature, which is where our initial efforts were horribly insufficient. This haunted our project throughout the year, hampering our progress in a great number of areas.

Our chitosan experiment is a prime example of this failure. Impressed by the wound healing potential of chitosan-based materials, we sought an application of electrospinning the polymer in the online literature. Experiments detailed in a few journal articles indicated that chitosan had been electrospun using acetic acid and trifluoroacetic acid (TFA); satisfied prematurely, we neglected to investigate the credibility of these accounts or continue reading elsewhere. In hindsight, our failure to electrospin a chitosan solution is obvious – the journals had poorly grounded reputations and the experiments lacked replication elsewhere – and in our untrained hands the experiment was doomed to failure. After discussion with our advisor, we conducted a more thorough search and found substantive accounts of appropriate polymer solutions for electrospinning.

The real catastrophe of this ordeal, however, was not the dismal collections of each electrospinning trial, but the time lost in futile experimental work. We dumped tens of hours into futile solution preparation and electrospinning – time quickly transformed into squandered potential. Therefore, the overall progress of our project could have been improved fantastically had we adopted the proper approach to our initial literature search.

A few other minor improvements could have aided our progress and experiment quality. Some of the experiments in the literature used chemical equipment and glassware which our lab did not possess, and easier access to these would have alleviated a temporal setback. The project orientation in its early stages could have been improved had I more reliably logged my weekly progress reports in the first semester. My lack of familiarity with the operation of laboratory equipment and safe practices in material handling were sometimes a source of confusion and frustration, likely remediable by a more thorough literature investigation of electrospinning and polymer laboratory work. I learned, if nothing else, that negligence shackles progress in research.

B.) Conclusion

In light of the shortcomings and faults of our project, the enamoring journey undeniably transformed my approach to the fields of engineering and medicine, and to the field of science in its totality. I must remark that my primary area of growth as a scientist through this project was in my ability to perform a literature search. Before this project I had no idea how to investigate topics in the realm of scientific literature – I knew that the resources were available to me but I did not know how to make them my tools. Now, with those tools under my belt, I feel empowered by the boundless world of scientific findings. I have already implemented this newfound utility, for example, for the sake of my sickly brother as we try to arm ourselves with knowledge to combat his rare disease. I hope to do endless good for my employers, myself, and others in my application of scientific truth as I navigate through my life and career.

I think that there may be an underlying academic issue worth addressing that surfaced as a result of my project. In retrospect, our failure to conduct a sufficient literature search might be the manifestation of a problem that prevails among many science students: an inability to wield scientific literature. Upon presentation of our meager progress at a laboratory meeting, our advisor noted that student inexperience with such literature is quite common at the school. Briefly, we tossed around the prospect of offering a class that introduces literature search techniques, and I mention this here because I think that such a class could prove invaluable for students like me in the future. I may recommend such a class to the CEAS administration.

I would like to thank Prof. Hunter and Dr. Wei for their efforts this year in coordinating senior design in Dr. Kosel's absence, especially given the strenuous circumstances of the last month. I would also especially like to thank our advisor, Dr. Esfandiari, for her guidance and high expectations which kept me on track and challenged me to do my absolute best.

8. References

Please note: below are the references for all of our experiments and the report content above. The review paper references are listed at its end.

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Review Paper

Mimicking the ECM and Optimizing Cell-Surface Interactions in Polymeric Scaffolds for Dermal Wound Healing: A Review

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Abstract

Solutions to healing chronic wounds have been explored in the field of tissue engineering, culminating in the design of biodegradable polymer scaffolds. In order for these scaffolds to promote wound healing effectively, they must be an appropriate stand-in for the fibrous extracellular matrix (ECM) and play the part of interacting with surrounding cells in order to promote sufficient cell migration and proliferation. The ECM is a nanofibrous structure that can be effectively mimicked by polymer scaffolds which can jump-start the healing process if recognized by the surrounding cells. This review discusses the fabrication methods and biocompatible properties of these scaffolds, and the techniques used to impart biochemical properties onto them which facilitate cell behavior crucial for wound healing.

Introduction

Dermal wound healing is a complex process with discrete stages of hemostasis, inflammation, proliferation, and maturation. Wounds arrested in the inflammation phase of healing are termed "chronic"; in these, high levels of matrix metalloproteinases (MMPs) degrade the extracellular matrix and growth factors. This in turn dampens necessary fibroblast activity in the wound [46]. Chronic wounds burden patients and the healthcare system quite substantially. The slow healing process of chronic wounds relative to acute wounds entails great pain and often does not lead to successful recovery. In many instances, the only option left is to amputate the affected limb to prevent morbidity of the patient. Furthermore, scarring of wounds after current treatments leaves a lot to be desired in improvement in the aesthetics of the wound post-healing. Caring for these chronic wounds costs developed countries about 2-3% of their healthcare budgets [47]. Therefore, it is abundantly evident that chronic wound healing poses a pressing challenge; in response to this challenge, a multitude of wound healing strategies have been explored in the field of bioengineering.

A viable strategy to address chronic wounds is dressing them with tissue regeneration scaffolds that mimic the extracellular matrix (ECM) to allow for optimal cell interactions with the polymer surface. Three dimensional scaffolds made from different polymers are an example of a bottom-up approach, since the scaffold is first placed on the wound site [45]. In theory, this promotes tissue regeneration via regeneration of the extracellular matrix, proliferation and differentiation of cells, migration of cells into the wound site, and degradation after provision of support. This methodology is in contrast to a top-down approach, in which cells are first seeded onto a scaffold and allowed to proliferate and create their own ECM; however, lack of vasculature and structure for biomolecule transport prevails as a significant complication [45].

There are a multitude of methods to fabricate scaffolds for wound healing. Hydrogels are one type of 3D scaffold made from different polymers that are named such because they contain 90-99% fluid [49]. These materials, often constructed from polysaccharide polymers such as alginate and chitosan, consist of a network of hydrophilic fibers and feature high biocompatibility, low cytotoxicity, ease of functionalization, and tunable physicochemical properties[48]. Unfortunately, hydrogels are relatively insusceptible to spatial and temporal control, rendering cell migration and proliferation difficult to moderate [49].

Solvent casting is another method used to create 3D scaffolds: polymer solution is poured into a mold of a specific shape, and upon evaporation of the solution a solvent film is left behind as a cast. While the consistency of this film is suitable for tissue regeneration applications, this method's success is limited by the shapes of possible molds, residual solvents, and denaturation of proteins [45].

Some tissue engineering methods are designed foremostly to mimic the nanofibrous quality of the ECM. One such method is electrospinning, an automated process in which a solution of a polymer is loaded into a needle syringe, ejected using a high voltage-induced electrostatic force, then forming a polymer nanofiber upon ejection which is collected on an electrically grounded surface (collector). As it is ejected, the solution forms a distinctive shape ("Taylor cone") and travels quickly as a "jet" towards the collector, causing the solvent to evaporate. The polymer is collected as a porous nanofiber "mat" (scaffold) which can serve as a scaffold [46]. Several characteristics of the fibers, including orientation, diameter, and porosity, can be tuned for specific applications upon modulation of various electrospinning process parameters. The tunability, versatility, and automation of the nanofiber scaffold electrospinning technique render it an extremely attractive tissue engineering method for wound healing applications.

Another method to generate a nanofibrous polymer scaffold is chemical phase separation, in which varying heat intensities cause a homogeneous polymer solution to separate into polymer-rich and solvent-rich phases. Upon evaporation of the solvent phase, a nanofibrous scaffold with a porous 3D arrangement remains. While this structure can be adjusted via phase separation parameter modulation (similar to the electrospinning method), this method is relatively more complex and allows for limited fiber arrangement optimization [45].

The aforementioned techniques are those which have been explored most extensively in wound healing applications of tissue engineering but there are others, less common, that occupy intriguing niches. "Rapid prototyping", for example, employs a computerized ink-jet printing process to generate 3D polymeric scaffolds with precisely designed structures and porous features [45]. In "lyophilization," polymer samples are frozen and the solvent is removed via sublimation under vacuum. No toxic organic solvents are used, and a porous structure is yielded [45].

Studies done on all of the above methodologies, in vitro and in vivo, have allowed the scientific community to gain insight on the properties most necessary in three-dimensional biopolymeric scaffolds for them to promote tissue regeneration and wound healing. This review seeks to summarize those findings and provide researchers with more direction on how to optimize chronic wound healing applications, specifically in mimicking the extracellular matrix and promoting surface interactions between polymeric scaffolds and cells.

Biomimetic Materials: Mimicking the ECM

In order for a polymer scaffold to function as tissue in wound healing, it must be synthesized to achieve properties similar to the ECM, yet different enough to aid a more expedited healing process. These properties include (but are not limited to) biodegradability, fibrosity/surface area to volume ratio, and porosity [1,2,3].

Biodegradability

A shift in the approach to repairing/regenerating damaged tissue has occurred in the last few decades - from using permanent biologically inert materials and devices to instead using biodegradable material to help the body regenerate the damaged tissue. This material must "support the tissue regeneration process while providing mechanical support and eventually degrade to non-toxic products with little or no harm to the body" [17].

Some common natural polymers of tissue engineering are collagen [19], elastin [20], chitosan [5], and fibrin [21]; some such synthetic polymers are polyglycolide (PGA) [22], polylactide (PLA) [23], poly(lactide-co-glycolide) (PLGA) [24], and polycaprolactone (PCL) [25].

Suitable natural and synthetic polymers possess a certain degree of biodegradability, and the degrading process is generally different for these two types [18]. In general, natural polymers undergo enzymatic degradation while synthetic polymers undergo hydrolytic degradation. The variable bioactivity of natural polymers combined with the unpredictable nature of enzymatic degradation starkly contrasts the relative biological inertness and tunable degradability of synthetic polymers [18]. In light of this disparity in characteristics, efforts to use natural and synthetic polymers in tandem to achieve a blend of their properties are gaining popularity in tissue engineering. Bhattarai et al. demonstrated that chitosan-PCL blended fibers facilitated significantly greater PC12 cell proliferation than PCL fibers [26].

Fibrous Structure

The ECM consists largely of fibrous bundles of collagen, ranging in diameter from 50 to 500 nm [27]. It is out of effort to mimic this structure that engineered tissue scaffolds are typically made from polymer nanofibers.

A crucial feature of small-diameter fibers is their high surface area-to-volume ratio. This exposes more of the fiber material to its surroundings, which is useful in several contexts. Chen and Ma demonstrated that nanofiber poly(L-lactic acid) (PLLA) foams degrade more quickly than solid-walled foams due to the greater surface area of the nanofiber material - the greater area exposes more ester bond sites for hydrolysis, resulting in greater hydrolytic degradation (superior biodegradability) [28].

Interactions between cells and polymer scaffold surfaces are integral to the scaffolds' ability to elicit desired cell responses, as cells interact with a surface primarily through proteins adsorbed from the surface [4]. The polymer must reach the cells to interact, and as the surface area-to-volume ratio of the polymer nanofibers increases, more of the polymer material reaches the cells. Surface modification, a technique used to guide cell-surface interactions by altering the features of the polymer scaffold surface [4], is the natural outgrowth of the high surface area feature of nanofiber scaffolds and will be explored later in this review.

Porosity

The physical structure of nanofibrous scaffolds for wound healing is not only characterized by its fibrosity. Such scaffolds possess a degree of porosity (amount of scaffold volume which is contained by pores), controllable through fiber diameter and packing density modulation [30]. Higher porosity generally results in superior cell proliferation [29,30]. The pore size best for tissue regeneration is different depending on the type of cells; for skin regeneration the size is roughly 20 to 125μm [29].

Other types of polymer scaffolds such as hydrogels are suitable for wound healing applications in large part due to their porous structure, in spite of their lack of nanofiber composition. The microporosity of hydrogel polymers renders them biocompatible as it enables them to absorb great amounts of biological fluid (enough so that 99% of the content of the hydrogel is biological fluid) [49].

Promoting Interactions between Scaffold Surface and Cells for Wound Healing

Cell interactions are mediated by the binding of cell surface receptors to ligands on other surfaces [12]. These receptors are different proteins that are adsorbed by the surface with which the cell interacts. This process is dependent on different variables, especially the hydrophilicity of the other surfaces and the presence of biologically active proteins, and can be manipulated in several ways to affect cell-surface interactions.

Hydrophilic Modification and Copolymer Grafting

Cell attachment is best on materials with moderate levels of wettability (hydrophilicity), but most polymers are somewhat hydrophobic [11] and relatively unattractive to cells due to their scarcity of bioreactive functional groups. To improve hydrophilicity, polarized functional groups are often introduced using one of a variety of different methods, to serve either as a hydrophilic surface component or to facilitate copolymer grafting. If the latter, typically the copolymer is grafted from a relatively inert initial polymer scaffold and a more hydrophilic polymer. For example, Datta et al. chemically grafted a copolymer from partially phosphorylated polyvinyl alcohol (PPVA) and polylactic acid (PLA); PVA (the unphosphorylated polymer) and PLA are hydrophilic and hydrophobic polymers, respectively [15]. The resultant copolymer (PPVA-g-LA) demonstrated a contact angle greater than PLA and less than PVA, indicating a wettability more moderate than that of the original polymers.

The degree of hydrophilicity is often determined by water contact angle measurements. Water contact angle is the angle between the polymer surface and the interface between the gas and liquid phases (air and water). The size of the water contact angle indicates the hydrophobicity of the surface, so in general the contact angle is lowered in efforts to increase hydrophilicity. While the grafting of PPVA and PLA by Datta et al. was only a wet chemistry process, copolymer grafting is often initiated by the introduction of radicals or peroxide groups onto the original material [2]. Besides wet chemical procedures, there are several methods of introducing these radicals, two of which - plasma modification and photo-oxidation - are described below.

In plasma-oxidation the plasma used is composed of highly excited gaseous species (typically oxygen). It causes radicalization of the polymer chains which combine with the gas radicals to make functionalized polymers with improved hydrophilicity [13]. Yan et al. demonstrated that PCL nanofiber meshes experienced a substantial decrease in water contact angle as a result of plasma treatment with various gases and facilitated cell growth more effectively than untreated meshes [14].

To introduce peroxide groups to the surface, the material can be immersed in hydrogen peroxide solution under UV irradiation [2, 31 32] . This process, coined “photo-oxidation” will impart hydroperoxide groups to the surface over time; Ma et al. found that the maximum hydroperoxide content on a PLLA surface was present after 40 minutes [31]. These groups render the surface hydrophilic and allow for grafting with other polymers [31,32].

After treatment to incorporate hydrophilic groups on the polymer surface, copolymer grafting is often performed, combining the features of two contrasting polymers and possessing sufficient hydrophilicity for wound healing applications. Chen et al grafted PCL, a FDA-approved, biocompatible, highly hydrophobic synthetic polymer [34], to chitosan, a natural polymer derived from chitin with established wound healing properties [5]. The copolymer was synthesized using argon gas as the radical initiator and then electrospun to nanofiber mats. The copolymer nanofiber mats demonstrated superior L929 fibroblast cell proliferation compared to PCL mats [33]. The copolymer contact angle was 0◦, compared to the 132◦ contact angle of PCL, indicating a very high hydrophilicity as well - in spite of the consensus on moderate hydrophilicity as mentioned above, some researchers have concluded that a very high hydrophilicity is superior to a very high hydrophobicity [32,33]

Immobilization of Ligands

As mentioned, the signals interpreted by cells that control their behavior come from cell surface receptors’ interactions with surrounding ligands [12]. Ligands that are known to regulate cell behavior can be immobilized either within or on the surface of polymers.

Covalent bonding is a prevalent method of surface immobilization [2,39]. Typically, this is accomplished by first improving the polymer's hydrophilicity (as mentioned previously) and then treating the polymer surface and/or ligand with an activating solution. Often, this solution is a carbodiimide (such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, or EDC/EDAC) in order to impart amine activation to carboxyl groups. Upon appropriate combination of the ligand, surface, and carbodoiimide the ligand will be amide-bonded to the polymer [36-39]. Similarly, rather than a carbodiimide a compound with a ligand-reactive group and a photoreactive phenyl azide group such as sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sulfo-SANPAH) can crosslink the ligand with the polymer surface [39-41]. First, the sulfo-SANPAH amide-bonds with the ligand, and when the product is coated on the polymer substrate and subjected to UV irradiation (or shaken [40]) it will covalently bond to the substrate.

Epidermal growth factor (EGF) is the protein that has been most demonstrated to affect cell behavior upon immobilization with potential for wound healing applications [39-43] EGF is integral in wound closure as it stimulates epithelial cell activity and reduces scarring [39,41] and has been found deficient in chronic wounds compared to normal acute wounds [43]. Stefonek and Masters found that the migration speed and cumulative migration distance of human fibroblast cells increased with EGF concentration on EGF-immobilized 2D polystyrene surfaces [42]. The migration and proliferation of fibroblast cells seem to be affected by the medium of EGF presentation; Puccinelli et al. demonstrated that soluble EGF (EGF in reduced-serum medium) induces high proliferation and low migration, while immobilized EGF induces low proliferation and high migration [41].

While immobilized EGF obviously influences fibroblast cell behavior, the literature regarding immobilized EGF on polymer scaffolds for tissue engineering is relatively sparse. In one study, mice with diabetic symptoms were inflicted identical wounds and treated with nanofiber scaffolds immobilized (conjugated) with human-recombinant EGF (rhEGF), which demonstrated superior wound closure rates after 7 but not 14 days compared to controls [38]. The authors suggested that this might be because mRNA expression of EGF-family factors seems to be greatest during the first 24h after administration of one factor to fibroblasts [44].

A potential issue of surface immobilization is the susceptibility of the ligands to unwanted degradation or reactions. Rather than immobilization to the surface of polymers, the ligands can be loaded to a polymer structure for isolation and delayed release. This "entrapment" is especially relevant for applications of EGF, which is subject to proteolytic degradation in wounds [50]. Hu et al. demonstrated that hydrogel polymers with loaded (entrapped) EGF exhibited a substantially slower release of EGF compared to unloaded hydrogel with EGF spray. Hydrogels are especially suited for this method of slowing release because of their

interconnected porosity and, unlike nanofiber scaffolds, propensity to swell. The loading of EGF also displayed no significant effects on the hydrogel's mechanical properties [51].

Conclusion

Polymer scaffolds for tissue regeneration constructed using a variety of methods are designed to interact favorably with native tissue cells and mimic the surrounding connective material. They must be designed carefully and thoughtfully to be accepted by the nearby tissue. This truism is perhaps most relevant in wound healing applications as, unlike in internal organ tissue regeneration, the quality of this incorporation affects not only the biological or physical function of the tissue but also the appearance of patients themselves. The degree of scarring can be mitigated with appropriate ECM-mimicking scaffolds that possess bioactive angiogenic factors [52]. This confirms the importance, two-fold, of wound healing applications and their necessary design features discussed in this review. Perhaps, for this reason, wound healing applications serve as one of the most demanding tests in all of tissue engineering.

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