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Engineered Extracellular Matrix: Current Accomplishments and Future Trends

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ABSTRACT

There have been many papers published about engineered extracellular matrix, however there is no tailored construct that can mimic all the extracellular matrix components and aspects perfectly while preserving all functionalities as needed. Current work has been partially successful; however, a combination of different approaches may be more successful in designing an optimized engineered extracellular matrix.

In this review, we articulate the successes in tissue engineered extracellular matrix and project the ideal engineered construct of the future.

Keywords:

Extracellular matrix, tissue engineering, electrospinning, hydrogel

1. Extracellular Matrix (ECM)

Extracellular Matrix (ECM) as a network of secreted macromolecules[1] is composed of basement membrane(BM) and interstitial matrix(IM)[2]. The basement membrane is the fusion of two lamina, the basal lamina and the reticular lamina (or lamina reticularis)[3]. The epithelium and endothelium is constantly in contact with the BM and is associated with its functions. Several large glycoproteins make up the structure of the BM. These glycoproteins assemble into an organized scaffold that provides structural support to the tissue and also influences cellular function. The human body contains several types of collagen, with collagen I being the most abundant. In the BM, the most abundant protein is collagen type IV. .

Basement membrane (BM) provides some cells a place for attachment. Vertebrate cells cannot survive without the BM, except in the case of some pathological conditions (e.g. cancer)[4]. There are multiple binding sites that exist on BM proteins for interaction with cell adhesion molecules, and several of these serve as ligands for cell surface receptors. Intracellular signaling pathways

are activated with the binding of cell surface receptors to the BM. This in turn influences the cellular behavior. The BM can be remodeled over time and this is accomplished through *de novo* deposition of BM proteins, self-assembly, and BM network formation. The process involves the manipulation of cell behavior by way of altered BM composition as well as exposed cryptic binding sites. The changes to the BM promote cellular activities that aid in repair of tissue via recruitment of immune cells and activation of fibroblasts.

The IM functions to fill in voids and determines the main characteristics of any given connective tissue. It is present between the intercellular spaces of a variety of animal cells. Gels of polysaccharides and fibrous proteins(IM) fill the interstitial space and act as a compression buffer against the stress placed on the ECM[5].

As other targets, many studies have strived to mimic ECM and clearly it has been the most popular component in tissue engineering (TE) studies[1]. The reason behind this intention for making synthetic ECM is that cells, after isolation and culture, are not able to make their own ECM and they need a synthetic ECM to give them a chance to anchor [6,7]. The scaffold allows cells to be functional (adherence, proliferation, migration and differentiation) [8] while renting them a place to make their own ECM [9-11]. Replicating the complete function of the ECM is a very critical point toward generating ideal engineered scaffold for ECM replacement [12]. The ideal engineered ECM should mimic both BM and interstitial matrix wtihout interfering with the normal cell-cell and ECM-cell interactions [13,14]. Successfully made ECM will be delivered to a particular site *In vivo* and provide a 3-D environemnt for the new tissue or organ formation (or healing) [15].

2. What electrospun fibers and hydrogel are mimicking?

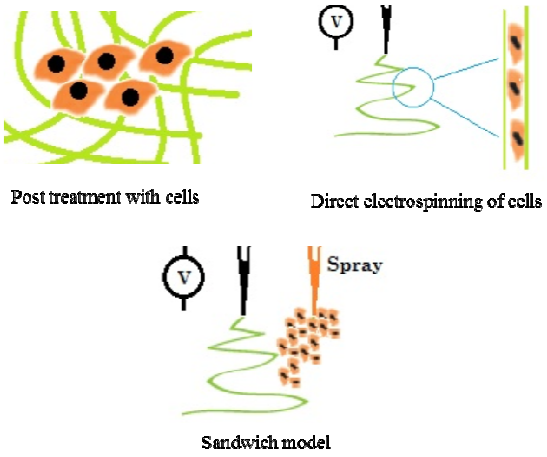
A better understanding of ECM leads us to the question “what can mimic this structure?” A technique known as electrospinning has been explored for the formation of nano-sized fibers for utilization in tissue engineering applications. Electrospinning is a promising method for mimicking the ECM due to the ability to control the parameters in constructing the continuous nanofiber scaffold. .

There are a number of publications focused on optimizing the size, morphology, porosity and topography of fibers that fall in the range of natural ECM components (e.g. collagen and elastin) [16-23]. Another feature of the electrospun fibers is their ability to carry proteins and drugs [24-29], but there are not a lot of publications specifically for targeting ECM and that is reasonable because ECM targeted drug delivery is a new concept. After fabricating the electrospun fibres, cells then need to be cultured within. Homogenous cell culture on fibers is always desirable but one of the common problems is poor adhesion and infiltration of cells onto fibers. [30,31]. In 2006, the first attempt to encapsulate live cells into electrospun fibers aimed to incorporate cells within fiber fabrication and removing post cell culture treatment stage[32].

A group has shown that it is possible to electrospin cells in coaxial fibers with low electrical conductivity (10-15 S m-1)[33]. After that, another study looked at the porcine vascular and rabbit

aorta smooth-muscle cells incorporated in a coaxial needle[34]. After those attempts, another group showed that spraying cells on a sheet of fibers while electrospinning is less harmful for cells[35]. A study compared direct and indirect cell-incorporated electrospinning. It was shown that direct electrospinning causes fibroblasts to die due to high voltage, overstretching, and dehydration. Another method used with limited success was to spray cells and make a sandwich model of fibers and cells that improved viability [30]. Summary of current strategies are shown in **Error! Reference source not found.**1.

This concept transcends cell electrospinning. New approaches include live organisms and heterogeneous cell electrospinning[36,37].



The new concept of organism electrospinning does not provide a hydrated environment for cells to survive and so there is a demand for incorporating a gel-like carrier into electrospun fibers that we will discuss in the next part.

Hydrogels are cross-linked hydrophilic three-dimensional polymer networks that contain a high water content and emulate the physical properties of soft tissues [38-42]. Hydrogels have widely been used in various medical and pharmaceutical applications. Among others, hydrogels have been used extensively as scaffolds for soft tissue engineering and regenerative medicine. Recent applications have utilized hydrogels as a vehicle to deliver cells in a three-dimensional environment that is conducive to reconstituting new tissues and organs [43,44]. Hydrogels are being used in tissue engineering because they emulate key features of native ECM [42,45]. The chemical and mechanical stability of hydrogels provides a structure that is similar to the natural ECM. These properties can influence the cell viability and function similarly to the way biochemical signals effect cells. Understanding how these signals work is crucial for tailoring hydrogels to accurately mimic ECM. Designing functional tissue is dependent on the structural environment and the interaction between cells and the biomaterial. Hydrogels must provide these chemical and mechanical properties in a manner that leads to native cellular behavior and function. In some respects, hydrogels provide an advantage in tissue engineering because they can be tailored to produce scaffolds that are analogous to native ECM. Adjusting the mechanical properties such as the stiffness and tensile strength plays a large role in the way that cells interact with a biomaterial.

Properties that can be varied include type of polymer, concentration of polymer, and cross-linking density. These changes have an effect on the degradation time and pore size because of the close relationship between the mechanical properties and the microenvironment of the hydrogel. Depending on the application, hydrogels can be synthesized to degrade over a known period of time that coincides with the degree of new tissue formation. The incorporation of a temporary scaffold provides the necessary support for the organization and development of native tissue. The development of new tissue and ECM components is dependent on the cells ability to adhere to an ECM substrate. Ideally, cells will replace the existing hydrogel structure with their own ECM components, particularly collagen [46] and Glycosaminoglycans (GAGs) [47]. Matrix metalloproteinases (MMPs) are a class of proteolytic enzymes responsible for degrading several ECM connective tissue components that are crucial for proper restructuring of the ECM. MMPs of particular importance include collagenases, gelatinases, and stromelysins [48-50] The rate of matrix turnover has been shown to be highest in areas of greatest cell proliferation [51]. It is critical that cells eventually begin to produce their own ECM, which can be achieved when they adhere to a substrate. The amount of ECM secreted from cells depends greatly on the pore size [52]. Although the microarchitecture is responsible for ECM deposition, it also controls the cellular interactions by organizing cell distribution and interconnectivity, as well as facilitating mass transfer. Hydrogels exhibit a highly hydrated environment not unlike GAGs that are found in native ECM. This highly hydrated macromolecule-based material serves as a means for proper nutrient transfer, gas and metabolic waste exchange, and signal transduction, which all occur primarily through diffusion [42,53,54]. The ability to closely mimic the mechanical, chemical, and biological characteristics of ECM is essential to developing native tissue. A wide range of different natural, synthetic, and hybrid polymers has been used to fabricate ECM-mimicking hydrogels [55].The most common natural polymer hydrogels currently in use include collagen [56,57], hyaluronic acid, chondroitin sulfate, fibrin [48,58], fibronectin, alginate [54,59,60], agarose, chitosan, and silk [23,42]. Natural polymers are often considered bioactive or bioresponsive because they possess ideal biological functions for tissue engineering such as cell adhesion and biodegradation [61]. However, the use of animal derived polymers as ECM mimics has raised concerns over their immunogenicity and tendency to cause infection [62,63]. The mechanical properties of natural polymers lack the necessary qualities for ECM constructs and have led to increased use of synthetic polymer hydrogels in tissue engineering. Although some of the most common synthetic hydrogels made of poly acrylic acid (PAA), polyethylene glycol (PEG), poly vinyl alcohol (PVA), polyacrylamide (PAAm), poly lactic acid (PLA), poly glycolic acid (PGA), poly lactic-glycolic acid (PLGA), and polypeptides lack the biological interaction of natural polymers, they have emerged as the preferred option because of their highly modifiable properties [42,62]. Synthetic hydrogels are advantageous because of their ability to be photopolymerized, and the relative ease of modifying their surface chemistry and mechanical properties. Changes in the polymer chain length and cross-linking density allow for customization of the degradation rate [64], scaffold architecture, and mass transport. Despite the benefits of customizable modifications, synthetic hydrogels exhibit little or no biological activity. It is the limitations of natural and synthetic hydrogels that have led to the fabrication of hybrid hydrogels. Utilizing the best aspects of natural and synthetic hydrogels, hybrid hydrogels offer a substrate that more closely mimics the ECM because of the ideal combination of biological and mechanical properties[65-68]. New methods for mimicking and regenerating ECM using hydrogels are currently being investigated due to the limitations of the current methods. Nevertheless, exciting new advances in material science and

bioengineering are paving the path for hydrogels to become an ideal substrate for ECM tissue engineering.

3. Current strategies for mimicking Collagen, Elastin and GAGs, ECM triangle

ECM Components are formed by local cells [69]. When secreted, they aggregate and are composed of fibrous proteins (Fibers) and GAGs. Heparin sulfate, chondroitin sulfate, keratan sulfate and hyaluronic acid (HA), which is a non-proteoglycan polysaccharide, are members of GAGs. GAGs are carbohydrates that attach to ECM proteins to form proteoglycans, and with some exceptions attract positively charged ions. Collagen and elastin are the most important fibrous components of ECM, but it also consists of other fibers like fibronectin and laminin [70,71].

GAGs, mostly in the form of proteoglycans, play a big role in binding and modulating enzymes, protenase inhibitors and cytokines [72,73]. Growth factors can bind to GAG-mimetics to elicit a biological response *in vivo* [74-76]. The applications of GAGs in tissue engineering include using GAGs independently or combining GAGs with other natural or synthetic scaffolds. Injectable hydrogels made from GAGs have been used for controlled release of growth factors *In vivo* [77]. Besides proteoglycans, another important type of GAGs is HA. Incorporating HA into polymer scaffolds showed improved biocompatibility and also yielded a new class of controllable, degradable materials [78].

Using GAGs in combination with synthetic and natural scaffolds may be the most promising approach to an improvement in tissue engineered ECM. Electrospun collagen-GAG nanofibers showed similar structure to native ECM [79]. Attachment of GAGs to collagenous matrices showed that GAGs have an effect on the inflammatory response in rats [80]. Type I collagen-GAG hydrogels showed to be a good candidate for mitral tissue engineered heart valve [81].
Collagen, a fiber of ECM, is a highly organized 3-D architecture for cell-ECM interaction [82]. There are two approaches to mimic collagen: a natural collagen scaffold derived from animal or human tissue and a synthetic biomaterial scaffold. Collagen scaffolds have advantages for both cell-free and cell-induced applications in tissue engineering [83]. Decellularized aortic collagen scaffolds are biodegradable and have good cell infiltration and viability [84]. Cell attachment, proliferation and migration would be affected by the mean pore size of collagen scaffolds [85]. Bioreactors have been used to enhance histogenesis in collagen scaffolds [86]. Cell-seeded collagen scaffolds have been used for articular cartilage tissue engineering [87]. Besides pure collagen scaffolds there are many collagen based complex scaffolds that have been investigated for specific purpose, such as collagen–hydroxyapatite composite scaffold [88] for cartilage, collagen–glycosaminoglycan scaffold for bone [85], chitosan/gelatin complex scaffolds for cartilage [89], nanohydroxyapatite/collagen/poly(L-lactide) for bone [90], and collagen/chitosan porous scaffolds for skin [91]. Promising synthetic biomaterials that are used to mimic collagen include: peptide-amphiphile (PA) nanofibers [92] and nanofibrillar gels [52].

In order to mimic the natural collagen micro-structure, techniques have been developed including electrospinning [93,94], phase separation [95], and self-assembly [96]. These techniques have achieved

success in tissue engineering applications but have poor cells attachment [97]. To address this issue, several surface modification methods have been developed. For example, biomacromolecules could were incorporated into 3D nanofibrous scaffolds by surface charge modification during the process of self-assembly [98].

To summarize, currently both natural derived collagen scaffold and synthetic biomaterials have been investigated to mimic collagen's ECM role. These biomaterials have some drawbacks pertaining to over-simplified natural collagen structure and biocompatibility. The next trend in collagen-mimetic material may be combining the natural derived collagen with synthetic biomaterials by using the techniques mentioned above. For example combination of collagen-and biodegradable polymer has been used as a potential grafts in vascular tissue engineering [99].

Tissues and organs derive their elasticity from elastin. It has an important role to play in tissue biomechanics with its ability to impart flexibility in combination with strength to the tissue, in addition to influencing a myriad of cellular and protein interactions [100]. Materials with properties similar to that of elastin have been designed and used in tissue engineering, such as hydrogels [66], elastin-mimetic polymers [101], polyhydroxyalkanoates [102] and decellularized elastin matrices [103]. This illustrates the importance of elastin and its major properties to the field of tissue engineering. In spite of this, current tissue-engineered grafts are mostly lacking in elastin [104]. Incorporation of elastin is significant in tissue-engineered grafts because its elasticity and biological effects can be exploited to good effect. However, there are some challenges that arise from the use of native elastin. It is highly insoluble and highly cross-linked and therefore not easily processable [105]. Development of easily processable elastin-based biomaterials may aid in advancing the design of functional extracellular matrices for tissue engineering. Elastin-based biomaterials need not be identical to native elastin. It has been proven that tropoelastin is deposited into the surrounding matrix, if an intact and adequate scaffold is present (for example, an already deposited ECM), not only live cells [106]. Another important property of tropoelastin, which helps greatly in elastic fiber formation, is its ability to self-assemble through a process called coacervation [107]. As such, tropoelastin may be a preferred component for tissue engineering, as it mimics elastin's physical and biological properties [108]. Elastin is used in different forms in biomaterials. They made be broadly divided into those derived from naturally occurring elastin and from biosynthetic elastin. Decellularized tissue [109,110], purified elastin preparations [111-113], and hydrolyzed elastin (in its soluble form) [114,115] are some forms of elastin, derived from its naturally occurring form, that are used in the design of tissue engineering scaffolds. Synthesized soluble elastins, like recombinant human tropoelastin(rhTE)[116], α-elastin, sequence-based variants of elastin such as recombinant elastin-like polypeptides(block polymers) [117], and recombinant human elastin polypeptides[118]are also used in biomaterials. Hybrids of elastin with other molecules (forming block polymers) are also used for engineering the extracellular matrix, such as silk-elastin [119], elastin-fibronectin [120] and elastin-synthetic polymers [121].

rhTE can be used in a host of biomaterial applications. It forms elastic hydrogels and fine microfibers, depending on the method of manufacture and can also be used as a surface coating. It retains the key properties of elastin like elasticity, enhanced cellular interactions, and biocompatibility, making it viable for biomaterial development. As such, biocompatibility (of both polymer and metal medical implants) may be improved by coating them with soluble elastin

derivatives, more specifically with rhTE due to its capacity to mimic elastin and its useful properties. However, the immunogenic response to these synthetically constructed materials is an unexplored area. Therefore, the scope of widespread application of such materials is restricted by some inherent challenges faced during the synthesis of recombinant proteins like the cost of production, scale-up, and endotoxin (present residually) [102].

Cross-linked α-elastin materials were found to have elastic moduli comparable to that of recombinantly produced elastin-like polypeptides. They also found that the cross linked α-elastin materials were fragile, like other elastin-based materials. Since the α-elastin materials have moduli lesser than native elastin, such materials can act as matrices for tissue remodeling *in vitro* [122,123]. Electrospinning of soluble fibers (collagen and elastin) is a useful method to produce ECM scaffolds with high surface area for small-diameter blood vessel tissue engineering. A useful advantage of electrospinning collagen and/or elastin is it results in the preparation of scaffolds with more surface area and higher porosity, both of which are central for tissue engineering. Currently, investigations are being done to produce fibers in which collagen and elastin proteins cannot be distinguished from each other (produced from a mixture of the proteins, collagen and elastin). This may result in the formation of fibers with excellent mechanical properties.

4. Combination of hydrogel and electrospun fibers: Toward future Engineered ECM

Research gaps exist regarding the use of hydrogel-electrospun fibers in tissue engineering, and some key issues are summarized below.

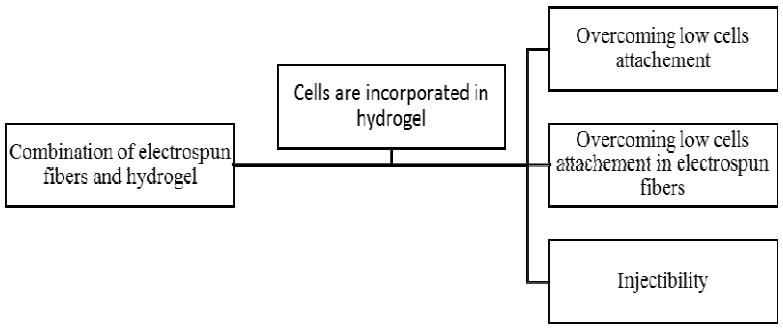
As discussed, it is common to use electrospun fibers or hydrogels exclusively for mimicking ECM. However, their clinical usage is restricted because of short term cell survival and poor mechanical properties. The combination of electrospun fibers and hydrogels may have potential for therapeutic usage [124].

There are a small number of research groups that are focused on finding an ideal combination of electrospun fibers and hydrogels. The importance of this combination is its high similarity to ECM, wherein the electrospun fibers act as proteins (soluble and insoluble) and hydrogels as a filler. Thus, they are good replacements for interstitial matrix and basement membrane, respectively. Different publications have been reviewed and the related studies were classified based on their goals of applying this combination. Incorporation of a small amount of poly (ε-caprolactone) (PCL) nanofibers fabricated by electrospinning has a reinforcing effect and changes the rheological properties of hydrogels to be similar to the human nucleus pulposus (NP) [125]. It has been proven in another study that hydrogels composed of poly(ethyleneglycol) (PEG) and poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) in combination with electrospun PCL can be a

future candidate for composite materials because cell attachment and proliferation can be optimized by these two components [126]. Moreover, three different scaffolds were made: poly (ε-caprolactone)/collagen (mPCL/Col) that was electrospun with PEO or gelatin, mPCL/Col meshes with micron-sized fibers, and mPCL/Col microfibers sprayed with Heprasil. Cell attachment for all three scaffolds was increased while cell diffusion was better in mPCL/Col microfibers[127]. Also, it is possible to inject the combined fibers and hydrogels directly at the site of the injury. For example electrospun hyaluronan and methylcellulose have been used for neural stem/progenitor cell delivery injected in the site of the injured spinal cord [128].

Recent advancements in portable medical technology have led to development of a direct healing tool that utilizes electrospun fibers as a scaffold for localized wound healing. This ready-to-use device is filled with the appropriate materials to provide an in-situ healing patch when injury occurs. However, this is only in the proof of concept stage and has limitations [129].

Moreover, hydrogels gives us a protected carrier for proteins, cells, and organisms during the electrospinning process. Keeping living organisms, proteins and cells undamaged is a very important aspect that can be achieved by fabricating a droplet of cell encapsulating hydrogel and incorporating it into a polymer slurry that needs to be electrospun [130]. Finally, it is valuable to briefly mention the different methods for making this composite because it affects the properties. To date, combining hydrogels and electrospun fibers have been attempted by encapsulation [131,132], dual spray-electrospinning [133] and coating [134].



To summarize, the benefits of combining electrospun fibers with hydrogel are illustrated in **Error! Reference source not found.**2.

5. Conclusion

The current literature mentions the use of electrospun fibers or hydrogels about tissue engineered ECM ECM, but rarely about their combination. Based on our review on different strategies for ECM mimicking, we believe that the new generation of scaffolds will be a composite of cells, hydrogel, and electrospun fibers.

This new advancement of incorporating cells into electrospun fibers needs a gel like carrier to protect cells from damage. It can be achieved by a cell encapsulating hydrogel, which polymerizes during the process of electrospinning. The advantage of this system is the homogenous cell distribution while preventing cells from dehydration and overstretching in the process of electrospinning.

The future of this concept resides in the use of different cells, organisms and lab grown tissue, although the latter remains far from reality.

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