Assessment of Collagen Based Polymer Scaffolds for Tissue Engineering

by

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ABSTRACT

The use of collagen-glycosaminoglycan copolymers in tissue engineering scaffolds has seen promise to date in skin regeneration templates, hollow nerve guides for peripheral nerve regeneration, and conjunctiva regeneration. Future applications appear to be directed toward both *in vivo* and *in vitro* engineering of complex cellular systems, with significant progress being prevented by a lack of biological knowledge concerning cell-matrix interactions, cell-cell interactions, and matrix-cytokine interactions. Despite this lack of knowledge, collagen based polymers still hold several benefits over competing technologies such as degradable synthetic polyesters, pseudo-poly amino acids, fibrin based polymers, and tissue derived matrices.

Development of key patents in the field since the 1970's has rooted the fundamental technology for developing bioactive scaffolds in the hands of the Massachusetts Institute of Technology. The short-term profitability of this technology can be realized by developing and marketing tissue engineering devices such as matrix filled nerve guides, where as long term profitability may be found in identifying material characteristics that confer bioactivity, and licensing this technology to facilitate commercialization.

Thesis Supervisor: Ioannis V. Yannas
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1. Introduction

1.1. Tissue Engineering

Mention of tissue engineering arouses the dream of one day having progressed well enough into knowledge and experience, and being able to synthesize complex biological systems from specific ingredients. Although the field of tissue engineering moves steadily toward this eventuality, that day still remains in the distance. For the present, tissue engineering is still confined to relatively simple systems involving limited cell types, with a great deal of work remaining before the dream becomes reality. Most notably, a gap exists between the expertise of biologists and engineers; this thesis will address a piece of this gap, specifically how an engineered polymer substrate can be used to modify cellular behavior. Throughout this thesis, tissue engineering will refer to the broad scope of possibilities where an attempt is made to cultivate conditions that facilitate tissue growth in a controlled manner, encompassing both *in vivo* and *in vitro* organ synthesis.

It is convenient to consider tissue engineering from an organ regeneration perspective. The question is how conditions can be tailored to facilitate growth of organ tissues in a controlled manner to produce desired results. To better illustrate the considerations taken in attempting to promote organ regeneration, the example of a wound will be used extensively in this thesis. A wound in this case is damage sustained by a physiologically normal tissue, and can be thought of as, for instance, a scrape on the knee or an incision caused by surgery; typically, the damage results in the loss of function in a portion of the organ, for instance, missing skin resulting from abrasion. Immediately after sustaining a wound, the body begins to close the damaged area via one or more of the following three processes: contraction, scar formation, and regeneration. The sum of the active processes governing wound closure produces the final healed wound; arresting contraction and scar formation can therefore presumably promote regeneration of the missing or damaged organ.

1.2 The Wound

A characteristic of the wound being discussed here is full penetration through all of the tissues comprising an organ: epithelia, basement membrane, and the underlying supporting tissue, the stroma (Figure 1). Epithelia is present on body surfaces and cavities (for instance, the

epidermis and the cells lining the respiratory system and gastrointestinal tract), and with few exceptions such as hepatocytes in the liver, are separated from the underlying stroma with a continuous basement membrane. Epithelium is characterized by the lack of both direct vascularization and a supporting protein extracellular matrix. The basement membrane is acellular and avascular, supporting the epithelia by diffusion of nutrients from the underlying vascular stroma. It is interesting to note that in these tissue layers, the basement membrane, which is capable of regenerating over undamaged stroma, separates a tissue that will spontaneously regenerate (epithelia) and one that does not (stroma).[1]

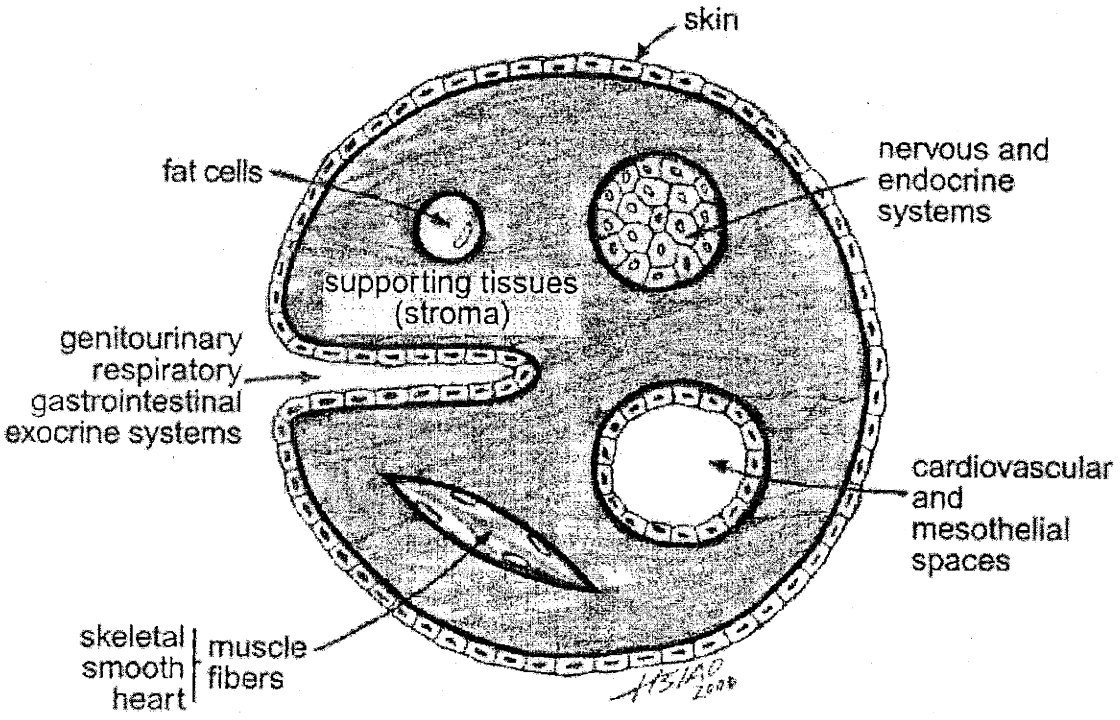

Figure 1: A schematic of the tissue triad: epithelia is depicted above as individual cells, stroma is depicted as the shaded supporting tissues, and basement membrane is shown as a bold line seperating the two. Figure reproduced from [1].

1.3 The Problem

The problem with wound recovery in adult humans is the distinct lack of regenerative capacity. The term regeneration is used in contrast to repair, where the former involves restoration at the site of injury in a way such that original function and appearance is restored.

The growth of a severed limb on a newt provides an excellent example of regeneration. This case in point is remarkable because of the organism's ability to distinguish injuries proximal and distal to the elbow and regenerate accordingly, restoring original appearance and functionality.

Repair is the response more commonly associated with injury, characterized by the formation of dysfunctional scar tissue and local contraction of the wound. This is the response normally elicited in adult humans following the formation of a wound, and is undesirable because the result is most often scar tissue that no longer exhibits normal physiological behavior; for instance, in skin, the normally random orientation of the collagen extracellular matrix is replaced by highly oriented collagen in scar tissue.[2] In severe cases of wound contraction in skin, especially in close proximity to joints, the tautness developed by contraction can severely inhibit a normal range of motion. Given this apparent problem in adult wound recovery, the solution would be to stimulate regeneration while preventing contraction and scar formation. Because these three processes alone lead to wound closure, the solution lies in manipulating the relative contributions from each of these processes; this can be achieved by manipulating the processes governing cellular behavior and response.

1.4 Unit Cell Processes

Cellular processes can be modeled using an equation format similar to that used to describe chemical reactions, known as a unit cell process (Figures 2 and 3). On the reactant side are the cells involved in the process, soluble regulators (cytokines), and insoluble regulators (an extracellular matrix, abbreviated ECM). On the product side are the cellular result and often the release of other cytokines, either to stimulate the next process in a pathway or as a feedback mechanism. As a practical analogy related by Professor I.V. Yannas, the unit cell process can be made analogous to human behavior. Cells represent individuals, cytokines represent a language allowing the cells to communicate with one another and modify others' behavior, and the ECM represents furniture, dictating the behavior of the cells (for example, people will sit if a couch is present).

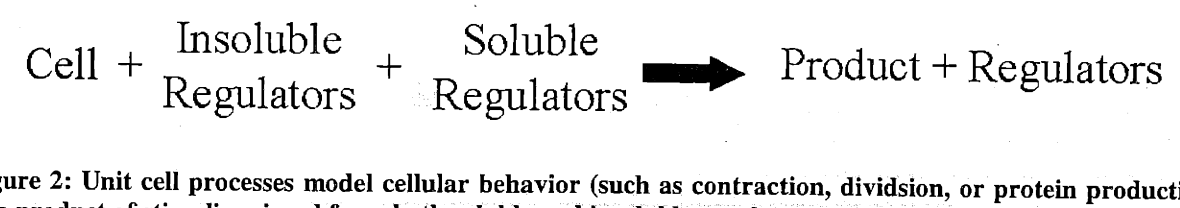

Figure 2: Unit cell processes model cellular behavior (such as contraction, dividsion, or protein production) as a product of stimuli recieved from both soluble and insoluble regulators.

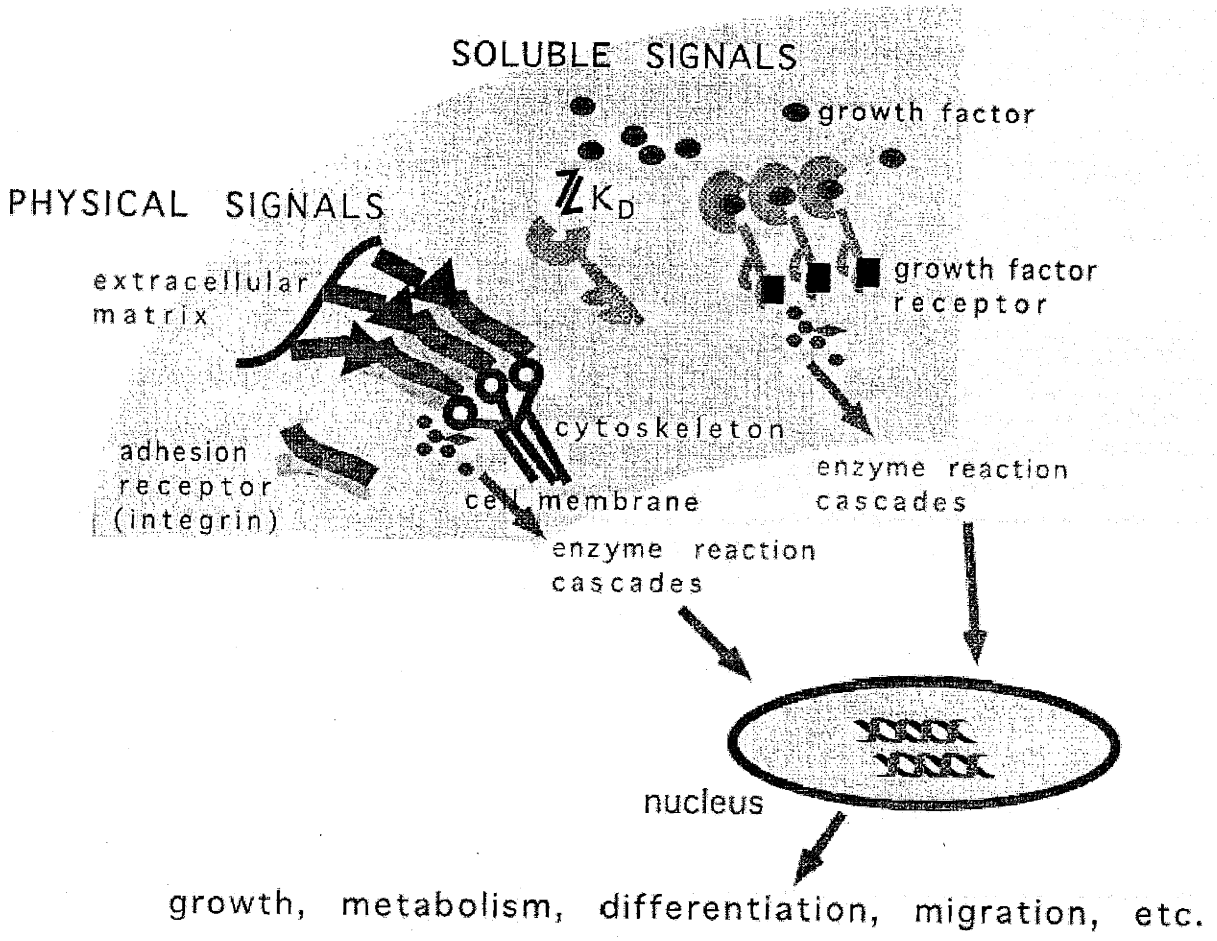

Figure 3: Cell behavior is driven by interactions with insoluble regulators (extracellular matrix) and soluble regulators (cytokines). Figure reproduced from [36].

The sustained trauma at a wound creates damage to the ECM and elicits the release of cytokines from affected cells. By abruptly disrupting the reactant side of physiologically normal unit cell process, cellular results such as contraction and scar tissue are elicited. Understanding the interaction between products and reactants allows the development of something that can modulate the natural healing response. Presumably, blocking contraction and scar formation

provides a chance for regeneration to occur. The solution to the problem is therefore to develop a product that can modify the unit cell processes leading to contraction and scar formation and prevent (or at the minimum, delay) their formation.

1.5 The Solution

The solution that has been investigated in Yannas' research over the past twenty years has focused on the development of an extracellular matrix replacement that delays contraction at the wound site and promotes regeneration of the injury. Specifically, a collagen/glycosaminoglycan (GAG) copolymer (or for brevity, CG copolymer) scaffold is currently used as an analogue to the natural extracellular matrix, preferred because of its inherent properties. Collagen is present in the body in abundance; consequently, the likelihood of a collagen-based scaffold eliciting an undesirable response such as inflammation in the recipient is quite small. Second, because collagen is readily available in the body, cells readily respond to its presence by binding to recognition sites (ligands) via proteins present on the cellular membrane (integrins). This latter property contributes to the ability of CG copolymer scaffolds to inhibit wound contraction and eventual scar formation. This effect is thought to occur by randomizing the preferred orientations of contractile cells (myofibroblasts) responsible for wound contraction, consequently preventing these cells from exerting a cooperative contractile force. Consequently, cells in the wound area are stimulated to resume physiologically normal activity.

Two important features of the scaffold dictate its effectiveness: porosity and degradation rate. Adequate bioactivity is achieved only by creating scaffolds with properly sized open cell pores to promote cell migration and binding. An upper limit on pore size is established by the requirement for a specific surface area (area per volume) to ensure a sufficient amount of binding surface. The lower limit is constrained by the cell size, so as to allow cells to migrate freely through the scaffold. The degradation rate establishes the length of time the scaffold is resident at the wound site. Because collagen is degraded by enzymes normally present in the body (collagenase), tailoring degradation rate allows an opportunity for the body to replace the CG copolymer scaffold with physiologically normal extracellular matrix during healing, resulting in a final healed wound site free of exogenous material.

Based on these concepts, the effectiveness of CG copolymer scaffolds have been demonstrated on organs such as skin[6, 22], peripheral nerves[23], and conjunctiva[24]. However,

efficacy of the scaffold as treatment requires establishing a consistent control. This is addressed through the use of anatomically well-defined defects on animal models that are allowed to recover with and without the presence of a scaffold. In skin, nerve, and conjunctiva models, CG copolymer scaffolds have shown regenerative promise, more so than synthetic polymer counterparts such as silicone and degradable polyesters. These results not only demonstrate the potentially wide applicability of CG copolymers for use in tissue reconstruction, but also imply that the same mechanism by which CG polymers influence cellular behavior in these three applications may also be applicable to other organs and tissues as well.

2. Technology

2.1 Collagen

Collagens are a family of related proteins similar in structure and composition.[3] Chemically, the amino acid sequence of a basic collagen constituent is the Gly-X-Y triplet sequences (X and Y representing various other amino acids, typically hydroxylated proline and lysine). The glycine present in roughly every third position imparts a right-handed twist to the polypeptide, with three of these chains forming left-handed triple helix in the final collagen fibril. Collagens comprise approximately one third of the total protein in a human, with a prominent presence in the extracellular matrix defining tissue properties such as strength and elasticity. Of the over fourteen known types of collagen, types I, II, and III comprise the bulk of native matrix in most tissues. Type I collagen is the single most abundant form in the body, found in quantity in tissues such as skin, bone, tendon, and scar. Type II is the major constituent in cartilage, and type III is normally found in pliable tissues such as blood vessels, uterus, and the gastrointestinal tract. Type IV collagen, by contrast, does not form fibers but rather, forms a membrane together with various glycoproteins such as laminin and fibronectin. Type IV collagen is found exclusively in the basement membrane, an amorphous structure composed of at least one layer which separates epithelial cells from the underlying and non-regenerative endothelia.

Despite being among the less abundant forms of collagen in the body, type IV collagen (and consequently basement membrane) is found separating almost all epithelia from the

underlying stroma; basement membranes separate the epidermis from the dermis, surrounds Schwann cells in peripheral nerves, and surrounds all muscle cells. Additionally, this membrane appears to play a crucial role in the development and organization of cells.[4]

2.2 CG Copolymer Synthesis

CG copolymers are typically synthesized from type I collagen and a variety of glycosaminoglycans such as chondroitin 6-sulfate, heparan sulfate, heparin, dermatan sulfate, and keratan sulfate. Of the various chemistries, collagen-graft-chondroitin 6-sulfate copolymers have been studied the most extensively, due to availability of both constituents. GAG chains can be grafted to collagen by forming a coprecipitate of collagen and GAG, and treating the condensed state to promote covalent bonding between the two components.[2] Coprecipitation is carried out in acidic solution (such 0.05M acetic acid) and requires sulfate groups to be present on the GAG. (Hyaluronic acid is the only GAG lacking a sulfate group and does not cause collagen to precipitate out of solution.) The coprecipitate formed is an ionic complex and is soluble at neutral pH; to combat this latter problem, the collagen-GAG coprecipitate must be cross-linked covalently.

Cross-linking can be achieved either through drastic dehydration or chemical treatment with aldehydes. Removal of water below 1 wt.% causes the coprecipitate to become insoluble by promoting a condensation reaction to form inter-chain amide links.[5] This is achieved either by heating the coprecipitate in excess of 105°C for several hours under atmospheric pressure, or exposure to high vacuum at 25°C, and can cross-link at least 10 wt. % GAG, creating average molecular weights between cross-links of between 2.5 to 25 kDa.[6] Care must be taken with this process such that the conditions utilized to promote cross-linking to not simultaneously facilitate denaturation of the triple-helical collagen structure.

Treatment with aldehydes, particularly gluteraldehyde, facilitates what is thought to be covalent cross-linking between lysine side chains. Exposure of the collagen-GAG coprecipitate to an aqueous solution of gluteraldehyde at neutral pH produces a low yield, again due to the solubility of the ionic coprecipitate complex. At pH 3, up to about 3 wt. % GAG can be incorporated; through control of the collagen source, gluteraldehyde concentration, and exposure time, average molecular weight between cross-links of 5 to 40 kDa can be achieved.[6] Alternatively, cross-linking can be induced by exposure to gaseous formaldehyde.[7] Because

aldehydes are toxic, care must be taken to ensure that an implant is sufficiently free of residue prior to implantation; previously, no adverse effects have been shown in patients receiving CG copolymer scaffolds manufactured using gluteraldehyde as a cross-linking agent[8], suggesting that gluteraldehyde crosslinking is a relatively safe practice.

Polymeric scaffolds can be formed in one of five ways, each producing a characteristically different three-dimensional structure: fiber bonding, 3D printing, solvent casting/particulate leaching, gas foaming, and phase separation. Fiber bonding produces an unwoven mesh of polymer fibers that are bonded together either by welding, where individual fibers are melted and joined at their cross points, or with an adhesive such as a polymer solution. 3D printing technology can be thought of as an extension of fiber bonding, allowing for both better shape variability as well as finer dimensioning. These scaffolds can be used in soft tissues but are mechanically weak and therefore not suitable for hard tissue.[9] Although this technique produces scaffolds with an interconnected and highly porous structure suitable for tissue engineering[10], the processes required to create these unwoven meshes subject the bulk sample to either high temperatures or potentially toxic solvents. For collagen-based polymers, the former can denature the active triple helical structure, where as the latter is shunned because of the potential for toxic compounds to be released after implantation.

Solvent casting/particulate leaching involves casting a polymer/porogen (typically a soluble salt such as NaCl) mixture followed by leaching to produce a foam.[11] The solvent used during casting must be chosen such that the polymer can be dissolved but the salt cannot. After mixing a porogen into the dissolved polymer, the solvent is allowed to evaporate. The bulk sample is then immersed for a sufficient amount of time in a second solvent to leach out the porogen. This process lends well to the control of porosity and pore size, as these characteristics can be tailored by salt volume and crystal size. Although this process is able to create scaffolds with variable pore properties, organic solvents are typically used which raises concern for toxicity after implantation.

Gas foaming utilizes a gas as a porogen; solid polymer discs are first formed, typically though compression in a heated mold, and exposed to high-pressure carbon dioxide for several days. Consequently, the pressure is rapidly decreased to atmospheric, allowing trapped gases in the bulk polymer to expand and create pores.[12] A variation of this process utilizes some aspects of particulate leaching by incorporating ammonium bicarbonate to the polymer prior to molding.

The bulk is then either exposed to vacuum or immersed in warm water; vacuum causes the ammonium bicarbonate to sublime, where as immersion into warm water causes gas evolution as well as particulate leaching of the ammonium bicarbonate.[13, 14] Both processes are novel because organic solvents are not used so toxicity is largely not a problem. However, high processing temperatures required to mold solid polymer discs prohibit the use of temperature sensitive molecules such as collagen. Additionally, the resulting closed pore structure of pure gas foaming does not facilitate cell migration or nutrient exchange.

Phase separation encompasses the techniques of emulsification/freeze drying, and liquid-liquid phase separation.[15, 16] The first requires the formation of an emulsion between the polymer and solvent, mixed with an amount of water. The mixture is then injected into a mold and freeze-dried to remove the water content, leaving an open-pore polymer scaffold. Liquid-liquid phase separation requires the polymer to be dissolved in a low melting point solvent. The solution is then cooled below the melting point and exposed to vacuum for several days to ensure complete sublimation of the solvent. Below the critical temperature, phase separation occurs by a nucleation and growth mechanism, creating spheroidal domains; at temperatures below the spinodal curve on a phase diagram, separation occurs via spinodal decomposition creating a structure of interconnecting cylinders.

Of the five scaffold forming processes, phase separation using emulsification/freeze drying appear to be most compatible with CG copolymers because it does not require exposure to temperatures that can denature the triple helical structure of the collagen. Additionally, this method is well suited to the entire manufacturing process; collagen is dissolved in acidic solution and is induced to precipitate with the addition of a sulfated GAG. The coprecipitate suspension is then injected into a mold and freeze dried to produce a porous structure with the desired shape. This preliminary scaffold is then processed to increase cross-link density by drastic dehydration followed by an aldehyde treatment. Subsequent rinsing in a buffered saline solution removes excess aldehyde residue. It should be noted that each step in this process requires one or more days to complete, and is somewhat prone to defect formation; producing an active CG copolymer scaffold from collagen and GAG requires a time scale on the order of a week.

2.3 Bioactivity

Producing a bioactive scaffold requires carefully tailoring the polymer micro and macro structural properties. The term bioactivity is used in contrast to being inert; bioactive materials are intended to illicit a response from the host tissue that would not otherwise be observed. For instance, a scaffold designed to facilitate dermal regeneration can arrest wound contraction, behavior not normally observed in the untreated wound. These responses are not intended to be adverse, and differ from negative responses such as inflammation and immunologic reactions. Specifically, parameters such as the degradation rate (or half-life, as defined by cross link density), porosity (including pore size, orientation, and percent pore volume), and the crystallinity must be carefully tailored for bioactivity. Degradation rate and porosity in particular most directly influence the scaffold efficacy.

The half-life of the copolymer scaffold can be tailored by adjusting cross-link density; this is usually accomplished by tailoring the cross-linking process for either higher temperatures or longer exposure times. The implant must reside for a sufficient amount of time so that its function can be properly served. The antagonist to residence time is the enzyme collagenase, produced by local cells as part of their physiologically normal behavior of remodeling their ECM. This enzymatic cleavage is specific to the triple helical structure of collagen; once cleaved, the collagen fragments become highly susceptible to degradation from other less specific enzymes present in the bloodstream. In normal wound recovery, the ECM is constantly being degraded and synthesized; an implanted scaffold must therefore dwell for a sufficient time so that when its integrity is lost, enough natural ECM has already been synthesized to support the tissue, structurally and functionally.

Porosity is a crucial component to bioactivity and includes the aspects of pore size, pore volume percent, and pore orientation. These properties are defined during phase separation; lower temperature has the effect of decreasing pore size, while pore orientation is determined by the magnitude of the heat flux vector during freezing.[2] The importance of pore orientation can be seen in experimentation with CG copolymer matrix filled nerve guides, where pores oriented along the nerve axis show significant performance over pores oriented perpendicular to the nerve axis.[17, 18] A lower limit on pore size is placed by the steric requirements of infiltrating cells, so that migration and movement is not hindered; an upper limit, however, is placed by the requirement for a minimum pore volume fraction. The pore volume fraction defines a specific

volume (porous area per volume of scaffold), which must be sufficient such that the scaffold presents enough surface area to bind an adequate amount of cells. Given the combination of both, average pore diameters on the order of 10 to 100 micrometers are required for best activity.

Crystallinity of the CG copolymer refers to the tertiary and quaternary structure of the collagen. Briefly, protein structures are formally divided into four levels: the primary structure is considered to be the basic amino acid sequence of the protein (for collagen, this is the Gly-X-Y repeating unit); the secondary structure is the conformation adopted by the polypeptide chain, typically defined as an alpha helix or beta pleated sheet (right handed alpha helix in collagen); the tertiary structure is the conformation adopted by the interaction of secondary structures (in collagen, this is the left handed triple helix formed by three individual collagen alpha helices); the quaternary structure is the conformation adopted by interactions between tertiary structures. For example, the type I collagen quaternary structure involves five collagen triple helices, aggregated to form a subunit. These subunits are then aggregated laterally to form a bundle, which are consequently aggregated longitudinally to form a type I collagen fiber. This aggregation creates the banded pattern observed in type I collagen fibers under microscopy (Figures 4 and 5). Type IV collagen, however, does not form this banded fibers but rather, develops into an interconnected amorphous matrix.

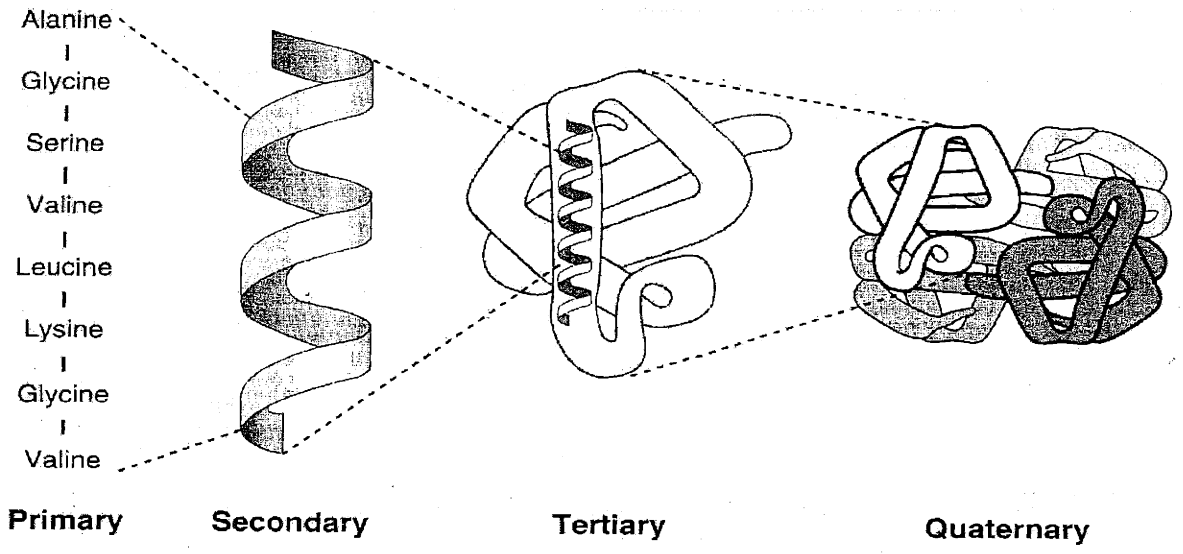

Figure 4: Protein structure is divided into four levels; primary structure refers to the amino acid sequence; secondary structure refers to the initial conformation adopted by the amino acid chain; tertiary structure describes the structure of the protein as a whole; quaternary structure describes the structure adopted by several folded proteins.

Figure 5: Banding can be observed on type I collagen under microscopy.

It is interesting to note that processed type I collagen used in scaffold production bears some resemblance to type IV collagen. Immersion of type I collagen into a solution below pH 4.25 ± 0.30, conditions readily achieved in acetic acid solution, disrupts the quaternary structure while leaving the tertiary triple helical structure intact.[2] Consequently, the fibrils of type I collagen become non-banded and adopt a structure more similar to type IV collagen. It has been found that this non-banded collagen does not aggregate platelets where as banded type I collagen fibrils do; the ability for a scaffold to avoid platelet aggregation may therefore be of importance to bioactivity.

Developing the proper set of characteristics for any given application requires experimentation to determine the optimal properties of a scaffold. Porosity and degradation rate are parameters that depend on the properties of the tissue attempting to be regenerated or engineered, and consequently must be tailored to suit specific needs. Similarly, the roles of crystallinity and even composition (combinations of various types and amounts of collagen and GAG) may be significant depending on the tissue type.

3. Applications

3.1 History

Degradable polymeric biomaterials have been in use since the late 1960's and have seen their applications grow in the thirty years since. Progress is best illustrated by investigating how applications of degradable polymers have changed with time, reflecting a transition toward more sophisticated uses.

Degradable synthetic polymers such as polyglycolic acid (PGA) and polylactic acid (PLA) were first developed in the 1950's, and by the 1970's, found use in applications replacing traditional non-degradable biomaterials, such as in sutures[19] and later in orthopedic plates and screws.[20] Key to the success in these applications were sufficient material strength, degradation rate compatible with the rate of healing, and non-toxic degradation products. The focus in this early period (from approximately 1960 to 1980) was on basic material properties, validating the structural integrity of these polymers under physiological conditions, and on biocompatibility, more specifically ensuring that the polymers and their degradation products would not elicit adverse effects from the host.

Between approximately 1980 and the present, this focus has gradually shifted toward a focus on bioactivity, or the ability for an implanted material to bind cell integrins and facilitate physiological cellular processes, typically growth and differentiation. The engineering development of collagen-based natural polymers occurred in the mid 1970's[21], with applications for skin regeneration investigated by 1980[6, 22], nerve in 1985[23], and more recently, conjunctiva[24]. Within approximately ten years of their respective initial investigations, both skin and nerve regeneration applications were being tested with good results.[25, 26]

Following these early successes, attempts were made into more sophisticated applications featuring more complex cell-to-cell interactions and morphologies. By approximately 1990, the merits of a filled nerve conduit were being investigated, [23, 27, 28, 29, 30, 31] as were applications for bone and cartilage regeneration.[32] The most notable progress made over the earlier scaffold applications with skin and nerve was the addition of a significant third dimension for cell propagation. Skin and nerve regeneration templates developed early in this period can be characterized as having thin sections (for example, a thin sheet used for dermal regeneration and a thin walled hollow cylinder used for nerve regeneration). These latter applications are set apart

by the presence of a significantly larger distance required for cells to traverse into the scaffold, as well as more complex scaffold morphologies.

A key characteristic in this second period is extension beyond basic material properties to recognize the interaction between the cell and the substrate material. Most notable was the identification of the arginine-glycine-aspartic acid (RGD) recognition sequence, which provided insight into the mechanism by which extracellular binding proteins influence cell behavior, including migration and differentiation.[33] The advances in this second period, namely the development of scaffolds for regeneration of skin, nerve, and conjunctiva, rely on the ability for the polymeric scaffold to specifically bind and modify cellular responses and elicit regeneration.

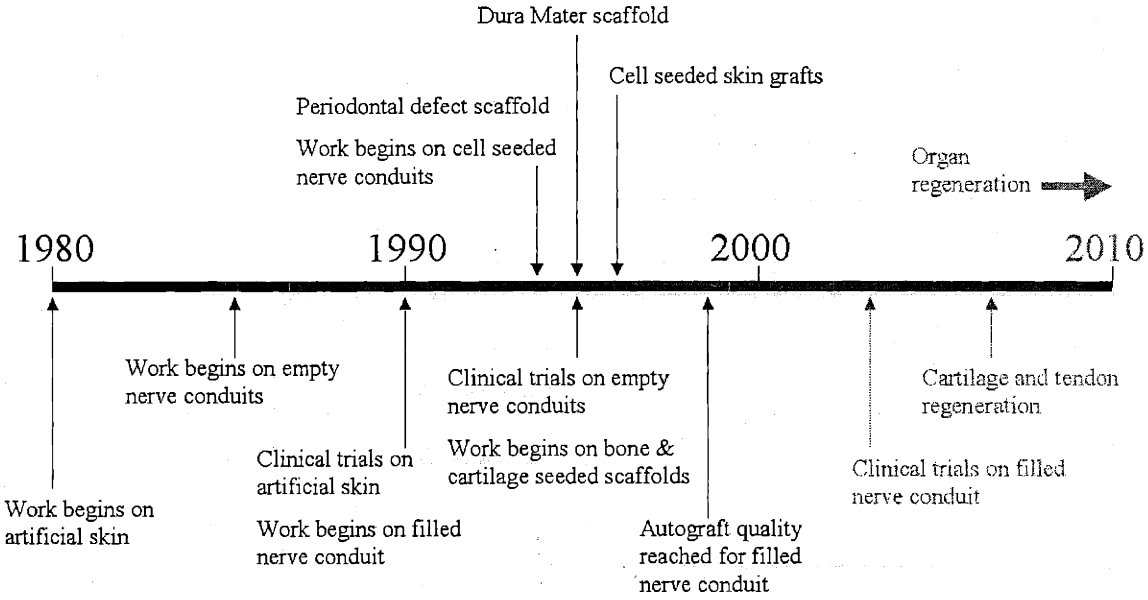

Figure 6: An approximate timeline showing development of scaffolds and their applications reveals a gestation period of approximately ten to fifteen years for a new application to become marketable.

A timeline showing the approximate progress made in scaffold development (Figure 6) reveals a gestation period of approximately ten to fifteen years between the initial work on a scaffold application to a product mature enough for clinical trials. This has been true with CG copolymers in skin, empty nerve guides, and at current progress, appears to be true with optimized and filled nerve conduits for peripheral nerve regeneration. In this latter application, injury to peripheral nerves resulting in a significant gap in nerve bundles, sustained either through surgery or trauma, can be joined using a CG copolymer tube filled with a properly

optimized CG copolymer matrix. This optimized filled nerve guide has the most potential to be the next to market because of progress to date and success comparable to an autograft, the current gold standard. Filled nerve guides differ from empty guides currently being marketed, through the use of a matrix filling which is optimized for chemistry (composition), degradation rate, and porosity (encompassing pore size, shape, and orientation). Preliminary work in filled nerve guides began in 1985, and by 1998, trials conducted on the rat sciatic nerve produced results comparable to that achieved from an autograft, the current gold standard.[7] An autograft requires the creation of a donor site in the patient and by harvesting a donor segment of peripheral nerve, creates a new gap in an otherwise healthy nerve bundle. Use of a filled CG copolymer nerve guide can potentially provide effective treatment for severed peripheral nerves while eliminating the added risks associated with obtaining autograft tissue.

3.2 Current Barriers

The current barrier to development of more complex tissue engineering applications is a lack of detailed knowledge of cell-material interactions. From a biological perspective, the mechanisms of cell behavior in response to external stimuli are far from well understood. Despite advances in the past decade identifying specific ECM ligands[34], many still remain to be discovered. A similar condition exists for cytokines, for which several have been specifically identified but still comprise an incomplete list. Moreover, interactions between cytokines, ligands on an ECM, and the cell itself have to be well understood to facilitate effective development on the materials aspect. Little is understood about ligand spacing or density, the grouping of several different ligands on a single matrix, and interactions between cytokines and the matrix. Matrix production and regulation have also remained unexplored. From the materials science perspective, the biological knowledge must be utilized to drive development of materials featuring specific ligands in proper spacing and combinations, proper interaction with cytokines, as well as methods of characterizing and matching the mechanical properties of engineered and native tissue.[35, 36] Until more significant progress is made toward overcoming these barriers, immediate tissue engineering applications will follow similar to the developments made in the second period, relying on inherently bioactive polymeric scaffolds such as CG copolymers to elicit desired cellular response.

4. Competing technology assessment

Present alternatives to collagen-based scaffolds include both synthetic polymers and natural polymers. Synthetic polymers relevant to tissue engineering can be grouped into two categories: degradable polyesters such as polyglycolic acid (PGA), polylactic acid (PLA), and poly-e-caprolactone (PCL); and pseudo-poly(amino acids). Relevant natural polymers are proteins, based either on collagen or fibrin, and tissue derived ECM.

4.1 Synthetic Polymers

Synthetic degradable polyesters were first adopted for surgical applications in the 1970's, and have an extensive history of FDA approval. PGA, PLA, and PCL are used in surgical applications as sutures[37] and bone fixation devices[20]; they tend to be relatively stiff materials[38] and benefit from easy manufacturing and adjustable degradation rates. Except for PGA, these polymers are soluble in organic solvents and can be processed using either thermal or solvent-based methods.[36] The polymers are formed from their respective monomers (glycolide, lactide, and caprolactone) through a condensation reaction, and deteriorate via hydrolysis of the polymer backbone. Consequently, degradation is reduced with increasing crystallinity[39, 40], increasing hydrophobicity of the monomers[41], and increasing bulk dimension of the polymer.[42] Polymer life can be adjusted to a time scale ranging from weeks to years by modifying molecular weight, or by varying a copolymer composition to adjust crystallinity and monomer concentrations. Typically, copolymers severely disrupt the crystallinity of the pure polymer, leading to degradation rates higher than that of the pure polymer.[43]

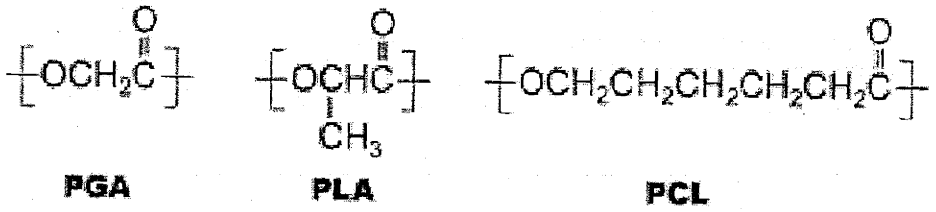

Figure 7: Synthetic degradable polyesters: poly(glycolic acid), poly(lactic acid), and poly(caprolactone).

Drawbacks of degradable polyesters however are the lack of cell specific binding sites, unpredictable mechanical properties during degradation, and adverse tissue interactions caused by the release of monomers. Synthetic polymers generally lack ligands that can bind specifically to cell integrins, omitting the ECM component of unit cell processes. Consequently, infiltrating cells must first synthesize a bioactive matrix over the polymer scaffold. Degradation in these polyesters occurs by random hydrolysis in the polymer backbone, and typically large reduction in molecular weight and strength occur before a significant loss of bulk mass is observed. Additionally, the crystallinity of chiral L-PLA tends to increase during degradation, allowing highly crystalline material to persist *in vivo* long after implant degradation.[44] Perhaps the most adverse drawback for synthetic polyesters is a result of the acidic monomers released following hydrolysis. These byproducts tend to induce inflammation of surrounding tissue, and have been implicated in adverse reactions in bony sites.[45] The resulting lower pH in the vicinity of these materials can further aggravate scaffold degradation and reduce scaffold efficacy.

Pseudo-poly(amino acid) polymers have properties similar to the degradable polyesters. In this case however, the monomer unit is an amino acid linked by nonamide bonds such as ester, carbonate, and iminocarbonate linkages. By incorporating amino acids as the monomer unit, these polymers possess the beneficial properties of degradable polyesters, such as ease of manufacture, controlled degradation, and relatively high stiffness. In particular, tyrosine based polymers show promise in bone fixation applications. Because of the hydrophobicity of the tyrosine monomer unit, degradation occurs on a time scale of months to years.[46] The key benefit enjoyed by pseudo-poly(amino acids) over their polyester counterparts concerns the products of degradation, with pseudo-poly(amino acid) monomers showing improved behavior over the acidic monomers of the polyesters.[47]

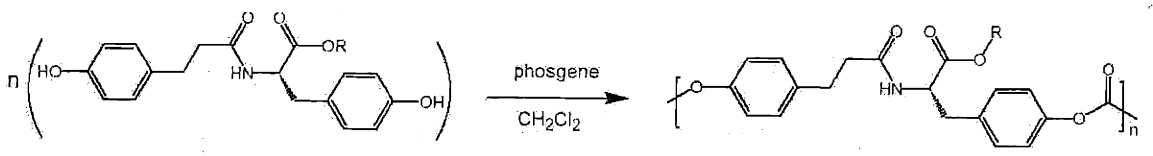

Figure 8: Pseudo-poly(amino acid) polymers (such as tyrosine polycarbonate, shown above), exhibit mechanical properties similar to polyesters. In the structure above, R represents ethyl, isopropyl, butyl, hexyl, or octyl.

4.2 Natural Polymers

Natural fibrin polymers for tissue engineering applications have been investigated in the past twenty years and address the lack of bioactivity in synthetic polymers. A fibrin polymer scaffold is the primary component of a physiological blood clot, serving to develop a solid substrate to promote tissue remodeling at the wound site. Fibrin based scaffolds used in tissue engineering applications are derived from the human plasma proteins fibrinogen and thrombin, which when combined, elicit polymerization in a mechanism simulating the final stages of blood clot formation. Key advantages of fibrin polymers are their inherent bioactivity, compatibility with tissues, and their ease of use. A polymerized fibrin clot enhances biological responses such as healing[48] and cell migration[49], all with very little risk of eliciting adverse tissue reactions[50]. The fibrinogen and thrombin are kept in solution and are typically mixed during injection directly into a wound site or a mold.

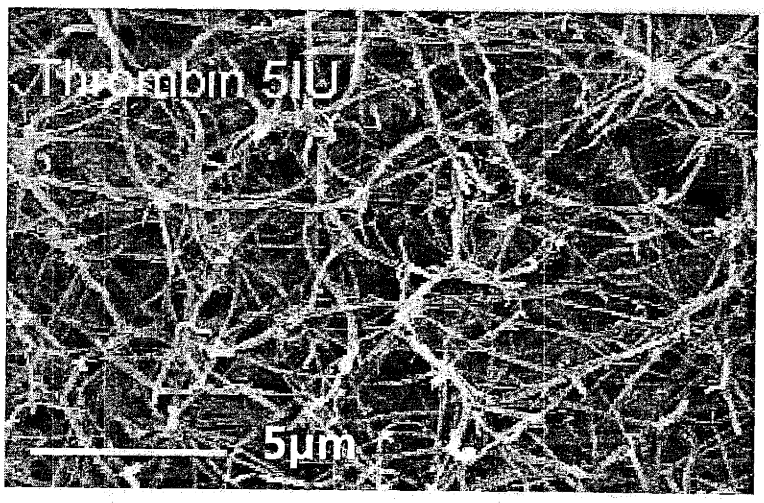

Figure 9: Fibrin scaffolds are produced by combining both fibrinogen and thrombin, producing an open pore scaffold suitable for cell infiltration.

Drawbacks of fibrin however are fast degradation, poor mechanical strength, and the possibility of transferring infectious agents. Fibrin is a provisional scaffold in physiologically normal tissue and does not by itself constitute normal ECM. When used in tissue regeneration applications, infiltrating fibroblasts must first degrade the provisional fibrin matrix and synthesize ECM native to the wound site. A fibrin scaffold degrades on a time scale of two weeks, which severely limits potential applications for tissue engineering. The mechanical

properties of fibrin are similarly poor compared to other synthetic and natural polymer alternatives, limiting applications to those where strength is not essential. Additionally, because the components of fibrin are derived from human plasma, some risk is present for the transfer of infectious agents.

Tissue based scaffolds provide yet another alternative to collagen based polymers. These materials are derived from living tissues that are processed in such a manner so that cellular components are removed leaving the ECM intact. The benefit of these natural polymers is that the physiological matrix chemistry is preserved, implying that active ligands are preserved to facilitate cell binding. Drawbacks include morphology limited by that of the donor tissue, as well as the risk of propagating infectious agents. A well-documented case is the transfer of Creutzfeldt-Jakob Disease from cadaveric dura mater grafts.[51]

Currently, the major drawbacks of collagen-based polymers lie in its manufacture. Type I collagen is typically derived from bovine sources such as hide and tendon and although low, some amount of risk does exist for the transfer of infectious agents. Uniformity of the derived collagen also suffers when compared to that of synthetic polymers. The production of scaffolds with various morphologies utilizes techniques that are labor intensive. Additionally, the time required to form these scaffolds are not compatible with high production volumes, versus methods such as injection and compression molding for solid synthetic polymers.[36, 46]

4.3 Merits of Collagen-based Polymers

However, the merits of collagen lie beyond these manufacturing limitations. Collagen comprises the largest fraction of protein in the human body. CG copolymer scaffolds, based on type I collagen, hold the advantage over synthetic polymer [degradable polyesters and pseudo-poly(amino acids)] scaffolds because of inherent bioactivity, enzymatic degradation, and benign degradation products. As a simple chemical analog of native ECM, CG copolymers exhibit bioactivity via exposed regions of the collagen triple helix[34], and do not rely on cellular synthesis of native ECM for this property. By mimicking natural ECM, cells are provided with the insoluble regulators necessary to promote beneficial unit cell processes.

Degradation of the bulk polymer *in vivo* occurs by specific enzymatic degradation by collagenase rather than through non-specific hydrolysis. Consequently, degradation is more predictable with respect to non-specific degradation, and can be specifically tailored by altering

the cross-link density. The products resulting from the degradation of collagen are small peptide fragments that can undergo further enzymatic degradation and pose little risk of eliciting adverse reactions in surrounding tissues. Progress has also been made to address both the issues of collagen uniformity and risk of infectious agents; recombinant DNA techniques can be used to synthesize several types of collagen with potentially higher uniformity and purity (FibroGen, Inc., San Francisco, CA). Recombinant technology may also provide the added benefit of making production of various collagen types; for example, type IV collagen is found in amounts to small for practical extraction from natural sources. The ability to synthesize this and other types of collagen may reveal the significance of collagen types in determining bioactivity.

4.4 Economic Potential of Collagen-based Polymers

The pursuit of collagen based polymer scaffolds stands to be profitable because of both the progress made to date and the inherent merits of the material. Promising results have already been shown in applications in skin grafts[52], peripheral nerve repair[7], and conjunctiva regeneration[24]. Additionally, results from skin and peripheral nerve regeneration suggest that some regenerative similarity exist between the two[53] and may be applicable to other tissues and organs as well. Consequently, future applications involving other organs, with particular interest in the kidney and liver, seem feasible. CG copolymer scaffolds for tissue regeneration can potentially realize a vastly expanded market as their efficacy in other organs and tissues are pursued and demonstrated.

Because the principles of unit cell processes govern cellular behavior *in vivo* or *in vitro*, applications for CG copolymer scaffolds can reach beyond tissue regeneration within the body. The market for these materials can again be expanded in the future by pursuing possible applications in both *in vitro* tissue engineering as well as stem cell research. In both cases, the attempt to culture cells, tissues, and even complete organs outside the body requires a matrix to support growth. In these applications, CG copolymer scaffolds provide the insoluble regulator required for normal cell proliferation and behavior that, when coupled with a growth support system, can produce physiologically normal, functional tissues and organs.

A more direct and short-term application for CG copolymer scaffolds may be found in cosmetic and reconstructive procedures. An example of this is the removal of scar formation around visible regions of the body and replacement with a scaffold that can promote normal-

looking regeneration; by reducing unsightly scar in this manner, it is hoped that the quality of life for the patient can be improved. FDA approval of these devices for implantation under non life-threatening conditions remains unclear, however, and is conditional on the demonstrated safety of these devices. The debate is enveloped by a question of whether the cosmetic benefits of these procedures are worth any the associated risks.

In the field of drug delivery, synthetic polymers [for instance, poly(ortho esters)[54]] stand to be preferable to natural polymers such as collagen. The focus in developing these applications lies more in controlling degradation rates and patterns rather than developing specific cell binding activity. Consequently, uniform surface degradation (rather than bulk degradation) and high potency drugs are of more importance that the concentration and arrangement of cell binding ligands. Uniform surface degradation is preferred because it provides better structural integrity and allows more importantly, allows uniform drug release over bulk degradation.[54] High potency drugs translate to minimal volumetric requirements of the implant, and could allow for the controlled release of potentially toxic drugs directly into affected tissues, minimizing undesired side effects. Synthetic polymer drug delivery applications already exist (Lupron Depot for prostate cancer treatment, TAP Pharmaceuticals Inc., Lake Forest, IL; Gliadel Wafer for brain cancer treatment, Guilford Pharmaceuticals Inc., Baltimore, MD), and are likely to be expanded in the future.

In any tissue engineering application, however, collagen directly addresses the hurdles currently facing synthetic polymers and, in the short term, profitability lies in the development of scaffolds based on CG copolymers for *in vivo* tissue engineering. Until further knowledge is gained about how complex interactions between insoluble and soluble regulators affect cellular behavior, collagen will remain the most logical choice for tissue engineering scaffold material. On a longer time scale, the study of a natural polymer ECM such as collagen may serve to better elucidate the materials science behind bioactive polymer scaffolds and define their crucial specifications (for instance, developing methods for recognizing and optimizing spacing and combinations of ligands, and bulk polymer interactions with soluble regulators). Long-term profitability of collagen polymers therefore appears to rely on exploiting the properties of collagen for the development of methods for: identifying optimal ligand spacing and combinations; quantitatively evaluating the bioactive efficacy of engineered materials; and synthesizing these materials.

The scope of potential applications for collagen-based polymer scaffolds suggests that a large market for the technology is impending. It does not seem unfathomable that these scaffolds can one day find application in applications as mundane as a self-administered bandage for abrasion wounds. As new applications for these scaffolds are developed, production infrastructure must scale accordingly; most notable are not only production facilities for the scaffolds themselves, but for the production of the raw materials such as collagen and chondroitin-6-sulfate. Given the time required to manufacture scaffolds, a substantial amount of invested capital is required to significantly raise production capacity over the next few decades. Similarly, because a significant amount of the raw materials used in production are derived from natural sources, either extraction must be increased or synthetic production must be further refined to guarantee adequate quantities for the growing market.

5. Patent History

Having seen development for over twenty years now, the basic CG copolymer technology has matured quite considerably. Patents filed in the United States were used as the basis for determining developments that have been filed. Although the scope of this search is rather limited, the results can still deliver a decent view of both processing techniques as well as potential applications that have been considered for collagen-based materials.

Searches were conducted from the United States Patent and Trademark Office database over all available years. Searches were conducted primarily on patent abstracts, with patents listed as citations also considered. The results were grouped into three general categories, relating to technology involving collagen-based polymers, other competing natural and synthetic polymer technologies, and applications for collagen based polymers; a listing of these patents can be found in the appendix. Focusing on patents filed specifically for collagen based materials and their applications, two types of progress can be seen: one defining processing technologies, and the other, applications of the resulting material.

5.1 Key Patents

Briefly, investigation of the patents issued for collagen based scaffolds for tissue engineering reveal that among the most prominent patent licensees are the Massachusetts Institute of Technology, Ethicon, Inc. of Somerville NJ, and Advanced Tissue Sciences of La Jolla, CA. The latter currently holds a large number of active patents concerning *in vitro* tissue engineering using collagen-based scaffolds, such as the *in vitro* cultivation of living cell seeded skin grafts; ten patents filed by Advanced Tissue Sciences since 1995 relate directly to the in vivo culture of various cell types on a substrate. Both Ethicon, Inc. and the Massachusetts Institute of Technology, however, hold several patents concerning basic porous scaffolds. Ethicon, Inc. holds the oldest patent found, U.S. Pat. No. 3157524 issued in 1964, relating to the manufacture of collagen-based sponges for surgical use. Twenty-two patents found were issued to the Massachusetts Institute of Technology since 1977, related to three-dimensional cell culturing both *in vivo* and *in vitro*.

5.2 General Technology Patents

Between approximately 1950 and 1990, the bulk of patents issued define a great deal of the basic technology required to synthesize and form devices from collagen-based polymers. Of these, the earliest found is U.S. Pat. No. 2610625 issued in 1952, which describes the preparation of a surgical sponge. This early patent describes the techniques of treating non-specific animal derived collagen with acid to form a gel, which is then freeze dried to form a porous mass of collagen. Durability of this resulting sponge in a wound site can be adjusted by treatment with any of a variety of tanning agents such as formaldehyde, tannic acid, or phosphoric acid. From approximately 1960 to 1970, variations of the processing themes set fourth in 1952 prevailed, relating primarily to producing collagen based mats or sponges for use as a wound dressing (U.S. Pat. Nos. 3071483, 3157524, 3272204, 3471598, 3491760). The first mention found of incorporating glycosaminoglycans with collagen is in U.S. Pat. No. 3527225, issued in 1970, which describes the use of protein fibers cross-linked with 1 to 6% glycosaminoglycans by weight.

The majority of patents relating to their current methods of preparation and applications are most broadly covered by a series patents assigned to the Massachusetts Institute of Technology. U.S. Pat. No. 4060081 issued in 1977 for a multiplayer membrane (synthetic skin)

presented the first iteration of CG copolymer scaffolds as they are today. This patent defines the application of a simple wound dressing formed by a two-layer membrane: a layer of engineered CG copolymer to contact the wound itself, and a layer of synthetic polymer on the exterior side to control moisture loss from the wound site. Key features of the collagen membrane set fourth in this patent are the use of a GAG to form cross-links, adjustment of the amount of GAG used to vary molecular weight between cross links, and a well-defined membrane thickness. The processing techniques described earlier in this thesis were first observed in U.S. Pat. No. 4280954, issued in 1981 for collagen-GAG composite materials. Claimed within this patent are the processes of first soaking the collagen in acidic solution, addition of a GAG to the solution to produce a coprecipitate, and crosslinking the resulting product through either dehydration or chemical treatment. The importance of porosity to cell migration and division were first set forth in U.S. Pat. No. 4458678, issued in 1984 for seeding cells in fibrous lattice. It is noted nonspecifically within the claims of this patent that the fibrous lattice used must have tailored porosity, pore size, and permeability to facilitate cell migration and division. References to specific pore sizes are not mentioned until U.S. Pat. No. 4787900, issued in 1988 for a blood vessel prosthesis. Within the claims, average pore sizes are established for CG copolymer scaffolds used in different locations in the device.

During the same time period, patents issued by other private and academic institutions extend the basic CG copolymer, the majority of these adapting the utility of collagen to synthetic polymers. These include (U.S. Pat. Nos. 4291013, 4563490, and 4829000 respectively) copolymers of collagen and glycolide or lactide, crosslinked copolymers of collagen and acrylate or methacrylate, as well as additives to the basic collagen-GAG to enhance similarity to basement membrane (addition of various proteins and GAG such as type IV collagen, laminin, and heparan sulfate). Additionally, patents adapting components of natural polymers to their synthetics counterparts have been issued. These include the attachment of specific tetrapeptides and polypeptides to a substrate to confer bioactivity to synthetic polymer substrates (U.S. Pat. Nos. 4578079 and 4589881), attachment of active peptides to a synthetic polymer backbone (U.S. Pat. No. 5399665), overlaying a synthetic scaffold with a material to enhance cell adhesion (U.S. Pat. No. 5770417), and incorporation of biologic growth factors to a polyethylene molecule conjugated to a natural polymer (U.S. Pat. No. 5863984). Patents related to the fabrication of polymer scaffolds can also be found describing the processes of particulate leaching, woven

scaffolds, and seemingly an adaptation of the prevalent method of producing CG copolymer scaffolds, an emulsification/freeze-drying process for synthetic polymers (U.S. Pat. Nos. 5677355, 5711960, and 5723508 respectively).

5.3 Applications Patents and Future Development

It was mentioned previously that a CG scaffold filled nerve conduit for the bridging of gaps in peripheral nerve appears to have high potential to become commercialized in the short term. The first mention found of a nerve guide made of natural polymers can be found in U.S. Pat. No. 3551560 issued in 1970 and assigned to Heinrich F. Thiele. In this is described the process of reconstructing protein extracted from tendons, cartilage, and nerve sheaths, and forming the extract into the tissue to be reconstructed. The first practical mention of nerve guides found, however, is in U.S. Pat. No. 4955893 issued to the Massachusetts Institute of Technology in 1990 for a nerve regeneration prosthesis. The claims of this patent describe the processing technique for forming a tubular nerve prosthesis with a preferential pore orientation and a pore diameter sufficient for axonal regeneration. A similar patent (U.S. Pat. No. 4963146), issued a month after the previous patent, lists as its claims specifically a hollow nerve guide with characteristic pore diameters and formed by a spinning technique. The concept of a filled nerve guide seems to fall under the first patent and does not appear to be blocked.

Concurrent with advances in CG copolymers, a myriad of applications have been proposed and appears to encompass most apparent uses of the material. These include skin (U.S. Pat. No. 4060071), nerve (U.S. Pat. Nos. 4955893, 4963146), tendon (U.S. Pat. No. 5171273), meniscus (U.S. Pat. No. 4880429), intervertebral disc (U.S. Pat. No. 5258043), bone (U.S. Pat. No. 6309670), cartilage (U.S. Pat. No. 6352558), and drug delivery (U.S. Pat. No. 4291013) including gene therapy (U.S. Pat. No. 5770417). The relevance of these patents however still relies on the basic technology, without which these applications cannot be realized. The strongest patents developed thus far rely on the discovery of a characteristic that confers bioactivity, such as degradation rate and porosity. Because these characteristics are fundamental to the efficacy of a scaffold, they must be included in any application; consequently the patents that are able to define these crucial parameters will hold an upper hand.

In this respect, it is my belief that many of the intricacies of collagen that remain to be discovered rest in the biological mechanisms governing and mediating cell behavior, and that

future prominent patents will cover characteristics of more complex biology. More specifically, relationships between insoluble and soluble regulators in a recovering wound site and their significance to cellular behavior, along with the issues previously identified as barriers to future progress and applications. Patents issued for tailoring yet undiscovered characteristics of CG copolymer scaffolds that are essential to bioactivity will likely be dominant, and will have the potential to secure future developments.

6. Cost Model

Although matrix-filled nerve guides appear to be the most likely application of collagen-based polymers, substantial investment must be made before the device can be marketed as a commercial product. The nature of the device however requires two separate areas to be addressed prior to commercialization. Namely, some amount of research and development must still be completed to thoroughly refine the nerve guide parameters and specifications. In addition, FDA approval must be granted to the device before it can be sold commercially. Although the costs associated with the former can be estimated with relative certainty, the latter is certainly more vague.

6.1 Research and Development

Research and development costs encompass the materials, equipment, and labor required to initially manufacture the device. Developing precise specifications for optimized efficacy of the device requires verification in animal models, typically in rats; research and development costs therefore must also account for the maintenance of control and experimental animals as well as the expertise required for the device implantation, evaluation of efficacy, and possible dissection. Materials cost per device is small relative to the cost of equipment and labor, with estimated costs shown in Figure 10. Briefly, most costs are associated with the labor involved. Assuming a basic research team of four full time researchers, assisted by a surgeon only when animal surgery is required, labor costs would amount to approximately a half million per year. Equipment costs would amount to roughly forty thousand initially, and the costs of approximately seventy animal models would amount to roughly ten thousand. Further assuming

that sufficient data can be generated over the course of a year, a rough estimation of total research and development costs amount to $550,000, neglecting the costs associated with housing an outfitted laboratory.

Item	Cost	Unit
Type I Collagen	$ 5.02	g
Chondroitin-6-Sulfate	$ 3.30	g
Gluteraldehyde	$ 0.97	mL
Phosphate Buffered Saline	$ 0.91	L
Vacuum Oven	$ 10,000.00	
Freeze Dryer	$ 10,000.00	
Sterile Hood	$ 10,000.00	
Miscellaneous	$ 10,000.00	
Researchers	$ 100,000.00	Individual/Year
Surgeon	$ 100,000.00	Individual/Year
Lewis Rat (176-200g)	$ 28.95	Individual
Animal Support	$ 100.00	per Individual/Year

Figure 10: Approximate item costs for research and development; these include materials, equipment, labor, and animal model costs.

6.2 FDA Approval and Clinical Trials

Costs associated with obtaining FDA approval cannot be readily estimated in this thesis largely due to my lack of understanding of the certification process. However, what is understood are the requirements placed on various devices depending on the degree of regulation the given device is perceived to require. Devices submitted for approval fall into one of three categories: Class I defines devices for which general control standards are sufficient for ensuring the effectiveness and safety of a device, with items such as latex gloves and crutches falling into this category. Class II encompasses devices for which some amount of extra control is required to ensure safety and effectiveness, either through the use of standardized testing protocols or clinical data. Class III specifies the most stringent category for devices, and typically applies to devices and their applications that are entirely new and have insufficient information to qualify either safety or effectiveness. Additionally, class III devices usually have no similar counterpart on the market and are therefore subject to greater scrutiny.[55]

The difficulty in assigning costs for obtaining certification is based on the uncertainty of how a matrix filled nerve guide will be categorized. Although filled nerve guides are essentially a new device, their basis has been demonstrated in hollow nerve guides previously certified for use. Because the CG copolymer has been previously certified for use in hollow nerve regeneration guides, safety of the device should not require extensive testing. There is a good possibility that this device will be categorized as a class II device, in which case enough information provided from laboratory and animal testing should already exist and suffice to demonstrate the effectiveness of the device, readily facilitating commercialization.

Categorization as a class III device however will require some amount of clinical data to demonstrate safety and efficacy of the device. This would most likely entail multi-center clinical trials and would require a substantial separate investment. Costs associated with this scenario cannot be predicted with any certainty, but should encompass the costs of administering such a trial (including the costs of any support staff required to facilitate collection of data, planning and logistics, and legal counsel), compensation to both the physicians administering these trials as well as the centers hosting the trials, possible compensation to be provided to participating patient subjects, as well as the costs associated with staffing and maintaining a small production facility to supply the trial with a sufficient number of devices.

A rough estimation can be made of the costs involved: assuming that five years of research is required to develop a product, research and development costs alone will amount to over $4.5 million. Further, assume that the cost of a clinical trial depends on the complexity of the device, which for simplicity is reflected by development costs. If the costs of clinical trials amount to five times the development cost, an investment of approximately $20 million is required to produce a device that can be marketed.

6.3 Production

Currently, the methods employed to produce CG copolymer matrices remain very labor and time intensive. Production on a larger scale would therefore require investment in large production equipment that can process adequately sized batches compatible with production losses and demand. Similarly, pursuit of FDA approval can potentially require a significant time and monetary investment before profit can be realized. These two factors suggest that although research and development may be manageable for a small development team, commercialization

is better suited for a company commanding significant resources to handle clinical trials if necessary and produce these devices on a large enough scale. The most effective solution for matrix-filled nerve guides (and more generally, for most medical devices being developed) therefore appears to be for small a small research team to develop significant and protected technology outlining key characteristics of the device, and to license the technology to a firm with sufficient resources to bring the device to market.

The kinetics of realizing profit on this device relies heavily on both the expenditures required for FDA approval, investment in infrastructure, and the potential patient population for the device. Production costs such as labor and materials are not heavily weighted in this profit model because of its potentially small role; after significant expenditure in research and clinical trial and investment in efficient production capability, the low cost of reagents and labor used to fabricate each device becomes negligible. An advantage in pricing options is that as a medical device, a matrix-filled nerve guide can significantly improve the quality of life for a patient. When compared to the current gold standard treatment, this advantage becomes even clearer; rather that rely on an autograft for repair, a polymeric scaffold can restore some nervous function to the patient with minimal risk that loss of function will occur at the autograft donor site. Pricing can therefore be set at a level compatible with a patient's perceived value of the treatment offered by the device. The time required to become profitable then depends on the product of patient population and pricing, taken in relation to the development expenditures.

Observations made from financial reports of two moderately sized companies marketing tissue engineering products (Integra LifeSciences Holdings Corporation, Plainsboro, NJ, and Advanced Tissue Sciences, La Jolla, CA) reveal that in the past three years, both experienced significant operating losses. Examination of both businesses reveals that both are involved simultaneously in research and development as well as marketing and sales. It is my opinion that the most efficient approach to realizing profit on matrix-filled nerve guides is therefore to separate research from sales; research can be conducted by a small team working efficiently. Conversely, a medical device corporation can more effectively manage production, marketing, and sales. The former can realize profit by licensing technology and knowledge to the latter. The latter can realize profits by streamlining production to reduce costs, and establishing a pricing strategy conducive to recovering the investment made in production equipment and obtaining FDA certification.

7.0 Conclusion

Collagen based polymeric scaffolds have shown promising results in organ regeneration in the past twenty years. One of the major hurdles currently limiting more effective materials is the lack of applicable knowledge regarding the interactions between cells, the extracellular matrix, and soluble growth factors. However, as a major component of natural ECM, collagen inherently facilitates proper interactions between the three to promote regeneration. Consequently, continued research in collagen based polymeric scaffolds stands to be profitable; for the present, collagen provides substantial evidence of efficacy in three applications, suggesting that further applications can be developed. On a larger time scale, research into collagen scaffolds can stand to elucidate critical features that a scaffold must possess for bioactivity; securing patents for such discoveries can therefore be quite important for developing future devices. From a business standpoint, however, consideration must be given to the expenditures associated with both research and development, and FDA approval. Because research and development costs for a specific device is relatively manageable, profitability as a business therefore depends on the costs required to gain FDA approval. Similarly, substantial investment into production infrastructure is necessary to support market growth. Yet, the nature of the scaffold as a medical device translates into a pricing advantage, since higher margins per unit sold can be realized.

Because substantial efficacy has already been demonstrated with several tissue types, it is intriguing to postulate where other uses may be found. Further research may reveal additional characteristics crucial to bioactivity, and with time, may drive this technology ever closer to making organ replacement a routine procedure. With this will come new hope for the large patient population awaiting donor organs, and an improved quality of living for the general population.

8. Appendix - Relevant Patents

Patent Number	Assignee	Date Issued	Title	Abstract
2610625	Armour and Company (Chicago, IL)	9/16/1952	Surgical Sponge and the Preparation Thereof	This invention relates to a surgical sponge and to the preparation thereof. The Invention is particularly useful in the preparation of a sponge from collagen, and the resulting product is unusually effective as a sponge for various types of surgical and other techniques.
3071483	United Shoe Machinery Corporation (Boston, MA)	1/1/1963	Manufacture of collagen products	This invention relates to the preparation of tanned collagen fiber masses and particularly to the preparation of a leather-like sheet material.
3157524	Ethicon, Inc. (NJ)	11/17/1964	Preparation of collagen sponge	This invention relates collagen sponges and the preparation thereof. The invention is particularly useful in the preparation of a high density sponge which is valuable for surgical uses.
3189401	Ethicon, Inc.	6/15/1965	Simultaneous Aldehyde, Chrome and Aromatic Alcohol or Quinone Tannage of Spun Collagen Fiber	
3272204	Ethicon, Inc. (NJ)	9/13/1966	Absorbable collagenprosthetic implant with non-absorbable reinforcing strands	The present invention relates to reinforced collagen prostheses adapted to be placed permanently in the human body, and to a method of making the same. More particularly, this incvention relates to collagen articles reinforced with non-absorbable fabrics.
3443261	FMC Corporation, Philadelphia PA	5/13/1969	Prosthetic structures from micro-crystalline collagen	
3471598	FMC Corporation (Philadelphia, PA)	10/7/1969	Method of producing absorbent mats	A water-insoluble, highly absorbent body or mat is formed by preparing an aqueous acidic dispersion of water-insoluble microcrystalline collagen, introducing the dispersion into a mold of the desired configuration and freeze drying the dispersion.
3491760	B. Braun International G.m.B.H. (Liestal, Switzerland)	1/27/1970	Wound coverings	A heteroplastic skin, i.e., wound covering comprising a first tanned collagen gel layer containing about 10 to 25 weight percent of a polyol plasticizer and adhered to one surface of the gel layer a second tanned collagen gel layer of substantially the same composition as the first gel layer but having a lower water content than the first gel layer

3526224	Johnson & Johnson	9/8/1967	Dressing	
3527225	David F. Smith	9/8/1970	Resorbable Surgical Sutures from Fibrous Proteins	
3551560		12/29/1970	Process of reconstructing cartilage, nerve sheaths, and products	
3628974	FMC Corporation (Philadelphia, PA)	12/21/1971	Microcrystalline collagen, an ionizable partial salt of collagen and foods, pharmaceuticals and cosmetics containing same	A new physical form of collagen termed microcrystalline collagen is a water-insoluble but water dispersible ionizable, partial salt of collagen and is formed by treating undenatured collagen with dilute acid solutions having a pH between about 1.6 and 2.6. Mechanically disintegrating the treated collagen in aqueous liquids until at least 10 percent by weight has
3742955	Avicon, Inc.	8/29/1970	Fibrous Collagen Derived Product Having Hemostatic and Wound Binding Properties	
3800792	Johnson & Johnson	4/17/1972	Laminated Collagen Film Dressing	
3810473	Avicon, Inc.	12/4/1972	Liquid-Lain, Non-Woven, Fibrous Collagen Derived Surgical Web Having Hemostatic and Wound Sealing Properties	
3826678	United States	7/30/1974	Method for preparation of biocompatible and biofuntional materials and product thereof	

3875937	American Cyanamid Company	5/31/1973	Surgical Dressings of Absorbable Polymers	
3896802	American Cyanamid Company	4/19/1974	Flexible Flocked Dressing	
3903882	American Cyanamid Company	4/19/1974	Composite Dressing	
3949073	The Board of Trustees of Leland Stanford Junior University (Stanford, CA)	4/6/1976	Process for augmenting connective mammalian tissue with in situ polymerizable native collagen solution	A method for augmenting hard or soft connective tissue, such as skin, tendon, cartilage, bone or interstitium, in a living mammal comprising implanting a proteolytic enzyme-solubilized, purified, native, in situ polymerizable collagen solution into the mammal at the augmentation site. The solution polymerizes at the site into a stable, non-
3955012	Zaidan Hojin, Seisan Kaihatsu Kagaku Kenkyusho (Kyoto, JA)	5/4/1976	Method for manufacturing medical articles composed of silicone rubber coated with collagen	Medical articles composed of silicone rubber coated with collagen to be used in living body, are manufactured by subjecting a surface of shaped articles composed of silicone rubber to a spark discharge, coating the thus treated surface with an acidic aqueous solution of collagen and then drying said surface to form collagen layer and irradiating the shaped article
4060081	Massachusetts Institute of Technology (Cambridge, MA)	11/29/1977	Multilayer membrane useful as synthetic skin	A multilayer membrane, which is useful as synthetic skin, is disclosed herein. A first layer is formed from a material which does not provoke an immune response and which is also insoluble and nondegradable in the presence of body fluids and/or body enzymes. Preferred materials for the first layer are crosslinked composites of collagen and a
4233360	Collagen Corp.	11/11/1980	Non-antigenic collagen and articles of manufacture	
4280954	Massachusetts Institute of Technology (Cambridge, MA)	7/28/1981	Crosslinked collagen-mucopolysaccharide composite materials	Composite materials are disclosed which are formed by contacting collagen with a mucopolysaccharide and subsequently covalently crosslinking the resultant polymer. These composite materials have a balance of mechanical, chemical and physiological properties which make them useful in surgical sutures and prostheses of controlled

4291013	Merck Patent Gesellschaft Mit Beschrankter Haftung (Darmstadt, DE)	9/22/1981	Medicinally useful, shaped mass of collagen resorbable in the body	A shaped mass resorbable in the body, comprises collagen and a bioresorbable binding agent for collagen, the binding agent being selected, e.g., from polymers of C.sub.2-16 .alpha.-hydroxyalkanoic acids, polymers of natural amino acids, hydrolyzed collagen or hydrolyzed elastin.
4304866	Massachusetts Institute of Technology (Cambridge, MA)	12/8/1981	Transplantable sheets of living keratinous tissue	A method of producing transplantable sheets of living keratinous tissue by culturing keratinocytes in a culture vessel and subsequently enzymatically detaching a sheet of keratinous tissue employing an enzyme, such as Dispase, is disclosed herein
4326532	Minnesota Mining and Manufacturing Company (St. Paul, MN)	4/27/1982	Antithrombogenic articles	A medical article having a layered antithrombogenic surface is useful as a polymeric implant. The article comprises a polymeric substrate coated with chitosan to which is appended an antithrombotic agent. A process for rendering the surface of a polymer antithrombogenic is disclosed.
4350629	Massachusetts Institute of Technology (Cambridge, MA)	9/21/1982	Procedures for preparing composite materials from collagen and glycosaminoglycan	This invention relates to crosslinked collagen and glycosaminoglycan materials, and to procedures for preparing such materials. It has been discovered that if collagen fibrils in an aqueous acidic solution are contacted with a crosslinking agent before being contacted with glycosaminoglycan, the materials produced have extremely low levels of thrombogenicity
4352883	Damon Corporation (Needham Heights, MA)	10/5/1982	Encapsulation of biological material	A core material such as living tissue, individual cells, hormones, enzymes or antibodies is encapsulated in a semipermeable membrane that is permeable to small molecules for contact with the core material but is impermeable to potentially deleterious large molecules. Encapsulation may be carried out by suspending the core material in an aqueous
4416814	Battista; Orlando A. (3725 Fox Hollow Rd., Fort Worth, TX 76109)	11/22/1983	Protein polymer hydrogels	Protein hydrogel structures formed from natural proteins having an average molecular weight of about 100,000 or less by dissolving the protein in an aqueous acidic solution, subjecting the protein to vapor phase crosslinking with at least two crosslinking agents, and air drying to a moisture content not exceeding 10 percent. The drying may be effected by treatment with a
4418691	Massachusetts Institute of Technology (Cambridge, MA)	12/6/1983	Method of promoting the regeneration of tissue at a wound	This invention comprises the use of centrifugal force to introduce viable cells into a fibrous lattice, as well as fibrous lattices that are seeded with cells by the use of centrifugal force. A variety of fibrous lattices may be seeded by the methods of this invention, such as a highly porous lattice comprising collagen fibers crosslinked with glycosaminoglycan
4448718	Massachusetts Institute of Technology (Cambridge, MA)	5/15/1984	Method for the preparation of collagen-glycosaminoglycan composite materials	A process for preparing a crosslinked collagen-glycosaminoglycan composite material which comprises forming an uncrosslinked composite material from collagen and a glycosaminoglycan and contacting the uncrosslinked composite with a gaseous aldehyde until a crosslinked product having an M sub n of from about 800 to about 60,000 is

4458678	Massachusetts Institute of Technology (Cambridge, MA)	7/10/1984	Cell-seeding procedures involving fibrous lattices	This invention relates to the introduction of viable cells into a fibrous lattice by surgical, force-utilizing, or other manipulative techniques, all of which are referred to herein as "seeding." One embodiment comprises an autografting technique which involves intact tissue. Other embodiments which involve the distribution of an aqueous suspension of cells
4485096	Massachusetts Institute of Technology (Cambridge, MA)	11/27/1984	Tissue-equivalent and method for preparation thereof	A tissue-equivalent, useful in the treatment of burns or other skin wounds and in the fabrication of prostheses, is disclosed which is prepared from a hydrated collagen lattice contracted by a contractile agent, such as fibroblast cells or blood platelets, to form tissue-equivalent. In one embodiment, a skin-equivalent can be fabricated by growing a layer
4485097	Massachusetts Institute of Technology (Cambridge, MA)	11/27/1984	Bone-equivalent and method for preparation thereof	A bone-equivalent, useful in the fabrication of prostheses, is disclosed which is prepared from a hydrated collagen lattice contracted by fibroblast cells and containing demineralized bone powder.
4505266	Massachusetts Institute of Technology (Cambridge, MA)	4/19/1985	Method of using a fibrous lattice	This invention relates to the introduction of viable cells into a fibrous lattice by surgical, force-utilizing, or other manipulative techniques, all of which are referred to herein as "seeding." One embodiment comprises an autografting technique which involves intact tissue. Other embodiments which involve the distribution of an aqueous suspension of cells
4520821	The Regents of the University of California (Berkeley, CA)	6/4/1985	Growing of long-term biological tissue correction structures in vivo	A mesh or gauze of a bioabsorbable material is used to temporarily correct a defect in a living body. The mesh is of a construction sufficient so that biological tissue in the area of the defect can grow into it and form a long-term biological tissue correction structure before the mesh is completely bioabsorbed. The long-term biological tissue correction structure forms a
4522753	Massachusetts Institute of Technology (Cambridge, MA)	6/11/1985	Method for preserving porosity in porous materials	A method for preserving the porosity of porous materials is disclosed. In this method, the porous material is subjected to elevated temperature and vacuum conditions to thereby produce a dimensionally-stable, non-collapsible porous material.
4553272	University of Pittsburgh (Pittsburgh, PA)	11/19/1985	Regeneration of living tissues by growth of isolated cells in porous implant and product thereof	A method of repair of patient tissues by implant including providing a living cell sample which is introduced into an implant member having a porous open structure. The cell sample may be cultured in the implant. The implant is secured to the patient, as by surgical implantation. In one embodiment, the implant portion which receives the cells preferably has a pore size of
4563490	Czechoslovenska akademie ved of Praha (Prague, CS)	1/7/1986	Composite polymeric material for biological and medical application and the method for its preparation	The invention relates to a composite polymeric material suitable for biological and medical applications and to the method for preparation thereof. The composite material consists of 1-99 wt. % of hydrophilic polymer or copolymer based on methacrylic or acrylic esters, 1-99 wt. % of fibrillar collagen, and up to 2.5 wt. % of a crosslinking agent based on both polymeric

4570629	University of Illinois Foundation (Urbana, IL)	2/18/1986	Hydrophilic biopolymeric copolyelectrolytes, and biodegradable wound dressing comprising same	Hydrophilic biopolymeric copolyelectrolytes comprising (a) a water-soluble linear anionic protein polyelectrolyte component derived from keratin and (b) a water-soluble linear cationic biopolymer polyelectrolyte component derived from at least one biopolymer selected from the group consisting of collagen and a glucosaminoglycan. Hydrogel membranes
4578079	La Jolla Cancer Research Foundation (La Jolla, CA)	3/25/1986	Tetrapeptide	The peptide X-Arg-Gly-Asp-R-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Thr or Cys, or other amino acid, having the same cell-attachment activity as fibronectin and the peptide X-Arg-Gly-Asp-Ser-Y, wherein X and Y, having said activity are disclosed
4589881	La Jolla Cancer Research Foundation (La Jolla, CA)	5/20/1986	Polypeptide	A polypeptide having the cell-attaching activity of fibronectin. The polypeptide has 108 amino acid residues and the formula: H-Ile-Gly-Gln-Gln-Ser-Thr-Val-Ser-Asp-Val-Pro-Arg-Asp-Leu-Glu-Val-Val-Ala- Ala-Thr-Pro-Thr-Ser-Leu-Leu-Ile-Ser-Trp-Asp-Ala-Pro-Ala-Val-Thr-Val-Arg-Tyr -Tyr-Arg-Ile-Thr-Tyr-Gly-Glu-Thr-Gly-Gly-Asn-Ser-Pro-Val-Gln-Glu-Phe-Thr-Val-Pro-Gly
4614794	Johnson & Johnson (New Brunswick, NJ)	9/30/1986	Protein/polysaccharide complexes	Complexes of polyanionic plant polysaccharides with biodegradable proteins, or proteolytic degradation products thereof, are useful in the formation of wound dressings and surgical implants, such as sutures, blood vessel grafts, and artificial organs. The biodegradable protein is preferably collagen, and the polysaccharide is preferably sodium alginate
4657548	Helitrex, Inc. (Princeton, NJ)	4/14/1987	Delivery system for implantation of fine particles in surgical procedures	A delivery system for implantation of fine particles in surgical procedures made of a collagen tube filled with the fine particles, the tube being made of a cast collagen film having holes in it larger than the size of the particles to allow cell migration. The system is particularly useful for alveolar ridge augmentation.
4725671	Collagen Corporation (Palo Alto, CA)	2/16/1988	Collagen membranes for medical use	Collagen membranes with desired properties are prepared by using a variety of gel-forming techniques in combination with methods for converting the gels to solid forms. The properties of these membranes or other solid forms may be further altered by cross-linking the collagen preparation either after formation of the membrane or gel, or most preferably by
4776890	Collagen Corporation (Palo Alto, CA)	10/11/1998	Preparation of collagen hydroxyapatite matrix for bone repair	An improved process for obtaining a matrix of mineral particles in reconstituted atelopeptide collagen comprises reconstituting a mixture of mineral particles with collagen in solution. This process results in a matrix of collagen containing the mineral particles which, when wetted, is malleable and retains its integrity.
4787900	Massachusetts Institute of Technology (Cambridge, MA)	11/29/1988	Process for forming multilayer bioreplaceable blood vessel prosthesis	Process for forming a multilayer blood vessel prosthesis. Each layer is formed from bioreplaceable materials which include those produced by contacting collagen with an aminopolysaccharide and subsequently covalently crosslinking the resulting polymer, polymers of hydroxyacetic acid and the like. Cross flow filtration molding and wet extrusion

4795467	Collagen Corporation (Palo Alto, CA)	1/3/1989	Xenogeneic collagen/mineral preparations in bone repair	A composition for use in bone repair, in particular, in onlay procedures, which comprises calcium phosphate mineral particles in admixture with atelopeptide reconstituted fibrillar collagen preparations is disclosed. This composition is non-immunogenic and encourages the fusion of host bone with new bone growth through the implant. Additional
4801299	University Patents, Inc. (Westport, CT)	1/31/1989	Body implants of extracellular matrix and means and methods of making and using such implants	A sterile body implant is derived from a body structure having as its major protein component collagens in the form of extracellular matrix. The body structure is treated to remove cellular membranes, nucleic acids, lipids and cytoplasmic components. Such structures are implanted internally in the body or externally on the body in a variety of medical uses
4829000	The United States of America as represented by the Secretary of the (Washington, DC)	5/9/1989	Reconstituted basement membrane complex with biological activity	The present invention discloses a biologically active basement membrane composition. When polymerized under physiological conditions, the composition forms gel-like structures whose ultrastructure resembles interconnected thin sheets of the lamina densa zone of basement membrane. The major components of the composition include laminin, type IV collagen
4880429	Stone; Kevin R. (133 Retiro Way, San Francisco, CA 94123)	11/14/1989	Prosthetic meniscus	A prosthetic meniscus is disclosed which can be implanted in a humanoid knee, and which can act as a scaffold for regrowth of native meniscal tissues. The meniscus comprises a three dimensional array of collagen fibers interspersed with glycosaminoglycan molecules. The collagen fibers are present at a concentration of about 65 to 98 percent by dry
4902289	Massachusetts Institute of Technology (Cambridge, MA)	2/20/1990	Multilayer bioreplaceable blood vessel prosthesis	Process for forming a multilayer blood vessel prosthesis. Each layer is formed from bioreplaceable materials which include those produced by contacting collagen with an aminopolysaccharide and subsequently covalently crosslinking the resluting polymer, polymers of hydroxyacetic acid and the like. Cross flow filtration molding and wet extrusion
4902295	Hana Biologics, Inc. (Alameda, CA)	2/20/1990	Transplantable artificial tissue	A transplantable artificial tissue matrix structure containing viable cells which is suitable for insertion into the body is made by polymerizing precursors in an aqueous solution to form a shape retaining solid matrix comprising viable cells, matrix polymer and reversible gel polymer. The solution contains a matrix polymer precursor, a reversible gel polymer
4947840	Massachusetts Institute of Technology (Cambridge, MA)	8/14/1990	Biodegradable templates for the regeneration of tissues	This invention relates to porous, biodegradable materials in which the pore size, biodegradation rate, and pore volume fraction are controlled and within values at which skin contraction rates around an implant-containing wound are delayed or slowed.
4955893	Massachusetts Institute of Technology (Cambridge, MA)	9/11/1990	Prosthesis for promotion of nerve regeneration	A method for producing a biodegradable polymer having a preferentially oriented pore structure and a method for using the polymer to regenerate damaged nerve tissue is disclosed. The preferentially oriented pores are produced by an axial freezing process and serve to promote proper vascularation and regeneration of the damaged nerve. Preferably, the

4963146	Colla-Tec Incorporated (Plainsboro, NJ)	10/16/1990	Multi-layered, semi-permeable conduit for nerve regeneration	The present invention is directed to hollow conduits whose walls are comprised of Type I collagen and are characterized by having a multi-layered, semi-permeable structure, which conduits are used to promote nerve regeneration across a gap of a severed nerve. Methods of making the nerve regeneration conduit are also disclosed
4963489	Marrow-Tech, Inc. (La Jolla, CA)	10/16/1990	Three-dimensional cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
4969912	Kelman; Charles D. (269 Grand Central Pkwy., Floral Park, NY 11005); DeVore; Dale P. (3 Warwick Dr., Chelmsford, MA 01824)	11/13/1990	Human collagen processing and autoimplant use	Production of a chemically modified, crosslinkable, telopeptide-containing, naturally crosslinked, solubilized collagen from tissue obtained from a sole human donor, for implanting in the same donor, by chemically modifying the tissue, e.g. by acylation and/or esterification, to form an autoimplantable, crosslinkable, telopeptide containing naturally
4970298	University of Medicine and Dentistry of New Jersey (Newark, NJ)	11/13/1990	Biodegradable matrix and methods for producing same	This invention relates to a biodegradable collagen matrix having a pore size and morphology which enhances the healing of a wound. It further relates to a process for preparing the matrix. One embodiment of the invention comprises a biodegradable matrix which comprises collagen, hyaluronic acid and fibronectin. Other embodiments include a
4997443	Hana Biologics, Inc. (Alameda, CA)	3/5/1991	Transplantable artificial tissue and process	A transplantable artificial tissue matrix structure containing viable cells which is suitable for insertion into the body is made by polymerizing precursors in an aqueous solution to form a shape retaining solid matrix comprising viable cells, matrix polymer and reversible gel polymer. The solution contains a matrix polymer precursor, a reversible gel polymer
5041138	Massachusetts Institute of Technology (Cambridge, MA); Children's Hospital (Boston, MA)	8/20/1991	Neomorphogenesis of cartilage in vivo from cell culture	Methods and artificial matrices for the growth and implantation of cartilaginous structures and surfaces are disclosed. In the preferred embodiments, chondrocytes are grown on biodegradable, biocompatible fibrous polymeric matrices. Optionally, the cells are proliferated in vitro until an adequate cell volume and density has developed for the cells to survive and
5112354	Northwestern University (Evanston, IL)	5/12/1992	Bone allograft material and method	A textured, demineralized, and unitary mammalian bone section for providing a rigid, foraminous, collagen scaffold for allogenic skeletal reconstruction. The allograft is prepared by treating a section of cadaver bone to remove all soft tissue, then texturing the bone surface to produce a pattern of holes of selected size, density, and depth, and finally
5133755	THM Biomedical, Inc. (Duluth, MN)	7/28/1992	Method and apparatus for diodegradable, osteogenic, bone graft substitute device	Device and method for treating mammalian bone deficiencies, defects, voids and conformational discontinuities produced by congenital deformities, osseous and/or soft tissue pathology, traumatic injuries and functional atrophy is described. The device is a one piece molded body member composed of four substances, each of which contributes to

5158881	Brown University Research Foundation (Providence, RI)	10/27/1992	Method and system for encapsulating cells in a tubular extrudate in separate cell compartments	Methods and systems are disclosed for encapsulating viable cells which produce biologically-active factors. The cells are encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension and a polymeric solution through a common port to form a tubular extrudate having a polymeric outer
5162430	Collagen Corporation (Palo Alto, CA)	11/10/1992	Collagen-polymer conjugates	Collagen, particularly atelopeptide collagen, exhibits improved handling characteristics when chemically conjugated and/or crosslinked with a synthetic hydrophilic polymer.
5171273	University of Medicine and Dentistry of New Jersey (Newark, NJ)	12/15/1992	Synthetic collagen orthopaedic structures such as grafts, tendons and other structures	The invention provides graft, prothesis, orthopaedic structures, implants and like body replacement parts which are constituted of synthetic collagen fibers, an embodiment of which is a tendon or a ligament prosthesis, graft or implants. These body parts have a combination of very useful properties, particularly high tensile strength combined with
5258043	ReGen Corporation (San Francisco, CA)	11/2/1993	Method for making a prosthetic intervertebral disc	A prosthetic intervertebral disc is disclosed which can be implanted in the human skeleton, and which can act as a scaffold for regrowth of intervertebral disc material. The disc includes a dry, porous, volume matrix of biocompatible and bioresorbable fibers which may be interspersed with glyscosaminoglycan molecules. The matrix is adapted to have in
5263984	ReGen Biologics, Inc. (San Francisco, CA)	11/23/1993	Prosthetic ligaments	Disclosed is a prosthetic ligament comprising a Plurality of substantially aligned, elongated filaments. Each filament is a dry, porous, volume matrix of biocompatible and bioresorbable fibrils, at least some of which are crosslinked. The fibrils are short segments of longer fibers of polymeric connective tissue components, or analogs thereof. Each filament
5266476	Yeda Research & Development Co., Ltd. (Rehovot, IL)	11/30/1993	Fibrous matrix for in vitro cell cultivation	There is provided a matrix and cultivation system for anchorage dependent cells. The matrix is characterized by a substantially increased available effective surface area for cell attachment which is attained by resorting to the use of a fiber network or open-pore foams with suitable pore size. Attachment of the cells can be enhanced by modifying the surface of
5266480	Advanced Tissue Sciences, Inc. (La Jolla, CA)	11/30/1993	Three-dimensional skin culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5283187	Brown University Research Foundation (Providence, RI)	2/1/1994	Cell culture-containing tubular capsule produced by co-extrusion	Living cells such as animal cells which produce biologically active factors are encapsulated within a semipermeable, polymeric membrane such as polyacrylate by co-extruding an aqueous cell suspension and a polymeric solution through a common port having at least one concentric bores to form a tubular extrudate having a polymeric membrane which

5284761	Brown University Research Foundation (Providence, RI)	2/8/1994	Method of encapsulating cells in a tubular extrudate	Methods and systems are disclosed for encapsulating viable cells which produce biologically-active factors. The cells are encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension and a polymeric solution through a common port to form a tubular extrudate having a polymeric outer
5354736	Regents of the University of California (Oakland, CA)	10/11/1994	Synthetic compounds and compositions with enhanced cell binding	Compositions of the invention include composites comprising a biomaterial carrying compounds with enhanced cell binding with respect to collagen. These composites are useful for soft and hard tissue repair or reconstruction. Suitable compounds with enhanced cell binding include synthetic peptides that mimic the conformation
5399665	Massachusetts Institute of Technology (Cambridge, MA); Children's Hospital (Boston, MA)	3/21/1995	Biodegradable polymers for cell transplantation	Polymers more suitable for use in organ transplantation are formed by coupling biologically active moieties to the free amino groups of polymers formed by incorporation of .alpha. amino acids into polymers formed of alpha hydroxy acids such as lactic acids. In the preferred embodiment, the peptides are coupled to the free amino groups
5443950	Advanced Tissue Sciences, Inc. (La Jolla, CA)	8/22/1995	Three-dimensional cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5487889	The MetroHealth System (Cleveland, OH); The University of Akron (Cleveland, OH); Case Western Reserve University (Cleveland, OH)	1/30/1996	Bandage for continuous application of biologicals	The present invention provides a biological bandage, comprising an envelope enclosing cells which secrete biologically active cellular products such as growth factors, which promote the healing of wounds. The envelope is further comprised of a permeable bottom membrane through which the cellular product diffuses, and a top membrane. Preferably the
5489304	Brigham & Women's Hospital (Boston, MA); Shriners Hospital for Crippled Children (Tampa, FL); Massachusetts Institute of Technology	2/6/1996	Method of skin regeneration using a collagen-glycosaminoglycan matrix and cultured epithelial autograft	The present invention relates to a method of skin regeneration of a wound or burn in an animal or human. This method comprises the steps of initially covering the wound with a collagen glycosaminoglycan matrix, allowing infiltration of the grafted GC matrix by mesenchymal cells and blood vessels from healthy underlying tissue and applying a
5512475	Advanced Tissue Sciences, Inc. (La Jolla, CA)	4/30/1996	Three-dimensional skin cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5516680	Advanced Tissue Sciences, Inc. formerly Marrow-Tech (La Jolla, CA)	5/14/1996	Three-dimensional kidney cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal

5516681	Advanced Tissue Sciences, Inc. (La Jolla, CA)	5/14/1996	Three-dimensional pancreatic cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5518915	Advanced Tissue Sciences, Inc. (La Jolla, CA)	5/21/1996	Three-Dimensional mucosal cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5541107	Advanced Tissue Sciences, Inc. (La Jolla, CA)	7/30/1996	Three-dimensional bone marrow cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5567612	Massachusetts Institute of Technology (Cambridge, MA); Children's Medical Center Corporation (Boston, MA)	10/22/1996	Genitourinary cell-matrix structure for implantation into a human and a method of making	Methods and artificial matrices for the growth and implantation of urological structures and surfaces are disclosed in which urothelial cells are grown in culture on biodegradable, biocompatible, fibrous matrices formed of polymers, such as polyglycolic acid, polylactic acid, or other polymers which degrade over time. The cells can be cultured in vitro until an
5578485	Advanced Tissue Sciences, Inc. (La Jolla, CA)	11/26/1996	Three-dimensional blood-brain barrier cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5580781	Advanced Tissue Sciences, Inc. (La Jolla, CA)	12/3/1996	Three-dimensional tumor cell and tissue culture system	The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal
5607474	Board of Regents, University of Texas System (Austin, TX)	3/4/1997	Multi-phase bioerodible implant/carrier and method of manufacturing and using same	A carrier and method of manufacturing and using the same is provided for receiving supporting replenished tissue growing into a diseased or damaged area within a physiological system. The carrier can be implanted in the interface region between tissue having different mechanical properties to support the growth and regeneration of differing
5610241	Cornell Research Foundation, Inc. (Ithaca, NY)	3/11/1997	Reactive graft polymer with biodegradable polymer backbone and method for preparing reactive biodegradable polymers	Graft polymers with reactive groups for linking to peptides for use in tissue engineering and to drugs to provide drug delivery systems and useful per se to release amino acids and useful, for example, for wound closure devices, pins, screws, anastomosis rings, and surgical implants, consist essentially of biodegradable homopolymer or copolymer backbone joined at

5624840	Advanced Tissue Sciences Inc. (La Jolla, CA)	4/29/1997	Three-dimensional liver cell and tissue culture system	The present invention relates to a three-dimensional cell and tissue culture system. In particular, it relates to this culture system for the long term culture of liver cells and tissues in vitro in an environment that more closely approximates that found in vivo. The culture system described herein provides for proliferation and appropriate liver cell
5660857	Johnson & Johnson Medical Inc. (Arlington, TX)	8/26/1997	Biopolymer composites	A process for preparing a composite comprising an insoluble protein matrix and an oleaginous material, which is useful as a material for surgical dressings and biomedical implants, and as a cosmetic material for application to the skin. The process comprises the steps of mixing a protein, the oleaginous material and water to form an emulsion of the
5677355	Smith & Nephew, Inc. (Memphis, TN)	10/14/1997	Continuous open-cell polymeric foams containing living cells	A polymeric foam with continuous, open-cell pores containing living cells suitable for medical applications and methods for preparing these foams. The microporous foams are of controlled pore size that may be utilized in a variety of applications. In general, the foams are characterized in that the pores are continuous and open celled. In preparing the
5711960	Takiron Co., Ltd. (Osaka, JP)	1/27/1998	Biocompatible implant material comprising a tri-axial or more three-dimensional fabric	This invention provides an implant material which has high mechanical strength and durability in three-dimensional directions and a function to synchronize with deformation characteristics of surrounding biological tissues, is capable of being penetrated by biological tissues into its fabric space, and does not cause foreign body reaction or pain
5716413	OsteoBiologics, Inc. (San Antonio, TX)	2/10/1998	Moldable, hand-shapable biodegradable implant material	This invention provides molded, biodegradable porous polymeric implant materials having a pore size distribution throughout the material which is substantially uniform. These materials can be molded into implants of any desired size and shape without loss of uniformity of pore size distribution. The implants are useful as biodegradable scaffolds for cell growth in
5723508	Northwestern University (Evanston, IL)	3/3/1998	Method of fabricating emulsion freeze-dried scaffold bodies and resulting products	Scaffold bodies and methods for their fabrication are disclosed, and, more particularly, fabrication of scaffold bodies by freeze-drying emulsion of polymer solutions are disclosed.
5755792	THM Biomedical, Inc. (Duluth, MN)	5/26/1998	Method and apparatus for biodegradable, osteogenic, bone graft substitute device	Device and method for treating mammalian bone deficiencies, defects, voids and conformational discontinuities produced by congenital deformities, osseous and/or soft tissue pathology, traumatic injuries and functional atrophy is described. The device is a one piece molded body member composed of four substances, each of which contributes to
5759830	Massachusetts Institute of Technology (Boston, MA); Children's Medical Center Corporation (Boston, MA)	6/2/1998	Three-dimensional fibrous scaffold containing attached cells for producing vascularized tissue in vivo	A cell-scaffold composition is prepared in vitro for implanting to produce functional organ tissue in vivo. The scaffold is three-dimensional and is composed of fibers of a biocompatible, biodegradable, synthetic polymer. Cells derived from vascularized organ tissue are attached in vitro to the surface of the fibers uniformly throughout the scaffold in an amount effective

5769899	Matrix Biotechnologies, Inc. (Melville, NY)	6/23/1998	Cartilage repair unit	A bio-absorbable cartilage repair system for regenerating damaged or destroyed articular cartilage on the surface of a bone by establishing a chondrogenic growth-supporting matrix between an area of removed damaged or destroyed articular cartilage and the adjacent healthy cancellous bone. The system is at least one assembly of a bio-absorbable delivery unit
5770193	Massachusetts Institute of Technology Children's Medical Center (Cambridge, MA)	6/23/1998	Preparation of three-dimensional fibrous scaffold for attaching cells to produce vascularized tissue in vivo	Fibers of a biocompatible, biodegradable or non-biodegradable, synthetic polymer are provided, and are formed into a three-dimensional scaffold. The fibers of the scaffold may have a branched configuration extending outwardly from a central stem. The fibers provide sufficient surface area to permit attachment to the scaffold in vitro of an amount
5770193	Massachusetts Institute of Technology Children's Medical Center (Cambridge, MA)	6/23/1998	Preparation of three-dimensional fibrous scaffold for attaching cells to produce vascularized tissue in vivo	Fibers of a biocompatible, biodegradable or non-biodegradable, synthetic polymer are provided, and are formed into a three-dimensional scaffold. The fibers of the scaffold may have a branched configuration extending outwardly from a central stem. The fibers provide sufficient surface area to permit attachment to the scaffold in vitro of an amount
5770417	Massachusetts Institute of Technology Children's Medical Center (Cambridge, MA)	6/23/1998	Three-dimensional fibrous scaffold containing attached cells for producing vascularized tissue in vivo	A cell-scaffold composition is prepared in vitro for implanting to produce functional organ tissue in vivo. The scaffold is three-dimensional and is composed of hollow or solid fibers of a biocompatible, synthetic polymer which is biodegradable or non-biodegradable. The fibers of the scaffold may have a branched configuration extending outwardly from a
5770417	Massachusetts Institute of Technology Children's Medical Center (Cambridge, MA)	6/23/1998	Three-dimensional fibrous scaffold containing attached cells for producing vascularized tissue in vivo	A cell-scaffold composition is prepared in vitro for implanting to produce functional organ tissue in vivo. The scaffold is three-dimensional and is composed of hollow or solid fibers of a biocompatible, synthetic polymer which is biodegradable or non-biodegradable. The fibers of the scaffold may have a branched configuration extending outwardly from a
5842477	Advanced Tissue Sciences, Inc. (La Jolla, CA)	12/1/1998	Method for repairing cartilage	The present invention relates to methods of making and/or repairing cartilage in vivo comprising implanting into a patient, at a site of cartilage damage or loss, a biocompatible, non-living three-dimensional scaffold or framework structure in combination with periosteal/perichondrial tissue that can be used to hold the scaffold in place and provides a
5858747	CytoTherapeutics, Inc.	1/12/1999	Control of cell growth in a bioartificial organ with extracellular matrix coated microcarriers	Methods and compositions are provided for controlling cell distribution within an implantable bioartificial organ by exposing the cells to a treatment that inhibits cell proliferation, promotes cell differentiation, or affects cell attachment to a growth surface within the bioartificial organ. Such treatments include (1) genetically manipulating cells, (2) exposing the
5863984	Universite Laval, Cite Universitaire (Quebec, CA)	1/26/1999	Biostable porous material comprising composite biopolymers	Biomaterials like collagen can be designed for use as scaffolds for connective tissue reconstruction. It is known that proteins conjugated with PEGs exhibit a decrease in their biodegradation rate and their immunogenicity. Different concentrations and molecular weights of PEGs (PEG-750 and PEG-5000) were conjugated by chemical or

5891558	Tissue Engineering, Inc. (Boston, MA)	4/6/1999	Biopolymer foams for use in tissue repair and reconstruction	Single and double density biopolymer foams, composite biopolymer foams including both single and double density foams, and methods of preparing these foams and composite foams are described. Also described are biocompatible constructs which include single or double density biopolymer foams and extracellular matrix particulates and methods of
5916265	Hu; Jie (1752 Ashley Hall Rd., Charleston, SC 29407)	6/29/1999	Method of producing a biological extracellular matrix for use as a cell seeding scaffold and implant	Tissue is procured from a human or animal donor. The tissue may be fixed by application of a fixative. Cellular components which would cause rejection are removed from tissue by a chemical treatment that allows the extracellular matrices (ECM) to retain their original shapes, biological structures and ultrastructures, locations and durability. The resulting ECM
5922025	Bristol-Myers Squibb Company (Skillman, NJ)	7/13/1999	Soft tissue augmentation material	A permanent, biocompatible material for soft tissue augmentation. The biocompatible material comprises a matrix of smooth, round, finely divided, substantially spherical particles of a biocompatible ceramic material, close to or in contact with each other, which provide a scaffold or lattice for autogenous, three dimensional, randomly oriented, non-scar soft
5928945	Advanced Tissue Sciences, Inc. (La Jolla, CA)	7/27/1999	Application of shear flow stress to chondrocytes or chondrocyte stem cells to produce cartilage	Mammalian cells capable of producing cartilage are cultured under a shear flow stress of about 1 to about 100 dynes/cm.sup.2 to produce artificial cartilage for surgical transplantation to replace damaged or missing cartilage. Shear flow stressed cells display enhanced maintenance of chondrocyte phenotype and produce an extracellular matrix containing an
5939323	Brown University (Providence, RI)	8/17/1999	Hyaluronan based biodegradable scaffolds for tissue repair	A hyaluronic acid derivitized scaffold and method of forming are disclosed. The scaffolds are useful for various medical purposes such as tissue repair, tissue reconstruction and wound healing. In order to enhance these processes the scaffolds may be engineered to incorporate biologically active molecules such as BMP
6110487	Keraplast Technologies Ltd. (San Antonio, TX)	8/29/2000	Method of making porous keratin scaffolds and products of same	Methods for producing thin keratin films, sheets, and bulk materials, and products formed using these methods. One method includes providing hair, reducing the hair such that the disulfide linkages are broken and free cysteine thiol groups formed, separating out a more soluble keratin fraction in solution, forming a thin layer from the more soluble
6124265	Keraplast Technologies, Ltd. (San Antonio, TX)	9/26/2000	Method of making and cross-linking keratin-based films and sheets	Methods for producing thin keratin films, sheets, and bulk materials, and products formed using these methods. One method includes providing hair, reducing the hair such that the disulfide linkages are broken and free cysteine thiol groups formed, separating out a more soluble keratin fraction in solution, forming a thin layer from the more soluble
6143293	Carnegie Mellon (Pittsburgh, PA); University of Pittsburgh (Pittsburgh, PA)	11/7/2000	Assembled scaffolds for three dimensional cell culturing and tissue generation	A three-dimensional scaffold for tissue generation. Mechanical fasteners allow layered and volumetric scaffold sections, which may be pre-seeded with cells and/or growth factors, to be assembled into a heterogeneous generated tissue for implantation.

6159495	Keraplast Technologies, Ltd. (San Antonio, TX)	12/12/2000	Porous and bulk keratin bio-polymers	Methods for producing thin keratin films, sheets, and bulk materials, and products formed using these methods. One method includes providing hair, reducing the hair such that the disulfide linkages are broken and free cysteine thiol groups formed, separating out a more soluble keratin fraction in solution, forming a thin layer from the more soluble
6165486	Carnegie Mellon University (Pittsburgh, PA); University of Pittsburgh (Pittsburgh, PA)	12/26/2000	Biocompatible compositions and methods of using same	Blends of biodegradable polymers, preferably poly(caprolactone) and poly(D,L-lactic-co-glycolic) acid are discussed as well as their applications in the medical field, particularly with regard to bone tissue engineering. Preferably, hydroxyapatite ("HA") granules are incorporated into the blends and the resulting blends have desirable mechanical, physical
6218182	Advanced Tissue Sciences (La Jolla, CA)	4/17/2001	Method for culturing three-dimensional tissue in diffusion gradient bioreactor and use thereof	A tissue engineering bioreactor is disclosed for growing three-dimensional tissue. Cells are seeded onto a mesh and provided with two media flows, each contacting a different side of the cells. The media flows contain different concentrations of nutrients, allowing nutrients to be delivered to the cells by diffusion gradient. The bioreactor can be used to grow liver tissue
6228117	IsoTis B.V. (Bilthoven, NL)	5/8/2001	Device for tissue engineering bone	A device for bone tissue engineering is described which comprises a scaffold material consisting of a bioactive, osteoconductive and bone-bonding segmented thermoplastic copolyester and cultured osteogenic or osteoprogenitor cells, especially bone cells. The copolyester consists essentially of a multiplicity of recurring long chain ester units
6281256	The Regents of the University of Michigan (Ann Arbor, MI)	8/28/2001	Open pore biodegradable matrices	The invention is directed to a process for preparing porous polymer materials by a combination of gas forming and particulate leaching steps. The invention is also directed to porous polymer material prepared by the process, particularly having a characteristic interconnected pore structure, and to methods for using such porous polymer material
6306424	Ethicon, Inc. (Somerville, NJ)	10/23/2001	Foam composite for the repair or regeneration of tissue	The present patent describes a biocompatible composite made of a first fibrous layer attached to a three-dimensional inter-connected open cell porous foams that have a gradient in composition and/or microstructure through one or more directions. These composites can be made from blends of absorbable and biocompatible polymers. These biocompatible
6328990	The Trustees of the University of Pennsylvania (Philadelphia, PA)	12/11/2001	Bioactive, degradable composite for tissue engineering	Bioactive, degradable composite material and composite material microspheres were produced using a new solid-in-oil-in-water method. Compositions and methods for tissue engineering and drug delivery are provided.
6333029	Ethicon, Inc. (Somerville, NJ)	12/25/2001	Porous tissue scaffoldings for the repair of regeneration of tissue	The present patent describes a three-dimensional inter-connected open cell porous foams that have a gradient in composition and/or microstructure through one or more directions. These foams can be made from a blend of absorbable and biocompatible polymers that are formed into foams having a compositional gradient transitioning from

6365149	Ethicon, Inc. (Somerville, NJ)	4/2/2002	Porous tissue scaffoldings for the repair or regeneration of tissue	The present patent describes a three-dimensional interconnected open cell porous foams that have a gradient in composition and/or microstructure through one or more directions. These foams can be made from a blend of absorbable and biocompatible polymers that are formed into foams having a compositional gradient transitioning from

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