DOWNLOAD PDF GROWING AN ECM SCAFFOLD

Chapter 1 : Stephen Badylak Laboratory | McGowan Institute for Regenerative Medicine

The slow degradation rate of PCL allows the scaffold to provide support for growing cells for a longer period of time enabling more dense tissue to form. For cases such as closed fractures (broken bone that has not penetrated the skin), PLGA could be a potential candidate for bone regeneration.

The results of such efforts are often much less than satisfactory. There are limited existing treatment options for loss of large masses of muscle tissue domain. Regenerative medicine that could restore functional muscle-tendon tissue, nerves, and blood vessels would be an ideal treatment of traumatic tissue injury. Replace the missing soft tissue. Help restore site appropriate functional tissue. Tissue engineering of lost muscle due to injury uses extracellular matrix ECM. ECM is a natural material with structural proteins that provide mechanical strength and structural support. It also has functional molecules with diverse bioactive properties. Rehab exercises likely provide the needed mechanical signals to encourage cell migration and site-specific differentiation in the temporal framework required for constructive remodeling. Fusion of the clinical and rehab expertise with tissue engineering and regenerative medicine technologies led to the incredible outcomes shared below. Marine Corporal Isaias Hernandez photographed by Scott Lewis, Discover Magazine , then 19, got injured by shrapnel when an enemy mortar exploded nearby. Worst hit was his upper right leg, which was so damaged he could hardly use it. After multiple surgeries using traditional procedures, Corporal Hernandez was considering having the leg amputated. He thought he could function better with a prosthetic leg. Instead, he had the chance to take part in the ECM-based therapy trial. Today, he rides mountain bikes and takes part in other rigorous activities. Sergeant Ron Strang was a Marine with a huge divot in his upper thigh where the quadriceps muscle had been. Walk easily and run on a treadmill. Think of a post-military career as a police officer. Early results with Sergeant Strang and some other patients showed that the animal scaffolding was spurring muscle growth.

Chapter 2 : Growth of human breast tissues from patient cells in 3D hydrogel scaffolds

Acellular ECM scaffold. A commercially available intact porcine small intestinal submucosal extracellular matrix (SIS-ECM) (CorMatrix-ECM, CorMatrix Cardiovascular Inc., Roswell, Georgia) was used.

Received Oct 28; Accepted Jan This article has been cited by other articles in PMC. Abstract Background Three-dimensional 3D cultures have proven invaluable for expanding human tissues for basic research and clinical applications. In both contexts, 3D cultures are most useful when they 1 support the outgrowth of tissues from primary human cells that have not been immortalized through extensive culture or viral infection and 2 include defined, physiologically relevant components. Here we describe a 3D culture system with both of these properties that stimulates the outgrowth of morphologically complex and hormone-responsive mammary tissues from primary human breast epithelial cells. Methods Primary human breast epithelial cells isolated from patient reduction mammoplasty tissues were seeded into 3D hydrogels. The hydrogel scaffolds were composed of extracellular proteins and carbohydrates present in human breast tissue and were cultured in serum-free medium containing only defined components. The physical properties of these hydrogels were determined using atomic force microscopy. Tissue growth was monitored over time using bright-field and fluorescence microscopy, and maturation was assessed using morphological metrics and by immunostaining for markers of stem cells and differentiated cell types. The hydrogel tissues were also studied by fabricating physical models from confocal images using a 3D printer. The mature tissues contained luminal, basal, and stem cells in the correct topological orientation and also exhibited the complex ductal and lobular morphologies observed in the human breast. The expanded tissues became hollow when treated with estrogen and progesterone, and with the further addition of prolactin produced lipid droplets, indicating that they were responding to hormones. Ductal branching was initiated by clusters of cells expressing putative mammary stem cell markers, which subsequently localized to the leading edges of the tissue outgrowths. Ductal elongation was preceded by leader cells that protruded from the tips of ducts and engaged with the extracellular matrix. Conclusions These 3D hydrogel scaffolds support the growth of complex mammary tissues from primary patient-derived cells. We anticipate that this culture system will empower future studies of human mammary gland development and biology. Electronic supplementary material The online version of this article doi: Background The ability to grow human tissues in three-dimensional 3D cultures has proven useful, both for regenerative medicines and for studies of tissue development. For mammary tissue, collagen matrices were first introduced four decades ago for growing mammary spheroids from primary mouse epithelial cells [5 , 6]. Subsequently, Barcellos-Hoff and colleagues developed a basement membrane Matrigel culture in which mouse epithelial cells generated ducts and lobules, enabling the first studies of mammary morphogenesis in vitro [7]. While these and similar 3D cultures have contributed valuable insights [8 â€” 13], the biology of mouse mammary tissue is known to differ in significant ways from its human counterpart [14 , 15]. To address this issue, investigators have developed 3D cultures that support organoid growth from human cell lines that have been immortalized by transduction with viral oncogenes [16 â€” 18]. However, growing tissues from primary human mammary cells has proven to be more challenging. Ductal growth was also limited in 3D cultures of primary human cells seeded into collagen or basement membrane Matrigel [20 , 21]. The extracellular matrix ECM plays a critical role in regulating the development and maintenance of epithelial tissues. The ECM of human breast tissue is a complex mixture of protein fibrils interwoven within a network of glycosaminoglycan carbohydrate chains. From a structural perspective, the protein components, including laminins, fibronectin, and collagens, provide resistance to tensile forces, while the carbohydratesâ€”composed primarily of hyaluronan chainsâ€”chelate water and provide resistance to compressive forces. To more fully reflect this complexity, we engineered a hydrogel scaffold that incorporated both the protein collagen, laminins, and fibronectin and carbohydrate components hyaluronan of human breast tissue. When seeded into these hydrogels, primary mammary epithelial cells isolated from patient breast tissues self-organized, expanded, and differentiated to form mature mammary tissues. We anticipate that these cultures will prove useful in future investigations of human mammary tissue morphogenesis and biology.

Methods Ethics statement Primary tissues that would otherwise have been discarded as medical waste following surgery were obtained in compliance with all relevant laws, using protocols approved by the institutional review board at Maine Medical Center. All tissues were anonymized before transfer and could not be traced to specific patients; for this reason, this research was provided exemption status by the Committee on the Use of Humans as Experimental Subjects at the Massachusetts Institute of Technology. All patients enrolled in this study signed an informed consent form to agree to participate in this study and for publication of the results. Epithelial clusters were disrupted by trituration, washed, and depleted for fibroblasts. The identical procedure was used to prepare mouse mammary epithelial tissues. Preparation of hydrogels Hydrogels were composed of 1. Structures were passaged from one hydrogel to another by dissolving the pad with collagenase and reseeding the structure as if it were a primary tissue fragment. Lentivirus production Lentivirus production was performed as previously described [22]. Lentiviral gene ontology vectors were kindly provided by Kristoffer Riecken [23]. Immunofluorescence and immunohistochemistry Immunofluorescence was performed as previously described [24]. Results Design of hydrogels with features of human breast tissue Because we were interested in engineering a 3D scaffold that could stimulate the growth of human breast tissues, we explored hydrogel formulations that contained protein and glycosaminoglycan components found in the ECM of human breast tissue. We focused our efforts on ECM hydrogels with defined components and evaluated various hydrogel formulations by assessing their ability to support the growth of primary human breast tissue fragments. The seeded tissue fragments contained 50â€” cells per fragment and were harvested by dissociating breast tissues from patient reduction mammoplasties Additional file 2: Through heuristic optimization, we established a novel hydrogel formulation that supported the growth of human breast tissues Fig. These hydrogels had several features that were important for supporting breast tissue growth:

Chapter 3 : An ECM-Mimicking, Mesenchymal Stem Cell-Embedded Hybrid Scaffold for Bone Regeneratio

Although MM cells grown on the DBT-TMS have richer ECM protein support than the cells on the other types of scaffolds tested, they may need to adapt to the tissue ECM condition to establish a fit cancer cell ECM environment.

Cells as building blocks[edit] Stained cells in culture Tissue engineering utilizes living cells as engineering materials. Examples include using living fibroblasts in skin replacement or repair, cartilage repaired with living chondrocytes , or other types of cells used in other ways. Cells became available as engineering materials when scientists at Geron Corp. Extraction[edit] From fluid tissues such as blood , cells are extracted by bulk methods, usually centrifugation or apheresis. From solid tissues, extraction is more difficult. Usually the tissue is minced, and then digested with the enzymes trypsin or collagenase to remove the extracellular matrix ECM that holds the cells. After that, the cells are free floating, and extracted using centrifugation or apheresis. Digestion with trypsin is very dependent on temperature. Higher temperatures digest the matrix faster, but create more damage. Collagenase is less temperature dependent, and damages fewer cells, but takes longer and is a more expensive reagent. Types of cells[edit] Mouse embryonic stem cells Cells are often categorized by their source Autologous cells are obtained from the same individual to which they will be reimplanted. Autologous cells have the fewest problems with rejection and pathogen transmission, however in some cases might not be available. For example, in genetic disease suitable autologous cells are not available. Also very ill or elderly persons, as well as patients suffering from severe burns, may not have sufficient quantities of autologous cells to establish useful cell lines. Moreover, since this category of cells needs to be harvested from the patient, there are also some concerns related to the necessity of performing such surgical operations that might lead to donor site infection or chronic pain. Autologous cells also must be cultured from samples before they can be used: Recently there has been a trend towards the use of mesenchymal stem cells from bone marrow and fat. These cells can differentiate into a variety of tissue types, including bone , cartilage , fat , and nerve. A large number of cells can be easily and quickly isolated from fat, thus opening the potential for large numbers of cells to be quickly and easily obtained. Allogeneic cells come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for in vitro studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin. Xenogenic cells are these isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants. Syngenic or isogenic cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models. Primary cells are from an organism. Secondary cells are from a cell bank. Stem cells are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells. According to their source stem cells are divided into "adult" and "embryonic" stem cells, the first class being multipotent and the latter mostly pluripotent ; some cells are totipotent , in the earliest stages of the embryo. While there is still a large ethical debate related with the use of embryonic stem cells, it is thought that another alternative source - induced stem cells may be useful for the repair of diseased or damaged tissues, or may be used to grow new organs. Scaffolds[edit] Scaffolds are materials that have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues for medical purposes. Scaffolds mimic the extracellular matrix of the native tissue, recapitulating the in vivo milieu and allowing cells to influence their own microenvironments. They usually serve for at least one of the following purposes: In , an interdisciplinary team led by the thoracic surgeon Thorsten Walles implanted the first bioartificial transplant that provides an innate vascular network for post-transplant graft supply successfully into a patient awaiting tracheal reconstruction. Carbon nanotubes are among the numerous candidates for tissue engineering scaffolds since they are biocompatible , resistant to biodegradation and can be functionalized with biomolecules. However, the possibility of toxicity with non-biodegradable nano-materials is not fully understood. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is often an essential factor since scaffolds should preferably be

absorbed by the surrounding tissues without the necessity of a surgical removal. The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation: Injectability is also important for clinical uses. Recent research on organ printing is showing how crucial a good control of the 3D environment is to ensure reproducibility of experiments and offer better results. Materials[edit] Many different materials natural and synthetic, biodegradable and permanent have been investigated. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen and some polyesters. New biomaterials have been engineered to have ideal properties and functional customization: PuraMatrix, originating from the MIT labs of Zhang, Rich, Grodzinsky and Langer is one of these new biomimetic scaffold families which has now been commercialized and is impacting clinical tissue engineering. A commonly used synthetic material is PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid , a naturally occurring chemical which is easily removed from the body. While these materials have well maintained mechanical strength and structural integrity, they exhibit a hydrophobic nature. This hydrophobicity inhibits their biocompatibility, which makes them less effective for in vivo use as tissue scaffolding. By combining the two different types of materials, researchers are trying to create a synergistic relationship that produces a more biocompatible tissue scaffolding. Proteic materials, such as collagen or fibrin , and polysaccharidic materials, like chitosan [23] or glycosaminoglycans GAGs , have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Among GAGs hyaluronic acid , possibly in combination with cross linking agents e. Functionalized groups of scaffolds may be useful in the delivery of small molecules drugs to specific tissues. Recently a range of nanocomposites biomaterials are fabricated by incorporating nanomaterials within polymeric matrix to engineer bioactive scaffolds. Upon deconstruction, these sheets can be useful in cell-based high-throughput screening and drug discovery. Each of these techniques presents its own advantages, but none are free of drawbacks. Nanofiber self-assembly[edit] Molecular self-assembly is one of the few methods for creating biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix ECM , a crucial step toward tissue engineering of complex tissues. Textile technologies[edit] These techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers. In particular, non-woven polyglycolide structures have been tested for tissue engineering applications: The principal drawbacks are related to the difficulties in obtaining high porosity and regular pore size. Solvent casting and particulate leaching[edit] Solvent casting and particulate leaching SCPL allows for the preparation of structures with regular porosity, but with limited thickness. First, the polymer is dissolved into a suitable organic solvent e. Such porogen can be an inorganic salt like sodium chloride , crystals of saccharose , gelatin spheres or paraffin spheres. The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast the solvent is allowed to fully evaporate, then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen: Once the porogen has been fully dissolved, a porous structure is obtained. Other than the small thickness range that can be obtained, another drawback of SCPL lies in its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold. Gas foaming[edit] To overcome the need to use organic solvents and solid porogens, a technique using gas as a porogen has been developed. First, disc-shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold. The discs are then placed in a chamber where they are exposed to high pressure CO2 for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge-like structure. The main problems resulting from such a technique are caused by the excessive heat used during compression molding which prohibits the incorporation of any temperature labile material into the polymer matrix and by the fact that the pores do not form an interconnected structure. Emulsification freeze-drying[edit] This technique does not require the use of a solid porogen like SCPL. First, a synthetic polymer is dissolved into a suitable solvent e. Before the two phases can separate, the emulsion is cast into a

mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure. While emulsification and freeze-drying allow for a faster preparation when compared to SCPL since it does not require a time consuming leaching step , it still requires the use of solvents. Moreover, pore size is relatively small and porosity is often irregular. Freeze-drying by itself is also a commonly employed technique for the fabrication of scaffolds. In particular, it is used to prepare collagen sponges: Thermally induced phase separation[edit] Similar to the previous technique, the TIPS phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example, dioxane could be used to dissolve polylactic acid, then phase separation is induced through the addition of a small quantity of water: Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent, a porous scaffold is obtained. In a typical electrospinning set-up, a solution is fed through a spinneret and a high voltage is applied to the tip. The buildup of electrostatic repulsion within the charged solution, causes it to eject a thin fibrous stream. A mounted collector plate or rod with an opposite or grounded charge draws in the continuous fibers, which arrive to form a highly porous network. The primary advantages of this technique are its simplicity and ease of variation. At a laboratory level, a typical electrospinning set-up only requires a high voltage power supply up to 30 kV , a syringe, a flat tip needle and a conducting collector. For these reasons, electrospinning has become a common method of scaffold manufacture in many labs. By modifying variables such as the distance to collector, magnitude of applied voltage, or solution flow rateâ€”researchers can dramatically change the overall scaffold architecture. Historically, research on electrospun fibrous scaffolds dates back to at least the late s when Simon showed that electrospinning could be used to produced nano- and submicron-scale fibrous scaffolds from polymer solutions specifically intended for use as in vitro cell and tissue substrates. This early use of electrospun lattices for cell culture and tissue engineering showed that various cell types would adhere to and proliferate upon polycarbonate fibers. It was noted that as opposed to the flattened morphology typically seen in 2D culture, cells grown on the electrospun fibers exhibited a more rounded 3-dimensional morphology generally observed of tissues in vivo. First, a three-dimensional structure is designed using CAD software. The porosity can be tailored using algorithms within the software. LaBP arranges small volumes of living cell suspensions in set high-resolution patterns. As of this study, only human skin tissue has been synthesized, though researchers project that "by integrating further cell types e. Engineered tissues generally lack an initial blood supply, thus making it difficult for any implanted cells to obtain sufficient oxygen and nutrients to survive, or function properly.

Chapter 4 : Multi-Spheroid Assays - Applications | Essen Bioscience Applications | IncuCyte System

The extracellular matrix (ECM) plays a critical role in regulating the development and maintenance of epithelial tissues. The ECM of human breast tissue is a complex mixture of protein fibrils interwoven within a network of glycosaminoglycan carbohydrate chains.

This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Abstract Articular cartilage lesions are a particular challenge for regenerative medicine due to cartilage low self-ability repair in case of damage. Hence, a significant goal of musculoskeletal tissue engineering is the development of suitable structures in virtue of their matrix composition and biomechanical properties. The objective of our study was to design in vitro a supporting structure for autologous chondrocyte growth. Introduction Cartilage degeneration, due to congenital abnormalities or disease and trauma, represents a major health problem of great clinical consequence [1 , 2]. In case of damage, cartilage is not capable of healing as it is an avascular and aneural tissue; moreover, its cellular components, chondrocytes, have low mitotic ability [3 , 4]. Cartilage lesions are generally believed to progress to severe forms of osteoarthritis [5 , 6], leading to pathologic changes in the joints with consequent pain, inflammation, and functional disability [7 , 8]. Injuries which reach the subchondral bone may induce a systemic reaction and generate reparative tissue. Although type II collagen may be produced by this reparative tissue, it consists predominantly of type I collagen, resulting in the formation of fibrocartilage which does not have the biomechanical properties of articular cartilage [9]. The poor regenerative potential of cartilage and the unsatisfactory current clinical therapies have led to the research of strategies providing solutions to the treatment of focal defects [10 , 11]. An emerging and promising field for the generation of tissue substitutes is tissue engineering. The basic approach to tissue engineering depends upon the interaction between cells, scaffolds, and signalling factors to create in vitro a biological tissue construct to implant in vivo mimicking the tissue of interest; engineering cartilage is no exception to this approach [1 , 12 , 13]. Amongst synthetic biomaterials, hydrogels have demonstrated their ability to simulate human tissue better than any other class. In particular, physically cross-linked poly vinyl alcohol PVA hydrogels are attractive tools in cartilage tissue engineering as they have a viscoelastic behaviour comparable with that of articular and meniscal cartilage. PVA hydrogels are physically cross-linked through freeze-thaw FT cycles: PVA chains come into close contact with each other and crystallite formation as well as hydrogen bonding occurs. These interactions remain intact after thawing and create a nondegradable 3D hydrogel network. It is possible to tailor mechanical properties of the hydrogel acting on the number of FT cycles [15]. However, despite PVA biocompatibility, its low protein adsorption property results in low cell adhesion compared with other hydrogels [3]. In the body, cells are embedded in the extracellular matrix ECM which is made up of protein fibres interwoven in a network of glycosaminoglycan GAG chains. The ECM influences cellular responses like survival, development, and behaviour by interacting with cellular adhesion molecules, growth factors, binding proteins, proteolytic enzymes, and enzyme inhibitors [16]. Hence, ECM has been successfully used as a scaffold for constructive remodelling of multiple tissues in both preclinical studies and in human clinical applications [17]. However, despite ECM, derived scaffolds offer promising regenerative responses in many settings; in some applications, more robust and long lasting mechanical properties are necessary [18]. A composite scaffold, strong and bioactive, may represent an interesting solution to this problem. In this work, we have investigated how to realize a scaffold able to sustain articular cartilage regeneration. Materials and Methods 2. Scaffold Manufacture Three different scaffold groups were investigated to analyse their ability in sustaining chondrocytes adhesion and proliferation: The PVA solution was then slowly cooled down to room temperature. Finally, a volume of 0. For composite scaffolds, ECMs were gained from umbilical cord and cartilage samples collected after obtaining informed consent of donors. Fragments were then decellularized according to the detergent-enzymatic method by Meezan and collaborators [19]. This stage was led in an ice bath. For total protein quantitation, 1 mL of each homogenate was analysed as described in Section 2. A

freeze-thaw treatment was used to physically cross-link the hydrogel and to embed the lyophilized matrix upon it. The samples were cut with a rectangular shape and size of. The samples were fixed to the machine by means of clamps. Samples were fixed with 2. The total protein amount was determined using a standard curve for bovine serum albumin BSA. In parallel, acellular samples were stained with Movat pentachromic and Masson trichromic kits to assess the maintenance of structural properties. Cartilage Harvest and Chondrocyte Isolation Noncalcified human articular cartilage samples were collected from 3 donors who underwent total knee arthroplasty; only tissue from joints without signs of degenerative changes was used. The resulting cell suspension was collected and centrifuged at rpm for 5 min. Isolated cells were then seeded on 25 cm2 flasks BD Falcon at high density with complete medium as described below. The medium was changed at the sixth day and then every days. Finally, band intensities were quantitated by densitometry, using Image J software. Immunophenotype Characterization Flow cytometry analysis was performed to identify chondrocyte specific immunophenotype. Labeling occurred in 15 minutes at RT, in the dark. Isotypic antibodies served as controls. Data were analysed by Flowing Software 2 and results were expressed as percentage of positive cells compared to the isotype negative control. Antibodies used for flow cytometry. Chondrocyte Culture on Scaffolds Primary human chondrocytes from passage 1, isolated and cultured as previously described, were used for seeding on scaffolds. Evaluation of Proliferative Activity After 24 h and 7 and 14 days from seeding on scaffolds, cells were treated with 3- 4,5-dimethylthiazolyl -2,5-dimethyltetrazolium bromide MTT 0. Formazan precipitates were dissolved in 2-propanol acid 0. Results were expressed as number of cells grown on seeded surface. Statistical Analysis -test to determine the statistical significance of the data. The stretching and relaxation curves of both biomaterials are represented by stress-strain profiles in Figure 1. No statistically significant difference was found between samples of each study group Figure 3. Histological evaluation of decellularized ECMs b, d, f, h, l, and n versus native tissues a, c, e, g, i, and m. In particular, both matrices mainly consist of collagen fibers and mucus, as shown by the green staining of native Figures 4 c and 4 i and decellularized Figures 4 d and 4 l tissues. Moreover, in native AC Figure 4 m and acellular ECMs Figures 4 f and 4 n , blue and yellow colors indicate the presence of mucins and collagen fibers, respectively. Chondrocyte Monolayer Cultures Freshly isolated chondrocytes were small and round and they were initially grown as a suspension culture. Six days after AC enzymatic digestion, adherent cells were observed to spread across the flask and demonstrated clear boundaries and distinct nuclei Figure 5 a. In the subcultures, at a subconfluence state, chondrocytes showed the classic round or polygonal shape with small membrane extroflessions Figure 5 b. Chondrocytes were expanded in culture up to passage 4; hereafter, their proliferation rate started to decrease and their morphology changed to elongated fibroblast-like phenotype. Morphological analysis by optical microscopy of human AC chondrocytes at passages 0 a, c and 4 b, d at a subconfluent a, b and confluent c, d state. Characterization of Isolated Chondrocytes Before seeding on 3D scaffolds, isolated human chondrocytes were characterized for the expression of specific cartilage markers. As shown in Figure 6 b , densitometry quantitated band intensities were corrected for loading using housekeeping gene HPRT1 as a control and graphed as a ratio of HPRT1. Relative expression of target genes is referred to HPRT1 expression. To define the immunophenotype of AC chondrocytes, cell surface molecules expressed on cells obtained from 3 different donors age range 32â€”85; mean Chondrocytes of each donor were cultured for 2 weeks in monolayer and passages 1 and 2 were investigated. The analysed cell surface molecules were classified into different categories according to their function: Chondrocytes subcultures were positive for CD44 Immunophenotype evaluation of AC chondrocytes by flow cytometry. Data are expressed as percentage of positive cells blue profile compared to isotypic control black profile. According to SEM micrographs Figure 8 , on PVA scaffolds, any cell was visible since 24 h from seeding Figure 8 a ; even at days 7 and 14 Figures 8 d and 8 g , no cell adhesion and proliferation was observable. At day 14, chondrocytes extensively colonised both scaffold surfaces, forming a homogeneous monolayer Figures 8 h and 8 i. Evaluation of AC chondrocyte growth on 3D scaffolds by scanning electron microscopy. Cell cultures were analysed 24 h a, b, c , 7 d d, e, f , and 14 d g, h, i from seeding. Cell growth on tissue culture-treated polystyrene plates was considered as internal proliferation control Ctrl. Data are average of three independent experiments: Discussion Articular hyaline cartilage is a soft tissue; it sustains the pressure between the hard ends of bones and it is subjected to

particularly complex loads affecting its development and maintenance in the body [20 , 21]. Because of its limited self-healing capacity, as it is an avascular and aneural tissue, even minor cartilage defects lead to mechanical joint instability and progressive damage [21 , 22]. Cartilage damage is difficult to treat. Until now, many approaches have been investigated: A general drawback of these therapeutic strategies is that the newly formed tissue lacks the structural organization of cartilage; it has inferior mechanical properties compared to native tissue, and it is, therefore, prone to failure [21 , 24]. Hence, the goal is to produce a repair tissue that has the same functional and mechanical properties of hyaline articular cartilage [25]. Cartilage restoration represents a challenge of musculoskeletal tissue engineering; despite that, the use of matrix scaffolds has paved the way for the use of functional tissue substitutes in the treatment of cartilage defects [22]. A wide range of natural and synthetic materials have been investigated as scaffolding for cartilage repair [26]. Natural scaffolds may face problems of immunogenic compatibility and batch inconsistency, while the properties offered by synthetic matrices provide much promise in the future of articular cartilage repair [25]. Amongst synthetic biomaterials, physically cross-linked PVA hydrogels become suitable for soft tissue applications: Mechanical properties of the gel can be modulated acting on different variables: As proved by stress-strain profiles presented, it did not maintain the residual strain when subjected to tensile strength, revealing high elasticity. However, cell adherence on PVA hydrogels is inhibited by its highly hydrophilic nature [28]. Many authors demonstrated ECM-based scaffold efficacy in creating a more suitable microenvironment to sustain cellular adhesion. Extracellular matrix is a reservoir of structural and functional proteins like collagens, glycoproteins, proteoglycans, mucins, and elastic fibres as well as a known repository for a variety of growth factors. As in vivo it is progressively degraded by proteinases, it can result in the exposure of new recognition sites with potent bioactivity [29]. Our aim was to provide a supportive biomimetic microenvironment for chondrocytes to produce articular cartilage, taking advantage of both PVA and ECM. In particular, we considered an alternative matrix source: The research of a new biological ECM useful in cartilage restoration arises from the need to identify an easily available resource suitable in sustaining chondrocytes adhesion and proliferation, even if not specific. Every tissue and organ contains an ECM with unique composition that consists of the secreted products of resident cells [29].

Chapter 5 : CAA1 - Biodegradable scaffold with ecm material - Google Patents

Research on Using an ECM Scaffold Implant to Restore Tissue. Grow new muscle. Walk easily and run on a treadmill. Think of a post-military career as a police officer.

Hereby it is possible to combine the range of physical properties the scaffold can offer with the reconstructive properties of the ECM. The optimal amount of discrete ECM material for each application is disclosed and this concentration is equally distributed in the dressing hence avoiding unnecessary high concentrations of ECM. In addition to the effect of the ECM, the porous structure of the base material provides the cells with a structure for in-growth. To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell growth and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is essential since scaffolds need to be absorbed by the surrounding tissues without the necessity of a surgical removal. Many different materials natural and synthetic, biodegradable and permanent have been investigated for use as scaffolds. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen or some linear aliphatic polyesters. However, when testing laboratory made scaffolds in vivo, it is often seen, that the cells do not grow readily into these scaffolds, maybe due to the fact that no biological signal molecules, e. In order to improve the biological properties of the scaffolds and to accelerate wound healing, several labs have added growth factors to a synthetic scaffold and seen beneficial effects on wound healing. In all of these publications a single growth factor has been incorporated in a sheet or hydrogel. Acellular extracellular matrices ECM from warm-blooded vertebras are used extensively in tissue engineering and plastic surgery 8. It has been shown that acellular ECM contains several growth factors ECMs contain a lot of biologic molecules and it has been shown that cells readily populate these sheets of concentrated ECM 12; The ECMs on the market today are of human or porcine origin. The cells are removed from the tissue and the tissue is subsequently lyophilized and cut into sheets. The sheets of porcine origin come in different sizes. The price of these sheets is very high. The sheets are fairly stiff when un-hydrated. An example is the sheets from the company Acell. These products are in the form of sheets or hydrogels. The sheets provide both a scaffold as well as a complex mixture of proteins to the cells of the wound. We demonstrate that when using scaffolds containing ECM material, higher concentrations of ECM surprisingly do not give better cell morphology. In addition it is shown that by varying the concentration of discrete ECM material in scaffolds the physical characteristic of the scaffold changes but that the changes are depending on the material of the scaffold. The present application takes this knowledge to the patient by showing a sterilisation strategy that maintains the biological activity of the ECM material after sterilisation. By adding discontinuous regions of ECM to a scaffold it is possible to combine the range of physical properties e. In addition, the price of such scaffold will be lower than other ECM scaffolds both because the powder is a waste-product from the production of acellular ECM sheets and because the optimal amount of discrete ECM material for each application can be determined and equally distributed in the dressing hence avoiding unnecessary high concentrations of ECM. In one embodiment a discontinuous region of ECM is obtained by adding discrete ECM material, such as particles, flakes, fibres or powder. A discrete phase of ECM material means material of ECM that is distinguished in their form and density from the ground material that they are embedded in. This can be demonstrated by histology sections as seen in example 5 or by scanning electron microscope SEM seen in example 6. As shown in the examples e. It is preferred, that the ECM material is added to the scaffold before scaffold formation e. In this way, the ECM material is homogeneously distributed in the scaffold. That is, in the time it takes to solidify the scaffold e. This is a huge clinical advantage as there is nothing to remove from the wound. Thus, the newly formed tissue is not disturbed or stressed by removal of the temporary scaffold. It is typically preferred that the scaffold is broken down during 1 day to 10 weeksâ€”depending on the application. For open wound applications, it is preferred that the scaffold is broken down during days, such as days. In one aspect of the invention, the scaffold is biodegradable. In one embodiment the scaffold is a continuous scaffold. That is a scaffold of a continues

phase. A continuous scaffold with discontinuous regions results in a composite material. As with other composite materials, this is an engineered material made from two or more constituent materials with significantly different physical or chemical properties and which remains separate and distinct within the finished structure. Extracellular matrix ECM is the non-cellular portion of animal or human tissues. The ECM is hence the complex material that surrounds cells. Consequently, it is preferred that the discontinuous regions of ECM are cell free regions. Layers of cells can be removed physically by e. Detergents and enzymes may be used to detach the cells from one another in the tissue. Water or other hypotonic solutions may also be used, since hypotonicity will provoke the cells in the tissue to burst and consequently facilitate the decellularization process. Another way to obtain cell free regions is by adding the ECM powder discontinuous regions of ECM to the scaffold matrix. A cell-free product minimizes the risk any immune rejection once implanted, since components of cells may cause an immunogenic response. In broad terms there are three major components in ECMs: ECMs are known to attract cells and to promote cellular proliferation by serving as a reservoir of growth factors and cytokines 9; A temporary scaffold containing particulate ECMs used in a wound will be populated by cells both from the wound edges as well as cells from the circulating blood. As the cells invade the scaffold, the scaffold material will be degraded and eventually the scaffold will be replaced with new tissue. The skin of humans comprises an upper layer of epidermis, formed by inter alia keratinocytes. Below epidermis is dermis, formed by inter alia fibroblasts, but also endothelial cells. When promoting growth of fibroblasts, the present examples e. The wound dressing of the present invention may comprise multiple layers. These layers could include 1 or more layers of biodegradable material, which all optionally comprise ECM. If ECM is incorporated in more than one layer the dose may vary across the layers. In another embodiment, the scaffold is designed for growth stimulation of different cell-types. It is our experience, that when promoting growth of fibroblasts, the growing fibroblasts will excrete growth factors inducing growth of keratinocytes. Hereby, fibroblast growth is promoted such that keratinocyte growth is subsequently promoted and the wound is healed. In a dissolvable scaffold e. After freezedrying, the material is weighted. In a non-dissolvable scaffold the material is embedded in an appropriate embedding material e. Using image analysis the amount of ECMs are calculated in relation to scaffold. The ECM may include the basement membrane, which is made up of mostly type IV collagen, laminins and proteoglycans. The ECM material of the invention is preferably prepared from tissue harvested from animals raised for meat production, including but not limited to, pigs, cattle and sheep. Other warm-blooded vertebrates are also useful as a source of tissue, but the greater availability of such tissues from animals used for meat production makes such tissue preferable. Pigs that are genetically engineered to be free of the galacatosyl, alpha 1,3 galactose GAL epitope may be used as the source of tissues for production of the ECM material. In a preferred embodiment the ECM will be of porcine origin. The ECM material can be obtained from any animal. It could be derived from, but not limited to, intestinal tissue, bladders, liver, spleen, stomach, lymph nodes or skin. Human tissue is preferably avoided to minimize transfer of diseases. Thus, in a preferred embodiment the discontinuous regions of ECM are obtained from animal tissues. Due to species similarity, it is preferred to use ECM from warm-blooded mammal. One aspect of the invention is to provide a scaffold with constant dosing of growth factors. One property of the scaffold used in the present invention is to distribute the discontinuous regions of ECM within the porous base material, such that the ECM is accessible for the cells. When the cells migrate through the scaffold matrix, the discontinuous regions of ECM are exposed to protease activity and degraded which are believed to result in release of the biologically active components from the discontinuous regions of ECM Thus, the release of biologically active components can be kept somewhat constant throughout the period of use, thereby providing a somewhat constant dosing to the wound bed and cells. In one embodiment, the discontinuous regions of ECM are equally distributed within the temporary scaffold. ECM comes in several micronized forms: All of these are considered discontinuous regions of ECM, i. This is determined by a Mastersizer from Malvern Instrument for volume weighted mean. By distributing the discontinuous regions of ECM in a porous scaffold, it is possible to optimise the physical properties e. In order to obtain both the beneficial effect of the ECMs, and the physical properties a porous scaffold can offer, particulate ECM can be included in a wound dressing such as a scaffold and be used for tissue engineering e. This porous scaffold

should preferably be of a material that is biodegradable. The temporary scaffold may be either in a lyophilised form, in a fibrous form woven or non-woven , in a foamed form or as a film. The material used for the scaffold may be any biodegradable material, from both synthetic and of natural sources. Of the scaffolds constructed from natural materials, particular preferred are those based on derivatives of the extracellular matrix. Examples of such materials are protein materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans GAGs. In one embodiment the biodegradable scaffold is made of protein containing substances. This will enable degradation by proteolytic enzymes. Such scaffolds are preferably made of proteins such as collagen, keratin, fibrin, elastin, laminin, vimentin, vitronectin, fibronectin, fibrinogen and derivatives of these and the like or denaturated proteins such as gelatin. By making scaffolds using polymer materials such as gelatin, fibrin, hyalouronic acid, collagen, chitin, chitosan, keratin, alginate, PLA and PLGA it is possible to vary the scaffolds physical characteristics strengths, softness, flexibility through combinations and modifications. This will enable degradation by hydrolysis and enzymatic degradation of the polysaccharides.

Chapter 6 : Custom-Made Body Parts: Advances in Tissue Engineering - Science in the News

To facilitate rapid clinical translation of an ECM scaffold approach to esophagus reconstruction, a study was designed to specifically evaluate the efficacy of an ECM scaffold in a surgical procedure that is currently performed, and accepted, namely the "gastric pull-up" procedure [5].

By Dr Kulwinder S. Similar surgery is also required for children born with long-segment esophageal atresia. Re-growing the esophagus to restore luminal and functional continuity would be ideal for these patients. De-novo organogenesis is possible using principles of regenerative medicine, as validated mostly in animal studies [2]. Researchers have successfully re-grown the human urinary bladder and the trachea [4, 5]. These techniques can be demanding in terms of expertise, cost, regulations, and time. Attempts to re-grow the human esophagus have so far been limited to only partial-thickness defects, like after endoscopic sub-mucosal dissection ESD or to patchy, non-circumferential transmural defects like perforations and leaks []. To re-grow the mucosa, Okhi et al. Small disks of these autologous cell-sheets were endoscopically implanted onto the raw esophageal surface immediately after ESD. Only one of the 9 patients in this cohort developed a stricture [7]. Extracellular matrix ECM and autologous stromal cell therapy have also been successfully used to prevent stricture formation after ESD [6, 8, 10, 11]. Similar to partial-thickness defects, full-thickness patchy defects like perforation and leaks have also been successfully treated in human beings with devices like expandable stents, clips and suturing [9, 12, 13]. Interestingly, the majority of these lesions heal with minimal need for additional measures, such as applying tissue matrix with or without autologous pluripotent cells. Re-growing the human esophagus after long-segment, full-thickness, circumferential LFC defects, such as those present after an esophagectomy, has not been attempted until recently [14]. All previous studies concerning re-growth of LFC esophageal defects were done in animal models. The basic principles involved were transplanting ECM molded into a tubular configuration into the recipient animal after populating it with autologous pluripotent cells with or without using a non-biological scaffold for example, a silicone stent ; to maintain the three-dimensional configuration of the esophagus during the slow process of regeneration. Since the pluripotent cells are autologous, immunosuppression to prevent organ rejection is not required. Whether allogenic or xenogeneic, ECM is ideal for tissue re-generation. They provide a biological scaffold on which pluripotent cells can spatially mature into site-specific phenotypic cells resulting in de-novo organogenesis. Suggested mechanisms include release of peptides, ligands and bioactive molecules to attract endogenous stem-cells, alter immune response, induce mitosis, and provide signals to local and migrant cells to mature into a site-specific organ [2, 15, 16]. Porcine derived ECM molded into a tubular shape was used to bridge the defects [17]. Dogs were sacrificed at various intervals and histology confirmed re-growth of all the layers of the esophageal wall. Until recently, no attempts have been made to re-grow the human esophagus using principles of regenerative medicine as validated in animal models. Availability of less than ideal alternatives like gastric pull-up conduits and the ethical concern on the uncertainty of outcomes using experimental techniques may have been the reason for this. Recently, Dua et al. The year-old patient presented with a life-threatening abscess that led to a direct communication between the hypopharynx and the mediastinum. Earlier in his life, the patient had been in a car accident that required stabilization of the cervical spine with metal plates. The anterior metal plate had eroded into the hypopharynx that resulted in the infection and abscess formation. Several attempts at surgical repair failed and gastric pull-up was not possible due to the high location of the esophageal defect. On compassionate grounds, the authors attempted to re-grow a 5 cm LFC defect in the esophagus that extended up to the upper esophageal sphincter level. They used off-the-shelf, readily available, FDA approved for-human-use material. The three-dimensional configuration of the esophagus was maintained by three non-biological fully-covered expandable esophageal stents with the upper end of the proximal stent extending into the hypopharynx. The dermal side of the matrix that attracts blood was oriented towards the mediastinum to facilitate neo-vascularization and the epithelial side that repels blood was oriented towards the lumen. Being autologous PRP, there was no risk of transmitting blood-borne infections. Platelets are known to stimulate growth and regeneration, by releasing platelet-derived growth

factors, transforming growth factors, and vascular endothelial growth factors that attract mesenchymal stem-cells, endothelial cells and epidermal cells, which express receptors for these growth factors []. Initially, the patient refused to give permission to remove the stents for fear of fistula and stricture formation. Eventually all the stents were removed over three-and-half years after placement. After almost 5 years since the stents were removed, the patient is now eating a normal diet and maintaining his weight. An added attraction of this case was that rather than using expensive tissue-engineering techniques, using non-FDA approved material followed by surgical transplantation, the authors used readily available, off-the shelf FDA approved materials implanted in-vivo. Research is actively going on in testing various biomaterials seeded with autologous pluripotent cells. Based on the lessons learnt and questions raised from this case, large-scale animal studies, followed by phase I and II clinical trials, will be required. If the results can be replicated, it will have a large impact on patients requiring esophagectomy [14]. J Am Coll Surg, Gilbert, Extracellular matrix as a biological scaffold material: Aliment Pharmacol Ther, Tyndal, Treatment of upper gastrointestinal leaks with a removable, covered, self-expanding metallic stent. Surg Laparosc Endosc Percutan Tech, Ann Thorac Surg, Badylak, The host response to allogeneic and xenogeneic biological scaffold materials. J Tissue Eng Regen Med, J Surg Res, J Oral Maxillofac Surg, Growth factor enhancement for bone grafts.

Chapter 7 : A new dimension to fighting cancer | Washington State Magazine | Washington State University

Natural scaffolds are obtained from animal (including human) organs through a process called decellularization, whereby the cellular compartment of the organ in question is destroyed and cell remnants are cleared from the remaining extracellular matrix (ECM) scaffold.

 Abstract While biologically feasible, bone repair is often inadequate, particularly in cases of large defects. The search for effective bone regeneration strategies has led to the emergence of bone tissue engineering TE techniques. When integrating electrospinning techniques, scaffolds featuring randomly oriented or aligned fibers, characteristic of the extracellular matrix ECM , can be fabricated. In parallel, mesenchymal stem cells MSCs , which are capable of both self-renewing and differentiating into numerous tissue types, have been suggested to be a suitable option for cell-based tissue engineering therapies. This work aimed to create a novel biocompatible hybrid scaffold composed of electrospun polymeric nanofibers combined with osteoconductive ceramics, loaded with human MSCs, to yield a tissue-like construct to promote in vivo bone formation. Characterization of the cell-embedded scaffolds demonstrated their resemblance to bone tissue extracellular matrix, on both micro- and nanoscales and MSC viability and integration within the electrospun nanofibers. Subcutaneous implantation of the cell-embedded scaffolds in the dorsal side of mice led to new bone, muscle, adipose, and connective tissue formation within 8 weeks. This hybrid scaffold may represent a step forward in the pursuit of advanced bone tissue engineering scaffolds. Introduction Bone regeneration is a complex physiological process, which occurs continuously during adult life, as well as during normal fracture healing. However, there are complex clinical situations, such as bone loss due to trauma, infection, or disease, in which large quantities of bone regeneration are required [1 , 2]. Currently, there are several clinical approaches to address insufficient bone repair and regeneration, including bone grafting techniques which apply autografts, allografts, and alloplastic bone grafts [2]. Although the current treatment strategies have been shown to improve bone repair and are commonplace in orthopedic surgery, none features the full gamut of ideal characteristics, such as high osteoinductive and angiogenic potentials, biological safety, low patient morbidity, scalability, extended shelf-life, and cost-effectiveness [3]. For example, using the current strategies, it is difficult to obtain the quantities of tissue necessary to replace large bone defects. Bone tissue engineering BTE has evolved to fill this unmet need [1 , 2]. The classic BTE process involves a 3-dimensional 3D scaffold that mimics the natural bone extracellular matrix niche, osteogenic cells, which deposit bone tissue matrix, morphogenic signals, which trigger differentiation of the osteogenic cells to the phenotypically desirable cell type, and sufficient vascularization to meet the growing tissue nutrient supply and clearance demands [3]. In the case of bone regeneration, the materials must demonstrate biocompatibility, osteoinductivity, to promote osteoblastic differentiation, osteoconductivity, to support new bone growth, osteointegrativity, to provide biological fixation of scaffold to bone, angiogenesis, to ensure long-term functionality of the graft, and mechanical compatibility with native bone [4 â€” 6]. Generally, the scaffold is comprised of polymers, ceramics, or a composite of the two, depending on the intended application of the scaffold [5 , 7]. Polymers display a range of physical and mechanical properties, degradation times, and modes, and they have vast design flexibility, allowing for tailoring of graft composition and structure to specific needs. In contrast, ceramics, which are formed from inorganic, nonmetallic materials that can take on a crystalline structure, are ideal scaffolding candidates as the inorganic component of bone, due to its close resemblance with native apatite of the human skeleton. Ceramics that are composed of hydroxyapatite have the ability to chemically bind live bone tissue and to enable osteoblast adhesion and proliferation [7 , 8]. However, they are nondegradable in a biological environment and display limited processability. Therefore, they are disadvantageous for tissue engineering applications [7 , 9]. Composite polymer and ceramic materials can significantly synergize with each other to reduce the overall brittleness of ceramics and to increase the porosity, bioactivity, and the osteoconductivity of the polymeric scaffold [7]. Some of the most promising

research efforts in the field of regenerative medicine have focused on the use of stem cells, which display both self-renewing and broad differentiation capacities [10 , 11], alongside accessibility and expansibility. Mesenchymal stem cells MSCs comprise a subtype of multipotent stem cells, and they are highly sought after in research due to their ease of isolation [12]. This study examined the potential of a 3D multilayered, hybrid scaffold composed of osteoconductive ceramic particles and polymeric polycaprolactone PCL nanofibers, to support human MSC proliferation and differentiation into bone tissue when subcutaneously implanted into an ectopic mouse model. Materials and Methods 2. Pro Osteons provide continuous pathways for bony ingrowth through their interconnected porosity and are consisted of small granules 0. Their architecture and chemical composition are similar to human bicortical bone. To produce a hybrid scaffold of PCL nanofibers and Pro Osteon particles, an electrospinning apparatus was built consisting of syringe pump, high-voltage power supply, and rotated collector Figure 1. To create mat of the hybrid scaffold, rotated flat aluminum collector was located 10 cm below the needle tip. This ratio was determined based on our previous studies that optimize the suitable ratio for highest scaffold porosity. Apparatus for fabrication process of the hybrid scaffold. An electrospinning apparatus was built consisting of syringe pump, high-voltage power supply, and rotated collector. MSCs were isolated from human adipose tissue, according to a protocol established by Zeng et al. Human adipose tissue, extracted by liposuction, was cut into pieces and then allowed to adhere to the walls of culture plates. On the third day of culture, MSCs were released from the edges of adipose tissue. MSCs were cultured in inductive conditions before being seeded on the scaffold. Medium was changed every 3 days. Unseeded hybrid scaffolds were sputter-coated with gold palladium while seeded scaffolds were fixed in 0. They were then dehydrated in graded ethanol solutions, sputter-coated with gold palladium. All samples were photographed using a Phenom scanning electron microscope PhenomWorld. Porosity To evaluate the relation between mass and porosity of the hybrid scaffolds, scaffold mass was manipulated by dispersing different amounts of ceramic particles and by that changing the ratio between them and the PCL nanofibers. Three samples were cut from each hybrid mat. Permeability Permeability tests were performed using a permeability rig, as previously described [14]. Fluorescence was recorded using a FLUOstar galaxy fluorescence reader, at an excitation wavelength of nm and emission wavelength of nm BMG Labtech. The same procedures were performed to determine the proliferation rate of cells on the time points of 3, 7, 14, and 21 days. After discarding the supernatant, the cells were incubated with mouse anti-human phycoerythrin- PE- conjugated antibodies: Marker expression was analyzed using FlowJo software FlowJo and is presented as the percentage of fluorescence-positive cells. Cell Differentiation Assay After 28 days of incubation in osteoinductive medium, cultured cells were rinsed twice in PBS, fixed in 0. Alternatively, cells were rinsed twice with PBS, fixed with 0. Alkaline phosphatase stains cells blue-violet if they contain active ALP. Staining was determined using an inverted light microscope Olympus. The cell-seeded scaffolds were then incubated for 70 min in the incubator, with slow rotation, and then soaked in inductive medium for one week. On the implantation day, scaffolds were stabilized with a fibrin clot coat, composed of 1: All of the described surgical procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee IL Five groups of 6-week-old, nude female mice per group, Harlan Laboratories were anesthetized using a 0. Cell-seeded constructs were then subcutaneously implanted in the dorsal side of the mice. Acellular scaffolds and cell-seeded osteoconductive particles Pro Osteon, 40 mg that were also precoated with a fibrin clot were subcutaneously implanted as negative and positive controls, respectively. Tissue samples of the construct area were extracted for histological analysis 8 weeks after implantation. Statistical Analysis All experiments included 4 or 5 replicates. All statistical analyses were performed using the GraphPad Prism 5 software. Scaffold Design and Characterization Initial research efforts focused on designing an adequate ECM-like scaffold model prepared from electrospun polymeric nanofibers and osteoconductive ceramics. SEM images Figures 2 c and 2 d revealed the 3D meshed structure of the hybrid scaffold, comprised of multilayers of PCL fibers and osteoconductive particles dispersed throughout. Images of the electrospun composite scaffold. In order to examine the effect of combining Pro Osteon particles with the PCL fibers, two physical parametersâ€”permeability and porosityâ€”were measured. Scaffold porosity proved mass-dependent, demonstrating that scaffolds with lower masses, as a result of dispersing more Pro Osteon particles and

changing the ratio of PCL nanofibers and Pro Osteon particles, exhibited higher porosity, indicating a positive effect of Pro Osteon particles on porosity Figure 3 b. Therefore, low-mass scaffolds were used in further assessments. Physical characteristics of the electrospun scaffold and PCL-only scaffold. Human MSC morphology resembled that of fibroblasts, with a distinct spindle-like shape that was retained for up to 7 days in basic growth medium Figure 4 a. Following culturing of up to 28 days in inductive conditions, the cells began to form clusters Figure 4 b. Isolated cells were cultured in basic or inductive medium and their morphology in culture was examined using an inverted microscope. Flow cytometry analysis of MSCs cultures. The osteogenic differentiation potential of the isolated MSCs, which were cultured in osteogenic conditions in culture plate, was demonstrated by expression of both early and late osteogenic markers ALP and calcium Figures 6 a and 6 b , resp. Validation of osteogenic potential of MSCs. MSCs were cultured in osteoinductive medium for 28 days and then stained with alkaline phosphatase and alizarin red to evaluate their osteogenic differentiation. MSC Expansion and Attachment to the Hybrid Scaffold Histological analysis demonstrated extensive MSC expansion within the meshed structure of the hybrid scaffold, after 7 days of incubation in inductive medium Figure 7. MSC-embedded hybrid scaffolds were cultured in inductive medium for 7 days. MSCs were extensively expanded within the hybrid scaffold: PCL fibers and cell nuclei are indicated by yellow and blue arrows, respectively. In contrast to histological sections, wherein the ceramics are dissolved through a demineralization process, SEM imaging allows for observation of the Pro Osteon particles. SEM images demonstrated MSC attachment Figure 8 , blue arrows to the scaffold components, and their growth along the PCL fibers Figure 8 a , green arrows and the osteoconductive particles Figure 8 , yellow arrows. These observations confirmed the ability of the scaffold components in support of the seeded cells. SEM images of cell-embedded hybrid scaffolds. Then, they were fixed, coated with gold, and analyzed using SEM. The Hybrid Scaffolds Supported MSCs Proliferation In order to assess whether the hybrid scaffolds provide adequate biological support for growing cells, the proliferation rate of seeded cells was monitored. Seeded cells demonstrated proliferative capacities which increased with culture time Figure 9. Of note, the proliferation rate of the MSCs only, cultured on a tissue culture dish control , was greater than that of the MSCs grown on scaffolds Figure 9. This difference could be related to the favorable MSCs initial adherence to plastic flasks rather than to PCL fibers taking into consideration the 2D versus 3D scaffold characteristics of culturing. Cells adhere easily to 2D flasks, while when seeded onto scaffolds they should integrate and adhere before being washed. In addition, cells cultured onto 2D surfaces are exposed more to nutrients and oxygen and thus grow faster compared to cells cultured on 3D scaffold. MSC proliferation on hybrid scaffolds. MSC-embedded hybrid scaffolds were cultured in basic growth medium for 21 days. MSC proliferation was determined on days 1, 3, 7, 14, and 21 days thereafter, using Alamar Blue assay, and was compared to that of MSCs cultured alone. Cells were well integrated within the implanted scaffolds Figure 10 and succeeded in forming several tissue types in each scaffold, including muscle tissue, blood vessels, adipose tissue, connective tissue, and bone tissue, as identified by their histological structure. Several tissue types were identified: To determine the osteogenic potential of the hybrid scaffolds, scaffolds were subcutaneously implanted in the dorsal side of mice and tissue samples were extracted for histological analysis 8 weeks after implantation. New bone tissue formation was observed within the MSCs-seeded scaffolds Figure 11 ; yellow arrows and margins , in addition to muscle, adipose, and connective tissues.

Chapter 8 : Re-growing the human esophagus | World Endoscopy Organization (WEO)

A porous PCL scaffold promotes the human chondrocytes redifferentiation and hyaline-specii¬•c extracellular matrix protein synthesis N. Garcia-Giralt,1 R. Izquierdo,2 X. NogueÂ´s,1 M. Perez-Olmedilla,3 P. Benito,1 J. L. GoÂ´mez-Ribelles,3,4.

Hereby it is possible to combine the range of physical properties the scaffold can offer with the reconstructive properties of the ECM. The optimal amount of discrete ECM material for each application is disclosed and this concentration is equally distributed in the dressing hence avoiding unnecessary high concentrations of ECM. In addition to the effect of the ECM, the porous structure of the base material provides the cells with a structure for in-growth. Description Biodegradable Scaffold with ECM Material Field of the invention The present invention relates to a scaffold comprising a biodegradable layer having ECM material in the form of flakes, fibres, particles, powder or the like incorporated in the biodegradable layer. Background Scaffolds are structures used to guide the organization, growth and differentiation of cells in the process of forming new functional tissue. To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell growth and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is essential since scaffolds need to be absorbed by the surrounding tissues without the necessity of a surgical removal. Many different materials natural and synthetic, biodegradable and permanent have been investigated for use as scaffolds. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen or some linear aliphatic polyesters. However, when testing laboratory made scaffolds in vivo, it is often seen, that the cells do not grow readily into these scaffolds, maybe due to the fact that no biological signal molecules, e. In order to improve the biological properties of the scaffolds and to accelerate wound healing, several labs have added growth factors to a synthetic scaffold and seen beneficial effects on wound healing. In all of these publications a single growth factor has been incorporated in a sheet or hydrogel. Acellular extracellular matrices ECM from warm-blooded vertebras are used extensively in tissue engineering and plastic surgery 8. It has been shown that acellular ECM contains several growth factors The ECMs on the market today are of human or porcine origin. The cells are removed from the tissue and the tissue is subsequently lyophilized and cut into sheets. The sheets of porcine origin come in different sizes. The price of these sheets is very high. The sheets are fairly stiff when un-hydrated. An example is the sheets from the company Acell. These products are in the form of sheets or hydrogels. The sheets provide both a scaffold as well as a complex mixture of proteins to the cells of the wound. Examples of non-scaffold products containing ECM proteins on the market, is Xelma from Molnlycke, which is a hydrogel that contains a protein extract from ECM of developing pig teeth. Summary The present application discloses that the growth promoting effects of ECM is maintained if the ECM is incorporated into a scaffold. We demonstrate that when using scaffolds containing ECM material, higher concentrations of ECM surprisingly do not give better cell morphology. In addition it is shown that by varying the concentration of discrete ECM material in scaffolds the physical characteristic of the scaffold changes but that the changes are depending on the material of the scaffold. The present application takes this knowledge to the patient by showing a sterilisation strategy that maintains the biological activity of the ECM material after sterilisation. Detailed Disclosure The present invention relates to a temporary composite scaffold comprising discrete ECM particles. By adding discontinuous regions of ECM to a scaffold it is possible to combine the range of physical properties e. In addition, the price of such scaffold will be lower than other ECM scaffolds both because the powder is a waste-product from the production of acellular ECM sheets and because the optimal amount of discrete ECM material for each application can be determined and equally distributed in the dressing hence avoiding unnecessary high concentrations of ECM. In one embodiment a discontinuous region of ECM is obtained by adding discrete ECM material, such as particles, flakes, fibres or powder. A discrete phase of ECM material means material of ECM that is distinguished in their form and density from the ground material that they are embedded in. This can be demonstrated by histology sections as seen in example 5 or by

scanning electron microscope SEM seen in example 6. As shown in the examples e. It is preferred, that the ECM material is added to the scaffold before scaffold formation e. In this way, the ECM material is homogeneously distributed in the scaffold. That is, in the time it takes to solidify the scaffold e. This is a huge clinical advantage as there is nothing to remove from the wound. Thus, the newly formed tissue is not disturbed or stressed by removal of the temporary scaffold. It is typically preferred that the scaffold is broken down during 1 day to 10 weeks - depending on the application. For open wound applications, it is preferred that the scaffold is broken down during days, such as days. In one aspect of the invention, the scaffold is biodegradable. In one embodiment the scaffold is a continuous scaffold. That is a scaffold of a continues phase. A continuous scaffold with discontinuous regions results in a composite material. As with other composite materials, this is an engineered material made from two or more constituent materials with significantly different physical or chemical properties and which remains separate and distinct within the finished structure. Extracellular matrix ECM is the non-cellular portion of animal or human tissues. The ECM is hence the complex material that surrounds cells. Consequently, it is preferred that the discontinuous regions of ECM are cell free regions. Layers of cells can be removed physically by e. Detergents and enzymes may be used to detach the cells from one another in the tissue. Water or other hypotonic solutions may also be used, since hypotonicity will provoke the cells in the tissue to burst and consequently facilitate the decellularization process. Another way to obtain cell free regions is by adding the ECM powder discontinuous regions of ECM to the scaffold matrix. A cell-free product minimizes the risk any immune rejection once implanted, since components of cells may cause an immunogenic response. In broad terms there are three major components in ECMs: ECMs are known to attract cells and to promote cellular proliferation by serving as a reservoir of growth factors and cytokines 9; A temporary scaffold containing particulate ECMs used in a wound will be populated by cells both from the wound edges as well as cells from the circulating blood. As the cells invade the scaffold, the scaffold material will be degraded and eventually the scaffold will be replaced with new tissue. The skin of humans comprises an upper layer of epidermis, formed by inter alia keratinocytes. Below epidermis is dermis, formed by inter alia fibroblasts, but also endothelial cells. When promoting growth of fibroblasts, the present examples e. The wound dressing of the present invention may comprise multiple layers. These layers could include 1 or more layers of biodegradable material, which all optionally comprise ECM. If ECM is incorporated in more than one layer the dose may vary across the layers. In another embodiment, the scaffold is designed for growth stimulation of different cell-types. It is our experience, that when promoting growth of fibroblasts, the growing fibroblasts will excrete growth factors inducing growth of keratinocytes. Hereby, fibroblast growth is promoted such that keratinocyte growth is subsequently promoted and the wound is healed. In a dissolvable scaffold e. After freezedrying, the material is weighted. In a non-dissolvable scaffold the material is embedded in an appropriate embedding material e. Using image analysis the amount of ECMs are calculated in relation to scaffold. The ECM may include the basement membrane, which is made up of mostly type IV collagen, laminins and proteoglycans. The ECM material of the invention is preferably prepared from tissue harvested from animals raised for meat production, including but not limited to, pigs, cattle and sheep. Other warm-blooded vertebrates are also useful as a source of tissue, but the greater availability of such tissues from animals used for meat production makes such tissue preferable. Pigs that are genetically engineered to be free of the galacatosyl, alpha 1,3 galactose GAL epitope may be used as the source of tissues for production of the ECM material. In a preferred embodiment the ECM will be of porcine origin. The ECM material can be obtained from any animal. It could be derived from, but not limited to, intestinal tissue, bladders, liver, spleen, stomach, lymph nodes or skin. Human tissue is preferably avoided to minimize transfer of diseases. Thus, in a preferred embodiment the discontinuous regions of ECM are obtained from animal tissues. Due to species similarity, it is preferred to use ECM from warm-blooded mammal. One aspect of the invention is to provide a scaffold with constant dosing of growth factors. One property of the scaffold used in the present invention is to distribute the discontinuous regions of ECM within the porous base material, such that the ECM is accessible for the cells. When the cells migrate through the scaffold matrix, the discontinuous regions of ECM are exposed to protease activity and degraded which are believed to result in release of the biologically active components from the discontinuous regions of ECM

Thus, the release of biologically active components can be kept somewhat constant throughout the period of use, thereby providing a somewhat constant dosing to the wound bed and cells. In one embodiment, the discontinuous regions of ECM are equally distributed within the temporary scaffold. ECM comes in several micronized forms: All of these are considered discontinuous regions of ECM, i. Preferably particles with a mean diameter of approximately pm. This is determined by a Mastersizer from Malvern Instrument for volume weighted mean. For example, a surface weighted mean of pm, can have the smallest particles of 3pm, the largest particles of pm. A volume weighted mean would, in this case be pm. By distributing the discontinuous regions of ECM in a porous scaffold, it is possible to optimise the physical properties e. In order to obtain both the beneficial effect of the ECMs, and the physical properties a porous scaffold can offer, particulate ECM can be included in a wound dressing such as a scaffold and be used for tissue engineering e. This porous scaffold should preferably be of a material that is biodegradable. The temporary scaffold may be either in a lyophilised form, in a fibrous form woven or non-woven , in a foamed form or as a film. The material used for the scaffold may be any biodegradable material, from both synthetic and of natural sources.

Chapter 9 : Tissue engineering - Wikipedia

Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological tissues.

Treatment options include a variety of techniques to resect the affected tissue but these strategies have had limited success and invariably have high complication and mortality rates. Extracellular matrix ECM -based biomaterials have shown promise for esophageal reconstruction. In early pre-clinical studies ECM scaffolds were used to repair partial and full thickness esophageal defects encompassing various portions of the total esophageal circumference. This initial study showed well organized, fully re-epithelialized site-specific tissue that was contiguous with the native esophagus. However, full circumferential defects healed with stricture within 19 days after surgery [1]. Subsequent studies were designed to show that a critically sized, full circumferential esophageal defect could be repaired with minimal stricture formation if adjacent autologous muscle tissue was placed in direct apposition to the ECM scaffold at the time of surgery [2]. The ECM scaffolds provide an ideal substrate for epithelial layer development and this, combined with the presence of skeletal muscle cells, may have facilitated the re-epithelialization necessary to prevent or minimize tissue contracture [3]. The study also showed spontaneous motility of the muscle within the remodeled section via esophograms and endoscopy, although the motility did not appear to be synchronous with the native esophagus. Immunolabeling at the site of ECM-facilitated remodeling showed mature and regenerating nerves within the newly formed muscle tissue [4]. Tubular ECM scaffold used to repair esophageal defects [2] Figure 2: Depiction of surgical procedure used to treat human patients with diseased esophageal muscosa. In an animal model, transections were made in the cervical esophagus and at the gastroesophageal junction and a few centimeters of mucosa were also resected. The ECM was placed at the site of the endomucosal resection to reinforce the anastomosis. The shape of the device was customized to the shape of the anatomy i. The remodeling of the ECM reinforced anastomoses was compared to an experimental control in which the endomucosal resection was performed at the time of anastomosis, and a clinical control in which the mucosa was left intact. After two months, the presence of UBM-ECM resulted in less stenosis and less contracture of the cervical and distal esophagus compared to the control. Furthermore, the site of ECM remodeling showed restoration of a more mature epithelium and regeneration of islands of muscle that bridged the gap between the native muscle tissues on either side of the surgical transection. This study suggests that the use of an ECM scaffold during the gastric pull-up surgery may substantially decrease the rate of complications i. Diagnostic biopsy top row , postoperative biopsy second row. The diagnostic biopsies all show adenocarcinoma. The postoperative biopsies show replacement of the ECM scaffold with mature, differentiated squamous epithelium. Scale bars represent mm. Naturally, in order to develop better regenerative medicine therapies for esophageal reconstruction, a more thorough understanding of esophageal disease progression and the mechanisms by which bioscaffolds mitigate the default tissue response to injury is required. The Badylak laboratory is currently working on these two separate but related questions: Specifically, we aim to determine the mechanism by which normal, inflammatory, and cancerous ECM dynamically, and reciprocally, instructs the behavior of esophageal cells and supporting cells e. The Badylak laboratory is uniquely equipped to conduct this study for its pioneering work in decellularization of mammalian tissues and development of organ-specific hydrogels [8,9]. We are currently working to develop the first hydrogel from inflammatory and cancerous ECM. Furthermore, we aim to temporally resolve the microenvironment-specific tissue-remodeling response sequence after biologic scaffold placement in a rat model of esophageal adenocarcinoma. Resorbable bioscaffold for esophageal repair in a dog model. Esophageal reconstruction with ECM and muscle tissue in a dog model. The basement membrane component of biologic scaffolds derived from extracellular matrix. Reinforcement of esophageal anastomoses with an extracellular matrix scaffold in a canine model. An extracellular matrix scaffold for esophageal stricture prevention after circumferential EMR. Evidence of innervation following extracellular matrix scaffold mediated tissue remodeling. J Tissue Eng Regen Med. Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the

setting of superficial cancer: Tissue Eng Part A. A hydrogel derived from decellularized dermal extracellular matrix. Epub Jul Hydrogels derived from central nervous system extracellular matrix. Epub Nov