CHRONIC WOUNDS

Chronic wounds are defined as those that have failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity of the injured site. Often disguised as a comorbid condition, chronic wounds represent a silent epidemic that affects a large fraction of the world population and poses major and gathering threat to the public health and economy of the United States. In the United States, chronic wounds affect around 6.5 million patients. It is claimed that an excess of $25 billion is spent annually on treatment of chronic wounds and the burden is growing rapidly due to increasing health care costs, an aging population and a sharp rise in the incidence of diabetes and obesity worldwide. (Sen C, et al. Wound Repair Regen 2009;17:763-771.)

WOUND HEALING

Wound healing is a dynamic process involving interactions between cells, extracellular matrix and growth factors that reconstitutes tissue following injury. (Clark RA, Ghosh K, Tonnesen MG. Tissue engineering for cutaneous wounds. J Invest Dermatol 2007; 127: 1018-29.) The normal wound repair process consists of three phases--inflammation, proliferation, and remodeling that occur in a predictable series of cellular and biochemical events. Wounds are classified according to various criteria: etiology, lasting, morphological characteristics, and biochemical events. Wounds are classified according to various criteria: etiology, lasting, morphological characteristics, communications with solid or hollow organs, the degree of contamination. (Komarcevic A, et al. Med Pregl 2000 Jul-Aug;53(7-8):363-8.)

While regeneration describes the specific substitution of the tissue, i.e. the superficial epidermis, mucosa or fetal skin, skin repair displays an unspecific form of healing in which the wound heals by fibrosis and scar formation. The first stage of acute wound healing is dedicated to hemostasis and the formation of a provisional wound matrix, which occurs immediately after injury and is completed after some hours. Furthermore, this phase initiates the inflammatory process. The inflammatory phase of the wound healing cascade gets activated during the coagulation phase and can roughly be divided into an early phase with neutrophil recruitment and a late phase with the appearance and transformation of monocytes. In the phase of proliferation the main focus of the healing process lies in the recovering of the wound surface, the formation of granulation tissue and the restoration of the vascular network. Therefore, next to the immigration of local fibroblasts along the fibrin network and the beginning of reepithelialization from the wound edges, neovascularization and angiogenesis get activated by capillary sprouting. The formation of granulation tissue stops through apoptosis of the cells, characterizing a mature wound as avascular as well as acellular. During the maturation of the wound the components of the extracellular matrix undergo certain changes. The physiological endpoint of mammalian wound repair displays the formation of a scar, which is directly linked to the extent of the inflammatory process throughout wound healing. (Reinke JM et Sorg H. Eur Surg Res 2012;49:35-43.)

The extracellular matrix plays an important role in tissue regeneration and is the major component of the dermal skin layer. The composition of ECM includes proteoglycans, hyaluronic acid, collagen, fibronectin and elastin. As well as providing a structural support for cells, some components of the ECM bind to growth factors, creating a reservoir of active molecules that can be rapidly mobilized following injury to stimulate cell proliferation and migration. In many chronic wounds, increased levels of inflammatory cells lead to elevated levels of proteases that appear to degrade the ECM components, growth factors, protein and receptors that are essential for healing. Recognition of the importance of the ECM in wound healing has led to the development of wound products that aim to stimulate or replace the ECM. These tissue-engineered products comprise a reconstituted or natural collagen matrix that aims to mimic the structural and functional characteristics of native ECM. When placed in the wound bed, the three-dimensional matrix provides a temporary scaffold or support into which cells can migrate and proliferate in an organized manner, leading to tissue regeneration and ultimately wound closure.

OVERVIEW OF AVAILABLE TREATMENTS

There is no definitive paper or guideline on the use of acellular matrices in acute and chronic wounds, according to “International Consensus. Acellular Matrices for The Treatment of Wounds. An expert working group review. London: Wounds International, 2010.” The team of authors for the paper reviewed current knowledge of acellular matrices and their rationale for use. They noted that acellular matrix products can be used in various applications, including burns and reconstructive surgery, soft tissue and abdominal wall repair and as internal implants for orthopedic use in joint resurfacing and tendon repair.

EXTRACELLULAR MATRIX IN WOUND HEALING

One management strategy for wound healing that has been studied recently is the application of an intact ECM material to the chronic wound bed.

The extracellular connective tissue matrix of the skin is a complex aggregate of distinct collagenous and non-collagenous components. Optimal quantities and delicate interactions of these components are necessary to maintain normal physiologic properties of skin. (J Invest Dermatol 1989;92:615-775.)

As described by Agren M, et al. (Int J Low Extrem Wounds 2007;6:82-97), the ECM is defined in Dorland’s Medical Dictionary as “any material produced by cells and excreted to the extracellular space within the tissues. It takes the form of both ground substance and fibres and is mostly made up of fibrous elements, proteins involved in cell adhesion, glucosaminoglycans (GAGs) and other space-filling molecules. The ECM serves as a
scaffolding to hold tissues together, its form and its composition help to determine tissue characteristics. In epithelia, it includes the basement membrane."

The ECM undergoes dynamic interactions with cells that are pivotal for cell adhesion, motility, growth, differentiation, ECM synthesis, in addition to offering passive support to the cells. A proportion of chronic lower extremity wounds mostly of venous etiology appear to get “stuck” in the nonhealing phase that could be described as follows: a persistent chronic inflammation associated with copious exudate production, unhealthy granulation tissue, and impaired epithelialization. It is fundamentally important to understand that the complex molecular mechanisms that prevent healing of chronic wounds are crucial in the development of optimal interventions. Elevated levels of chemokines and proinflammatory cytokines lead to an abnormal secretion and activation of different proteinases, which may result in a suboptimal ECM composition that diminishes the action of growth factors via reduced integrin binding.

Immunohistochemical staining patterns and intensities for collagens of types I and III, laminin and tenasin in diabetic foot ulcers and venous leg ulcers are not remarkably different from those seen in acute wounds. Hemrick et al. (Am J Pathol 1992;141:1085-95) reported that fibronectin was absent in the base of nonhealing venous leg ulcers, although histochemical studies on ulcers in the healing phase showed prominent immunostaining reaction to fibronectin. Agren M, et al. (Int J Low Extrem Wounds 2007;6:82-97) further noted that fibronectin deficiency may impede cellular migration and proliferation capable of inducing MMPs. It has also been reported that fibroblasts from venous leg ulcers can synthesize the full range of ECM molecules including fibronectin in vitro compared with normal, that is, “control” fibroblasts, indicating that fibronectin deficiency is related to increased degradation rather than to decreased synthesis. Fibronectin mRNA tissue levels are increased in venous leg ulcers. Degradation products of fibronectin, vitronectin and tenasin-C have also been found in wound fluid from venous ulcers. The degradation of these ECM molecules has been attributed to excessive activities of serine proteinases, such as neutrophil elastase and plasmin.

Fibronectin fragments are capable of inducing MMPs. In fact, increased MMP-1, MMP-2, MMP-8 and MMP-9 tissue levels but reduced concentrations of natural-occurring tissue inhibitor of metalloproteinase-2 (TIMP-2) were found in diabetic foot ulcers compared with traumatic wounds. The intrinsic ability of fibroblasts from the skin of insulin-dependent diabetic patients to produce MMP-2 is increased.

Interestingly, Staphylococcus aureus, a common pathogen in venous leg ulcers, produces a fibronectin-binding protein that facilitates infection of keratinocytes by interfering with endogenous cellular fibronectin self-assembly. In culture, a fragment of this protein was reported to impair keratinocyte migration.

IN VITRO EXAMINATION OF ECM SCAFFOLD

Recent data indicate that an extracellular matrix (ECM) secreted by human umbilical vein endothelial cells (HUVECs) assembled on gelatin coated plates overlaid by a mixed matrix secreted by human dermal microvascular endothelial cells (HDMECs) and human dermal fibroblasts provides a viable acellular scaffold for use in wound healing. (Solomon DE. Int J Exp Pathol 2002;83:209-16.) The extracellular secretions of HUVECs included in this analysis were type IV procollagen, fibronectin, and thrombospondin.

Trypsinized epidermal keratinocytes or colonies from Dispase-digested fresh and cadaver skin tissue adhered and proliferated on either HUVECs ECM/gelatin or mixed matrix overlaid on HUVECs ECM/gelatin. An epithelial–mesenchymal interaction, previously thought to be tissue-specific, was expressed as well as concomitant integrin versatility. In addition, heterologous HDMECs and dermal fibroblasts attached and proliferated on the mixed matrix as well as HUVECs ECM.

The conditioned medium from HUVECs (HUVECs CM) was found to neutralize the lingering after effects of Dispase, and could be used for the tissue culture of epidermal keratinocytes, HDMECs and dermal fibroblasts, which share related extracellular secretions.

“The conclusion to be drawn from these studies is that the proposed acellular scaffold composed in part of a ‘sandwich’ of two ECMs (with the mixed matrix uppermost) might be viable as a structural substitute for the lamina densa and lamina lucida, damaged or completely missing in some wounds and body burns,” the study authors wrote.

HUMAN PLACENTA-DERIVED ECM

In a separate study, a team of investigators evaluated full-thickness skin wound healing using human placenta-derived extracellular matrix (ECM) containing bioactive molecules. (Choi JS, et al. Tissue Eng Part A. 2012 Aug 15)

They noted that human placenta contains abundant ECM components and well-preserved endogenous growth factors. In the whole placenta, including the amnion, which contain collagen (types I, IV, VII and XVII), elastin, laminin, proteoglycans and adhesion proteins, play an important role in the maintenance of vessel walls and villous integrity. Moreover, many growth factors secreted from the mother during pregnancy, such as insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-beta), are delivered to the fetus through the placenta, suggesting that they have important roles in promoting the growth of the developing fetus. Furthermore, the biological properties of the placenta, such as anti-inflammatory, anti-bacterial, low immunogenicity, anti-scarring and wound protection, make it an ideal candidate to treat burned skin, leg ulcers and ophthalmic disorders.

Given that the compositional and biological properties of the placenta have the potential to provide a highly favorable environment for wound healing, the study authors conducted an analysis to further test its use. They fabricated highly porous, extracellular secretions from human placentas via homogenization, centrifugation, chemical and enzymatic treatments, molding, and freeze-drying. The tissue lysate from the placenta was used as a control.

Five human placentas were obtained with informed consent from normal or Caesarean deliveries at the Hanyang University Medical Center (Seoul, Korea). Thirty-six rats divided into 2 groups were wounded and then implanted with or without a human placenta-derived ECM sheet. Under general anesthesia, the dorsal area was completely depeleted, and a full-thickness circle wound (about 169 mm² in area) was created in the upper back area of each rat. Wounds were wrapped with plastic molds for protection. At 1, 2, and 4 weeks after treatment, 6 rats treated with ECM sheets and 6 control rats were sacrificed, and the skin including wounds were harvested for histological examination. The cutaneous wounds were photographed, and the wound areas were measured based on the image produced.

During postoperative day 1, the ECM sheet efficiently absorbed wound exudates and tightly attached to the wound surface. One week after the operation, scabs were observed in both groups. It is notable that the wounds of the control group were greatly reduced in size. At week 2, the scabs were falling off the wounds, and the wounds were mostly filled with restored skin. Complete wound closure was observed in both groups at 4 weeks. While the restored skin treated with an ECM sheet was similar to normal skin, an elongated scar was still observed in the healed skin of a control group due to excessive contraction. The difference
between the ECM sheet group and the control group was remarkable at 1 week after the operation (P<.05), but was not significant at 2 or 4 weeks.

Histological and immunofluorescence staining were used to assess the wound healing processes and the structure of the restored tissues. At week 1 postoperatively, the control and the ECM sheet-implanted groups exhibited abundant inflammatory cells. The wounds in the control group were distinguishable from adjacent tissues, and no clear keratin layer was observed.

Moreover, CD31 staining showed the vascularization of each experimental group at weeks 1 and 2 after operation and indicated a higher blood vessel density in the ECM sheet-implanted group than in the control group. “Our findings suggest that human placenta-derived ECM sheets could effectively promote the migration of keratinocytes and epithelial cells, as well as neovascularization due to a combination of physico-mechanical and compositional properties, thereby improving the quality of wound healing,” the researchers concluded.

**BIOLOGICAL ECM WOUND THERAPY**

A case series was conducted to evaluate the clinical effectiveness of a biological extracellular matrix that contains the following growth factors: transforming growth factor-beta1 (TGF-beta1), fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF). (Rando, T. J Wound Care 2009;18:70-74.)

The case series included four patients with diabetes, arterial vascular impairments or osteoarthritis, plus multiple comorbidities. Each patient had difficult-to-heal, chronic wounds that were treated with the test wound product after failing to progress with at least 8 weeks of standard wound care at specialist wound clinics. Preintervention standard wound care involved wound bed preparation, pressure offloading, diabetes control (when necessary), reduced graduated compression therapy (when necessary), advanced wound dressings, and nutritional and psychosocial support.

Before each application of the test wound therapy, the wound bed was prepared and non-viable tissue was debrided using conservative sharp wound debridement. The test product was cut slightly larger than the wound, placed on the wound bed, moistened with sterile normal saline and covered with a non-viable dressing. The test product was applied weekly. Primary and secondary dressings were placed over the product and were changed as necessary for the 11-week study duration or until complete wound healing occurred.

The first case was a 54-year-old female who had type 1 diabetes, peripheral arterial disease and anemia. She developed a 6.1 cm2 painless neuropathic, full-thickness, partially sloughed-based ulcer on her left lateral heel. Case No. 2 was an 83-year-old female with osteoarthritis, peripheral arterial disease, a history of infection with Staphylococcus epidermidis and previous necrotizing fasciitis of the wound area. She had a 23.1 cm2 ulcer on her right Achilles (posterior ankle) with a large amount of hemorheologic exudate that was causing maceration and irritation of the peri-wound skin. This full-thickness wound had been present for 2.25 years and had been unsuccessfully treated with a variety of management tools. The third patient was a 79-year-old male with peripheral arterial disease, chronic obstructive airways disease, hypertension, chronic atrial fibrillation, gout, chronic obstructive pulmonary disease and chronic renal impairment. He had a painless, neuropathic, full-thickness cavity wound measuring 0.4 cm2 on the amputation stump of his right foot. The wound had been present for 20 weeks. Case No. 4 was a 79-year-old male with type 2 diabetes, Huntington’s disease, peripheral arterial disease, chronic renal failure, rheumatoid arthritis and multiple foot wound infections. He had a left transmetatarsal amputation in November 2003. He had a history of Pseudomonas and meticillin-resistant

Staphylococcus aureus (MRSA) infections in his foot wounds, and a knee joint replacement which had become septic. The patient had a painless, neuropathic, full-thickness, heavily colonized wound, measuring 5.3 cm2, on his amputation stump. At presentation, it was of eight weeks’ duration.

Results showed that 11 weeks of treatment with the test product was effective in greatly reducing the size (n=2) or facilitating complete healing (n=2) of chronic wounds that had not responded to standard wound care. Rando T. noted this small case series provides limited evidence for the test product. However, he described two randomized clinical trials that investigated the test product and each found it was associated with higher healing rates than a comparator.

In one study, patients with venous leg ulcers were randomized to receive either weekly topical treatment with the test product plus compression (n=62) or compression alone (n=58). Healing was assessed weekly for 12 weeks and recurrences after six months were reported. After 12 weeks, 55% of the wounds in the group of patients who received the biological ECM wound therapy had healed compared with 34% in the other group.

No recurrences were reported in the test product group at six months. (Mostow E, et al. J Vasc Surg 2005;41:837-843.)

During a second study, patients with diabetic foot ulcers were randomized to the test product (n=37) or platelet derived growth factor therapy (n=36). The main outcome measure was the incidence of healing at 12 weeks. After that period of time, 49% (n=18) of patients achieved complete wound closure with the test product as compared with 28% (n=10) of those given the comparator.

In conclusion, Rando T. said, “The findings suggest that replacement of the extracellular matrix with [the test product] may be a viable treatment strategy that is potentially cost-effective.”

**PLATELET-RICH PLASMA- AND ECM-DERIVED PEPTIDES**

A team of researchers recently discovered that platelet-rich plasma derived peptides (PDP) and extracellular matrix-derived peptides (EDP) can be used as separate entities or in combination to stimulate cellular responses to injury both in vitro and in vivo. (Demidova-Rice T, et al. PLoS One 2012;7: e32146.) The investigators explained that the importance of endogenous platelets during the early phase of the course of wound healing had been known for decades. In fact, other data has suggested that exogenous platelets and platelet products, including platelet-rich plasma extracts, might be used for stimulating wound healing as well. (Borzini P et Mazzucco L. Curr Opin Hematol 2005;12:473-479.)

Given these findings, Demidova-Rice T et al. tested PDP and extracellular matrix-derived peptides (EDP) in several in vitro assays and in a mouse model of impaired wound healing.

During the in vitro endothelial morphogenesis, capillary endothelial cells or HMDEC 7x10(4) cells/cm2 were plated on growth factor reduced Matrigel at in DMEM supplemented with 1% BCS in the presence or absence of platelet extracts, PDP or EDP added directly to basal media or mixed with GFR Matrigel prior to gel polymerization 100 nM (combi) or 250 nM (UN- peptides). Purified recombinant basic FGF (FGF-2) or VEGF were used as positive controls.

During the in vivo wound healing studies, Balb/c mice were pretreated with two doses of cyclophosphamide (CY) dissolved in sterile saline and administered by IP injection: 150 mg/kg 4 days and 100 mg/kg 1 day before wounding in order to delay wound healing. Three control mice received full thickness cutaneous, excisional head wounds and were treated with daily application of carbomethylocellulose (CMC), which was used in the study as a vehicle for peptide application. Immediately after injury, wounds were dressed. The peptides were suspended in 3% CMC in PBS at concentration of 1 mg/ml (combi) or 284 mg/ml.

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(UN3) and injected under the dressings using 25G needle at 24 h post-injury.

Overall, results reveal that different lots of platelet extracts contained a complex mixture of more than a dozen proteins, including but not limited to albumin, immunoglobulins and cytoskeletal components, including myosin IIB. All three lots of the extracts stimulated proliferation of transformed keratinocytes (Hacat cells). Lot 1 preparation of the extracts added to cell culture media at 1 mg/mL induced a three-fold increase in keratinocyte proliferation as compared with serum-stimulated controls while lots 2 and 3 used at identical protein concentration only moderately enhanced cellular responses by 30% to 50%. This variability of the biological activity of platelet products correlated with variations in their chemical compositions, which were both observed in the study.

The researchers hoped to overcome lot variability of platelet extracts and identify novel biologically active moieties (peptides) possessing pro-angiogenic and wound healing properties. Specifically, they subjected platelet extracts to gel filtration and ion-exchange chromatography followed by mass spectrometry to allow for protein identification. In addition, to well-known platelet extract components, they identified 2 novel small fragments that they named UN1 and UN2. Moreover, they created a combinatorial peptide UN3, which contained amino acids present in both UN1 and UN2. In vitro testing of the peptides showed that all of them had stimulatory effects of cellular responses, but UN3 peptide was more biologically active compared with UN1 or UN2.

They further tested whether UN3 could be combined with extracellular matrix derived peptide comb1. Results demonstrated that both peptides used separately or in combination had stimulatory effects on cellular proliferation and morphogenesis in vitro.

Thereafter, they wanted to investigate if the peptides would retain their properties in vivo and this was done through the mouse model. The peptides, especially when used in combination, significantly improved wound healing in CY-treated mice.

Given that the investigators’ in vitro experiments with endothelial cells indicated the proangiogenic potential of the peptides, they examined whether similar effects could be achieved in vivo. They found that the peptides could stimulate blood vessel formation within the wound bed.

**ECM BIOMATERIALS FOR SOFT TISSUE REPAIR**

ECM biomaterials have been used in tendon repair procedures for more than a decade with clinical success. These materials are used to augment primary repair of tendons, to reinforce weakness, and to promote healing in a tissue that represents a significant clinical challenge. Tendons tend to have a limited vascular supply, and large tears, like those common in the rotator cuff, do not heal spontaneously, necessitating surgical intervention with high recurrence rates. In pediatric applications, these materials are commonly used as a wrap during Achilles tendon repair, tendon-lengthening procedures, and other foot and ankle tendon reattachment procedures. (Cornwell K, et al. *Clin Podiatr Med Surg* 26 (2009) 507-523.)

Cornwell K, et al. added that in podiatric medicine, one of the most common applications for ECM biomaterials is in dermal regeneration and the healing of difficult open wounds, although the mechanism of healing and repair is not equivalent to that of surgical implantation. Closing complex full-thickness wounds of the foot and ankle is challenging because of the varying causes and underlying conditions leading to pressure ulcers, most notably diabetes. Ulcers may persist for months to years, and practicing podiatrists may try repeated applications and multiple products before finding the right means of achieving wound closure.


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