Extracellular Matrices

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40.1 Introduction

The wounded skin heals through a series of events aimed at restoring the skin barrier properties. The healing process is divided into four main phases: hemostasis, inflammation, proliferation, and remodeling. The temporal progression between these phases is regulated by an interaction between the cells, the wound micro-environment, and the development of the extracellular matrix (ECM) [1]. During hemostasis, which follows within a few hours from the wound appearance, a fibrin clot is formed to control bleeding [2] and platelets trapped in the clot release coagulation factors and cytokines. The cytokines, in the inflammatory phase, have a chemotactic action on the cells towards the wound site. The first to respond are neutrophils that release TNF alpha, IL-1 beta, and IL-6 in order to amplify the immune response and protect the body from pathogens [3]. Monocytes are recruited at the site of the clot, where they differentiate into macrophages and phagocytize pathogens and cellular debris [4, 5]. Failure of this phase of cellular recall favors the chronicity of the lesion. In fact, macrophages activate and produce a variety of chemotactic factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). The proliferative phase is characterized by angiogenesis and by the synthesis of collagen and other extracellular proteins regulated by fibroblasts, and by a granulation tissue capable to support re-epithelization [6]. The granulation tissue matures and remodels in the following months, during which collagen density increases and the fibroblasts differentiate in myofibroblasts for the organization of the fibers and reinforce the microstructures. During the remodeling phase, the scar can become hypertrophic due to the excessive production and contraction of collagen fibers [7].

In chronic wounds, the response described above may be complicated by several factors such as prolonged inflammation, malnutrition, increased protease activity, possible superinfections, and reduction of cell activity [8]. In venous leg ulcers, the evaluation of the exudate has shown several fibroblast dysfunctions, an increase of matrix metalloproteinases (MMPs) and the activity of matrix metalloproteases inhibitors, a reduction of VEGF, and a reduction in collagen deposition [9–11]. These conditions are going to block the progression to normal ECM production. In a chronic wound, a condition is created in which the ECM stop to function normally, and therefore the normal wound healing process cannot be initiated. Thus, artificial substitutes of the dermal matrix aim to facilitate the restoration of the skin barrier by improving the wound environment and assist cell proliferation, differentiation, and engraftment.

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40.2 Extracellular Matrix in Chronic Wounds

ECM is a dynamic structure that provides physical and functional support to the underlying tissue. It is made of water, polysaccharides, and protein components such as collagen, fibronectin, elastin, and proteoglycans [12].

In chronic wounds, the ECM is altered and therefore normal healing is prevented. This is where artificial ECMs come into play. Substituted dermal matrices may function by different mechanisms including: stimulating the production of ECM components; donating ECM components; serving as supporting structure; also promoting, via cellular signals, cell migration and proliferation, facilitating lesion healing [13, 14]. Each of these roles is influenced by the structural composition of the dermal substitute matrix [15, 16].

The artificial ECM ideal for the healing of chronic wounds would be a structure similar to the natural ECM and in addition it should have the capacity to be gradually degraded and totally integrated with the host [13].

Each component of the extracellular matrix plays a fundamental role in modulating wound healing:

- Collagen is one of the most used components in the development of dermal substitutes, mainly because, as the main component of ECM, it plays a fundamental role in every phase of correct healing. In addition, collagen has a role in cellular communication [15–17]. Each ECM collagen molecule is constituted by three polypeptide chains of glycine, proline, and hydroxyproline arranged in a helix. In the extracellular space, the chains undergo a process of proteolysis that generates a series of cross-links with support functions and elastic resistance of the skin [18]. To date, more than 27 types of collagen have been identified. Type 1 collagen, which accounts for 75–80% in dry weight of the dermis, has above all a role in the adhesion, differentiation, and migration of fibroblasts and keratinocytes [29]. In vitro studies have shown that collagen 1 promotes the proliferation of fibroblasts and decreases reactive oxygen species (ROS) and the concentration of pro-inflammatory proteases and cytokines [19, 20]. Type 3 collagen plays a key role in the production of granulation tissue [21] and subsequent scar formation [22, 23]. Type 4 collagen is the main protein of the basement membrane and therefore it has a fundamental role in keratinocyte migration and in angiogenesis [7, 24, 25]. It has been shown that inserting Type I collagen into ECM scaffolds shows the lesion in a better physiological condition for healing [20].
- Among the other important components, (I) Elastin endows the ECM with greater resistance to stress [26, 27]. (II) Fibronectin, thanks to different binding sites for collagen, fibrin, proteoglycans, and integrins, facilitates cellular interaction with the ECM [27]. Fibronectin is essential for the deposition of type 1 collagen in the extracellular matrix [28, 29]. (III) Glycosaminoglycans and proteoglycans surround all the previous components. They are strongly hydrophilic molecules and therefore provide viscoelasticity to the fabric. Together they form a gel-like substance in which ions, hormones, and nutrients can move freely through the matrix [30]. (IV) Some glycosaminoglycans such as hyaluronic acid and chondroitin-6-sulfate play a fundamental role in guiding the cell to healing and are important because they activate growth factors.

40.3 The Ideal Extracellular Matrix

The collagen used for dermal skin substitutes can be obtained using two techniques: by decellularization of a native tissue or by means of extraction. Native collagen is regarded as advantageous because it is thought to preserve a higher level structure and therefore maintain better biological activities such as neoangiogenesis, cellular chemotaxis, and the ability to revitalize senescent fibroblasts and cause the upregulation of integrins involved in angiogenesis [18, 31].

Once the dermal substitute has been applied, the patient's skin tissue can give an incorporating response or non-incorporating response (Fig. 40.1a–b). The non-incorporative response

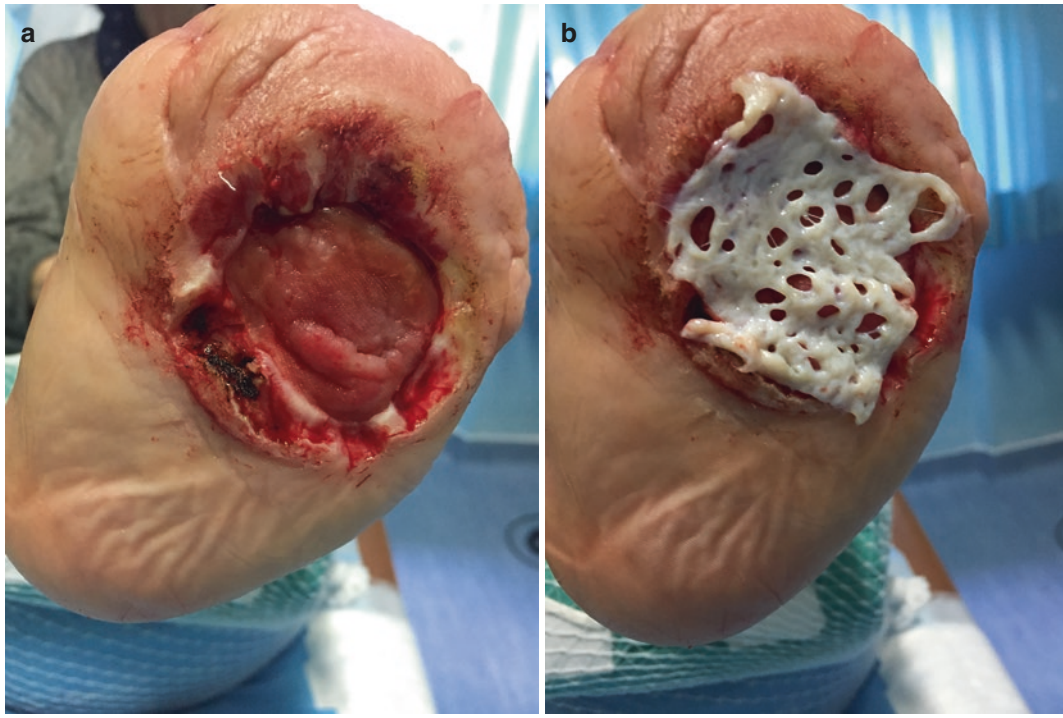

Fig. 40.1 **a–b** Amputation stump in a diabetic patient, before and after application of a dermal matrix

leads to encapsulation, and the host's fabric creates a wall around the implant. This answer is useful for permanent implants but is not ideal for healing of chronic wounds. The incorporating responses depend on how much the host's cells can penetrate the implant and the final result will depend on the inflammatory response. If the inflammation is excessive, it leads to the degradation and reabsorption of the substitute in a short time, giving rise to the deposition of disorganized tissue. On the other hand, if the inflammation is of medium intensity there will be a gradual degradation that can be equaled by the deposition of new tissue. A light inflammation causes the dermal substitute to serve as a guide to the deposition of new tissue within the original tissue [7, 15, 32].

Wound healing is a dynamic process that involves interactions between cells, ECM, and growth factors, all elements that restore the tissue after damage. ECM plays an important role in the tissue regenerative process and is the main component of the skin dermis. In addition to providing structural support to cells, some components of the ECM bind to growth factors, creating a reservoir of active molecules that can be rapidly mobilized after damage to stimulate cell proliferation and migration. In many chronic wounds, the increase in the number of inflammatory cells causes the levels of proteases to rise, which seems to be able to break down the components of the ECM, growth factors, proteins, and receptors essential for scarring. The recognition of the importance of ECM in the wound healing process has led to the development of products designed to stimulate or replace ECM (Fig. 40.2). Among these products intended to stimulate or replace the ECM are the dermal matrices.

40.4 Preparation of the Dermal Matrix

In order to use the extracellular matrix from the donor as a model for the growth of the new dermis, this tissue must first be adequately decellu-

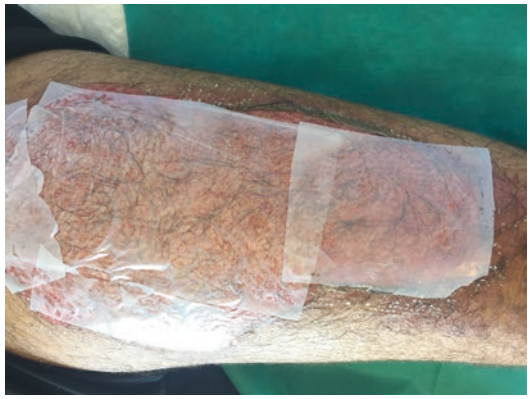

Fig. 40.2 Partial thickness burn on lower leg with an epidermal matrix applied

larized, due to antigenicity of the donor tissue components [33]. Otherwise there is a very strong immune response to the material and a problem with the biocompatibility can occur. Dermal substitutes may be derived from different species, e.g., cattle, horses, humans (corpses and placentas), fish, and plants. The properties of these extracellular matrix scaffolds depend on their tissue of origin. Knowing their characteristics, the practitioner can decide which one to choose according to the patient's needs.

An important feature is the porosity of the matrix scaffoldings. In fact, these must have pores in their internal architecture to allow for the passage of cells without altering the mechanical stability of the scaffolding. A porosity of adequate size not only facilitates cell migration but also the proliferation of these cells within the scaffolding [34].

Another important characteristic regards the cross-links. Regardless of the origin, ECMs are composed of polymers—especially collagen fibers. The term cross-links describes the chemical link between the various chains. Cross-links have a direct effect on the degradation and durability of the product.

The matrix must be sterilized, so it must be free of any living organism. Sterility is measured in sterility assurance level (SAL), which is the probability that a product contains microorganisms after sterilization. SAL is expressed in 10^{-n}, so that a lower SAL value corresponds to a higher sterility of the scaffold. Normally SAL range is between 10^{-3} and 10^{-6}, which are considered good sterilization values [35, 36].

40.5 Extracellular Matrices Registered

Since the ECM scaffolds were first used in the treatment of chronic wounds, a wide range has been developed and marketed. Available ECM scaffolds are derived from allogeneic and xenogeneic sources, or a combination of the two, or from biosynthetic routes.

40.6 ECM Derived from Allogeneic Skin

Ideal ECMs for wound healing should be similar in structure to the tissue that is to be replaced [13]. For this purpose, allogeneic skin can result in structure and composition very similar to the recipient's skin. ECMs derived from allogeneic skin such as Alloderm®, Graftjacket®, and Dermacell® come from human cadaver skin. For a better biocompatibility and bio-efficacy (recruitment and activation of the cell population), the matrix is decellularized.

40.7 ECM Derived from Human Placenta

The amniotic membrane, a thin avascular tissue, is considered as immune-privileged thanks to the absence of HLA (human leukocyte antigen), which codes for the major histocompatibility complex associated with the immune response/rejection [37]. Though the density of matrix protein is low, one of the benefits of using amniotic membranes is that they contain a wide range of growth factors and cytokines embedded in the membrane, which are thought to highly contribute to the healing process [38]. All amniotic tissues are suitable for repairing homologous fabrics. EpiFix® Amniotic Membrane Allograft is a tissue composed of human amniotic mem-

brane and chorion. EPIFIX® is not decellularized because it does not contain viable cells, thanks to the dehydration and sterilization processes. Similarly, BIOVANCE® and AMNIOEXCELV are tissues composed of amniotic membrane and the chorion layer is removed to reduce the passage of cellular debris and to eliminate the laterality of the transplanted tissue.

40.8 ECM Derived from Xenogen Fabrics

Typically for xenotransplants, tissues are decellularized to remove or reduce the immunogenic components of animal cells, and a final sterilization step is performed to minimize the risk of foreign bodies/infectious agents. They have the same indications as allogeneic ECM: for the management of diabetic and venous ulcers, full-thickness lesions, pressure, indeterminate, traumatic, abrasion and tearing injuries, and surgical or indeterminate wounds.

PriMatrix® is an ECM derived from bovine dermis that is processed, freeze-dried (frozen dried), and sterilized. The technology used for PriMatrix® exploits decellularization and preserves the dermal and biochemical structure of the ECM. PriMatrix® is highly biocompatible due to its ability to bind and trap human cells and growth factors [39]. MatriStem® Wound Care Matrix derives from a pig bladder matrix that is lyophilized in foils and irradiated with electron rays. The MatriStem® has a matrix with the intact basal lamina on one side and a thin layer of connective tissue on the other. MatriStem® contains various types of collagen and proteins that are reabsorbed and therefore promotes tissue remodeling.

OASIS® Wound Matrix derives from the submucosa of the fasting pigs, freeze-dried and sterilized with ethylene oxide. The smooth muscle and mucosa are removed and the tissue is decellularized forming a network of collagen, proteoglycans, GAG, fibronectin, and growth factors.

Kerecis ™ Omega3 is an acellular structure derived from fish skin that contains natural components of extracellular matrix and adds the benefit of bioactive lipids such as omega 3 and polyunsaturated fatty acids. Research has shown that the effects of bioactive lipid mediators (Omega3, EPA eicosapentaenoic acid, DHA docosahexaenoic acid) reduce the inflammatory response [40].

Endoform® Dermal Template is extracted from the submucosa of the ovine stomach. After decellularization and processing of the tissue that includes delamination, Endoform® preserves 90% of the original collagen (types I, III, IV) and also 10% of laminin, fibronectin, and GAG [41]. Endoform® was shown to have a broad spectrum of MMP inhibitors for collagenesis, MMP1 MMP8, also gelatinase, MMP2 and MMP9. This feature can be useful to protect and buffer from the harmful action of MMP in chronic wounds.

40.9 Biosynthetic ECM Scaffolds

In biosynthetic cellular matrices, the composition and the consistency of the reticular structure can be controlled and designed to be stable and biodegradable. Obviously, the sterilization and decellularization processes are not needed. The matrix is absorbed in about six weeks after application and replaced by autologous cells. It acts as a scaffold for tissue reconstruction (neoderm). Any skin substitute should maintain the three-dimensional structure for a minimum of three weeks to allow for fibroblast growth, neoangiogenesis, and epithelial cell coverage. Biodegradation begins after this period and the whole process should occur without significant foreign body reaction as this could lead to increased scarring.

INTEGRA® Dermal Regeneration Template, Omnigraft® Dermal Regeneration Matrix, INTEGRA® bilayer Matrix Wound Dressing, and Matriderm® are biosynthetic dermal regeneration matrices. These dermal laminae are composed of a porous three-dimensional matrix, with bovine collagen and chondroitin-6-sulfate, with a predefined degradation time. The temporary epidermal layer consists of a thin layer of silicone which provides immediate coverage of the lesion and controls fluid loss. The silicone

layer serves as a barrier to possible infections and mimics the normal regulation of liquids (sweating). This bio-engineered dermal layer is populated with cells from the wound bed and a granulation layer is formed while the revascularization process follows two to three weeks after the matrix is applied. Then the silicone layer can be removed and a re-epithelialization strategy can be set up.

40.10 Conclusions

Increasing availability and specific ECM is one more tool for treating chronic lesions; however, the biological response is difficult to be quantified or characterized. It can be assumed that specific ECMs are better in particular conditions (high or low MMP, re-epithelialization, etc.). Future research will lead to better understanding of the mechanisms of cellular integration and stimulation of the skin.

Conflict of Interest The authors declare no conflict of interest for this chapter.

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