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Amniotic membrane application for the healing of chronic wounds and ulcers

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ABSTRACT

Wound healing usually follows a predictable sequence and prognosis of events. Its evolutionary process is the result of a complicated interaction between patient-related factors, the wound, the treatment used and the skills and knowledge of the professionals who treat them. Only through a meticulous initial assessment of the wound is it possible to identify the factors that contribute to its complexity. The challenge for professionals will be to implement efficient therapies at the right time and in the most cost-efficient way in order to reduce associated problems, treat the symptoms and expectations of the patients and achieve adequate wound healing whenever possible. This is particularly evident in big chronic wounds with considerable tissue loss, which become senescent in the process of inflammation or proliferation losing the ability to epithelialize. Generally, chronic wounds do not respond to current treatments, therefore they need special interventions. AM is a tissue of particular interest as a biological dressing and it has well-documented reepithelialization effects which are in part related to its capacity to synthesize and release biological active factors. Our studies have demonstrated that amniotic membrane (AM) is able to induce epithelialization in chronic wounds that were unable to epithelialize. AM induces several signaling pathways that are involved in cell migration and/or proliferation. Additionally, AM is able to selectively antagonize the anti-proliferative effect of transforming growth factor-ß (TGF-β) by modifying the genetic program that TGF-β induces on keratinocytes. The combined effect of AM on keratinocytes, promoting cell proliferation/migration and antagonizing the effect of TGF-β is the perfect combination, allowing chronic wounds to move out of their non-healing state and progress into epithelialization.

1. Introduction

Skin is a complex organ that creates a safety barrier against infection and defends the body against external chemical, mechanical or physical insults. Skin participates in body temperature regulation, water exchange and electrolyte balance [1]. If skin structure breaks down, it does not perform these functions; therefore, it becomes peremptory to restore its wholeness as soon as possible. This breakage in the epithelial integrity of the skin is defined as wound, although the disruption could be deeper, extending to the dermis, subcutaneous fat, fascia, muscle or even the bone.

The tissue that has suffered a lesion compromising its morphological integrity is repaired through a biological process called wound healing, which is dynamic and well-ordered and involves soluble mediators, blood cells, the extracellular matrix, and parenchymal cells [2,3]. Wound healing produces a completely healed wound, usually seen after simple injury, which returns to its normal anatomical structure, function and appearance within an acceptable amount of time, and also presents the complete skin closure without drainage or dressing needs. In contrast to these, some wounds fail to heal in a regular sequence, ending in chronic, non-healing wounds. Chronic wounds can have several causes, including venous, arterial and neuropathic involvement, pressure, vasculitis and burns [1]. Important cellular, molecular and biochemical differences exist between the acute and the chronic wound environment [1]. In addition, extended traumatic deep

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wounds usually become chronic [4].

2. Acute wound healing

Acute wound healing implies four stages: hemostasis, inflammation, proliferation and tissue remodeling; that might overlap in time: [1,5].

Hemostasis, the first stage, occurs after tissue injury. The torn vessels immediately constrict and thromboplastic tissue antigens, mainly from the subendothelium, are exposed causing platelet aggregation and the initial hemostatic plug. Then, the activation of coagulation factors leads to the formation of a thrombus and wound hemostasis is achieved [6]. This clot, very diverse in its composition, provides the provisional matrix for cellular migration [7,8]. Concomitantly, previously aggregated platelets degranulate the alpha-granules releasing a cocktail of substances, which are potent chemoattractants for inflammatory cells [6].

The next stage, inflammation, begins with complement activation and the initiation of the classic molecular cascade that leads to infiltration of the wound with granulocytes or polymorphonuclear leukocytes (PMNLs) [1]. As a consequence of the environment created, the local endothelial cells then break due to cell-to-cell contact, which enhances the infiltration of inflammatory cells into the wound site [9]. More details about wound healing and the inflammation phase can be seen in Refs. [10] and [2].

Three days after wounding, the proliferative phase starts. Essentially, the replacement of fibrin/fibronectin matrix with newly formed granulation tissue takes place. At the beginning of that phase, there is a reposition of fibrin and fibrinogen matrix by local fibroblasts that are attracted by macrophages-released grow factors. Using the newly deposited fibrin and fibronectin matrix as a scaffold, fibroblasts migrate into the wound, becoming activated and increasing protein synthesis in preparation for cell proliferation, which makes them the prominent cell type within three to five days in clean, non-infected wounds [6]. Then, fibroblasts begin synthesis and secretion of extracellular matrix products. The formation of new blood vessels occurs concurrently during all stages of the healing process. During the hemostatic phase, platelet-produced TGF-β and platelets derived growth factor (PDGF) attract macrophages and granulocytes and promote angiogenesis. Furthermore, macrophages release a number of other angiogenic substances such as tumor necrosis factor-α (TNF-α) and fibroblastic growth factor-basic (FGF-b) inducing angiogenic capillary sprouts to invade the fibrin/fibronectin-rich wound clot. Later, a microvascular network would form throughout the granulation tissue [1,11]. In the granulation tissue, as collagen accumulates to yield scar tissue, blood vessels density decreases. The alteration of this delicate process may promote the development of chronic wounds [1,6]. New granulation tissue formation is accompanied by the proliferation of fibroblasts, the appearance of capillaries and the presence of tissue macrophages in a matrix of collagen, glycosaminoglycans (GAGs) including hyaluronic acid (HA), and fibronectin and tenascin glycoproteins [12,13]. The resurfacing of the wound with new epithelium, epithelialization, consists of both the migration and the proliferation of keratinocytes at the rim of the wound [14]. Morphological changes in keratinocytes, at the wound margin, are evident within hours from the injury. Marginal basal Keratinocytes enlarge and begin migrating over the wound healing defect; and they do not divide until epidermal continuity is restored [6,14]. Basal cells in a zone near the edge of the wound provide new epithelial cells that flatten and migrate over the wound matrix as a sheet [6,15]. After the re-establishment of the epithelial layer, the basement membrane is formed by keratinocyte/fibroblast secretion of laminin and type IV collagen [6,16]. Then, keratinocytes become columnar and divide re-establishing the stratified epithelium and re-forming a barrier against further contamination and moisture loss. The epithelial coverage rate increases when the basal lamina is intact, the wound is kept moist and there is no need for debridement [6].

Remodeling phase and its regulation are poorly understood, but simplistically, remodeling can be conceptualized as the balance between synthesis, deposition, and degradation. Matrix synthesis and the remodeling phase are initiated in parallel with granulation tissue development and continue over a prolonged period of time. Wound remodeling occurs when the underlying contractile connective tissue, which contracts due to the interactions between fibroblasts and ECM influenced by TGF-β, PDGF and FGF-b, brings the wound margins closer together. Highly disorganized early collagen deposition is organized by wound contraction [17]. With continued remodeling, the outgrowth of capillaries is halted, blood flow to the area is reduced and metabolic activity in the area declines. An acellular, avascular scar is the final result of an acute wound healing process.

3. Chronic wounds

Wound-healing course might be altered in severe pathological conditions [18,19]. In big wounds, with a noticeable loss of tissue affecting the skin, subcutaneous tissue, fascia or even muscle, the first phase of filling can lengthen for too long, generating a chronic wound [18,19]. Chronic wounds fail to proceed through an orderly and timely process in order to produce anatomic and functional integrity [2], because the normal process of healing is disrupted at one or more points of its phases [1,20], due to such varied different factors as: impaired vascularization/oxygenation, deficient cytokine levels, fibrotic and desiccated tissues, etc.

In most chronic wounds, the healing process is stuck in the inflammatory or proliferative phases. A long-lasting inflammatory process can be negative for wound healing due to the production of free radicals with detrimental effects upon healthy cells, finally causing a persistent destruction of tissue [21]. Wound fluid derived from chronic venous leg ulcers is rich in pro-inflammatory cytokines such as TNF-α, interleukin-1β (IL-1β), and TGF-β1 [22]. Inflammatory cells will be dispersed from the wound only when inflammation has decreased and the repair is complete [1]. Big complex traumatic wounds with important tissue loss become senescent in the process of inflammation or proliferation, losing the ability to epithelialize [18,19].

4. Surgical management of the complex traumatic wounds before epithelialization

We consider that a complex traumatic wound has devascularised tissues, a loss of substance in its soft tissues, wound border separation, a large inflammatory component, demonstrated infection, exudate, and local and systemic factors that directly affect its healing process.

In order to manage these wounds, it is necessary to adopt a comprehensive and dynamic approach, taking into account the complete assessment of the patient, to ensure that there is control of causal factors and general care and preparation of the wound bed [23].

Preparation of the wound bed, in each phase of the healing process, provides an adequate framework for a structured approach of the management of these wounds. The objective is to create an optimum environment for well-vascularised healing with a stable and balanced wound bed in terms of exudate, aimed at reducing healing time and facilitating the efficiency of other therapeutic measures [23].

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