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REVIEW

Can host reaction animal models be used to predict and modulate skin regeneration?

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Abstract

The study of host reactions in the biomedical and tissue engineering (TE) fields is a key issue but somehow set aside where TE constructs are concerned. Every day new biomaterials and TE constructs are being developed and presented to the scientific community. The combination of cells and biomolecules with scaffolding materials, as TE constructs, make the isolation and the understanding of the effect of each one those elements over the overall host reaction difficult. Eventually, all variables influence the host reaction and the performance of the constructs. For this reason, current assessment of the *in vivo* performance of TE constructs follows individual approaches, using specific animal models to independently provide insights regarding the contribution of the biomaterials/scaffolds towards the host reaction, and of all the constructs regarding their functionality. Skin wound healing progress into tissue regeneration or repair is highly dependent on the specificities of the inflammatory stage, as demonstrated by comparison between fetal and adult mechanisms. Thus, it would be expected that insights acquired from host tissue reaction evaluation to biomaterials/scaffolds would be explored to predict healing progression and improve the functionality of skin TE constructs. The rational of this review is to make a comprehensive analysis of to what extent the knowledge obtained from the evaluation of *in vivo* host reactions to implantable biomaterials/scaffolds has been used in the design of skin TE strategies, by promoting tissue regeneration rather than repair.

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1. Introduction

The main objective of skin wound closure is to re-establish tissue and organ homeostasis preventing the invasion of microorganisms and infections. It is obvious that the sooner the wound heals, the lower is the infection probability and the faster the organism may return to its 'normal' function. However, accelerated closure is mainly achieved by rapid interposition of fibroblasts that will form the scar tissue.

Many text books (Fantone and Ward, 1999; Frank and Kaempfer, 2003; Gamelli and He, 2003; Williams, 2001) and review papers (Gurtner *et al.*, 2008; Martin *et al.*, 2003; Martin and Leibovich, 2005; Monaco and Lawrence, 2003; Werner and Grose, 2003) have been compiling the acquired knowledge on the cascade of events subsequent to tissue injury. However, the mechanisms by which organisms repair or regenerate injured tissue are not completely disclosed. Important insights have been revealed by studying the regeneration of amputated appendages of amphibians, showing that differentiated cells from mature tissues surrounding the wound dedifferentiate into mononuclear blastemal cells, which then differentiate into multiple tissue lineages (Brockes and Kumar, 2002; Suzuki *et al.*, 2005). Also, data from developmental studies in the sponge *Amphimedon queenslandica* (Adamska *et al.*, 2007) and in *Drosophila melanogaster* (Woolner *et al.*, 2005) revealed remarkable

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similarity with human wound closure mechanisms, namely cell–cell interactions mediated by Wnt proteins and transforming growth factor-β (TGFβ). Furthermore, salamanders and humans share the molecular machinery to regenerate tissues but the main difference relies on the rapid deposition of fibrotic tissue in adult humans, limiting the regenerative potential (Gurtner *et al.*, 2008).

Human fetal skin wound healing also presents very distinct characteristics from adult healing, among which is reduced inflammatory response (Cowin *et al.*, 1998; Ferguson and O'Kane, 2004; Hopkinson-Woolley *et al.*, 1994). In fact, this is translated by the diminished number and persistence of inflammatory cells, such as monocytes, and by the lack of B lymphocytes (Cowin *et al.*, 1998), until the third trimester of gestation. In fetal skin wound healing, reduction in the number and type of inflammatory and immune system cells also represents, as compared to adult process, decreased levels of inflammatory signals, cytokines and growth factors, with rapid clearance from the wound site (Table 1) (Chen et al., 2005a, 2005b; Cowin *et al.*, 2001; Ferguson and O'Kane, 2004; Levine *et al.*, 1993; Liechty *et al.*, 1998, 2000a, 2000b). In addition, the difference in the inflammatory cells' presence/absence and interval of action also impact extracellular matrix formation, re-epithelialization and wound contraction (Ferguson and O'Kane, 2004). Lack of macrophages and polymorphonuclear neutrophils were shown to improve re-epithelialization and diminished scar formation (Martin *et al.*, 2003). In fact, the presence of a more persistent acute inflammation (Adzick *et al.*, 1985) and keratinization in adult wound healing leads to the formation of scabs, resulting in almost permanent scars (Ferguson and O'Kane, 2004). Conversely, the absence of an underlying cell growth, a sterile and relatively low moist environment, high oxygen tension (Hunt *et al.*, 1969), slow matrix deposition and epithelialization (Lorenz *et al.*, 1992) and extensive angiogenesis leads to a slower closure rate in adult skin healing (Ferguson and O'Kane, 2004), which might contribute to tissue regeneration.

Given the relevance of inflammatory cells and mediators within the repair/regeneration process, it is clear that the host tissue response to tissue-engineering (TE) constructs, resulting from the individual and, most importantly, the concerted action of all their elements, is of major significance in determining constructs functionality *in vivo*. Furthermore, the identification of major factors involved in scarless fetal wound healing, and the establishment of their corresponding role within host reaction mechanisms, might be a way to achieve tissue regeneration and functional skin tissue in particular.

2. Host reaction

Ideally, a TE construct aims to interact with and integrate host tissue, allowing functional re-establishment and complete recovery of the injured tissue. The resolution of inflammation, with concomitant integration of the transplant into the host tissue, precedes complete healing (Langer and Vacanti, 1993). In a TE construct, where the scaffolding material works as a temporary structure, constant change of the implanted material, due to degradation and interaction with the host environment, certainly influences the reaction from the host and, thus, tissue healing. Moreover, the presence of cells and bioactive agents in the constructs, also known as relevant players in the host reaction, raises additional concerns and further impedes understanding of their individual and concerted contributions, and consequently the achievement of ideal regeneration settings.

2.1. Host reaction models

Subcutaneous, intraperitoneal and intramuscular mouse and rat (Azab *et al.*, 2007; Christenson *et al.*, 1991; Kamath *et al.*, 2008; Krause *et al.*, 1993; Meinel *et al.*, 2005; Mendez *et al.*, 2004; Tang *et al.*, 1998) models are the most commonly used to assess the host response to newly developed biomaterials/scaffolds for different TE applications. Despite the well-known influence of the processing methodologies the surface properties of materials (Gomes *et al.*, 2004; Tuzlakoglu *et al.*, 2010, 2011) and their degradation behaviour (Azab *et al.*, 2007; Dagang *et al.*, 2008; Gomes *et al.*, 2008), host reaction models usually have the limitation of not dealing with the final shape of the device. Nonetheless, valuable considerations can be obtained with those models regarding acute (Azab *et al.*, 2007; Marques *et al.*, 2005; Rhodes *et al.*, 2007; Spargo *et al.*, 1994) or chronic inflammation (Azab *et al.*, 2007; Kim *et al.*, 2007; Marques *et al.*, 2005; Rhodes *et al.*, 2007), as well as long-term reactions with full integration of the biomaterials/scaffolds into the host tissue (Ishii *et al.*, 2009; Matthews *et al.*, 2005; Rhodes *et al.*, 2007; Schlosser *et al.*, 2002). Additionally, conclusions regarding the local and/or systemic effects of injectable or scaffolding materials over the host have been obtained (Azab *et al.*, 2007; De Souza *et al.*, 2009; Rhodes *et al.*, 2007; Tomazic-Jezic *et al.*, 2001). Subcutaneous and intramuscular models have mostly focused on assessing the direct effect of the biomaterial/scaffold over the implantation site. Due to muscle high degree of vascularization that assists the activation of complement and clot systems, intramuscular models can be considered more reliable for providing information on the fibrotic capsule formation and development throughout the implantation (Meinel *et al.*, 2005; Mendez *et al.*, 2004). Variations of classical subcutaneous implantations, such as subcutaneous air pouches (Hooper *et al.*, 2000; Krause *et al.*, 1993; Wooley *et al.*, 2002), dorsal skin fold chamber (Laschke *et al.*, 2005) or cage implants (Brodbeck *et al.*, 2002, 2003; Kao and Lee, 2001; Marchant *et al.*, Marchant *et al.* 1983, 1989; Rodriguez *et al.*, 2008), have also demonstrated reliable results regarding the interplay between direct and indirect surface reactions of materials. Conversely, intraperitoneal models have been useful in

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Table 1. Inflammatory cells and molecules in fetal vs adult skin wound healing

Molecule	Secreted by	Role	Fetus	Adult
EGF	Platelets Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Re-epithelialization Stimulation of collagen secretion by fibroblasts	–	mRNA decreases with gestational age (Peled *et al.*, 2001)
VEGF	Platelets Mast cells Neutrophils Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Angiogenesis	Unclear (Wilgus *et al.*, 2008)	–
PDGF	Platelets Mast cells Monocytes/macrophages Fibroblasts Endothelial cells	Fibroplasia Recruitment of fibroblasts to the wound site	High levels but rapid clearance from wound site (Chen *et al.*, 2005b)	–
FGF	Platelets Mast cells Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Matrix deposition, Re-epithelialization, angiogenesis, cell migration (endothelial, keratinocyte and fibroblast)	High expression of FGF2 (Chen *et al.*, 2005b) Downregulation of FGF7 and FGF10 (Dang *et al.*, 2003)	–
TGFβ3	Platelets Mast cells Neutrophils Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Infiltration of PMNs and MΦ, fibroplasia, matrix deposition Angiogenesis	Low levels and increased clearance (Cowin *et al.*, 2001; Levine *et al.*, 1993)	High levels and long intracellular signalling (Levine *et al.*, 1993)
IGF-I	Platelets Monocytes/macrophages Fibroblasts	Matrix deposition Scarring Re-epithelialization	Low proliferation and collagen synthesis (Werner and Grose, 2003)	High proliferation and collagen synthesis (Rolfe *et al.*, 2007)
IL-1β	Platelets Mast cells Neutrophils Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Perpetuation of inflammation Induction of proteolytic activity Vascularization	Induction of lower response of fibroblasts to contraction (*in vitro*) (Irwin *et al.*, 1998)	Induction of higher response of fibroblasts to contraction (*in vitro*) (Irwin *et al.*, 1998)
IL-4	Platelets Mast cells	Reduction of inflammation	–	Downregulation of inflammatory cytokines (Leonard *et al.*, 1993)
IL-6	Mast cells Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Re-epithelialization and granulation tissue formation	Increased expression but rapid clearance from wound site (Liechty et al., 2000a●)	Mitotic effect in keratinocytes and chemoattractive to granulocytes (Sato et al., 1999a●)
IL-8	Mast cells Neutrophils Monocytes/macrophages Keratinocytes Fibroblasts Endothelial cells	Re-epithelialization Inflammation induction Wound contraction reduction	Chemoatractant for granulocytes but rapid clearance from wound site (Liechty *et al.*, 1998)	Chemoatractant for granulocytes (Liechty *et al.*, 1998; Rennekampff *et al.*, 2000) Inhibition of keratinocyte proliferation (Rennekampff *et al.*, 2000)
IL-10	Mast cells Monocytes/macrophages	Fibrosis/scar formation and inflammation inhibition	Regulating the expression of proinflammatory cytokines (Liechty et al., 2000b●)	Inhibition of granulocytes and MΦ infiltration at the wound site (Sato et al., 1999b●)

evaluating the reactions of abdominal organs, such as spleen, liver, kidney, mesenteric lymph nodes and the adjacent adipose tissue (Azab *et al.*, 2007; De Souza *et al.*, 2009; Tomazic-Jezic *et al.*, 2001), translating the host systemic reaction to the biomaterial/TE scaffold. Additionally, intraperitoneal and intramuscular models also allow evaluation of the systemic recruitment of host inflammatory cells and secretion of molecules after transplantation/injection at short and long time periods of reaction (Busuttil *et al.*, 2004; Lozano *et al.*, 2002; Tang and Eaton, 1993; Tomazic-Jezic *et al.*, 2001; Schlosser *et al.*, 2002). However, cell recovery from intraperitoneal fluid is easier than from intramuscular models, also allowing kinetic studies. The antigenic/immunogenic potential of a biomaterial and the consequent acquired immunity by the host has also been studied after repeated implantations in either subcutaneous or intraperitoneal rat models (Schlosser *et al.*, 2002; van Luyn *et al.*, 2001) .

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2.2. Mechanistics of the host reaction to biomaterials/scaffolds

Among inflammatory cells, macrophages, always a major player in the host reaction, recognize and react with the proteins adsorbed to the material's surface (Anderson and Miller, 1984). Although some insight has been gained regarding this surface-dependent interaction (Kao, 1999; Kao and Lee, 2001; Keselowsky *et al.*, 2007; Schmidt and Kao, 2007), the mechanisms by which macrophages adhere and react to the different surfaces are still far from being revealed. Specific fibronectin peptide sequences, such as Pro–His–Ser–Arg–Asn (PHSRN) and Arg–Gly–Asp (RGD) were shown to elicit an early-stage foreign body giant cells (FBGCs) reaction (Kao and Lee, 2001). Thus, those domains were identified as important factors mediating macrophage adhesion to polyethyleneglycol-based surfaces and then in the formation of FBGCs (Kao and Lee, 2001). A transgenic mouse model, in which plasma fibronectin [p(FN)] was depleted (Keselowsky *et al.*, 2007), permitted further demonstration that, besides being an important regulator of FBGCs reaction, p(FN) plays a role in fibrotic capsule formation. Additionally, two other transgenic mouse models, where either fibrinogen or plasminogen was depleted, proved that leukocyte recruitment after the intraperitoneal implantation of polyethylene terephthalate is plasminogen-dependent, while leukocyte adhesion is fibrinogen-dependent (Busuttil *et al.*, 2004).

Ultimately, the specific cellular response to the material's surface determines the deposition of collagen by the tissue-repairing cells and consequently the extent of the fibrotic capsule deposited (Keselowsky *et al.*, 2007). Fibrotic tissue surrounding the implant hinders the interaction of the host with the implanted biomaterial (Tang and Eaton, 1995; Wynn, 2008). This diminished interaction may protect the host from eventual material debris but, more importantly, will not allow integration of the implant into the host tissue, therefore being highly detrimental in TE approaches.

Despite the achievements using subcutaneous, intramuscular and intraperitoneal models regarding the evaluation of host inflammatory/immune reactions to biomaterials /scaffolds, understanding of the mechanisms involved on the transition from an acute to a chronic reaction, which the existing animal models are not capable of answering, is a significant lack in this field. This issue is particularly relevant for skin regeneration, due to the need to restore its integrity after injury in a short time frame. Furthermore, as the cascade of events in inflammatory/immune reactions involves crucial host cells and molecules that influence the progression of skin healing, comprehension of these insights offers important cues to lead tissue regeneration that have been poorly used or disregarded by researchers.

2.3. Modulating the host response through modification of biomaterials

Classically, researchers in the biomedical/TE field tend to consider the material–host interface as a key issue in evaluating tissue reactions. In fact, great efforts have been made to develop materials whose surfaces are less antigenic/immunogenic (Hetrick *et al.*, 2007; Hickey *et al.*, 2002; Khouw *et al.*, 2001; Ravin *et al.*, 2001). The reduced number and lack of *in vitro* models to validate the antigenic/immunogenic potential of biomaterials still renders *in vivo* tests more reliable for testing the success of these approaches. The effect of key molecules, such as dexamethasone (Hickey *et al.*, 2002), nitric oxide (Hetrick *et al.*, 2007), tumour necrosis factor-α (TNFα), interferon-γ (IFNγ) (Khouw *et al.*, 2001), vascular endothelial growth factor (VEGF) and fibroblast growth factor-β (FGF-β) (Ravin *et al.*, 2001), has been tested in subcutaneous rat (Hetrick *et al.*, 2007; Hickey *et al.*, 2002) and mouse (Khouw *et al.*, 2001) models, as well as in an intramuscular rat model (Ravin *et al.*, 2001). However, due to given differences in the models and consequent differences in reaction mechanisms on the materials, and thus the surface properties, few remarks regarding the potential of the tested molecules in modulating the host response can be extracted.

In a TE context, these approaches easily find a parallel in the functionalization of scaffolding structures with different bioactive molecules; however, these have been mainly chosen to direct the differentiation of the transplanted cells towards a specific lineage (Altman *et al.*, 2009; Santos *et al.*, 2013) or to recruit progenitor cells (Aguirre *et al.*, 2012) responsible for new tissue formation. To consider the action of those molecules as an intricate signalling network that not only directs new tissue formation but also determines the host reaction still remains to be particularly addressed.

3. Skin healing

The ultimate goal of skin TE is to lead the regeneration of new skin tissue with all the sensorial and aesthetic functionalities restored. Trauma and surgical procedures are the main causes of acute skin lesions and their healing mechanisms are not necessarily similar. Incisional wounds heal by primary intention (Gamelli and He, 2003), meaning that tissue loss is not extensive, the inflammatory response is not exuberant and scar formation is not a relevant issue. In contrast, skin excisions involve high tissue removal and, thus, a secondary-intention healing process (Frank and Kaempfer, 2003), characterized by abundant inflammation and granulation tissue formation, resulting in significant scarring. Acute skin wounds follow a well-established pattern of progression through the inflammatory, proliferative and maturation phases (Baum and Arpey, 2005; Fantone and Ward, 1999; Williams, 2001). It is expected that an acute excisional wound will be closed within 2 weeks, although this time frame may vary depending on the extent of the excised tissue (Monaco and Lawrence, 2003). Conversely, chronic wounds take months to years to close or might never do so. In chronic wounds, healing progression is impaired

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due to arrest at one or more stages of the process, caused by local or systemic factors. Exuberant contamination, hypoxia, trauma, presence of foreign bodies, diabetes, malnutrition, immunodeficiency and medication (Komesu *et al.*, 2004; Robson, 1988; Williams and Harding, 2003) are among those.

3.1. Chronic skin wound-healing models

When an cute inflammatory reaction persists at the wound site, a chronic skin wound with delayed healing and inability to re-epithelialize within 6–8 weeks develops (Menke *et al.*, 2007; Schultz and Wysocki, 2009). A major problem of chronic wounds with associated impaired healing relies on its different aetiologies, such as diabetes, immunosuppression, blood supply or nourishing deficiencies, glucocorticoids administration and age (Davidson, 1998; Menke *et al.*, 2007), which do not represent a localized deficit.

Frequently, the established chronic animal models mimic delayed wound healing and not the impaired wound healing observed in the clinical condition of chronic wounds. Therefore, these models have been mainly used to try to understand the mechanisms underlying impaired healing, as the case of diabetes, in mice (Brown *et al.*, 1997) and rats (Chen *et al.*, 1999; Komesu *et al.*, 2004), instead of working as proper chronic wound models for testing skin regeneration strategies. An example of an animal model that may mimic a particular characteristic of chronic wounds, such as ischaemia, is the porcine split-thickness skin wound model. Even though it is not a specific model of chronic wound formation, its impaired blood supply allows testing the potential of TE constructs to trigger angiogenesis and to promote dermal regeneration (Markowicz et al., 2006b•). Contrarily, rabbit (Niitsuma *et al.*, 2003) and murine (Wassermann *et al.*, 2009) models of decubitus ulcers, although representing a specific type of chronic wound, have been mainly used to gather further knowledge on the mechanisms of pressure ulcer development and the assessment of basic healing mechanisms of chronic wounds.

3.2. Acute skin wound-healing models

Incisional full-thickness wound rat (Cho *et al.*, 1999; Hu *et al.*, 2003; Ono *et al.*, 2004) and mouse (Ishihara *et al.*, 2001; Repertinger *et al.*, 2004) models have been used to evaluate the effect of biomaterials *per se*, or of locally releasing growth factors over healing rates, considering skin breaking strength and bleeding cessation. However, these models are not able to offer valid information for skin TE, since healing by primary intention does not characterize the mechanisms involved in skin regeneration. In turn, partial or full-thickness excisional acute wound models are the most appropriate and the ones that were proved useful (Davidson, 1998) in predicting the biofunctionality of biomaterials for skin-related applications or of skin TE constructs. Full-thickness wound models are, contrarily to the partial-thickness models, essential to demonstrate the direct role of biomaterials (Cho *et al.*, 1999; Choi *et al.*, 2001; Hu *et al.*, 2003; Ishihara *et al.*, 2001; Noorjahan and Sastry, 2004; Sugihara *et al.*, 2000; Suzuki *et al.*, 1999) and TE constructs (Altman *et al.*, 2009; Inoue *et al.*, 2008; Markowicz et al., 2006a, 2006b•) over the healing mechanisms. Despite this, the panniculus carnosus muscle beneath the dermis is a main concern in rodents and lagomorphs models, due to tissue contraction. Moreover, the fast healing rate observed in small animals, even after the administration of steroids to impair wound healing (Saulis and Mustoe, 2001), compel researchers to adjust the rodent models, or to substitute small animals by larger animals (Ma *et al.*, 2007; Middelkoop *et al.*, 2004) with skin healing closer to humans. The resemblances between human and porcine skin (Metcalfe and Ferguson, 2007) support the reliability of these models. In fact, swine full-thickness skin excisional models have proved useful and reliable tools to evaluate skin regeneration after the grafting of skin tissue-engineering constructs. In fact, results concerning the involvement of transplanted cells (mostly autologous keratinocytes), acellular matrices and host cells and molecules (Butler and Orgill, 2005; Druecke *et al.*, 2004; Jones *et al.*, 2003; Melendez *et al.*, 2008; Wood *et al.*, 2007) obtained with this model were translated to the clinic. Therefore, these and the similarities between porcine and human skin healing counterbalances the high costs and demanding logistics of using this model, and support controversy about the relevance of the results obtained with the different acute skin wound healing rodent models.

Researchers are not prone to using comparative animal models to evaluate the functionality of the proposed approaches. Either the constructs are only tested in rodents or directly transplanted into porcine models. From our perspective, these models could complement each other. The possibility of directly comparing the healing microenvironment evaluated in skin-healing models with the reaction to the scaffolding material determined in host-response models could somehow surpass the limitation of the rodent models. Thus, a systematization of this comparative approach would contribute to better predicting the functionality of the constructs and refine the TE strategies to be tested in larger animal models afterwards.

4. Host reaction vs skin regeneration

Inflammation and wound healing share the extraordinarily important feature of restoring the homeostatic status of a living body. Inevitably, wound healing is preceded by inflammation; the key inflammatory cells and mediators also share functions in the progression of wound healing and tissue regeneration, especially in skin. Polymorphonuclear neutrophils (PMNs), macrophages

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(MΦ), mast cells, lymphocytes and platelets secrete a wide range of molecules that balance the inflammatory response, but are also involved in the stimulation or restraint of matrix deposition, cell infiltration, vascularization, angiogenesis and re-epithelialization during skin healing. A clear crosstalk between the inflammatory and connective tissue cells (e.g. fibroblasts) that, in a first stage, regulate the resolution of inflammation and then the restoration of the original tissue, demonstrates the existing interplay. After 6 weeks of implantation in full-thickness porcine wounds, mononuclear cells (MΦs and lymphocytes) and giant cells were found in direct contact with the collagen scaffold fibres of Integra. Although no signs of phagocytosis were observed, the number of giant cells was significantly higher than in healthy tissue (Druecke *et al.*, 2004; Melendez *et al.*, 2008). This decodes an activation of MΦs, inducing them to fuse without achieving the phagocytosis step. In a subsequent study, (Agrawal *et al.*, 2012) identified the presence of M1 and M2 type MΦs up to 42 days postimplantation of Integra (R), associated with a more constructive tissue remodelling response than for AlloDerm®. In this case, MΦs were predominantly M1 and a more inflammatory-type tissue remodelling outcome was observed. Thus, a balanced effect where, due to the persistent presence of the Integra (R) scaffold, M1 type MΦs influenced the formation of giant cells and the M2 MΦs led skin healing towards a fibrosis-free scar was potentially occurring. Thus, in the context of biomaterials, the host response and the progression to a wound-healing profile, the cytokines produced by the M2 polarized macrophages, known to have anti-inflammatory potential, are expected to aid tissue remodelling and vascularization and to inhibit fibrous tissue formation (Gordon and Martinez, 2010). The capacity of a biomaterial to modulate the expression of inflammatory mediators and the time course of cutaneous healing, particularly the relationship between IL-8 expression and re-epithelialization, was also demonstrated (Kleinbeck *et al.*, 2010). Thus, an intervention in the early stage of wound healing, particularly targeting the inflammatory mediators, is expected to trigger the skin tissue regeneration pathway, providing better outcomes than the current strategies, which have been mainly targeting the later phases of proliferation and remodelling.

The improvement of scarless skin healing was also associated with beneficial crosstalk between the transplanted keratinocytes and host fibroblasts (Melendez *et al.*, 2008). In fact, autologous keratinocytes in combination with Integra (R) were shown to enhance the epithelialization of porcine full-thickness wounds (Jones *et al.*, 2003; Melendez *et al.*, 2008; Wood *et al.*, 2007) and seemed to influence the progression of the inflammatory reaction towards a more remodelling-prone response.

Overall, it seems evident that, by identifying and studying the cells and molecules involved in the ongoing resolution of the host reaction to skin TE constructs, important insights could be drawn in the context of restoring homeostasis with skin tissue regeneration.

5. Final Remarks

From this overview, it becomes clear that inflammatory and immune cells, as well as the associated mediators (cytokines and growth factors) involved in the host reaction to material/TE scaffolds, are intrinsically related to the progression of skin tissue healing into scar formation (repair) or regeneration. Although demonstration is yet to be attained, it is likely that a material that elicits a less severe inflammatory response is more prone to trigger skin regeneration rather than repair. Nonetheless, the interplay and complexity of these phenomena significantly hinder the controlled and well-designed approaches that have yet to be proposed. Exploitation of the knowledge acquired from host tissue reaction models to predict and modulate skin tissue healing is seen as an extremely valuable approach to attain this. Ultimately, this complementarity will benefit the development of improved skin TE constructs capable of modulating the progression of the host reaction by targeting the key signalling pathways that rule skin regeneration.

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