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Extracellular matrix components of oral mucosa differ from skin and resemble that of foetal skin

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ABSTRACT

Objective: Wounds of both the oral mucosa and early-to-mid gestation foetuses have a propensity to heal scarless. Repair of skin wounds in adults, however, regularly results in scar formation. The extracellular matrix (ECM) plays an important role in the process of healing. The fate of scarless or scar forming healing may already be defined by the ECM composition, prior to wounding. In this study, the presence of several ECM components in oral mucosa (palatum) and skin was investigated.

Design: Immunohistochemical stainings of different ECM components were performed on skin, obtained from abdominal dermolipectomy surgery, and oral mucosa, derived after pharynx reconstruction.

Results: Expression of fibronectin, its splice variant ED-A, and chondroitin sulphate was elevated in oral tissue, whereas elastin expression was higher in skin. Tenascin-C, hyaluronic acid, biglycan, decorin, and syndecan-1 were expressed at similar levels in both tissues. Oral mucosa contained more blood vessels than skin samples. Finally, oral keratinocytes proliferated more, while dermal keratinocytes demonstrated higher differentiation.

Conclusions: Comparing ECM components of the skin and oral mucosa coincides with differences earlier observed between foetal and adult skin, and this might indicate that some ECM components are involved in the mode of repair.

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Abbreviations: α-SMA, alpha smooth muscle actin; ECM, extracellular matrix; fibronectin ED-A, fibronectin splice variant extra domain A; TGF-β, transforming growth factor-beta.

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1. Introduction

Wound healing can result in excessive scarring which is a great burden for patients.

Principally deep dermal wounds have the tendency to form hypertrophic scars, while superficial wounds heal with minimal scar formation. Dermal scar tissue differs in composition from normal skin as a result of excessive accumulation of extracellular matrix (ECM) components and a disturbed organization.[1] In addition, scars are less elastic and reach only about 70% tensile strength compared to intact skin.[2]

Contrary to skin wounds oral mucosal wounds heal faster, showing minimal scar formation. Thus far, exact mechanisms between scarless oral and scar forming dermal healing are unknown, although a few differences have been described. For instance, oral wounds contained lower number of immune cells.[3,4] As compared to dermal wounds reduced expression of the profibrotic factor transforming growth factor (TGF)-β1 was found in oral wounds, while antifibrotic TGF-β3 was elevated.[5,6] Finally, oral mucosa fibroblasts proliferated faster than the dermal counterparts.[7] In healthy tissue oral fibroblasts produced significantly more hepatocyte growth factor and keratinocyte growth factor, compared to dermal fibroblasts.[8] Contraction was enhanced in oral fibroblasts, although these cells appeared to be less susceptible to TGF-β1 with respect to alpha smooth muscle actin expression (α-SMA).[9]

Scarless healing is also observed in early-to-mid gestational foetal wounds. Fast reepithelialization, lack of immune mediators and complete regeneration are typical features of foetal wound repair.[10] Studies that investigated the mechanism of scarless healing mainly focused on processes during wound healing, though the fate of scarless or scar forming healing may already be found in the tissue architecture itself, prior to wounding. Coolen et al.[11] demonstrated increased expression of the ECM components fibronectin and chondroitin sulphate in foetal skin, while elastin was only present in adult skin.

The ECM plays a significant role in cell adherence, migration, proliferation and it directs cell phenotype. Therefore differential expression of ECM components may possibly contribute to scar forming or scarless repair. The ECM comprises proteoglycans (e.g. heparan sulphate, chondroitin sulphate, keratan sulphate), fibrous proteins (collagens, elastin, fibronectin, laminin), and functions as a reservoir for growth factors. ECM proteins are synthesized and secreted by fibroblasts and myofibroblasts. When comparing oral and dermal fibroblasts, several differences were found regarding ECM expression. For instance, hyaluronan synthase-3 was highly expressed by oral fibroblasts, but expression by dermal fibroblasts was low.[12] On the contrary, hyaluronan synthase-1 was expressed in dermal fibroblast while it was absent in oral fibroblasts. The oncofetal cytokine migration stimulating factor, which is a truncated form of fibronectin, was only produced by oral (gingiva) and foetal fibroblasts but not by healthy adult dermal fibroblasts.[13,14] This cytokine stimulates migration of fibroblasts, epithelial cells and endothelial cells, but also promotes angiogenesis and hyaluronic acid synthesis.[15] These data may imply an elevated hyaluronic acid expression in the oral mucosa, though a study by Pedlar[16] showed increased hyaluronic acid expression only in the palatum, when compared to the rat skin or gingiva. Also matrix metalloproteinases (MMPs) were shown to be differentially expressed: oral fibroblasts produced more MMP-2 and -3 compared to their dermal counterparts.[17,18] In foetal fibroblasts, an increased gelatinase activity was reported in contrast to adult fibroblasts, indicative for reduced collagen accumulation.[19]

Additionally, several ECM components were shown to be associated with the formation of fibroproliferative disorders. For example, mice deficient for the fibronectin splice variant extra domain A (ED-A), did not develop pulmonary fibrosis after challenge with a fibrotic agent.[20] On the contrary, fibronectin ED-A has been shown to be important for repair by means of participation in the reepithelialization process.[21] Levels of the small leucine-rich proteoglycan biglycan was significantly elevated in hypertrophic scars, compared to normal skin.[22] Expression of decorin and fibromodulin however, was lower in these scars. Addition of recombinant decorin downregulated cell proliferation, TGF-β1 production, and collagen synthesis in hypertrophic scar fibroblasts.[23]

In this study, we evaluated the location and deposition of several ECM components in skin and oral mucosa, as expression of various ECM components might be involved in the fate of healing.

2. Materials and methods

2.1. *Tissue samples*

Human skin was obtained from six healthy individuals (gender not registered, mean age 37 ± 18 years old) undergoing abdominal dermolipectomy. All donors provided informed consent according to institutional and national guidelines. Skin pieces of maximal 1 cm^2 were embedded in Tissue Tek® OCT™ Compound (Sakura Finetek, Alphen aan den Rijn, The Netherlands) and stored at −80 °C until sectioning. Oral mucosa was obtained after informed consent from six patients (5 females, one male; mean age 6 ± 3 years old) with a history of open cleft palate undergoing pharynx reconstruction. A small part of the palate was resected and processed as described for human skin. To overcome discrepancies due to age, we tested oral mucosal tissue from one male aged 27 years old (informed consent), and found identical results as with oral tissues derived from juveniles (data not shown).

2.2. *Immunohistochemistry*

Skin and oral mucosal cryosections (5 μm) were mounted on collagen coated glass slides and fixed in acetone for 10 min. Peroxidase was quenched with H_2O_2 and sections were incubated with primary antibodies (Table 1) for 1 h at room temperature. Subsequently, sections were incubated with Envision (Dako, Glostrup, Denmark) and detection was performed using diaminobenzidine (DAB) as chromogen substrate (Dako). Finally, sections were counterstained with haematoxylin, dehydrated in ethanol and embedded in