Oral Diseases (2007) 13, 1–10. doi:10.1111/j.1601-0825.2006.01314.x

http://www.blackwellmunksgaard.com

INVITED REVIEW

Non-epithelial oral mucosal progenitor cell populations

P Stephens[1], P Genever[2]

[1]*Wound Biology Group, Cardiff Institute of Tissue Engineering and Repair, Department of Oral Surgery, Medicine & Pathology, School of Dentistry, Cardiff University, Cardiff, UK;* [2]*Biomedical Tissue Research Group, Department of Biology (Area 9), University of York, York, UK*

This review considers the potential existence and role of stem or progenitor cell populations within the non-epithelial tissues of the oral mucosa. Currently, there is little published evidence supporting this hypothesis; however, because of the similarities in structure and function of the oral mucosa and skin, findings within the dermis of the skin may potentially reflect the situation within the oral mucosa. Over recent years, the identification of the skin as a local reservoir of adult stem cell populations and the idea that multipotent cell populations exist within the dermal tissues of skin has gained increasing credibility. Indeed, numerous multipotent progenitor cells have been identified within the dermis and resident appendages, all capable of differentiating into multiple cell lineages. Furthermore, a number of these cell populations have been implicated in the repair of these tissues following injury. There is increasing evidence suggesting that such populations of progenitor cells may also reside within the lamina propria. In this respect, the ability to isolate large numbers of multipotent progenitor cells from a tissue which when biopsied heals without a scar would be of great interest scientifically and commercially, particularly with respect to future therapeutic applications and the developing discipline of tissue engineering.
Oral Diseases (2007) **13**, 1–10

Keywords: oral mucosa; progenitor cell; dermis; fibroblast; vasculature

Introduction

The purpose of this review was to consider the existence and the role of stem or progenitor cell populations within the oral mucosa. There is a growing literature on stem cells in the epithelial tissues of the oral mucosa which is covered in another review within this series and therefore will not be considered here. Rather, this review will concentrate on the evidence supporting the existence of progenitor cells within non-epithelial tissues. Surprisingly, the published evidence for the potential existence of such populations within the non-epithelial tissues of the oral mucosa is extremely limited. However, the similarities in structure and function of the oral mucosa and skin means that the focus of this review, by default, will be on progenitor cells within the non-epithelial tissues of the skin with an eye on how these findings may be interpreted with respect to the oral mucosa.

Correspondence: Dr Phil Stephens, Wound Biology Group, Cardiff Institute of Tissue Engineering and Repair, Dept. Oral Surgery, Medicine and Pathology, School of Dentistry, Cardiff University, Cardiff, CF14 4XY, UK. Tel: +44 (0) 29 2074 2529, Fax: +44 (0) 29 2074 4509, E-mail: stephensp@cardiff.ac.uk
Received 8 March 2006; revised 26 June 2006; accepted 30 June 2006

Structure of the oral mucosa and skin

Both the oral mucosa and the skin play a crucial role in maintenance of the organism through the regulation of water and electrolyte balance, thermoregulation, initiation of immunological functions and by acting as a barrier to external noxious agents including microorganisms (Urmacher, 1990; Sloan *et al*, 1991). Both consist of a number of distinct layers (see Figures 1 and 2; Table 1) which includes: (i) an epithelium comprising of stratified squamous cells, (ii) an underlying layer of connective tissue (the lamina propria in the oral mucosa and the dermis in the skin) which is composed of dense fibro-elastic material and contains extensive vascular and neural networks, excretory and secretory glands and appendages such as the hair (skin only) and (iii) a deeper fat layer (the submucosa in the oral mucosa and the hypodermis or subcutis in the skin).

The epithelium

The epithelium of the oral cavity and the skin is stratified squamous throughout with the cells varying from cuboidal or low columnar at the connective tissue interface to flat squamous at the surface. Keratinocytes are the principal cell type within epithelial tissues and are so named because of the filamentous keratin proteins that are produced as the cells undergo epidermal differentiation. Within the oral cavity there are two types of epithelium: non-keratinized (soft palate, underside of tongue, alveolar mucosa, labial mucosa, buccal

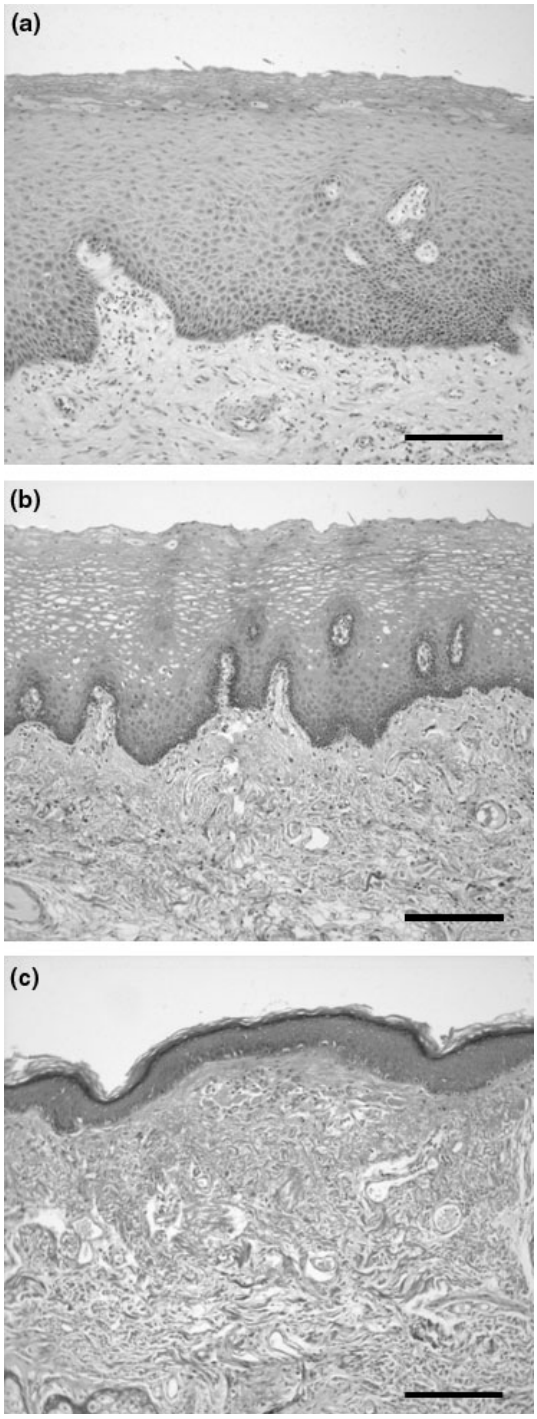

Figure 1 Hematoxylin and eosin-stained sections of: (**a**) keratinized oral mucosa, (**b**) non-keratinized oral mucosa and (**c**) skin demonstrating similarities in the overall composition of these tissues (despite some differences in epithelial thickness). Scale bar = 200 μm

mucosa) and keratinized (gingiva, hard palate). Whilst the non-keratinized epithelium has a structure somewhat different to that of skin [non-keratinized epithelial layers include the stratum (S.) basale, S. intermedium and S. superficiale], the structure of keratinized oral epithelium is very similar to skin (epithelial layers include the S. basale, S. spinosum, S. granulosum and S. corneum). The similarities in the structure of both the keratinized oral epithelium and skin are linked to similarities in function which include defence and resistance to shear stress/friction (provided by the cornified outer layers of the respective epithelial structures). The closely packed layers of the epithelium are produced by cell division within the S. basale cell layer which is thought to contain populations of progenitor cells (Brouard and Barrandon, 2003). At any point in time, 30% of these columnar cells are in the process of preparing for cell division after which the cell can remain within the basal layer or move to a suprabasal position and become committed to terminal differentiation and stratification. In addition to the keratinocytes, the epithelia can also contain a small number of melanocytes, Langerhans cells and Merkel cells. The epidermis extends into the dermis as broad folds or rete ridges, giving rise to a broadly undulating interface between the two layers.

The epithelial–connective tissue junction

This is the interface, also termed the basal complex/lamina (oral mucosa) or the basement membrane (skin), between the epithelium and the underlying lamina propria. It is composed of the lamina lucida, which is less dense towards the epithelial side and the lamina densa (basal lamina) which is composed of type IV collagen and laminin and located next to the lamina propria. Basal cells of the epithelium are not attached to the connective tissue proper, but rather form mechanical adhesions with the basal lamina/basement membrane. These attachments are hemidesmosomes which are composed of an attachment plaque that possesses intracellular modifications of tonofilaments. These penetrate the plasma membrane of the cell, terminating in the basal lamina. Fine collagen fibres (anchoring fibres composed of type VII collagen) attach to this lamina on the connective tissue side. This complex of fibres is found at intervals along the basal cell plasma membrane of the epithelial–connective tissue interface.

The lamina propria and the dermis

This is the connective tissue layer immediately below the epithelium, which can be divided into the papillary and reticular layers. The uppermost layer just beneath the epithelium is the papillary lamina propria/dermis which surrounds the adnexal structures. The superficial portion of the papillary layer is arranged into ridge-like structures, the connective tissue papillae, which contain microvascular and neural components that sustain the epidermis. The papillary layer is composed of a highly woven network of type I collagen mixed with type III collagen, elastic fibres, glycosaminoglycans, proteoglycans and glycoproteins. Located within this meshwork are capillaries and fibroblasts [the main producers of the extracellular matrix (ECM)]. Beneath the papillary layer is the thick reticular lamina propria/dermis which is composed of thick bundles of type I collagen and elastic fibres, glycosaminoglycans, proteoglycans, glycoproteins and other populations of fibroblasts. The reticular layer extends from this superficial vascular plexus to a

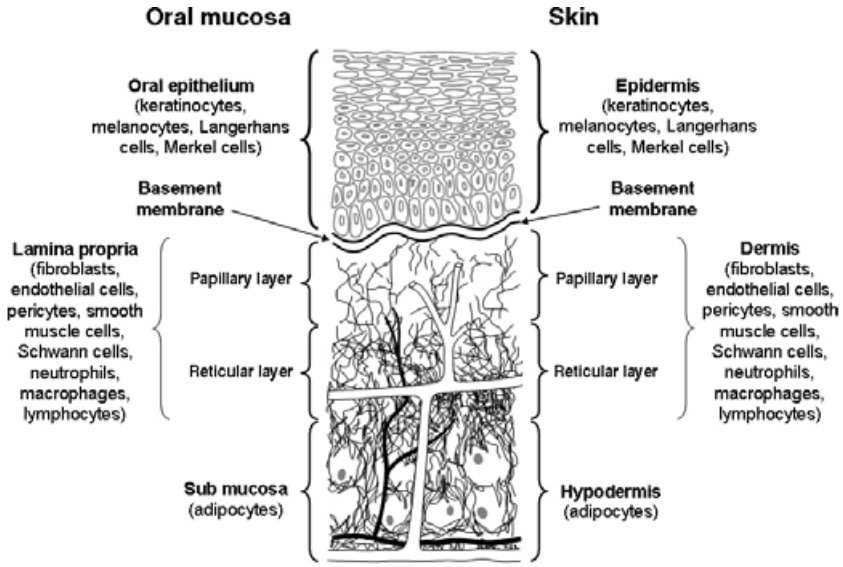

Figure 2 A schematic representation of the oral mucosa and skin demonstrating the similarities in the epithelial, dermal and subdermal layers

Table 1 A summary of the major similarities and differences between oral mucosal and skin tissues

	Oral mucosa	*Skin*
Major functions	Barrier function, regulation of water and electrolyte balance, thermoregulation, initiation of immunological functions	Barrier function, regulation of water and electrolyte balance, thermoregulation, initiation of immunological functions
Epithelial type	Stratified squamous epithelium (keratinized and non-keratinized mucosa)	Stratified squamous epithelium (keratinized)
Basement membrane	Basal complex/lamina	Basement membrane
Connective tissue layer	Lamina propria (some variation in extracellular matrix composition between keratinized and non-keratinized mucosa)	Dermis
Fat layer	Submucosa (non-keratinized mucosa only)	Hypodermis or subcutis
Appendages	Minor salivary and sebaceous glands	Hair follicles and sebaceous glands

deeper vascular plexus which serves as the boundary between the connective tissue and submucosa/hypodermis. In the lamina propria whilst the connective tissue structure and composition are broadly similar between keratinized oral mucosa and skin (ECM content, highly developed connective tissue papillae), the lamina propria of non-keratinized oral mucosa shows some differences (the ECM is loose and more elastic with fewer, more slender connective tissue papillae). The continuous structure of the oral mucosa is often interrupted by the presence of minor salivary and sebaceous glands whilst within the skin, hair follicles and sebaceous glands extend into and often through the reticular dermis to terminate in the hypodermis. In addition to the fibroblasts (of which there are distinct populations with the oral mucosa and the skin), cells of blood vessels (endothelial, pericytes and smooth muscle cells), peripheral nerves (Schwann cells) and the immune system (neutrophils, macrophages, lymphocytes) are also present in the lamina propria and skin.

The submucosa and the hypodermis

The submucosa and hypodermis are arranged into lobules of mature adipocytes which are separated by thin bands of dermal connective tissue that constitute the interlobular space. An extensive network of arteries, veins, capillaries, nerves and lymphatics extend through these structures and into the connective tissue layers where they supply nutrients and remove waste products. This layer also acts as a mechanical cushion and provides insulation against heat loss. Within the oral mucosa the presence or absence of a submucosa is dependent on the type of tissue under examination (i.e. in relation to function, keratinized oral mucosa has little or no submucosal layers, whereas non-keratinized oral mucosa can have a significant submucosa associated with it).

Stem cells

Whilst much has been documented about the toti/pluripotency of embryonic stem cells (Fortier, 2005; Lerou and Daley, 2005) recent attention has turned towards adult stem cells which were originally thought to have a restricted potential for generating new tissue. However, a growing body of evidence now supports the notion that a sub-population of mesenchymal stem cells (MSCs) exists within the bone marrow stromal compartment that are capable of self-renewal and can differentiate into several cellular phenotypes. These

MSCs, when cultured *ex vivo*, have been demonstrated to differentiate into numerous cell types, including osteoblasts, chondroblasts, adipocytes, fibroblasts, skeletal myoblasts and endothelial cells (Pittenger *et al*, 1999, 2000; Jiang *et al*, 2002; Reyes *et al*, 2002). It has been suggested that pluripotent stem cell populations persist in multiple organs even after birth and that when stimulated they proliferate and differentiate in response to local cues provided by the organs they are recruited to (Jiang *et al*, 2002).

Multipotent stem cells and the dermis

It has been suggested that skin stem cells are either unipotent (i.e. they generate a single lineage) or multipotent (i.e. they generate multiple lineages) and that at least six different stem cell populations are thought to exist within the skin including epidermal, mesenchymal, haematopoietic, neural and endothelial (Brouard and Barrandon, 2003). Between them they are thought to generate more than 25 lineages within the functional unit known as the skin (Brouard and Barrandon, 2003). Over the recent years, the identification of the skin as a local reservoir of adult stem cell populations and the idea that multipotent cell populations exist within the dermal tissues of skin has gained increasing credibility (Table 2) (Toma *et al*, 2001; Young *et al*, 2001; Lako *et al*, 2002; Chunmeng and Tianmin, 2004; Fernandes *et al*, 2004; Shi *et al*, 2004; Shi and Cheng, 2004; Toma *et al*, 2005). Indeed, labelled MSC transplantations into lethally irradiated C57BL/6 mice have now provided direct evidence that bone marrow-derived cells can give rise to functional skin cells and regenerate skin tissue (Deng *et al*, 2005). However, as a point of caution, extrapolation of findings within animal model systems may not be entirely consistent with what is found in human systems (Koestenbauer *et al*, 2006).

A multipotent stem cell population termed skin-derived progenitor (SKP) cells has been identified within the dermis (Fernandes *et al*, 2004; Toma *et al*, 2005). These cells demonstrate similarities with MSCs, differentiate into neural cell types and mesothelial derivatives, and are derived from a niche in the papillae of the hair follicle and maintain multipotency into adulthood (Sieber-Blum *et al*, 2004). Such SKPs have been isolated from the dermis of both juvenile and adult rodent skin and may represent a novel multipotent adult stem cell that may be less 'biased' than other adult stem cells (Toma *et al*, 2001). The authors further suggest that these SKPs exhibit properties similar to embryonic neural-crest stem cells and that they can first be isolated from skin during embryogenesis and persist into adulthood (Fernandes *et al*, 2004). This therefore suggested that SKPs represent an endogenous embryonic precursor cell that arises in peripheral tissues such as skin during development and maintains multipotency into adulthood. SKPs have also been isolated from neonatal human foreskin tissue (Toma *et al*, 2005). These human SKPs could be maintained in culture for long periods of time and could still differentiate into neurones, glia and smooth muscle cells, including cells with the phenotype of peripheral neurones and Schwann cells. Interestingly, a subpopulation of these dissociated primary foreskin cells could differentiate into neurones, a cell type never seen in skin. Together, these data indicate that SKPs are endogenous multipotent precursor cells present in human skin that can be isolated and expanded and can differentiate into both neural and mesodermal cell types.

Seruya *et al* (2004) isolated putative MSCs from the connective tissue of an adult rat. Exposure to non-specific differentiation culture medium revealed multilineage differentiation potential of adult rat MSC clones. Immunostaining confirmed the appearance of mesodermal phenotypes, including adipocytes, chondrocytes and skeletal myoblasts. Furthermore, Young *et al* (2001) isolated stem cells from the connective tissues of dermis and skeletal muscle derived from foetal, mature and geriatric humans. All populations contained lineage-committed myogenic, adipogenic, chondrogenic and osteogenic progenitor stem cells as well as lineage-uncommitted pluripotent stem cells capable of forming muscle, adipocytes, cartilage, bone, fibroblasts and endothelial cells. Others suggest that these MSC-like cells may be a source of wound-healing fibroblasts (Chunmeng *et al*, 2004).

Multipotent stem cells and hair follicles

Hair follicle stem cell biology is also the focus of increasing interest because the adult hair follicle has well-defined dermal and epithelial populations that display distinct developmental properties. Indeed, Lako *et al* (2002) have demonstrated that rodent hair follicle end bulbs as well as micro-dissected dermal papilla and dermal sheath cells actively produced cells of erythroid and myeloid lineages, but that follicle epithelial cells did not. This suggests that the dermal, but not epidermal, compartments of the adult hair follicle have much broader stem cell activities than previously described. Furthermore, it has been proposed that the repair of the skin dermis after injury is brought about by dermal sheath cells that become wound-healing fibroblasts (Jahoda and Reynolds, 2001; Gharzi *et al*, 2003; Richardson *et al*, 2005). In direct comparative analyses, we have recently demonstrated that the rate and extent of differentiation of hair follicle dermal stem cells was equivalent to bone marrow-derived MSCs obtained from adult rats (Hoogduijn *et al*, 2006). Cells expanded from the interfollicular dermis failed to differentiate. Hence, it is feasible that multipotent cell populations within the skin could be of hair follicle dermal cell origin.

Multipotent stem cells and adipose tissue

It is now well established that adipose tissue represents a plentiful reservoir of adult stem cells with predictions that stem cell frequency is significantly higher in adipose tissue compared with the bone marrow (2% *vs* 0.002%) (Strem and Hedrick, 2005; Strem *et al*, 2005). Populations of stromal cells have been obtained from adipose

Table 2 A summary of the available data for existing progenitor cell populations within non-epithelial skin structures

Progenitor cell name/source	*Potential tissue/cell type*	*Other information*	*Related references*
Skin-derived progenitor cells (SKPs) in the dermis	Neurones, glia and smooth muscle cells	Derived from a niche in the papillae of the hair follicle	Fernandes *et al*, 2004; Toma *et al*, 2005
Dermal putative mesenchymal stem cells	Adipocytes, chondrocytes and skeletal myoblasts		Seruya *et al*, 2004
Stem cells from the connective tissues of dermis and skeletal muscle	Muscle, adipocytes, cartilage, bone, fibroblasts and endothelial cells	May be a source of wound healing fibroblasts	Young *et al*, 2001; Chunmeng *et al*, 2004
Hair follicle end bulbs, dermal papilla and dermal sheath cells	Cells of erythroid and myeloid lineages	Repair of injured skin dermis brought about by dermal sheath cells that become wound healing fibroblasts	Jahoda and Reynolds, 2001; Lako *et al*, 2002; Gharzi *et al*, 2003; Richardson *et al*, 2005
Processed lipo-aspirate cells (PLAs) from adipose tissue	Adipogenic, osteogenic, chondrogenic and myogenic lineages	Secrete cytokines that support angiogenesis and the inhibition of apoptosis	Zuk *et al*, 2002; De Ugarte *et al*, 2003a,b; Rehman *et al*, 2004
Adipose tissue-derived cells (ADCs)	Adipogenic, osteogenic and chondrogenic lineages	Secrete cytokines that support angiogenesis and the inhibition of apoptosis	Rehman *et al*, 2004; Strem and Hedrick, 2005
Fibrocytes	May be predisposed to develop into wound healing myofibroblasts	Role in inflammation due to their ability to present antigen, interact with T cells and produce a wide variety of pro-inflammatory molecules (e.g. IL-1β). Produce a number of angiogenic factors which may contribute to new blood vessel formation	Bucala *et al*, 1994; Abe *et al*, 2001; Hartlapp *et al*, 2001; Quan *et al*, 2004
Endothelial progenitor cells from the bone marrow	New blood vessels	In response to cytokines and/or tissue ischemia, incorporate into sites of new blood vessel growth. Found during the early wound repair phases	Brouard and Barrandon, 2003; Guo *et al*, 2003; Tepper *et al*, 2003; Fathke *et al*, 2004; Roufosse *et al*, 2004; Garmy-Susini and Varner, 2005; Tepper *et al*, 2005
Pericytes	Osteogenic, adipogenic and chondrogenic potential		Doherty *et al*, 1998; Farrington-Rock *et al*, 2004

tissue and termed either processed lipo-aspirate cells (PLAs; Zuk *et al*, 2002; De Ugarte *et al*, 2003a,b) or adipose tissue-derived cells (ADCs; Strem and Hedrick, 2005). PLAs, like bone-marrow MSCs, have the capacity to differentiate along the adipogenic, osteogenic, chondrogenic and myogenic lineages. However, notably the surface phenotypes of PLA and bone marrow MSC cells were distinct for several cell adhesion molecules implicated in haematopoietic stem cell homing, mobilization and proliferation suggesting that these two cell types represent distinct cell populations. PLAs/ADCs could support tissue repair directly because it has been demonstrated that they secrete cytokines that support angiogenesis and the inhibition of apoptosis (Rehman *et al*, 2004).

Progenitor cells and wound healing in the skin

The wound repair process is an extremely complex yet highly orchestrated process which consists of successive but overlapping phases which include inflammation, new connective tissue formation, re-epithelialization, angiogenesis and tissue remodelling. Whilst in the skin the ultimate outcome of the process is the production of scar tissue, within various parts of the oral mucosa there is evidence of healing with little or no evidence of a scar. The increasing evidence of the existence of progenitor cell populations within the dermal tissues of the skin strongly suggests a potential role for these cells in the repair of wounded tissues as part of the normal healing response. Mackenzie and Flake (2001) have demonstrated that human MSCs persist, demonstrate site-specific multipotential differentiation and are present in sites of wound healing and tissue regeneration after transplantation into foetal sheep. Other studies by Chunmeng and Tianmin (2004) have demonstrated that both topical and systemic transplantation of dermis-derived multipotent cells accelerated the healing process in simple wounds made in rats; the promoting effect by topical transplantation occurring earlier than systemic transplantation. Furthermore, these dermis-derived multipotent cells were demonstrated to engraft into recipient wounded skin tissues after transplantation. Other reports of the successful regeneration/repair of the dermis using a variety of multipotent cells types are now emerging (Gharzi *et al*, 2003; Chunmeng *et al*, 2004; Satoh *et al*, 2004).

Fibroblast progenitor cells and wound healing

Whilst evidence about the self-renewing capacity of fibroblasts is slowly emerging, there is increasing evidence to suggest that there are several types of fibroblasts within the skin possibly with different functions (Fries *et al*, 1994; Falanga *et al*, 1995; Cook *et al*, 2000; Stephens *et al*, 2003; Martin-Ruiz *et al*, 2004; Middelkoop, 2005). Such different functionality could serve to direct how phenotypically distinct fibroblast populations respond to a wound-healing stimulus. Indeed, over the past few decades, much debate has raged over whether wounds are healed by the ingress of adjacent mesenchymal or fibroblast-like cells or by the entry of fibroblast precursors from the blood. Whilst the ingress of wound-healing fibroblasts or MSC-like cells from surrounding healthy tissue (Chunmeng *et al*, 2004) or from the dermal sheath of hair follicles (Jahoda and Reynolds, 2001; Gharzi *et al*, 2003; Richardson *et al*, 2005) is proven, the existence of blood-borne cell populations has also now gained support within the scientific community (Fathke *et al*, 2004).

One such bone marrow-derived cell type is the fibrocyte, first identified in 1994, and defined by its growth characteristics and unique surface properties (Bucala *et al*, 1994). Fibrocytes comprise approximately 0.5% of circulating leucocytes in peripheral blood from which they are morphologically distinct because of unique cytoplasmic extensions intermediate in size between microvilli and pseudopodia. Peripheral blood fibrocytes express CD34, CD11b, CD45, HLA-DR, CD71, CD80 and CD86 which hints at their haematologic origin (Quan *et al*, 2004). However, other markers have also been utilized to identify these cells, which include the production of type I collagen, vimentin and prolyl 4-hydroxylase, suggesting that these cells are a unique and distinct population (Aiba and Tagami, 1997; Quan *et al*, 2004).

Homing of injected fibrocytes to sites of cutaneous tissue injury *in vivo* is, at least in part, due to secondary lymphoid chemokine, a ligand of the CCR7 chemokine receptor which acts as a potent stimulus for fibrocyte chemotaxis (Abe *et al*, 2001). It has been suggested that fibrocytes play a role in the inflammatory phases of wound repair because of their ability to present antigen (Quan *et al*, 2004). This is enabled because they express each of the known surface components that are required for antigen presentation, including class II major histocompatability complex molecules (HLA-DP, -DQ and -DR), the costimulatory molecules CD80 and CD86 and the adhesion molecules CD11a, CD54 and CD58 (Chesney *et al*, 1997). Furthermore, at least *ex vivo*, cultured fibrocytes can differentiate from a $CD14^+$-enriched mononuclear cell population when in contact with T cells. Fibrocytes also produce a wide variety of growth factors and cytokines (including the pro-inflammatory molecule interleukin-1β) and so are likely to play an important role in the recruitment and activation of both inflammatory and connective tissue cells during the tissue repair response.

It has been demonstrated that transforming growth factor-β1 increases the differentiation and functional activity of cultured fibrocytes, increases the α-smooth muscle actin content of the cells and that such cells can reorganize collagen lattices efficiently *in vitro* suggesting that fibrocytes may be predisposed to develop into wound-healing myofibroblasts (Abe *et al*, 2001). It is interesting to note that the analyses of a purified population of bone marrow stromal cells described as $STRO\text{-}1^{BRIGHT}/VCAM\text{-}1^+$, identified that approximately 70% of these cells expressed α-SMA (Gronthos *et al*, 2003). These cells had colony-forming activity and were able to undergo multilineage differentiation. These findings again suggest that there may be a relationship between α-smooth muscle actin-positive myofibroblasts

during would repair and the emergence of a stem cell phenotype. Fibrocytes also produce a number of angiogenic factors which may contribute to new blood vessel growth during the healing process (Hartlapp *et al*, 2001; Quan *et al*, 2004).

Vascular progenitor cells and wound healing

In addition to the inflammatory cells and fibroblasts required to heal wounds, bone marrow also provides endothelial progenitor cells (Brouard and Barrandon, 2003; Guo *et al*, 2003; Roufosse *et al*, 2004; Garmy-Susini and Varner, 2005). The work of others supports this hypothesis in that transplanted adult haematopoietic stem cells (HSCs) differentiate into functional endothelial cells with donor-derived endothelial cells detected in the skin and gut of transplant recipients with a mean frequency of 2% (Bailey *et al*, 2004; Jiang *et al*, 2004). It would therefore seem plausible that bone marrow is a source of endothelial progenitor cells that are mobilized into the peripheral blood in response to cytokines or tissue injury. Fathke *et al* (2004) have demonstrated that endothelial progenitor cells were found early during the wound repair process but they were not observed after epithelialization was complete. Furthermore, it has been reported that bone marrow-derived endothelial progenitor cells are recruited to the systemic circulation and, in response to various cytokines and/or tissue ischemia, incorporate into sites of new blood vessel growth (Tepper *et al*, 2003, 2005). These findings have changed our understanding of adult neovascularization by demonstrating that both preexisting endothelial cells and endothelial progenitor cells can contribute to blood vessel formation during repair.

An accumulation of data suggests that pericytes and other cells associated with the vessel wall also have stem cell-like activities and can undergo multipotent differentiation and may actually be the source of MSCs in bone marrow. Initial studies reported the osteogenic capacity of pericytes (Doherty *et al*, 1998), which were subsequently supported by evidence of adipogenic and chondrogenic potential of pericytes both *in vitro* and *in vivo* (Farrington-Rock *et al*, 2004). As the dermal tissues have an extensive microvasculature network it is likely that resident pericytes and related vessel wall cells could be involved in wound repair activities.

Mobilization of progenitor cells

Mobilization and homing is thought to be a coordinated, multistep process, which involves signaling by stromal-derived factor 1 (SDF-1) and stem cell factor, activation of lymphocyte function-associated antigen 1, very late antigen 4/5 and CD44, cytoskeleton rearrangement, membrane type 1-matrix metalloproteinase activation and secretion of matrix metalloproteinase-2/9 (Lapidot *et al*, 2005). Furthermore, it is believed that SDF-1 influences HSC moblilization through the specific changes to the adhesion of the progenitor cells to the bone marrow microenvironment via alterations in the adhesion molecules such as very late antigen-4 (Peled *et al*, 2000). It is proposed that there is increased trans-endothelial migration of the progenitor cells towards a gradient of SDF-1 (Netelenbos *et al*, 2002) which in turn downregulates apoptosis in $CD34^+$ HSCs (Lataillade *et al*, 2002). Another growth factor implicated in this process is the hepatocyte growth factor (HGF) which is involved in the migration and differentiation of HSC (Kollet *et al*, 2003).

The potential for stem/progenitor cells within the lamina propria

So are there parallel mechanisms underpinning normal tissue turnover and repair within the lamina propria of oral tissues? Similarities in structure and cell populations would hint that similar progenitor populations could exist or could be recruited to injured or normal tissues (Figure 3). Whilst the growing knowledge about stem cell populations associated with hair follicles suggest that these structures are a major source of progenitor cells within the skin, these populations of multipotent cells would obviously have little direct, if any, role to play within the hairless oral mucosa (except potentially when skin is utilised for grafts within the oral mucosa). However, evidence is now accumulating which is starting to add support (albeit somewhat circumstantial at the current time) to the notion that progenitor cells may exist with the lamina propria. For example, the findings that HGF is involved in directing the migration

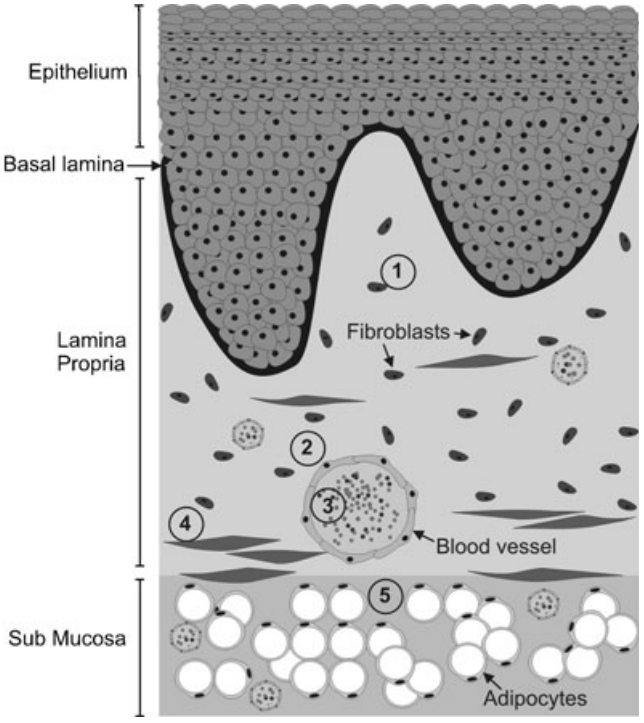

Figure 3 A schematic diagram showing putative non-epithelial stem cell sources in the oral mucosa. Whilst dermal cells (1) may have a role to play in normal homeostasis of the skin, vessel wall-derived cells including pericytes (2), blood-derived stem cells (3), muscle-derived stem cells (4) and adipose-derived stem cells (5) will probably be recruited into damaged tissues during wound repair

and differentiation of HSC (Kollet *et al*, 2003) is interesting in the light that oral mucosal fibroblast produce increased levels of bioactive HGF compared with patient-matched skin fibroblasts (Stephens *et al*, 2001b). Interestingly, Gruber *et al* (2004) have identified a population of STRO-1 positive cells in porcine sinus mucosa which were able to undergo osteogenic differentiation following exposure to bone morphogenetic proteins. Furthermore, it has been suggested that stem cell therapy could somehow reset the wound healing process resulting in healing reminiscent of foetal, scarless healing (Yang *et al*, 2003). Similar healing is already observed within the oral mucosa reflected by a reduced inflammatory response (Szpaderska *et al*, 2003) and the 'foetal-like' responses of oral mucosal fibroblasts which have been well documented (Irwin *et al*, 1994; Schor *et al*, 1996; Stephens *et al*, 1996, 2001a,b). Therefore, this represents some further indirect evidence for the existence of stem/progenitor cell populations within the lamina propria. These oral tissues that fail to scar are also supported by a generous fat layer from which these progenitor populations could also arise. What is evident is that work in this field is urgently needed as the ability to isolate large numbers of multipotent progenitor cells from a tissue which when biopsied will heal without a scar would be of great interest scientifically and commercially particularly with respect to future therapeutic applications and the developing discipline of tissue engineering.

Acknowledgements

The authors would like to thank Kath Allsopp for providing the images of the tissue sections.

References

Abe R, Donnelly SC, Peng T, Bucala R, Metz CN (2001). Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol* **166:** 7556–7562.

Aiba S, Tagami H (1997). Inverse correlation between CD34 expression and proline-4-hydroxylase immunoreactivity on spindle cells noted in hypertrophic scars and keloids. *J Cutan Pathol* **24:** 65–69.

Bailey AS, Jiang S, Afentoulis M *et al*. (2004). Transplanted adult hematopoietic stems cells differentiate into functional endothelial cells. *Blood* **103:** 13–19.

Brouard M, Barrandon Y (2003). Controlling skin morphogenesis: hope and despair. *Curr Opin Biotechnol* **14:** 520–525.

Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A (1994). Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* **1:** 71–81.

Chesney J, Bacher M, Bender A, Bucala R (1997). The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. *Proc Natl Acad Sci USA* **94:** 6307–6312.

Chunmeng S, Tianmin C (2004). Skin: a promising reservoir for adult stem cell populations. *Med Hypotheses* **62:** 683–688.

Chunmeng S, Tianmin C, Yongping S *et al*. (2004). Effects of dermal multipotent cell transplantation on skin wound healing. *J Surg Res* **121:** 13–19.

Cook H, Stephens P, Harding KG, Thomas DW (2000). Defective extracellular matrix reorganisation by chronic wound fibroblasts is associated with alterations in TIMP-1, TIMP-2 and MMP-2 activity. *Trends Biotechnol* **115:** 225–233.

De Ugarte DA, Alfonso Z, Zuk PA *et al*. (2003a). Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol Lett* **89:** 267–270.

De Ugarte DA, Morizono K, Elbarbary A *et al*. (2003b). Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs* **174:** 101–109.

Deng W, Han Q, Liao L *et al*. (2005). Engrafted bone marrow-derived flk-(1+) mesenchymal stem cells regenerate skin tissue. *Tissue Eng* **11:** 110–119.

Doherty MJ, Ashton BA, Walsh S, Beresford JN, Grant ME, Canfield AE (1998). Vascular pericytes express osteogenic potential in vitro and in vivo. *J Bone Miner Res* **13:** 828–838.

Falanga V, Zhou LH, Takagi H *et al*. (1995). Human dermal fibroblast clones derived from single cells are heterogeneous in the production of mRNAs for alpha 1(I) procollagen and transforming growth factor-beta 1. *J Invest Dermatol* **105:** 27–31.

Farrington-Rock C, Crofts NJ, Doherty MJ, Ashton BA, Griffin-Jones C, Canfield AE (2004). Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation* **110:** 2226–2232.

Fathke C, Wilson L, Hutter J *et al*. (2004). Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. *Stem Cells* **22:** 812–822.

Fernandes KJ, McKenzie IA, Mill P *et al*. (2004). A dermal niche for multipotent adult skin-derived precursor cells. *Nat Cell Biol* **6:** 1082–1093.

Fortier LA (2005). Stem cells: classifications, controversies, and clinical applications. *Vet Surg* **34:** 415–423.

Fries KM, Blieden T, Looney RJ *et al*. (1994). Evidence of fibroblast heterogeneity and the role of fibroblast subpopulations in fibrosis. *Clin Immunol Immunopathol* **72:** 283–292.

Garmy-Susini B, Varner JA (2005). Circulating endothelial progenitor cells. *Br J Cancer* **93:** 855–858.

Gharzi A, Reynolds AJ, Jahoda CA (2003). Plasticity of hair follicle dermal cells in wound healing and induction. *Exp Dermatol* **12:** 126–136.

Gronthos S, Zannettino AC, Hay SJ *et al*. (2003). Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. *J Cell Sci* **116:** 1827–1835.

Gruber R, Kandler B, Fuerst G, Fischer MB, Watzek G (2004). Porcine sinus mucosa holds cells that respond to bone morphogenetic protein (BMP)-6 and BMP-7 with increased osteogenic differentiation in vitro. *Clin Oral Implants Res* **15:** 575–580.

Guo H, Fang B, Liao L *et al*. (2003). Hemangioblastic characteristics of fetal bone marrow-derived Flk1(+)CD31(−)CD34(−) cells. *Exp Hematol* **31:** 650–658.

Hartlapp I, Abe R, Saeed RW *et al*. (2001). Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. *FASEB J* **15:** 2215–2224.

Hoogduijn MJ, Gorup E, Genever PG (2006). Comparative characterisation of hair follicle dermal stem cells and bone marrow mesenchymal stem cells. *Stem Cells Dev* **15:** 49–60.

Irwin CR, Picardo M, Ellis I *et al*. (1994). Inter-site and intra-site heterogeneity in the expression of fetal-like phenotypic characteristics by gingival fibroblasts – potential significance for wound-healing. *J Cell Sci* **107:** 1333–1346.

Jahoda CA, Reynolds AJ (2001). Hair follicle dermal sheath cells: unsung participants in wound healing. *Lancet* **358:** 1445–1448.

Jiang Y, Jahagirdar BN, Reinhardt RL *et al*. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418:** 41–49.

Jiang S, Walker L, Afentoulis M *et al*. (2004). Transplanted human bone marrow contributes to vascular endothelium. *Proc Natl Acad Sci USA* **101:** 16891–16896.

Koestenbauer S, Zech NH, Juch H, Vanderzwalmen P, Schoonjans L, Dohr G (2006). Embryonic stem cells: similarities and differences between human and murine embryonic stem cells. *Am J Reprod Immunol* **55:** 169–180.

Kollet O, Shivtiel S, Chen YQ *et al*. (2003). HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver. *J Clin Invest* **112:** 160–169.

Lako M, Armstrong L, Cairns PM, Harris S, Hole N, Jahoda CA (2002). Hair follicle dermal cells repopulate the mouse haematopoietic system. *J Cell Sci* **115:** 3967–3974.

Lapidot T, Dar A, Kollet O (2005). How do stem cells find their way home? *Blood* **106:** 1901–1910.

Lataillade JJ, Clay D, Bourin P *et al*. (2002). Stromal cell-derived factor 1 regulates primitive hematopoiesis by suppressing apoptosis and by promoting G(0)/G(1) transition in CD34(+) cells: evidence for an autocrine/paracrine mechanism. *Blood* **99:** 1117–1129.

Lerou PH, Daley GQ (2005). Therapeutic potential of embryonic stem cells. *Blood Rev* **19:** 321–331.

Mackenzie TC, Flake AW (2001). Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep. *Blood Cells Mol Dis* **27:** 601–604.

Martin-Ruiz C, Saretzki G, Petrie J *et al*. (2004). Stochastic variation in telomere shortening rate causes heterogeneity of human fibroblast replicative lifespan. *J Biol Chem* **279:** 17 826–17 833.

Middelkoop E (2005). Fibroblast phenotypes and their relevance for wound healing. *Int J Low Extrem Wounds* **4:** 9–11.

Netelenbos T, Zuijderduijn S, Van Den BJ *et al*. (2002). Proteoglycans guide SDF-1-induced migration of hematopoietic progenitor cells. *J Leukoc Biol* **72:** 353–362.

Peled A, Kollet O, Ponomaryov T *et al*. (2000). The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood* **95:** 3289–3296.

Pittenger MF, Mackay AM, Beck SC *et al*. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* **284:** 143–147.

Pittenger MF, Mosca JD, McIntosh KR (2000). Human mesenchymal stem cells: progenitor cells for cartilage, bone, fat and stroma. *Curr Top Microbiol Immunol* **251:** 3–11.

Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R (2004). Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol* **36:** 598–606.

Rehman J, Traktuev D, Li J *et al*. (2004). Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* **109:** 1292–1298.

Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM (2002). Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* **109:** 337–346.

Richardson GD, Arnott EC, Whitehouse CJ *et al*. (2005). Plasticity of rodent and human hair follicle dermal cells: implications for cell therapy and tissue engineering. *J Investig Dermatol Symp Proc* **10:** 180–183.

Roufosse CA, Direkze NC, Otto WR, Wright NA (2004). Circulating mesenchymal stem cells. *Int J Biochem Cell Biol* **36:** 585–597.

Satoh H, Kishi K, Tanaka T *et al*. (2004). Transplanted mesenchymal stem cells are effective for skin regeneration in acute cutaneous wounds. *Cell Transplant* **13:** 405–412.

Schor SL, Ellis I, Irwin CR *et al*. (1996). Subpopulations of fetal-like gingival fibroblasts: characterisation and potential significance for wound healing and the progression of periodontal disease. *Oral Dis* **2:** 155–166.

Seruya M, Shah A, Pedrotty D *et al*. (2004). Clonal population of adult stem cells: life span and differentiation potential. *Cell Transplant* **13:** 93–101.

Shi CM, Cheng TM (2004). Differentiation of dermis-derived multipotent cells into insulin-producing pancreatic cells in vitro. *World J Gastroenterol* **10:** 2550–2552.

Shi CM, Cheng TM, Su YP, Mai Y, Qu JF, Ran XZ (2004). Transplantation of dermal multipotent cells promotes the hematopoietic recovery in sublethally irradiated rats. *J Radiat Res (Tokyo)* **45:** 19–24.

Sieber-Blum M, Grim M, Hu YF, Szeder V (2004). Pluripotent neural crest stem cells in the adult hair follicle. *Dev Dyn* **231:** 258–269.

Sloan P, Picardo M, Schor SL (1991). The structure and function of oral mucosa. *Dent Update* **18:** 208–212.

Stephens P, Davies KJ, Al-Khateeb T, Shepherd JP, Thomas DW (1996). A comparison of the ability of intra oral and extra oral fibroblasts to stimulate extracellular matrix reorganization in a model of wound contraction. *J Dent Res* **75:** 1358–1364.

Stephens P, Davies KJ, Occleston N *et al*. (2001a). Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix reorganization and matrix metalloproteinase activity. *Br J Dermatol* **144:** 229–237.

Stephens P, Hiscox S, Cook H, Jiang WG, Zhiquiang W, Thomas DW (2001b). Phenotypic variation in the production of bioactive hepatocyte growth factor/scatter factor by oral mucosal and skin fibroblasts. *Wound Repair Regen* **9:** 34–43.

Stephens P, Cook H, Hilton J *et al*. (2003). An analysis of replicative senescence in dermal fibroblasts derived from chronic leg wounds predicts that telomerase therapy would fail to reverse their disease-specific cellular and proteolytic phenotype. *Exp Cell Res* **283:** 22–35.

Strem BM, Hedrick MH (2005). The growing importance of fat in regenerative medicine. *Trends Biotechnol* **23:** 64–66.

Strem BM, Hicok KC, Zhu M *et al*. (2005). Multipotential differentiation of adipose tissue-derived stem cells. *Keio J Med* **54:** 132–141.

Szpaderska AM, Zuckerman JD, DiPietro LA (2003). Differential injury responses in oral mucosal and cutaneous wounds. *J Dent Res* **82:** 621–626.

Tepper OM, Sealove BA, Murayama T, Asahara T (2003). Newly emerging concepts in blood vessel growth: recent discovery of endothelial progenitor cells and their function in tissue regeneration. *J Investig Med* **51:** 353–359.

Tepper OM, Capla JM, Galiano RD *et al*. (2005). Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood* **105:** 1068–1077.

Toma JG, Akhavan M, Fernandes KJ *et al*. (2001). Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* **3:** 778–784.

Toma JG, McKenzie IA, Bagli D, Miller FD (2005). Isolation and characterization of multipotent skin-derived precursors from human skin. *Stem Cells* **23:** 727–737.

Urmacher C (1990). Histology of normal skin. *Am J Surg Pathol* **14:** 671–686.

Yang GP, Lim IJ, Phan TT, Lorenz HP, Longaker MT (2003). From scarless fetal wounds to keloids: molecular studies in wound healing. *Wound Repair Regen* **11:** 411–418.

Young HE, Steele TA, Bray RA *et al*. (2001). Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. *Anat Rec* **264:** 51–62.

Zuk PA, Zhu M, Ashjian P *et al*. (2002). Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* **13:** 4279–4295.