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Soft tissue wound healing around teeth and dental implants

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Abstract

Aim: To provide an overview on the biology and soft tissue wound healing around teeth and dental implants.

Material and Methods: This narrative review focuses on cell biology and histology of soft tissue wounds around natural teeth and dental implants.

Results and conclusions: The available data indicate that:

(a) Oral wounds follow a similar pattern.
(b) The tissue specificities of the gingival, alveolar and palatal mucosa appear to be innately and not necessarily functionally determined.
(c) The granulation tissue originating from the periodontal ligament or from connective tissue originally covered by keratinized epithelium has the potential to induce keratinization. However, it also appears that deep palatal connective tissue may not have the same potential to induce keratinization as the palatal connective tissue originating from an immediately subepithelial area.
(d) Epithelial healing following non-surgical and surgical periodontal therapy appears to be completed after a period of 7–14 days. Structural integrity of a maturing wound between a denuded root surface and a soft tissue flap is achieved at approximately 14-days post-surgery.
(e) The formation of the biological width and maturation of the barrier function around transmucosal implants requires 6–8 weeks of healing.
(f) The established peri-implant soft connective tissue resembles a scar tissue in composition, fibre orientation, and vasculature.
(g) The peri-implant junctional epithelium may reach a greater final length under certain conditions such as implants placed into fresh extraction sockets versus conventional implant procedures in healed sites.

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Key words: alveolar mucosa; dental implants; gingiva; non-surgical periodontal therapy; periodontal surgery; soft tissue grafting; wound healing

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The last decades have improved our understanding of the mechanisms of oral wound healing and how this knowledge translates into today's clinical treatment concepts. This narrative review aims to provide an overview *on* the cellular aspects of soft tissue healing including the classical stages of wound repair and the implication for oral wound healing, *emphasizing* the role of TGF-β.

Wound healing in the oral cavity is not only restricted to healing following accidental trauma or surgery, but it also encompasses the biological events following a variety of pathological conditions such as cancer and infections (Gurtner et al. 2008).

Wound healing does not always result in a *restitutio ad integrum*, but it may end up with a scar tissue. This is not only true for the classical

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skin injury, but also relevant for the healing following periodontal surgery. Embryonic and foetal wounds, however, have a much higher potential to regenerate.

The aim of this review was to provide an overview on the most important biologic events during healing of soft tissue wounds in the oral cavity as related to teeth and dental implants. This review will, however, not address the biological events during healing of hard tissues associated with periodontitis or peri-implantitis.

Cell biology of soft tissue healing

Periodontal repair can be achieved, periodontal regeneration remains a challenge (Bosshardt & Sculean 2009). The overall goal is to provide patients with a less invasive, fast, save and predictable therapy that re-establishes a healthy periodontal situation to maintain the teeth. To achieve this goal, surgical techniques have been refined and biomaterials and growth factors are applied to support the natural process of wound healing and repair/regeneration (Susin & Wikesjo 2013, Bosshardt 2008, Sculean et al. 2008, Kaigler et al. 2011, Stavropoulos & Wikesjo 2012). Advances in periodontal therapy were usually based on the deep understanding of the fundamental cellular processes of periodontal regeneration and repair – basically, to provide optimal local conditions, thus enhancing the regeneration process. In the following a brief summary of the basic biological aspects of soft tissue healing is provided.

Most information on soft tissue healing comes from studies with epidermal also termed cutaneous or skin wound healing. Much less is known about the cellular aspects of soft tissue healing in the oral mucosa (see below). Therefore, it is currently assumed that wound healing represents a conserved process while the cellular processes of periodontal and peri-implant soft tissue repair resemble, at least partly, the healing of skin wounds (Wikesjö & Selvig 1999, Polimeni et al. 2006). This assumption might, however, provoke criticism, for example, because scar formation occurs less in the oral cavity than in skin wounds (see below). On the other hand, the examples provided here may offer the scientific basis to determine, if the respective mechanisms also account responsible for periodontal repair or even regeneration. Today, sophisticated preclinical models are available, for example allowing to understanding the involvement of a particular cell type in wound healing, or tracking one particular cell type in the ongoing process of wound healing. Novel developments in analytical methodology such as genomics and proteomics have opened the door for a better understanding of the molecular regulation of the complex wound healing process.

Summarizing the current knowledge on wound healing is beyond the scope of this review, here referring to the existing excellent literature (Martin 1997, Singer & Clark 1999, Gurtner et al. 2008, Shaw & Martin 2009, Nauta et al. 2011). Instead, we highlight some aspects that appear to be relevant for periodontal and peri-implant soft tissue healing – as it should help to understand the clinical consequences of current periodontal treatment concepts. Moreover, the cellular basics of general wound healing should provide inspiration to further improve or develop treatment strategies for soft tissue repair and regeneration as relates to teeth and dental implants. The next sections will focus on the following aspects:

- Provide a brief summary of the classical stages of wound repair
- Compare healing of oral soft tissues with "classical" skin wound healing
- Summarize the role of innate and adaptive immunity in soft tissue wound healing
- Provide our current understanding on the genetic basis of wound healing exemplified by TGF-β and associated genes.

Brief summary of the classical stages of wound repair

The *haemostatic phase* is initiated by tissue injury including defects after periodontal surgery (Dickinson et al. 2013). The defect site is sealed rapidly off by the forming blood clot that basically originated from blood coagulation. Extravasated platelets are activated and aggregate together with other blood-derived cells such as neutrophils and red blood cells in the blood clot, also termed blood coagulum. The main component of the extracellular matrix is the newly formed fibrin meshwork that also includes other proteins for cell adhesion such as fibronection and vitronetin (Clark et al. 2004, Reheman et al. 2005). This conglomerate of cells and the fibrin-rich matrix is frequently termed "provisional extracellular matrix" as it will be later replaced by the granulation tissue. The formation of the blood clot is also the kick-start for the recruiting of inflammatory cells into the defect site.

The *inflammatory phase* parallels the haemostatic phase. Neutrophils are attracted by chemokines, the complement system, and by peptides released during cleavage of fibrinogen (Kolaczkowska & Kubes 2013). Extravasation and migration of cells in the surrounding tissue is controlled by endothelial cells (Shi & Pamer 2011, Kolaczkowska & Kubes 2013). Neutrophils and monocytes appear at defect sites within one and 24 h respectively. Neutrophils clean the wound site as they kill invading bacteria and release proteases before they are removed via phagocytosis. Macrophages are a heterogeneous population, as they can be involved in inflammation (M1-macrophages) but also switch to an anti-inflammatory "M2a" phenotype (Mantovani et al. 2013, Novak & Koh 2013). In general, resolution of inflammation is a controlled process involving lipid mediators (Serhan et al. 2008). The primarily catabolic inflammatory process is transient, but crucial for the following steps of the anabolic phase of "new tissue formation".

The *phase of new tissue formation* is initiated by the formation of the "granulation tissue", a morphologic term that reflects the highly vascularized tissue made of fibroblast and an extracellular matrix. The transition of the catabolic to the anabolic phase requires activation of a complex process involving at least three cell types: endothelial cells, fibroblasts and the epithelial cells. The cellular origins are partially resolved: (i) Endothelial cells, required for the formation of new capillaries, can be derived from endothelial cells of the original blood

vessels but also from the circulating endothelial progenitors (Potente et al. 2011). (ii) Fibroblasts can be derived from the connective tissue in the wound edges, from monocyte-derived fibrocytes (Grieb et al. 2011, Reilkoff et al. 2011), from vessel-derived pericytes (Grieb et al. 2011) and possibly also by a process termed epithelial-mesenchymal transition (Weber et al. 2012). (iii) Epithelial cells originate from the keratinocytes at the wound edges, but at least in the skin, stem cells of the hair follicle can contribute to the re-epithelialization (Blanpain & Fuchs 2009, Cordeiro & Jacinto 2013). A proportion of the fibroblasts achieve a phenotype that resembles smooth muscle cells (Tomasek et al. 2013). These myofibroblasts can draw the wound edges together and are thus critical components of wound healing (Klingberg et al. 2013).

The long-term *remodelling phase* that ends up with scar tissue starts with the resolution phase. Most of the myofibroblasts, fibroblasts, endothelial cells and macrophages undergo apoptosis, leaving the collagen-rich extracellular matrix containing only a few cells. The signals leading to cell group suicide are not clear (Hinz 2007). Also, we have to accept that besides the aesthetic drawbacks of a scar tissue, also the biomechanical capacity is less than it was before injury. Scar tissue formation, also termed fibrosis, is the main pathological factor of a variety of pathologies linked with inflammation. Fibrosis, seen in the liver, lung, heart, kidney and skin, is a significant global disease burden. The pathophysiology of fibrosis, however, remains an enigma (Meneghin & Hogaboam 2007). Thus, great effort is paid on the control of scarring, basically to avoid scar formation (Wynn & Ramalingam 2012). In periodontal wound healing, subepithelial connective tissue grafts can end up with a dense tissue, which is considered to provide long-term stability of the area (Thoma et al. 2011, Santagata et al. 2012). Therefore, it is reasonable to suggest that a dense and stable soft tissue can bear clinical advantage.

Compare oral with "classical" skin wound healing

First, it is necessary to summarize studies comparing oral with skin wound healing. For example, mouse oral wound healing can be faster than skin wounds, at least following an experimental mucosa incision or tongue excision respectively (Sciubba et al. 1978, Szpaderska et al. 2003b). Also in pig models, injured oral mucosa showed reduced scar formation compared to skin incisions (Wong et al. 2009). Moreover, pig oral mucosal wounds showed similar molecular composition and clinical and histological scar scores to human oral mucosal wounds (Wong et al. 2009). However, in mouse models with punch biopsies in the scalp and palate, oral healing was slower than dermal repair, likely because of persisting inflammatory stimuli (Nooh & Graves 2003). In the latter study, epithelial and connective tissue bridging of excisional wounds was delayed compared to dermal wounds and also neutrophils were more abundant in the oral than in the skin wounds (Nooh & Graves 2003). Interestingly, the inflammatory cytokine IL-1 plays a role in oral wound healing, but not in dermal wound healing, likely because IL-1 is necessary to control the defence mechanisms against commensal bacteria in the oral cavity (Graves et al. 2001). Taken together, the majority of studies indicate that oral mucosa likely heals faster and with less scar tissue than do skin wounds. The underlying mechanisms remain a matter of speculation but may include aspects unique to the oral cavity such as the presence of saliva with its well-recognized biological activities (Zelles et al. 1995, Schapher et al. 2011). For example, the cutaneous and bone healing of sublingual sialadenectomized mice was slower than that of sham-operated controls (Bodner et al. 1991a, b). Importantly, palatal wound healing is delayed in desalivated rats and larger wounds are more sensitive to desalivation than smaller wounds (Bodner et al. 1992, 1993). Overall, the search for explanations why oral mucosa heals faster and with less scar tissue formation than skin wounds continues.

Summarize the role of innate and adaptive immunity in soft tissue wound healing

Mice lacking neutrophils or macrophages can efficiently heal skin wounds, as long as microbial infection is controlled (Martin et al. 2003). Thrombocytopenic mice exhibited altered wound inflammation but no delay in dermal wound healing (Szpaderska et al. 2003a). Mast cell-deficient mice are characterized by a decrease in neutrophils but no other aspects of wound healing (Egozi et al. 2003, Nauta et al. 2013). Taken together, it seems that neither of the inflammatory cells is essential for wound healing when the defects are not challenged by microbial or other contamination. Thus, the models not necessarily represent the clinical scenario of the wound site after periodontal surgery. Moreover, there may be functional redundancies between various cells types. Inflammation usually provokes slower healing and more scarring (Martin & Leibovich 2005). Thus, reducing the inflammatory response in a defect site might have a beneficial impact on wound healing (Martin & Leibovich 2005).

Wound healing was enhanced by the depletion of T helper and cytotoxic lymphocytes (Efron et al. 1990) and in athymic nude mice that lack a normally developed T cell (Barbul et al. 1989). T-cells are heterogeneous, suggesting the existence of a population that stimulates wound healing. For example, mice lacking a population of dendritic epidermal T cells exhibit a delay in wound closure (Jameson et al. 2002, Havran & Jameson 2010). B-cells are also involved in wound healing (Nishio et al. 2009). For a review on the mechanisms by which the immune system regulates wound healing see Park & Barbul (2004). Overall, there is increasing evidence that lymphocytes are involved in the control of wound healing – clearly more details are wanted, in particular with regard to periodontal soft tissue healing.

Genetic basis of wound healing exemplified by TGF-β and associated genes

Understanding wound healing at the molecular level provides the scientific basis to develop targeted strategies mainly with the intension to overcome delayed wound healing or to control excessive scar formation. For example, TGF-β1 has pleiotropic functions; including increased

collagen synthesis by fibroblasts and their conversion into myofibroblasts, also with gingival cells (Hong et al. 1999, Sobral et al. 2011). In TGF-β1-deficient mice, early skin wound healing proceeded almost normally (Brown et al. 1995). However, mice that lack Smad3, which transduces signals from TGF-β, show accelerated cutaneous and palatal wound healing compared with wild-type mice (Ashcroft et al. 1999, Jinno et al. 2009). Targeting of Smad3 with small interfering RNA also accelerates wound-healing and inhibits wound contraction in palatal mucoperiosteal wounds (Yoneda et al. 2013). Moreover, IFN-γ knockout mice exhibited an accelerated wound healing and there seems to be a crosstalk with TGF-β signalling pathways (Ishida et al. 2004). Overall, these and other (Liaw et al. 1998, Padmakumar et al. 2012, Zhang et al. 2012, Guo et al. 2013) genetic models have helped to reveal role of TGF-β in wound healing and to use these pivotal aspects to design targeted therapies that may also support periodontal wound healing. TGF-β is exemplified as one out of multiple factors that are key regulators of wound healing. For the other molecules involved in wound healing see the recent reviews (Martin 1997, Singer & Clark 1999, Gurtner et al. 2008, Shaw & Martin 2009, Nauta et al. 2011). It is not the intention of this part to review the clinical application of the respective molecules such as platelet-derived growth factor-BB (Hollinger et al. 2008) and basic fibroblastic growth factors (bFGF or FGF-2) (Murakami 2011).

Healing of soft tissue wounds at natural teeth

This section will attempt to provide the biological background of soft tissue healing around natural teeth and to give an overview on the most important histological events following non-surgical and various periodontal surgical procedures.

Role of connective tissue and sulcular environment in determining epithelial differentiation

The question whether the specificity of the epithelium is determined by hereditary mechanisms rather than by functional adaptation has been elegantly investigated in monkeys by Karring et al. (1971). Following excision of the buccal crevicular epithelium adjacent to the maxillary and/or mandibular premolars, mucoperiosteal flaps were raised, separate pedicles of gingiva and alveolar mucosa prepared and the flaps were transposed and sutured. Furthermore, free palatal grafts including epithelium and lamina propria, were transplanted to the maxillary and/or mandibular alveolar mucosa. The experimental procedures were designed to yield observation periods of 5 and 14 days, 1–8, 10 and 12 months.

The histological evaluation indicated that during the early wound healing period (e.g. 5-day grafts) the superficial layers of the epithelium were desquamated and an abundance of mitoses was present in the basal layers of gingival and alveolar mucosal transplants. Epithelial proliferation started from the edges of the grafts and the adjacent tissues, while regeneration of the supraalveolar connective tissue appeared to be initiated within the periodontal ligament. At 14 days the entire surface of the grafts was already covered with a thin layer of epithelium. At 30 days, the transplanted grafts were completely covered by a thin epithelium layer but displayed identical tissue characteristics to those of the corresponding control tissues. After 2 months, the grafted tissue demonstrated clinical and histological features identical to those of the respective donor tissues. Moreover, both the clinical and the histological findings have indicated that the tissue specificities of the gingival, alveolar and palatal mucosa were conserved after heterotopic transplantation, thus suggesting that the clinical and structural features of these tissues are rather genetically than functionally determined.

The finding that after complete excision of the keratinized tissues surrounding the teeth (e.g. free and attached gingiva) a zone of gingiva will always reform, was later corroborated in animals and humans (Wennström et al. 1981, Wennström 1983, Wennström & Lindhe 1983). In all these studies a narrow zone of gingiva was always observed to regenerate following complete excision (e.g. gingivectomy). Interestingly, at 9 months following surgery, complete excision of the gingival unit appeared to lead to a wider zone of attached gingiva compared to a "flap-excision" approach (Wennström 1983). This finding was explained by the more pronounced formation of granulation tissue originating from the periodontal ligament space following "gingivectomy" than following the "flap-excision" approach.

Further evidence for the pivotal role of gingival connective tissue in determining epithelial differentiation has been provided by a subsequent animal study where free connective tissue grafts were transplanted from either gingiva (test) or non- keratinized alveolar mucosa (controls), into areas of the alveolar mucosa (Karring et al. 1975b). The grafts were implanted into pouches created in the connective tissue of the alveolar mucosa as close as possible to the overlying epithelium. Following a period of 3–4 weeks, the transplants were exposed by removal of the overlying epithelium, thus allowing epithelialization from the surrounding non-keratinized alveolar mucosa. The clinical and histological evaluation after a healing period between 1 and 12 months has shown that the gingival connective tissue grafts became covered with keratinized epithelium, displaying the same characteristics as those of normal gingival epithelium, while the alveolar mucosa transplants were covered with non-keratinized epithelium. These findings indicate that the specificity of these epithelia is genetically determined and their differentiation is mainly dependent on stimuli from the underlying connective tissues. They also suggest that the granulation tissue proliferating from the alveolar mucosa will produce a non-keratinized epithelium, whereas that originating from the supra-alveolar connective tissue or from the periodontal ligament will lead to a keratinized epithelium. These observations in animals were later also confirmed in humans (Edel 1974, Edel & Faccini 1977).

In a first study, 14 free palatal connective tissue grafts without epithelium were transplanted to partial-thickness sites prepared in patients with an inadequate width of

attached gingiva (Edel 1974). It was reported that clinically, the graft surfaces appeared keratinized after already 2 weeks and an increase in the width of keratinized tissue occurred. A subsequent study has evaluated histologically 10 connective tissue grafts transplanted to partial-thickness sites (Edel & Faccini 1977). Eight grafts were completely free of epithelium, while two grafts had one thin layer of retained epithelium oriented at the apical edge of the graft. The grafts were placed in a way to be in contact with both keratinized and non-keratinized mucosa. The histological evaluation has demonstrated that at 24 weeks, the epithelium covering the gingival connective tissue grafts displayed keratinization with a normal architecture. Interestingly, in the two cases where a keratinized epithelial border was left on the grafts, an epithelial down-growth between the graft and the recipient site was observed indicating no apparent advantage of leaving a border of keratinized epithelium on a graft transplanted in an area where alveolar mucosa was originally present.

An important question, which still needs to be definitely clarified, is related to the possible differences inherent in deep and superficial connective tissue in determining epithelial keratinization. In a nicely designed experiment, a thick palatal epithelial-connective tissue graft was excised and split into two thinner grafts (e.g. one epithelial-connective tissue graft and one connective tissue graft) (Ouhayoun et al. 1988). The grafts were transplanted into contra-lateral areas lacking keratinized mucosa. Following a healing period of 3 months, biopsies were excised and examined by means of routine histology, immunofluorescence and gel electrophoresis. The results have shown that while the epithelial-connective tissue grafts displayed histological and biochemical characteristics of keratinized mucosa (e.g. gingiva) the deep connective tissue grafts expressed features belonging to both keratinized and non-keratinized mucosa. These observation appear to suggest that deep palatal connective tissue, may not have the same potential to induce keratinization of non-keratinized epithelial cell as the palatal connective tissue originating from an immediately subepithelial area. Comparable findings in humans were also reported by others indicating that palatal connective tissue grafts or free gingival grafts transplanted into areas of non-keratinized mucosa may not always develop the characteristics of keratinized mucosa (Bernimoulin & Schroeder 1980, Bernimoulin & Lange 1973, Lange & Bernimoulin 1974). On the other hand, it is interesting to note that despite the fact that the connective tissue underlying the epithelium appears to determine the characteristics of the overlying epithelium, the sulcular epithelium is not keratinized. The question whether the sulcular environment may influence keratinization of sulcular epithelium has been evaluated in two animal experiments (Caffesse et al. 1977, Caffesse et al. 1979b).

In a first experiment, 24 intrasulcular mucoperiosteal flaps were raised by blunt dissection on the buccal aspect of individual teeth including also the approximal papillae of the tooth (Caffesse et al. 1977). Subsequently, a split thickness flap was prepared in the alveolar mucosa apical to the flap, by removing the epithelium and a thin layer of connective tissue. The flaps were then folded and sutured in a way that the sulcular epithelium became exposed to the oral cavity. Following surgery, biopsies were taken to allow for observation periods of 1 h to 8 weeks. The findings have indicated that the sulcular epithelium has the potential for keratinization and that the contact to the tooth appears to determine the lack of keratinization of the sulcular epithelium. In another experiment, the influence of the sulcular environment on the keratinization of the outer surface gingival epithelium was evaluated (Caffesse et al. 1979b). Mucoperiosteal flaps were raised on the buccal aspect of experimental teeth, without including the approximal papillae and inverted in order to place the outer surface epithelium in contact with the tooth. The experimental time intervals varied from 1 h to 60 days. The results have shown that the outer surface epithelium may change its morphology to a non-keratinized epithelium devoid of deep rete pegs when placed in close contact with the tooth and displayed anatomical characteristics of sulcular epithelium. It thus appears that the sulcular environment has the capability of controlling the keratinizing potential of the outer surface gingival epithelium.

The question whether an inflammatory process may influence epithelial keratinization is still controversially discussed. Results from experimental studies in animals have failed to show that an experimentally induced acute or chronic inflammation of gingival connective tissue may modify the induced-keratinized sulcular epithelium, or the normally keratinized oral gingival epithelium, if bacterial plaque is removed regularly (Nasjleti et al. 1984, Caffesse et al. 1985). On the other hand, the reduction of gingival inflammation by means of systemic antibiotics, local plaque control and scaling and subgingival scaling may facilitate keratinization of sulcular epithelium (Bye et al. 1980, Caffesse et al. 1980, Caffesse et al. 1982). Furthermore, histological, immunofluorescence, electron microscopic observations in humans have also indicated that, in the presence of an inflammatory process, alterations of gingival epithelia may occur (Levy et al. 1969, Ouhayoun et al. 1990, Tonetti et al. 1994).

Taken together, the available data indicate that the granulation tissue originating from the periodontal ligament or from connective tissue originally covered by keratinized epithelium has the potential to induce keratinization. The question whether a deep palatal connective tissue has the same potential to induce keratinization of non-keratinized epithelial cell as the palatal connective tissue originating from an immediately subepithelial area may bear clinical relevance and should be clarified in further studies.

Soft tissue healing following non-surgical periodontal therapy

Several histological studies in animals and humans have evaluated the healing after root planing and soft tissue curettage. Novaes et al. (1969) have performed gingival curettage on the labial side of upper incisors in dogs and observed that the epithelial attachment was re-established after

6 days and remained at the cementum-enamel junction until the end of the experiment. Stahl et al. (1971) have treated 80 suprabony pockets in 60 adult patients suffering from periodontitis by means of curettage and root planing. The histological analysis has indicated that at one week after curettage, an epithelial lining was already present in all investigated specimens. The early stage after curettage was characterized by an increase in an acute inflammatory infiltrate. However, after a healing period of 8 weeks, the inflammatory infiltrate appeared to be similar in distribution and degree to that observed in non-treated control samples.

Waerhaug (1978) has studied the healing of the dento-epithelial junction following subgingival plaque control in 39 biopsies from 21 patients. Following removal of subgingival calculus and plaque and a healing period varying from 2 weeks to 7 months, block biopsies were harvested and analysed histologically. The histological analysis revealed that a normal dento-epithelial junction has been routinely reformed in areas from which subgingival calculus and plaque has been removed. The new dento-epithelial junction appeared to be completed within a period of 2 weeks.

The healing following periodic root planing and soft tissue curettage has been evaluated in a non-human primate model (Caton & Zander 1979). The soft tissue curettage of the pocket wall and marginal gingiva was extended apical to the bottom of the clinical pocket to remove the entire pocket epithelium. The procedure was repeated at 3, 6 and 9 months after the initial root planing and curettage. The histological evaluation has demonstrated that in all cases the healing occurred through formation of a long junctional epithelium along with no connective tissue attachment.

Similar findings have been recently reported in humans following non-surgical therapy performed with the aid of a dental endoscope. Besides the observation that at 6 months following therapy the healing was characterized by a long junctional epithelium, complete removal of calculus and plaque was associated with a lack of histological signs of inflammation (Wilson et al. 2008). Taken together, the available histological evidence indicates that the healing following non-surgical periodontal therapy is characterized by epithelial proliferation, which appears to be completed after a period of 7–14 days after treatment. Complete removal of calculus and plaque was associated with a limited or complete lack of inflammation.

Soft tissue healing following periodontal surgical procedures

Gingivectomy

The sequential healing events following gingivectomy have been evaluated by Novaes et al. (1969). Immediately after surgery, a haemorrhage is present. At 2 days, a thick clot covered the entire wound and a slight epithelial migration at the apical margin of the wound was observed. At 4 days, the blood clot still covered the major part of the wound surfaces, but the epithelial proliferation was clearly visible from the oral epithelium and epithelial attachment cells. At 1 week, the wound surface was usually completely epithelized and the sulcus reformed but keratinization and the reformation of rete pegs was only detected at 16 days. Wound maturation was still detectable until 38 days, when no differences between the treated areas and the pristine sites were detected.

Flap surgery

Several studies have evaluated the healing following full thickness and partial thickness flaps. Overall, the healing follows the same pattern and is characterized by the formation of a blood clot between the soft and hard tissues. The adherence mediated by the blood clot between the soft and hard tissues appears to be weak and does not seem to be able to hold them together (Kon et al. 1969). At 6 and 7 days, an inflammatory reaction and an increase in vascularization of the remaining connective tissue and flap can usually be observed. At this stage, the flap is still more prone to separation from the subjacent tissues when tension is applied. At 12 days, the flap is reattached to the bone and tooth, while the oral gingival epithelium appears to be keratinized. The rete pegs are of a normal shape. At around 4 weeks, the flap is re-attached to the tooth by dense, organized, connective tissue. At 5 weeks, the tissues appear to be completely regenerated and do not show differences compared to pristine sites. Comparable findings have been also reported by Caffesse et al. (1984) and Kon et al. (1984). Bone resorption always occurs following elevation of full thickness and partial-thickness flaps. Despite the observation that partial thickness flaps may result in less bone loss compared to the elevation of *full- thickness* flaps, they do not seem to completely prevent bone loss (Fickl et al. 2011).

The strength of the flap attachment to the tooth during healing following periodontal surgery was evaluated by Hiatt et al. (1968). At 2 and 3 days following surgery, 225 g of tension on the silk suture placed through the margin of the flap was needed to separate the flap from the tooth and the bone and increased to 340 g at 1 week. Once the epithelial attachment was severed, the fibrin beneath the connective tissue surface appeared to offer very limited mechanical resistance. At 2 weeks, the suture was pulled through the gingival margin with a force of 1700 g, which separated the flap only partially from the tooth. At 1 month, the flap could not be mechanically separated from the tooth, but a split within the epithelium at the point of stress was observed microscopically. These findings indicate that the initial attachment of the flap to the tooth was through the epithelium while the fibrin layer did not appear to significantly contribute to the retention of the flap. Moreover, a proper re-adaptation of the mucoperiosteal flap to the root surface appeared to inhibit epithelial proliferation and down-growth. The findings have also suggested that the strength of the epithelial attachment to the root is greater than the attachment between cells. These findings were later corroborated by Wikesjö et al. (1991) suggesting that connective tissue attachment to dentin is mediated by adsorption of plasma proteins to the surface and subsequent development and maturation of the fibrin clot (Wikesjö et al. 1991).

Taken together these data indicate that the tensile strength of the

tooth-soft tissue interface still appears vulnerable to mechanical trauma at 7-day post-surgery. At approximately 14 days post-surgery, a structural integrity of a maturing wound between a denuded root surface and a mucogingival flap, which can sufficiently withstand mechanical trauma, is achieved. These observations, in turn point to the critical role of passive flap adaptation and of suturing to allow undisturbed wound maturation.

Mörmann & Ciancio (1977) have evaluated the effect of various types of surgical procedures on the gingival capillary blood circulation by means of fluorescein angiography. The circulation changes observed suggested that flaps receive their major blood supply from their apical aspects. The full thickness incision in clinically healthy gingiva revealed that the blood supply had predominantly caudocranial direction from the vestibule to the gingival margin. Moreover, the findings have also indicated that flaps should be broad enough at their base to include major gingival vessels and pointed to the importance of ensuring a tension-free re-adaptation to avoid dehiscence. Furthermore, partial thickness flaps should not be too thin in order to include more blood vessels and avoid necrosis. Later studies have demonstrated that during the elevation of a mucoperiosteal flap the connection of the gingivo-periosteal plexus with the periodontal ligament vascular plexus is severed and significant vascular trauma is induced, especially in the inter-dental areas (Nobuto et al. 1989, McLean et al. 1995). These findings have been later confirmed in a series of studies evaluating gingival blood flow by means of laser Doppler flowmetry after different periodontal surgical procedures (Donos et al. 2005, Retzepi et al. 2007a,b).

The results have shown that the blood flow decreased immediately following anaesthesia and remained at lower values compared to baseline immediately following flap surgery. The gingival flow presented an overall increase in comparison to baseline values until the 7th days following surgery at the inter-dental and alveolar mucosa sites. At 15 days, however, the blood flow values were again similar to baseline. The findings have also indicated that the location of the incisions and the use of surgical techniques intending to preserve the inter-dental tissues (i.e. simplified papilla preservation flap) may be associated with faster recovery of the gingival blood flow post-operatively compared with the modified Widman flap (Donos et al. 2005, Retzepi et al. 2007a, b).

Healing following denudation techniques

The healing following full thickness and split thickness flaps used in mucogingival surgery to increase the width of attached gingiva has been evaluated in several animal experiments (Staffileno et al. 1966, Karring et al. 1975a, Pustiglioni et al. 1975, Kon et al. 1978). The origin and development of granulation tissue following periosteal retention and denudation procedures has been evaluated in monkeys by Karring et al. (1975a). The findings have shown that following periosteal exposure and denudation of the alveolar bone, the granulation tissue originated from the residual periosteal connective tissue, periodontal ligament, bone marrow spaces and the adjacent gingiva and alveolar mucosa. During the initial stages of healing, resorption of the labial and buccal bone took place and the amount of bone loss was influenced by the thickness and structure of the labial or buccal bone. Generally, the resorption was most severe following the denudation technique, while the loss of crestal bone was generally smaller with the periosteal retention procedure than following the complete exposure of bone. Moreover, with the denudation technique, a larger portion of the marginal areas was filled with granulation tissue deriving from the periodontal ligament. Granulation tissue derived from the remaining or adjacent gingival connective tissue or the periodontal ligament appeared to be covered by keratinized epithelium, whereas that originating from the connective tissue of the alveolar mucosa was covered by non-keratinized epithelium. After 1 month, the free gingiva was regenerated and exhibited a shallow gingival crevice. In the injured mucosa, delicate elastic fibres reappeared in the regenerated tissues after 1–2 months and displayed similar histological characteristics to those of the pristine alveolar mucosa and probably originated from the intact alveolar mucosa. Generally, no predictable increase in the width of the gingiva was found after any of the two methods.

Taken together, the results suggest that the success or failure to extend the width of keratinized tissue by surgical means is unpredictable and depends on the origin of the granulation tissue. It may thus be suggested that the use of transplants is a more predictable method for increasing the width of keratinized tissue.

Healing following soft tissue grafting

Free gingival grafts or free connective tissue grafts have been introduced in periodontal therapy to increase the width of the attached gingiva and to prevent or treat gingival recessions (Gargiulo & Arrocha 1967, Oliver et al. 1968, Sullivan & Atkins 1968, Staffileno & Levy 1969, Sugarman 1969, Edel 1974, Edel & Faccini 1977, Caffesse et al. 1979a). The sequential events of the healing and revascularization of free gingival grafts when placed over periosteum has been evaluated in monkeys by Oliver et al. (1968). Following preparation of a periosteal recipient bed in the maxillary and mandibular anterior region in monkeys, free gingival grafts were obtained from the buccal attached gingiva in the premolar area. The grafts were placed over the periosteum and sutured to the adjacent interproximal tissue, attached gingiva and interproximal tissue. Animals were sacrificed to allow observation periods at 0, 2 4, 5, 7, 8, 11, 14, 17, 21, 28 and 42 days. The histological evaluation revealed that the healing of free gingival grafts can be divided into three phases: (i) Initial phase (0–3 days) characterized by a thin layer of fibrin separating the periosteum from the graft and degeneration of epithelium and desquamation of the outer layers. (ii) Revascularization phase (4–11 days) characterized by minimal resorption of the alveolar crest, proliferation of fibroblasts into the area between the graft and periosteum. At 5 days all the graft epithelium was degenerated and desquamated. At the same time, a thin layer of new epithelial cells proliferated over the graft from the

adjacent tissues. At day 11, a dense fibrous union was observed between the graft and the periosteum. Granulation tissue was gradually replaced by fibroblastic proliferation and at day 11 the graft was completely covered by an epithelial layer, which was continuous with the marginal epithelium. Vascularization was evident, and capillary ingrowth was observed at the base of the graft. (iii) Tissue maturation phase (11–42 days). At 14 days of healing, the connective fibres within the graft were comparable in staining quality and appearance to the fibres in the control specimens. The thickness of the epithelium had developed more fully at 14 days but no keratinization was present. Keratinization was only detectable at 28 days. At 14 days, the number of vessels throughout the connective tissue of the graft was decreased but in the same time the connective tissue density increased. The pattern of vascularization did not show major changes after day 14.

The observations made by Oliver et al. (1968) are generally in agreement with those reported by Caffesse et al. (1979a,b) and Staffileno & Levy (1969) have evaluated the healing of free gingival grafts placed on either periosteum or on denuded bone in monkeys. In cases when the grafts were placed on bone, a delay in healing was observed. However, by 28 days, there were no differences in the rate of healing between grafts placed on bone or on periosteum. However, the periosteal bed appeared to favour better initial adaptation and nourishment of the graft. Grafts placed directly on bone showed initially more degenerative changes and a delay of epithelial migration. Furthermore, the epithelial coverage was restored in 7 days when the grafts were on periosteum and in 14 days when they were placed on bone. Keratinization was found in both groups at 28 days, while elastic fibres were only observed in cases where the grafts were placed on periosteum. One important aspect, which needs to be considered when using connective tissue grafts or free gingival grafts, is the shrinkage, which occurs during healing. It has been reported that the greatest amount of shrinkage occurs in the first postoperative month but can be followed up to 360 days and varies between 25% and 45% (Egli et al. 1975, James & McFall 1978, Rateitschak et al. 1979, Mörmann et al. 1981, Orsini et al. 2004). While re-vascularization appears to be more delayed in thicker grafts, less shrinkage was observed with increasing graft thickness (Egli et al. 1975, Rateitschak et al. 1979, Mörmann et al. 1981).

Soft tissue healing around implants

Dental implants are anchored in jawbone through a direct bonding between bone and the implant. Success and survival of an implant do, however, not depend solely on osseointegration. A soft tissue, which surrounds the transmucosal part of a dental implant, separates the peri-implant bone from the oral cavity. This soft tissue collar is called "peri-implant mucosa" (Lindhe et al. 2008). The attachment of the soft tissue to the implant serves as a biological seal that prevents the development of inflammatory peri-implant diseases (i.e. peri-implant mucositis and peri-implantitis). Thus, the soft tissue seal around implants ensures healthy conditions and stable osseointegration and therefore also the long-term survival of an implant.

Around teeth, a sophisticated soft tissue collar seals the tissues of tooth support (i.e. alveolar bone, periodontal ligament and cementum) against the oral cavity (Bosshardt & Lang 2005). While the soft tissue seal around teeth develops during tooth eruption, the peri-implant mucosa forms after the creation of a wound in oral soft and hard tissues. The wound healing phase may occur following the closure of a mucoperiosteal flap around the neck portion of an implant placed in a so-called one-stage procedure or after a second surgical intervention for abutment connections to an already installed dental implant (two-stage procedure). Since wound healing occurs in the presence of a biomaterial (i.e. a foreign body) at a critical region, interference of wound healing events with this biomaterial and adaptation of the soft tissue to this biomaterial have to be taken into consideration. The aim of this part was to review the anatomy and histology of the soft tissue seal around transmucosal biomaterials used to replace missing teeth and to summarize its morphogenesis during wound healing.

Nature and dimensions of peri-implant mucosa (quantity)

During the process of wound healing, the features of the peri-implant mucosa are established. Many biomaterial and surgical factors may have an influence on the outcome of soft tissue quantity, i.e. the length of the peri-implant mucosa. In an excellent pioneer study in dogs, Berglundh et al. (1991) examined anatomical and histological features of the peri-implant mucosa, which formed in a two-stage procedure, and compared these with those of the gingiva around teeth. The abutment consisted of titanium with a machined surface. Two months after abutment connection, the animals were enrolled in a careful and meticulous plaque control programme consisting of cleaning of the abutment once daily. Four months after abutment connection, clinical inspection and radiographic evaluation revealed healthy conditions. Histologically, the peri-implant mucosa consisted of a well-keratinized oral epithelium, which was located at the external surface and connected to a thin barrier epithelium (i.e. the equivalent to the junctional epithelium around at teeth, which will be referred to as the peri-implant junctional epithelium) facing the abutment. This peri-implant junctional epithelium terminated 2 mm apical to the coronal soft tissue margin and 1.0–1.5 mm coronal from the peri-implant bone crest. Thus, the mean biological width (including the sulcus depth) was 3.80 mm around implants and 3.17 mm around teeth. While there was no statistically significant difference in the height of the junctional epithelium and sulcus depth between implants and teeth, the height of the soft connective tissue was significantly greater around implants than around teeth. The peri-implant junctional epithelium and the soft connective adjacent to the abutment appeared to be in direct contact with the implant abutment surface. However, the precise nature of the epithelial and soft connective tissue attachments could not

accurately be analysed, since the "fracture technique" was applied, which included removal of the titanium implant before cutting of the histological sections. This important study in dogs showed that under the conditions chosen, the peri-implant mucosa has a comparable potential as the gingiva around teeth to prevent subgingival plaque formation and subsequent infection.

Effects of implant system on the peri-implant mucosa dimension

While in the above-mentioned study by Berglundh et al. (1991) the Branemark system (Nobel Biocare, Gothenburg, Sweden) was used, subsequent studies revealed that a similar mucosal attachment formed on titanium in conjunction with different implant systems (Buser et al. 1992, Abrahamsson et al. 1996) and around intentionally non-submerged and initially submerged implants (Arvidson et al. 1996, Weber et al. 1996, Abrahamsson et al. 1999). However, the peri-implant junctional epithelium was significantly longer in initially submerged implants to which an abutment was connected later than in intentionally non-submerged implants (Weber et al. 1996). The biological width was revisited in a further dog experiment after abutment connection to the implant fixture with or without a reduced vertical dimension of the oral mucosa (Berglundh & Lindhe 1996). While the peri-implant junctional epithelium was about 2 mm long, the supraalveolar soft connective was about 1.3–1.8 mm high. Interestingly, sites with a reduced mucosal thickness consistently revealed marginal bone resorption so that the biological width could be adjusted. Evaluating the biological width around one- and two-piece titanium implants that healed unloaded in either a non-submerged or a submerged fashion in dog mandibles, Hermann et al. (2001) suggested that the gingival margin is located more coronally and the biological width more similar to teeth in association with one-piece non-submerged implants compared to either two-piece non-submerged or two-piece submerged implants. These data were confirmed in a comparably designed dog study with another implant system (Pontes et al. 2008).

Effects of implant material on the peri-implant mucosa dimension

In a dog study, Abrahamsson et al. (1998) demonstrated that the material used for the abutment had a major impact on the location of the soft connective tissue compartment. Sintered ceramic material made of aluminium (Al_2O_3) lead to a peri-implant mucosal attachment comparable to that adjacent to titanium abutments. Gold alloy or dental porcelain, however, resulted in inferior histological outcome of the peri-implant mucosa. Kohal et al. (2004) and Welander et al. (2008) have demonstrated the same peri-implant soft tissue dimensions around titanium and zirconia implants installed in the maxilla of monkeys and dogs respectively.

Effects of implant surface characteristics on the peri-implant mucosa dimension

The effects of surface macro design, topography, hydrophilicity and various coatings on the peri-implant mucosa have been evaluated in numerous pre-clinical and clinical studies. Organic implant coatings will not be discussed in this review. Numerous in vitro studies have addressed the issue of surface modifications on mesenchymal and epithelial cell responses. However, these are not included, since it is beyond the scope of this article to review in vitro studies.

The impact of surface topography, often characterized by surface roughness measurements, on the peri-implant mucosa has been investigated in numerous studies. Cochran et al. (1997) noted no differences in the dimensions of the sulcus depth, peri-implant junctional epithelium and soft connective tissue contact to implants with a titanium plasma-sprayed (TPS) surface or a sandblasted acid-etched surface. Abrahamsson et al. (2001, 2002) observed similar epithelial and soft connective tissue components on a rough (acid etched) and smooth (turned) titanium surface. The biological width was greater on the rough surface, however, without a statistically significant difference to that around a smooth surface. In two studies with human biopsy material, less epithelial down-growth and a longer soft connective tissue were found in conjunction with oxidized or acid-etched titanium compared to a machined surface (Glauser et al. 2005, Ferreira Borges & Dragoo 2010). In a study in baboons, Watzak et al. (2006) could show that implant surface modifications had no significant effect on the biological width after 18 months of functional loading. After 3 months of healing in dog mandibles, nanoporous TiO_2 coatings of one-piece titanium implants showed similar length of peri-implant soft connective tissue and epithelium than the uncoated, smooth neck portion of the control titanium implants (Rossi et al. 2008). In a dog study, Schwarz et al. (2007) investigated the effects of surface hydrophilicity and microtopography on soft and hard tissue healing at 1, 4, 7, 14 and 28 days. The authors concluded that soft tissue integration was influenced by hydrophilicity rather than by microtopography. Using a new human model, Schwarz et al. (2013) investigated the peri-implant soft tissue dimensions after an 8-week healing period on specially designed healing abutments with different surface roughness and hydrophilicity. The length of the peri-implant junctional epithelium was in the order of 2 mm for all abutment types without statistically significant differences.

Immediate versus delayed implant loading

Studying immediate versus delayed loading of titanium implants placed in jawbone of monkeys, Siar et al. (2003) and Quaranta et al. (2008) could not detect any significant differences in the dimensions of the peri-implant sulcular and junctional epithelia and connective tissue contact to the implants.

Composition and structural organization of the peri-implant mucosa (quality)

Concerning composition, most studies focused on the soft connective tissue compartment of the peri-implant mucosa. In particular, amount and structural organization of fibroblasts, collagen, and blood vessels were determined. Fewer fibroblasts and more collagen fibres were observed in the bulk of the supra-crestal soft connective tissue around implants with a smooth abutment surface

than around teeth (Moon et al. 1999). However, very close to the implant surface the number of fibroblasts was high and they were interposed between thin collagen fibrils and oriented parallel to the implant surface (Moon et al. 1999). Surface roughness did not seem to have an influence on the number of fibroblasts (Abrahamsson et al. 2002).

In a zone close to the implant surface (i.e. 50–100 μm away), no blood vessels were found (Buser et al. 1992, Listgarten et al. 1992). Further away from the implant surface and adjacent to the barrier (junctional) and sulcular epithelia, blood vessels were observed (Buser et al. 1992). Thus, the number of blood vessels increased with increasing distance from the implant surface. Compared to teeth, there were less vascular structures in the supra-crestal soft connective tissue near the implant than at a corresponding location around teeth (Moon et al. 1999). Using a clearing technique to visualize carbon-stained blood vessels, Berglundh et al. (1994) showed that the vascular network of the peri-implant mucosa originates from one large supra-crestal blood vessel, which branches towards the implant abutment surface.

Influence of material on collagen fibre orientation

Comparing one-piece machined titanium necks with one-piece smooth zirconia implants, no difference was observed concerning collagen fibre orientation, that is, the majority of collagen fibres were oriented parallel or parallel-oblique to the implant surfaces (Tete et al. 2009).

Influence of surface topography on collagen fibre orientation

Collagen fibre orientation was found to be primarily parallel to implants with a smooth titanium surface and the site of fibre insertion into bone was at the bone crest in dogs (Berglundh et al. 1991, Buser et al. 1992, Listgarten et al. 1992). Parallel and uniform collagen fibre orientation was also found around smooth titanium grade 4 implants in a rat model at a very early healing phase of 4 and 7 days, whereas collagen fibre orientation on rough (alumina grit blasted) titanium grade 4 implants was more irregular (Yamano et al. 2011). While these findings corroborate with other reports demonstrating a perpendicular insertion of collagen fibres to the surface of porous plasma-sprayed titanium implants (Schroeder et al. 1981, Buser et al. 1989), and suggesting that the surface texture might affect the collagen fibre orientation (Schupbach & Glauser 2007), others concluded that surface roughness and different materials did not appear to influence fibre orientation (Listgarten et al. 1992, Comut et al. 2001) and amount of collagen (Abrahamsson et al. 2002).

Microgrooves are a sort of surface modifications that are different from topographic modifications intended to alter surface roughness characteristics. In a dog study, it was shown that collagen fibres were oriented perpendicularly to the laser ablated, microgrooved abutment surface, while collagen fibres on a smooth (machined) surface were oriented parallel to the abutment surface after a 3-month healing period (Nevins et al. 2010). Human histological evidence for this attachment of perpendicularly oriented collagen fibres to a microgrooved abutment surface was provided by four implants retrieved after a 6-month (Nevins et al. 2008) and a 10-week (Nevins et al. 2012) healing period.

Influence of surface topography on inflammation and defence

Concerning plaque accumulation and inflammatory cells, no relation was found to surface roughness after 4 weeks in a human model (Wennerberg et al. 2003). However, TiO_2-coated implants with a porous surface showed less inflammation and less epithelial detachment than uncoated implants with a smooth neck portion (Rossi et al. 2008). In contrast, human soft tissue biopsies retrieved from titanium healing caps after a 6-month healing period revealed more inflammation, higher microvessel density, and more proliferation epithelial cells around a rough (acid-etched) surface than adjacent to a smooth (machined) surface (Degidi et al. 2012).

Implant-tissue interfaces of the peri-implant mucosa

The study of tissue interactions with metallic or ceramic biomaterials hampers histological evaluation. If the biomaterial is not removed, only limited histological techniques and analyses are possible. On the other hand, if the biomaterial is removed, some information may irreversibly get lost or tissue artefacts may impede the analysis and consequently also the conclusions. Since in most studies examining the peri-implant mucosa, the "fracture technique" (Thomsen & Ericson 1985) was applied to remove the implant, the true composition and arrangement of the tissue-implant interface could not be analysed.

The ultrastructure of the interface between a metal implant and the peri-implant junctional epithelium was first reported on the basis of freeze-fractured preparations of vitallium implants (James & Schultz 1974). Using transmucosal epoxy resin implants, Listgarten & Lai (1975) noted the ultrastructural similarity of the intact epithelium-implant interface between implants and teeth. Subsequently, these findings were confirmed for titanium-coated plastic implants (Gould et al. 1984), freeze-fractured specimens from ceramic (alpha-alumina oxide in single crystalline form) implants (McKinney et al. 1985), and single-sapphire implants (Hashimoto et al. 1989). These studies revealed that the epithelial cells attach to different implant materials in a fashion comparable to that of the junctional epithelial cells to the tooth surface via hemidesmosomes and a basal lamina.

Analysing the intact interface between soft connective tissue and titanium-coated epoxy resin implants, the parallel orientation of collagen fibrils to the titanium layer was confirmed (Listgarten et al. 1992, Listgarten 1996). Implants normally lack a cementum layer that can invest the peri-implant collagen fibres. Thus, the attachment of the soft connective tissue to the transmucosal portion of an implant is regarded as being weaker than soft connective tissue attachment to the surface of a tooth root. Therefore, improving the quality of the soft tissue-implant interface is considered to be of paramount importance.

Wound healing and morphogenesis of the peri-implant mucosa after flap surgery in healed ridges

While the above studies primarily showed the dimensional and histo-

logical outcomes of the established epithelial and soft connective tissue components of the peri-implant mucosa under various conditions, the wound healing sequence leading to this establishment has only recently been evaluated. Berglundh et al. (2007) examined the delicate process of wound healing and morphogenesis in the mucosa around non-submerged commercially pure titanium implants in dogs. Two weeks after mucosal adaptation to the marginal portion of the implants, the sutures were removed and a rigid plaque-control program was initiated. Healing periods varied from 2 h to 12 weeks. The morphogenesis was analysed in histological sections and by means of histomorphometry.

Immediately after implant placement, a coagulum occupied the implant-mucosa interface. Numerous neutrophils infiltrated the blood clot and at 4 days an initial mucosal seal was established. In the next few days, the area with the leucocytes decreased and was confined to the coronal portion, whereas fibroblasts and collagen dominated the apical part of the implant-tissue interface. Between 1 and 2 weeks of healing, the peri-implant junctional epithelium was about 0.5 mm apical to the mucosal margin. At 2 weeks, the peri-implant junctional epithelium started to proliferate in the apical direction. After 2 weeks, the peri-implant mucosa was rich in cells and blood vessels. At 4 weeks of healing, the peri-implant junctional epithelium migrated further apically and occupied now 40% of the total soft tissue implant interface. The soft connective tissue was rich in collagen and fibroblasts and well-organized. The apical migration of the peri-implant junctional epithelium was completed between 6 and 8 weeks and the fibroblasts formed a dense layer over the titanium surface at that time. From 6 to 12 weeks, maturation of the soft connective tissue had occurred and the peri-implant junctional epithelium occupied about 60% of the entire implant soft tissue interface. Further away from the implant surface, the number of blood vessels was low and fibroblasts were located between thin collagen fibres running mainly parallel to the implant surface. From this study, it can be concluded that the soft tissue attachment to transmucosal (i.e. non-submerged) implants made of commercially pure titanium with a polished surface in the neck portion requires at least 6 weeks in this animal model.

Using a new human model, Tomasi et al. (2013) investigated the morphogenesis of the peri-implant mucosa during the first 12 weeks of healing. They observed that a soft tissue barrier adjacent to titanium implants developed completely within 8 weeks, which is in agreement with observations made in dogs (Berglundh et al. 2007, Schwarz et al. 2007, Vignoletti et al. 2009), but not with those from Glauser et al. in humans. Concerning stability of soft tissue dimensions over time, it can be concluded that the dimensions of the soft tissue seal (i.e. the biological width) around implants are stable for at least 12 (Cochran et al. 1997, Assenza et al. 2003) or 15 months (Hermann et al. 2000).

Wound healing and morphogenesis of the peri-implant mucosa after immediate implant placement into fresh extraction sockets

Vignoletti et al. (2009) described histologically and histomorphometrically the early phases of soft tissue healing around implants placed into fresh tooth extraction sockets in dogs. They observed a fast apical downgrowth of the peri-implant junctional epithelium within the first week of healing and a final biological width of approximately 5 mm with a peri-implant junctional epithelium measuring 3.0–3.5 mm at 8 weeks. Similar dimensional outcomes were reported by Rimondini et al. (2005) in minipigs (i.e. 3 mm epithelial length after 30 and 60 days) and by de Sanctis et al. (2009) around different implant systems in dogs (i.e. 2.33–2.70 mm epithelial length after 6 weeks). The above mentioned peri-implant soft tissue dimensions differ from those reported in other studies where implants were placed into fresh extraction sockets in dogs (Araujo et al. 2005, 2006) and from those reported after placement into healed ridges (see 3.4.). In summary, it can be concluded that when implants are placed into fresh extraction sockets there are conditions that appear to favour a fast apical migration of the peri-implant junctional epithelium and the establishment of a greater final biological width dimension, particularly as regards the epithelial component. The clinical consequences, the conditions that favour and the measures that reduce the formation of a longer peri-implant junctional epithelium on implants need to be determined.

Flap versus flapless healing of the peri-implant mucosa

In a dog experiment, teeth were removed either flapless or with flap surgery and implants were immediately placed (Blanco et al. 2008). After a 3-month healing period, the distance between the peri-implant mucosal margin and the first bone-implant contact was significantly greater in the flap group compared to the flapless group (3.69 mm *versus* 3.02 mm). At the edentulous site, the implantation region may be exposed using flap surgery (i.e. the bone crest is exposed by a crestal incision) or a flapless approach (i.e. the bone crest is exposed by a soft tissue punch). In a study in dogs by You et al. (2009), flat bone crests were created following tooth extractions. Three months later, implants were placed by either the flap or the flapless approach. Three months after implant installation, the height of the peri-implant mucosa and the length of the peri-implant junctional epithelium were significantly greater in the flap than in the flapless group. In other studies in dogs, a significantly longer peri-implant junctional epithelium formed at both 3 weeks and 3 months (Lee et al. 2010) and at 3 months (Bayounis et al. 2011) after implant placement, when the punch diameter was greater than that of the implant. This was probably also the reason for the formation of a longer peri-implant junctional epithelium after soft tissue punching as opposed to flap surgery (Bayounis et al. 2011). These findings suggest that the diameter of the soft tissue punch should be slightly smaller than that of the implant to obtain better peri-implant mucosa adaptation and subsequent healing.

The vascularity of the peri-implant mucosa was investigated after flap and flapless implant installation (Kim et al. 2009). Morphometric measure-

ments revealed that there was increased vascularity after the flapless procedure than after the flap approach. Mueller et al. (2010, 2011, 2012) evaluated marker molecules for inflammation, re-epithelialization, and the implant-epithelial junction in minipigs at 1, 2, 3 and 12 weeks after implant installation using the flapless approach or flap surgery. Flapless implant insertion resulted in less inflammation (Mueller et al. 2010), early re-epithelialization (Mueller et al. 2011) and significant higher expressions of integrin $\alpha 6\beta 4$ chain $\beta 4$ at 2 and 12 weeks and laminin $5\gamma 2$ chain for all healing periods (Mueller et al. 2012). A further conclusion was that a smooth neck portion is to be preferred for the flapless approach (Mueller et al. 2010). Using the flapless approach with and without immediate loading, Blanco et al. (2012) found similar soft tissue dimensions in dogs around titanium implants after a 3-month healing period.

In summary, it seems that the flapless approach has, at least in the short-term, some advantages over flap surgery, provided that the diameter of the soft tissue punch is below that of the transmucosal portion of the implant. The disadvantage of the flapless approach is that the bone volume may not accurately be determined. However, the clinical relevance of these histological findings remains to be determined.

Wound healing after probing around implants

Probing of the soft tissues around dental implants is an important parameter during clinical monitoring. The healing of the disrupted soft tissue seal as a result of clinical probing has been studied in dogs from 1 to 7 days post-probing around titanium plasma-sprayed implants (Etter et al. 2002). The probe initiated a separation of the peri-implant junctional epithelium from the implant surface. Although the probe went past the most apical cell of the peri-implant junctional epithelium, no tissue separation was detected in the soft connective tissue compartment at any time point. At day 0, the peri-implant junctional epithelium of five test sides was completely separated from the implant surface, while in one case tissue separation ended 0.3 mm apical to the apical termination of the peri-implant junctional epithelium. Primarily leucocytes filled the separation space. One day after probing, an initial new epithelial attachment was observed at the bottom of the separation space, while leucocytes were still present more coronally. The length of the epithelial attachment increased from 0.5 mm at day 1 to 1.92 mm at day 7 after probing. From this study, it was concluded that the healing of the epithelial attachment adjacent to dental implants was complete 5 days after clinical probing. Thus, probing does not seem to jeopardize maintenance of healthy conditions around dental implants. From another animal study, it was concluded that frequent clinical probing at short intervals during the healing phase caused dimensional and structural changes of the peri-implant mucosal seal (Schwarz et al. 2010). Surface roughness and surface chemistry did not influence the outcome in this dog study.

Based on the available data, it was concluded that:

(a) Wound healing in skin and oral wounds follows a similar pattern.
(b) The tissue specificities of the gingival, alveolar and palatal mucosa appear to be innately and not necessarily functionally determined.
(c) The granulation tissue originating from the periodontal ligament or from connective tissue originally covered by keratinized epithelium has the potential to induce keratinization. However, it also appears that deep palatal connective tissue, may not have the same potential to induce keratinization as the palatal connective tissue originating from an immediately subepithelial area.
(d) Epithelial healing following non-surgical and surgical periodontal therapy appears to be completed after a period of 7–14 days. A structural integrity of a maturing wound between a denuded root surface and a soft tissue flap is achieved at approximately 14 days post surgery.
(e) The formation of the biological width and maturation of the barrier function around transmucosal implants requires 6–8 weeks of healing.
(f) The established peri-implant soft connective tissue resembles a scar tissue in composition, fibre orientation and vasculature.
(g) The peri-implant junctional epithelium may reach a greater final length under certain conditions such as implants placed into fresh extraction sockets *versus* conventional implant procedures in healed sites.

References

Abrahamsson, I., Berglundh, T., Glantz, P. O. & Lindhe, J. (1998) The mucosal attachment at different abutments. An experimental study in dogs. *Journal of Clinical Periodontology* **25**, 721–727.

Abrahamsson, I., Berglundh, T., Moon, I. S. & Lindhe, J. (1999) Peri-implant tissues at submerged and non-submerged titanium implants. *Journal of Clinical Periodontology* **26**, 600–607.

Abrahamsson, I., Berglundh, T., Wennström, J. & Lindhe, J. (1996) The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. *Clinical Oral Implants Research* **7**, 212–219.

Abrahamsson, I., Zitzmann, N. U., Berglundh, T., Linder, E., Wennerberg, A. & Lindhe, J. (2002) The mucosal attachment to titanium implants with different surface characteristics: an experimental study in dogs. *Journal of Clinical Periodontology* **29**, 448–455.

Abrahamsson, I., Zitzmann, N. U., Berglundh, T., Wennerberg, A. & Lindhe, J. (2001) Bone and soft tissue integration to titanium implants with different surface topography: an experimental study in the dog. *International Journal of Oral and Maxillofacial Implants* **16**, 323–332.

Araujo, M. G., Sukekava, F., Wennström, J. L. & Lindhe, J. (2005) Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. *Journal of Clinical Periodontology* **32**, 645–652. doi:10.1111/j.1600-051X.2005.00726.x.

Araujo, M. G., Sukekava, F., Wennström, J. L. & Lindhe, J. (2006) Tissue modeling following implant placement in fresh extraction sockets. *Clinical Oral Implants Research* **17**, 615–624. doi:10.1111/j.1600-0501.2006.01317.x.

Arvidson, K., Fartash, B., Hilliges, M. & Kondell, P. A. (1996) Histological characteristics of peri-implant mucosa around Branemark and single-crystal sapphire implants. *Clinical Oral Implants Research* **7**, 1–10.

Ashcroft, G. S., Yang, X., Glick, A. B., Weinstein, M., Letterio, J. L., Mizel, D. E., Anzano, M., Greenwell-Wild, T., Wahl, S. M., Deng, C. & Roberts, A. B. (1999) Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nature Cell Biology* **1**, 260–266. doi:10.1038/12971.

Assenza, B., Scarano, A., Petrone, G., Iezzi, G., Thams, U., San Roman, F. & Piattelli, A. (2003) Crestal bone remodeling in loaded and unloaded implants and the microgap: a histologic study. *Implant Dentistry* **12**, 235–241.

Barbul, A., Shawe, T., Rotter, S. M., Efron, J. E., Wasserkrug, H. L. & Badawy, S. B. (1989) Wound healing in nude mice: a study on the regulatory role of lymphocytes in fibroplasia. *Surgery* **105**, 764–769.

Bayounis, A. M., Alzoman, H. A., Jansen, J. A. & Babay, N. (2011) Healing of peri-implant tissues after flapless and flapped implant installation. *Journal of Clinical Periodontology* **38**, 754–761. doi:10.1111/j.1600-051X.2011.01735.x.

Berglundh, T., Abrahamsson, I., Welander, M., Lang, N. P. & Lindhe, J. (2007) Morphogenesis of the peri-implant mucosa: an experimental study in dogs. *Clinical Oral Implants Research* **18**, 1–8. doi:10.1111/j.1600-0501.2006.01380.x.

Berglundh, T. & Lindhe, J. (1996) Dimension of the periimplant mucosa. Biological width revisited. *Journal of Clinical Periodontology* **23**, 971–973.

Berglundh, T., Lindhe, J., Ericsson, I., Marinello, C. P., Liljenberg, B. & Thomsen, P. (1991) The soft tissue barrier at implants and teeth. *Clinical Oral Implants Research* **2**, 81–90.

Berglundh, T., Lindhe, J., Jonsson, K. & Ericsson, I. (1994) The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. *Journal of Clinical Periodontology* **21**, 189–193.

Bernimoulin, J. P. & Lange, D. E. (1973) Effects of heterotopic connective tissue transplantation on epithelium regeneration. *Deutsche zahnärztliche Zeitschrift* **28**, 202–205.

Bernimoulin, J. P. & Schroeder, H. E. (1980) Changes in the differentiation pattern of oral mucosal epithelium following heterotopic connective tissue transplantation in man. *Pathology, Research and Practice* **166**, 290–312. doi:10.1016/S0344-0338(80)80136-1.

Blanco, J., Carral, C., Linares, A., Perez, J. & Munoz, F. (2012) Soft tissue dimensions in flapless immediate implants with and without immediate loading: an experimental study in the beagle dog. *Clinical Oral Implants Research* **23**, 70–75. doi:10.1111/j.1600-0501.2011.02183.x.

Blanco, J., Nunez, V., Aracil, L., Munoz, F. & Ramos, I. (2008) Ridge alterations following immediate implant placement in the dog: flap versus flapless surgery. *Journal of Clinical Periodontology* **35**, 640–648. doi:10.1111/j.1600-051X.2008.01237.x.

Blanpain, C. & Fuchs, E. (2009) Epidermal homeostasis: a balancing act of stem cells in the skin. *Nature Reviews Molecular Cell Biology* **10**, 207–217. doi:10.1038/nrm2636.

Bodner, L., Dayan, D., Oberman, M., Hirshberg, A. & Tal, H. (1992) Healing of experimental wounds in sialadenectomized rat. *Journal of Clinical Periodontology* **19**, 345–347.

Bodner, L., Dayan, D., Pinto, Y. & Hammel, I. (1993) Characteristics of palatal wound healing in desalivated rats. *Archives of Oral Biology* **38**, 17–21.

Bodner, L., Dayan, D., Rothchild, D. & Hammel, I. (1991a) Extraction wound healing in desalivated rats. *Journal of Oral Pathology and Medicine* **20**, 176–178.

Bodner, L., Knyszynski, A., Adler-Kunin, S. & Danon, D. (1991b) The effect of selective desalivation on wound healing in mice. *Experimental Gerontology* **26**, 357–363.

Bosshardt, D. D. (2008) Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels. *Journal of Clinical Periodontology* **35**, 87–105. doi:10.1111/j.1600-051X.2008.01264.x.

Bosshardt, D. D. & Lang, N. P. (2005) The junctional epithelium: from health to disease. *Journal of Dental Research* **84**, 9–20.

Bosshardt, D. D. & Sculean, A. (2009) Does periodontal tissue regeneration really work? *Periodontology* **51**, 208–219. doi:10.1111/j.1600-0757.2009.00317.x.

Brown, R. L., Ormsby, I., Doetschman, T. C. & Greenhalgh, D. G. (1995) Wound healing in the transforming growth factor-beta-deficient mouse. *Wound Repair Regeneration* **3**, 25–36. doi:10.1046/j.1524-475X.1995.30108.x.

Buser, D., Stich, H., Krekeler, G. & Schroeder, A. (1989) Faserstrukturen der Peri-Implantären Mukosa bei Titanimplantaten – Eine tierexperimentelle Studie am Beagle-Hund. *Zeitschrift für Zahnärztliche Implantologie* **5**, 15–23. doi:10.1111/j.1600-0501.2006.01380.x.

Buser, D., Weber, H. P., Donath, K., Fiorellini, J. P., Paquette, D. W. & Williams, R. C. (1992) Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. *Journal of Periodontology* **63**, 225–235. doi:10.1902/jop.1992.63.3.225.

Bye, F. L., Caffesse, R. G. & Nasjleti, C. E. (1980) The effect of different plaque control modalities on the keratinizing potential of the sulcular epithelium in monkeys. *Journal of Periodontology* **51**, 632–641. doi:10.1902/jop.1980.51.11.632.

Caffesse, R. G., Burgett, F. G., Nasjleti, C. E. & Castelli, W. A. (1979a) Healing of free gingival grafts with and without periosteum. Part I. Histologic evaluation. *Journal of Periodontology* **50**, 586–594. doi:10.1902/jop.1979.50.11.586.

Caffesse, R. G., Karring, T. & Nasjleti, C. E. (1977) Keratinizing potential of sulcular epithelium. *Journal of Periodontology* **48**, 140–146. doi:10.1902/jop.1977.48.3.140.

Caffesse, R. G., Kon, S., Castelli, W. A. & Nasjleti, C. E. (1984) Revascularization following the lateral sliding flap procedure. *Journal of Periodontology* **55**, 352–358. doi:10.1902/jop.1984.55.6.352.

Caffesse, R. G., Kornman, K. S. & Nasjleti, C. E. (1980) The effect of intensive antibacterial therapy on the sulcular environment in monkeys. Part II: inflammation, mitotic activity and keratinization of the sulcular epithelium. *Journal of Periodontology* **51**, 155–161. doi:10.1902/jop.1980.51.3.155.

Caffesse, R. G., Nasjleti, C. E. & Castelli, W. A. (1979b) The role of sulcular environment in controlling epithelial keratinization. *Journal of Periodontology* **50**, 1–6. doi:10.1902/jop.1979.50.1.1.

Caffesse, R. G., Nasjleti, C. E. & Kowalski, C. J. (1985) Carrageenan-induced inflammation and its effects on mitotic activity and keratinization of gingival epithelium. A histologic and autoradiographic study. *Journal of Periodontology* **56**, 132–138. doi:10.1902/jop.1985.56.3.132.

Caffesse, R. G., Nasjleti, C. J., Kowalski, C. J. & Castelli, W. A. (1982) The effect of mechanical stimulation on the keratinization of sulcular epithelium. *Journal of Periodontology* **53**, 89–92. doi:10.1902/jop.1982.53.2.89.

Caton, J. G. & Zander, H. A. (1979) The attachment between tooth and gingival tissues after periodic root planing and soft tissue curettage. *Journal of Periodontology* **50**, 462–466. doi:10.1902/jop.1979.50.9.462.

Clark, R. A., Lin, F., Greiling, D., An, J. & Couchman, J. R. (2004) Fibroblast invasive migration into fibronectin/fibrin gels requires a previously uncharacterized dermatan sulfate-CD44 proteoglycan. *The Journal of Investigative Dermatology* **122**, 266–277. doi:10.1046/j.0022-202X.2004.22205.x.

Cochran, D. L., Hermann, J. S., Schenk, R. K., Higginbottom, F. L. & Buser, D. (1997) Biologic width around titanium implants. A histometric analysis of the implanto-gingival junction around unloaded and loaded nonsubmerged implants in the canine mandible. *Journal of Periodontology* **68**, 186–198. doi:10.1902/jop.1997.68.2.186.

Comut, A. A., Weber, H. P., Shortkroff, S., Cui, F. Z. & Spector, M. (2001) Connective tissue orientation around dental implants in a canine model. *Clinical Oral Implants Research* **12**, 433–440.

Cordeiro, J. V. & Jacinto, A. (2013) The role of transcription-independent damage signals in the initiation of epithelial wound healing. *Nature Reviews Molecular Cell Biology* **14**, 249–262. doi:10.1038/nrm3541.

de Sanctis, M., Vignoletti, F., Discepoli, N., Zucchelli, G. & Sanz, M. (2009) Immediate implants at fresh extraction sockets: bone healing in four different implant systems. *Journal of Clinical Periodontology* **36**, 705–711. doi:10.1111/j.1600-051X.2009.01427.x.

Degidi, M., Artese, L., Piattelli, A., Scarano, A., Shibli, J. A., Piccirilli, M., Perrotti, V. & Iezzi, G. (2012) Histological and immunohistochemical evaluation of the peri-implant soft tissues around machined and acid-etched titanium healing abutments: a prospective randomised study. *Clinical Oral Investigation* **16**, 857–866. doi:10.1007/s00784-011-0574-3.

Dickinson, D. P., Coleman, B. G., Batrice, N., Lee, J., Koli, K., Pennington, C., Susin, C. & Wikesjo, U. M. (2013) Events of wound healing/regeneration in the canine supraalveolar periodontal defect model. *Journal of Clinical Periodontology* **40**, 527–541. doi:10.1111/jcpe.12055.

Donos, N., D'Aiuto, F., Retzepi, M. & Tonetti, M. (2005) Evaluation of gingival blood flow by the use of laser Doppler flowmetry following periodontal surgery. A pilot study. *Journal of Periodontal Research* **40**, 129–137. doi:10.1111/j.1600-0765.2005.00777.x.

Edel, A. (1974) Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva. *Journal of Clinical Periodontology* **1**, 185–196.

Edel, A. & Faccini, J. M. (1977) Histologic changes following the grafting of connective tissue into human gingiva. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **43**, 190–195.

Efron, J. E., Frankel, H. L., Lazarou, S. A., Wasserkrug, H. L. & Barbul, A. (1990) Wound healing and T-lymphocytes. *Journal of Surgical Research* **48**, 460–463.

Egli, U., Vollmer, W. H. & Rateitschak, K. H. (1975) Follow-up studies of free gingival grafts. *Journal of Clinical Periodontology* **2**, 98–104.

Egozi, E. I., Ferreira, A. M., Burns, A. L., Gamelli, R. L. & Dipietro, L. A. (2003) Mast cells modulate the inflammatory but not the proliferative response in healing wounds. *Wound Repair Regeneration* **11**, 46–54.

Etter, T. H., Hakanson, I., Lang, N. P., Trejo, P. M. & Caffesse, R. G. (2002) Healing after standardized clinical probing of the perlimplant soft tissue seal: a histomorphometric study in dogs. *Clinical Oral Implants Research* **13**, 571–580.

Ferreira Borges, P. & Dragoo, M. (2010) Reactions of periodontal tissues to biologic implant abutments. Clinical and histologic evaluation (a pilot study). *Journal GABD Online* **5**, 15–23.

Fickl, S., Kebschull, M., Schupbach, P., Zuhr, O., Schlagenhauf, U. & Hurzeler, M. B. (2011) Bone loss after full-thickness and partial-thickness flap elevation. *Journal of Clinical Peri-*

odontology **38**, 157–162. doi:10.1111/j.1600-051X.2010.01658.x.

Gargiulo, A. W. & Arrocha, R. (1967) Histo-clinical evaluation of free gingival grafts. *Periodontics* **5**, 285–291.

Glauser, R., Schupbach, P., Gottlow, J. & Hammerle, C. H. (2005) Periimplant soft tissue barrier at experimental one-piece mini-implants with different surface topography in humans: a light-microscopic overview and histometric analysis. *Clinical Implant Dentistry and Related Research* **7** (Suppl 1), S44–S51.

Gould, T. R., Westbury, L. & Brunette, D. M. (1984) Ultrastructural study of the attachment of human gingiva to titanium in vivo. *Journal of Prosthetic Dentistry* **52**, 418–420.

Graves, D. T., Nooh, N., Gillen, T., Davey, M., Patel, S., Cottrell, D. & Amar, S. (2001) IL-1 plays a critical role in oral, but not dermal, wound healing. *Journal of Immunology* **167**, 5316–5320.

Grieb, G., Steffens, G., Pallua, N., Bernhagen, J. & Bucala, R. (2011) Circulating fibrocytes–biology and mechanisms in wound healing and scar formation. *International Review of Cell and Molecular Biology* **291**, 1–19. doi:10.1016/B978-0-12-386035-4.00001-X.

Guo, F., Hutchenreuther, J., Carter, D. E. & Leask, A. (2013) TAK1 is required for dermal wound healing and homeostasis. *Journal of Investigative Dermatology* **133**, 1646–1654. doi:10.1038/jid.2013.28.

Gurtner, G. C., Werner, S., Barrandon, Y. & Longaker, M. T. (2008) Wound repair and regeneration. *Nature* **453**, 314–321. doi:10.1038/nature07039.

Hashimoto, M., Akagawa, Y., Nikai, H. & Tsuru, H. (1989) Ultrastructure of the peri-implant junctional epithelium on single-crystal sapphire endosseous dental implant loaded with functional stress. *Journal of Oral Rehabilitation* **16**, 261–270.

Havran, W. L. & Jameson, J. M. (2010) Epidermal T cells and wound healing. *Journal of Immunology* **184**, 5423–5428. doi:10.4049/jimmunol.0902733.

Hermann, J. S., Buser, D., Schenk, R. K., Higginbottom, F. L. & Cochran, D. L. (2000) Biologic width around titanium implants. A physiologically formed and stable dimension over time. *Clinical Oral Implants Research* **11**, 1–11.

Hermann, J. S., Buser, D., Schenk, R. K., Schoolfield, J. D. & Cochran, D. L. (2001) Biologic Width around one- and two-piece titanium implants. *Clinical Oral Implants Research* **12**, 559–571.

Hiatt, W. H., Stallard, R. E., Butler, E. D. & Badgett, B. (1968) Repair following mucoperiosteal flap surgery with full gingival retention. *Journal of Periodontology* **39**, 11–16. doi:10.1902/jop.1968.39.1.11.

Hinz, B. (2007) Formation and function of the myofibroblast during tissue repair. *Journal of Investigative Dermatology* **127**, 526–537. doi:10.1038/sj.jid.5700613.

Hollinger, J. O., Hart, C. E., Hirsch, S. N., Lynch, S. & Friedlaender, G. E. (2008) Recombinant human platelet-derived growth factor: biology and clinical applications. *Journal of Bone and Joint Surgery. American Volume* **90** (Suppl 1), 48–54. doi:10.2106/JBJS.G.01231.

Hong, H. H., Uzel, M. I., Duan, C., Sheff, M. C. & Trackman, P. C. (1999) Regulation of lysyl oxidase, collagen, and connective tissue growth factor by TGF-beta1 and detection in human gingiva. *Laboratory Investigation* **79**, 1655–1667.

Ishida, Y., Kondo, T., Takayasu, T., Iwakura, Y. & Mukaida, N. (2004) The essential involvement of cross-talk between IFN-gamma and TGF-beta in the skin wound-healing process. *Journal of Immunology* **172**, 1848–1855.

James, R. A. & Schultz, R. L. (1974) Hemidesmosomes and the adhesion of junctional epithelial cells to metal implants–a preliminary report. *Oral Implantology* **4**, 294–302.

James, W. C. & McFall, W. T., Jr (1978) Placement of free gingival grafts on denuded alveolar bone. Part I: clinical evaluations. *Journal of Periodontology* **49**, 283–290. doi:10.1902/jop.1978.49.6.283.

Jameson, J., Ugarte, K., Chen, N., Yachi, P., Fuchs, E., Boismenu, R. & Havran, W. L. (2002) A role for skin gammadelta T cells in wound repair. *Science* **296**, 747–749. doi:10.1126/science.1069639.

Jinno, K., Takahashi, T., Tsuchida, K., Tanaka, E. & Moriyama, K. (2009) Acceleration of palatal wound healing in Smad3-deficient mice. *Journal of Dental Research* **88**, 757–761. doi:10.1177/0022034509341798.

Kaigler, D., Avila, G., Wisner-Lynch, L., Nevins, M. L., Nevins, M., Rasperini, G., Lynch, S. E. & Giannobile, W. V. (2011) Platelet-derived growth factor applications in periodontal and peri-implant bone regeneration. *Expert Opinion on Biological Therapy* **11**, 375–385. doi:10.1517/14712598.2011.554814.

Karring, T., Cumming, B. R., Oliver, R. C. & Loe, H. (1975a) The origin of granulation tissue and its impact on postoperative results of mucogingival surgery. *Journal of Periodontology* **46**, 577–585. doi:10.1902/jop.1975.46.10.577.

Karring, T., Lang, N. P. & Loe, H. (1975b) The role of gingival connective tissue in determining epithelial differentiation. *Journal of Periodontal Research* **10**, 1–11.

Karring, T., Ostergaard, E. & Loe, H. (1971) Conservation of tissue specificity after heterotopic transplantation of gingiva and alveolar mucosa. *Journal of Periodontal Research* **6**, 282–293.

Kim, J. I., Choi, B. H., Li, J., Xuan, F. & Jeong, S. M. (2009) Blood vessels of the peri-implant mucosa: a comparison between flap and flapless procedures. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **107**, 508–512. doi:10.1016/j.tripleo.2008.08.003.

Klingberg, F., Hinz, B. & White, E. S. (2013) The myofibroblast matrix: implications for tissue repair and fibrosis. *Journal of Pathology* **229**, 298–309. doi:10.1002/path.4104.

Kohal, R. J., Weng, D., Bachle, M. & Strub, J. R. (2004) Loaded custom-made zirconia and titanium implants show similar osseointegration: an animal experiment. *Journal of Periodontology* **75**, 1262–1268. doi:10.1902/jop.2004.75.9.1262.

Kolaczkowska, E. & Kubes, P. (2013) Neutrophil recruitment and function in health and inflammation. *Nature Reviews Immunology* **13**, 159–175. doi:10.1038/nri3399.

Kon, S., Caffesse, R. G., Castelli, W. A. & Nasjleti, C. E. (1984) Revascularization following a combined gingival flap-split thickness flap procedure in monkeys. *Journal of Periodontology* **55**, 345–351. doi:10.1902/jop.1984.55.6.345.

Kon, S., Novaes, A. B., Ruben, M. P. & Goldman, H. M. (1969) Visualization of the microvascularization of the healing periodontal wound. IV. Mucogingival surgery: full thickness flap. *Journal of Periodontology* **40**, 441–456. doi:10.1902/jop.1969.40.8.441.

Kon, S., Pustiglioni, F. E., Novaes, A. B., Ruben, M. P. & de Araujo, N. S. (1978) Split thickness flap, apically replaced, with protected linear periosteal fenestration: a clinical and histological study in dogs. *Journal of Periodontology* **49**, 174–180. doi:10.1902/jop.1978.49.4.174.

Lange, D. E. & Bernimoulin, J. P. (1974) Exfoliative cytological studies in evaluation of free gingival graft healing. *Journal of Clinical Periodontology* **1**, 89–96.

Lee, D. H., Choi, B. H., Jeong, S. M., Xuan, F., Kim, H. R. & Mo, D. Y. (2010) Effects of soft tissue punch size on the healing of peri-implant tissue in flapless implant surgery. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **109**, 525–530. doi:10.1016/j.tripleo.2009.10.002.

Levy, B. M., Taylor, A. C. & Bernick, S. (1969) Relationship between epithelium and connective tissue in gingival inflammation. *Journal of Dental Research* **48**, 625–629.

Liaw, L., Birk, D. E., Ballas, C. B., Whitsitt, J. S., Davidson, J. M. & Hogan, B. L. (1998) Altered wound healing in mice lacking a functional osteopontin gene (spp1). *The Journal of Clinical Investigation* **101**, 1468–1478. doi:10.1172/JCI2131.

Lindhe, J., Wennström, J. L. & Berglundh, T. (2008) The mucosa at teeth and implants. In: Lindhe, J., Lang, N. P. & Karring, T. (eds). *Clinical Periodontology and Implant Dentistry*, 5th edition, pp. 69–85, ??????: Blackwell Munksgaard.

Listgarten, M. A. (1996) Soft and hard tissue response to endosseous dental implants. *Anatomical Record* **245**, 410–425. doi:10.1002/(SICI)1097-0185(199606)245:2%3c410:AID-AR20%3e3.0.CO;2-R.

Listgarten, M. A., Buser, D., Steinemann, S. G., Donath, K., Lang, N. P. & Weber, H. P. (1992) Light and transmission electron microscopy of the intact interfaces between non-submerged titanium-coated epoxy resin implants and bone or gingiva. *Journal of Dental Research* **71**, 364–371.

Listgarten, M. A. & Lai, C. H. (1975) Ultrastructure of the intact interface between an endosseous epoxy resin dental implant and the host tissues. *Journal de Biologie Buccale* **3**, 13–28.

Mantovani, A., Biswas, S. K., Galdiero, M. R., Sica, A. & Locati, M. (2013) Macrophage plasticity and polarization in tissue repair and remodelling. *Journal of Pathology* **229**, 176–185. doi:10.1002/path.4133.

Martin, P. (1997) Wound healing–aiming for perfect skin regeneration. *Science* **276**, 75–81.

Martin, P., D'Souza, D., Martin, J., Grose, R., Cooper, L., Maki, R. & McKercher, S. R. (2003) Wound healing in the PU.1 null mouse–tissue repair is not dependent on inflammatory cells. *Current Biology* **13**, 1122–1128.

Martin, P. & Leibovich, S. J. (2005) Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends in Cell Biology* **15**, 599–607. doi:10.1016/j.tcb.2005.09.002.

McKinney, R. V., Jr, Steflik, D. E. & Koth, D. L. (1985) Evidence for a junctional epithelial attachment to ceramic dental implants. A transmission electron microscopic study. *Journal of Periodontology* **56**, 579–591. doi:10.1902/jop.1985.56.10.579.

McLean, T. N., Smith, B. A., Morrison, E. C., Nasjleti, C. E. & Caffesse, R. G. (1995) Vascular changes following mucoperiosteal flap surgery: a fluorescein angiography study in dogs.

Journal of Periodontology **66**, 205–210. doi:10.1902/jop.1995.66.3.205.

Meneghin, A. & Hogaboam, C. M. (2007) Infectious disease, the innate immune response, and fibrosis. *The Journal of Clinical Investigation* **117**, 530–538. doi:10.1172/JCI30595.

Moon, I. S., Berglundh, T., Abrahamsson, I., Linder, E. & Lindhe, J. (1999) The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. *Journal of Clinical Periodontology* **26**, 658–663.

Mörmann, W. & Ciancio, S. G. (1977) Blood supply of human gingiva following periodontal surgery. A fluorescein angiographic study. *Journal of Periodontology* **48**, 681–692. doi:10.1902/jop.1977.48.11.681.

Mörmann, W., Schaer, F. & Firestone, A. R. (1981) The relationship between success of free gingival grafts and transplant thickness. Revascularization and shrinkage–a one year clinical study. *Journal of Periodontology* **52**, 74–80. doi:10.1902/jop.1981.52.2.74.

Mueller, C. K., Thorwarth, M., Chen, J. & Schultze-Mosgau, S. (2012) A laboratory study comparing the effect of ridge exposure using tissue punch versus mucoperiosteal flap on the formation of the implant-epithelial junction. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **114**, S41–S45. doi:10.1016/j.tripleo.2011.08.019.

Mueller, C. K., Thorwarth, M. & Schultze-Mosgau, S. (2010) Influence of insertion protocol and implant shoulder design on inflammatory infiltration and gene expression in peri-implant soft tissue during nonsubmerged dental implant healing. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **109**, e11–e19. doi:10.1016/j.tripleo.2010.01.003.

Mueller, C. K., Thorwarth, M. & Schultze-Mosgau, S. (2011) Histomorphometric and whole-genome expression analysis of peri-implant soft tissue healing: a comparison of flapless and open surgery. *International Journal of Oral and Maxillofacial Implants* **26**, 760–767.

Murakami, S. (2000) (2011) Periodontal tissue regeneration by signaling molecule(s): what role does basic fibroblast growth factor (FGF-2) have in periodontal therapy? *Periodontology* **56**, 188–208. doi:10.1111/j.1600-0757.2010.00365.x.

Nasjleti, C. E., Caffesse, R. G. & Kowalski, C. J. (1984) Dextran-induced inflammation and its effect on keratinized gingival epithelium in monkeys. *Journal of Periodontology* **55**, 531–535. doi:10.1902/jop.1984.55.9.531.

Nauta, A., Gurtner, G. & Longaker, M. T. (2011) Wound healing and regenerative strategies. *Oral Diseases* **17**, 541–549. doi:10.1111/j.1601-0825.2011.01787.x.

Nauta, A. C., Grova, M., Montoro, D. T., Zimmermann, A., Tsai, M., Gurtner, G. C., Galli, S. J. & Longaker, M. T. (2013) Evidence that mast cells are not required for healing of splinted cutaneous excisional wounds in mice. *PLoS ONE* **8**, e59167. doi:10.1371/journal.pone.0059167.

Nevins, M., Camelo, M., Nevins, M. L., Schupbach, P. & Kim, D. M. (2012) Connective tissue attachment to laser-microgrooved abutments: a human histologic case report. *The International Journal of Periodontics and Restorative Dentistry* **32**, 385–392.

Nevins, M., Kim, D. M., Jun, S. H., Guze, K., Schupbach, P. & Nevins, M. L. (2010) Histologic evidence of a connective tissue attachment to laser microgrooved abutments: a canine study. *The International Journal of Periodontics and Restorative Dentistry* **30**, 245–255.

Nevins, M., Nevins, M. L., Camelo, M., Boyesen, J. L. & Kim, D. M. (2008) Human histologic evidence of a connective tissue attachment to a dental implant. *The International Journal of Periodontics and Restorative Dentistry* **28**, 111–121.

Nishio, N., Ito, S., Suzuki, H. & Isobe, K. (2009) Antibodies to wounded tissue enhance cutaneous wound healing. *Immunology* **128**, 369–380. doi:10.1111/j.1365-2567.2009.03119.x.

Nobuto, T., Yanagihara, K., Teranishi, Y., Minamibayashi, S., Imai, H. & Yamaoka, A. (1989) Periosteal microvasculature in the dog alveolar process. *Journal of Periodontology* **60**, 709–715. doi:10.1902/jop.1989.60.12.709.

Nooh, N. & Graves, D. T. (2003) Healing is delayed in oral compared to dermal excisional wounds. *Journal of Periodontology* **74**, 242–246. doi:10.1902/jop.2003.74.2.242.

Novaes, A. B., Kon, S., Ruben, M. P. & Goldman, H. M. (1969) Visualization of the microvascularization of the healing periodontal wound. 3. Gingivectomy. *Journal of Periodontology* **40**, 359–371. doi:10.1902/jop.1969.40.6.359.

Novak, M. L. & Koh, T. J. (2013) Macrophage phenotypes during tissue repair. *Journal of Leukocyte Biology* **93**, 875–881. doi:10.1189/jlb.1012512.

Oliver, R. C., Loe, H. & Karring, T. (1968) Microscopic evaluation of the healing and revascularization of free gingival grafts. *Journal of Periodontal Research* **3**, 84–95.

Orsini, M., Orsini, G., Benlloch, D., Aranda, J. J., Lazaro, P. & Sanz, M. (2004) Esthetic and dimensional evaluation of free connective tissue grafts in prosthetically treated patients: a 1-year clinical study. *Journal of Periodontology* **75**, 470–477. doi:10.1902/jop.2004.75.3.470.

Ouhayoun, J. P., Goffaux, J. C., Sawaf, M. H., Shabana, A. H., Collin, C. & Forest, N. (1990) Changes in cytokeratin expression in gingiva during inflammation. *Journal of Periodontal Research* **25**, 283–292.

Ouhayoun, J. P., Sawaf, M. H., Gofflaux, J. C., Etienne, D. & Forest, N. (1988) Re-epithelialization of a palatal connective tissue graft transplanted in a non-keratinized alveolar mucosa: a histological and biochemical study in humans. *Journal of Periodontal Research* **23**, 127–133.

Padmakumar, V. C., Speer, K., Pal-Ghosh, S., Masiuk, K. E., Ryscavage, A., Dengler, S. L., Hwang, S., Edwards, J. C., Coppola, V., Tessarollo, L., Stepp, M. A. & Yuspa, S. H. (2012) Spontaneous skin erosions and reduced skin and corneal wound healing characterize CLIC4(NULL) mice. *American Journal of Pathology* **181**, 74–84. doi:10.1016/j.ajpath.2012.03.025.

Park, J. E. & Barbul, A. (2004) Understanding the role of immune regulation in wound healing. *American Journal of Surgery* **187**, 11S–16S. doi:10.1016/S0002-9610(03)00296-4.

Polimeni, G., Xiropaidis, A. V. & Wikesjö, U. M. (2000) (2006) Biology and principles of periodontal wound healing/regeneration. *Periodontology* **41**, 30–47. doi:10.1111/j.1600-0757.2006.00157.x.

Pontes, A. E., Ribeiro, F. S., Iezzi, G., Piattelli, A., Cirelli, J. A. & Marcantonio, E., Jr (2008) Biologic width changes around loaded implants inserted in different levels in relation to crestal bone: histometric evaluation in canine mandible. *Clinical Oral Implants Research* **19**, 483–490. doi:10.1111/j.1600-0501.2007.01506.x.

Potente, M., Gerhardt, H. & Carmeliet, P. (2011) Basic and therapeutic aspects of angiogenesis. *Cell* **146**, 873–887. doi:10.1016/j.cell.2011.08.039.

Pustiglioni, F. E., Kon, S., Novaes, A. B. & Ruben, M. P. (1975) Split thickness flap, apically replaced, with protected linear periosteal fenestration. *Journal of Periodontology* **46**, 742–744. doi:10.1902/jop.1975.46.12.742.

Quaranta, A., Piattelli, A., Scarano, A., Quaranta, M., Pompa, G. & Iezzi, G. (2008) Light-microscopic evaluation of the dimensions of peri-implant mucosa around immediately loaded and submerged titanium implants in monkeys. *Journal of Periodontology* **79**, 1697–1703. doi:10.1902/jop.2008.070631.

Rateitschak, K. H., Egli, U. & Fringeli, G. (1979) Recession: a 4-year longitudinal study after free gingival grafts. *Journal of Clinical Periodontology* **6**, 158–164.

Reheman, A., Gross, P., Yang, H., Chen, P., Allen, D., Leytin, V., Freedman, J. & Ni, H. (2005) Vitronectin stabilizes thrombi and vessel occlusion but plays a dual role in platelet aggregation. *Journal of Thrombosis and Haemostasis* **3**, 875–883. doi:10.1111/j.1538-7836.2005.01217.x.

Reilkoff, R. A., Bucala, R. & Herzog, E. L. (2011) Fibrocytes: emerging effector cells in chronic inflammation. *Nature Reviews Immunology* **11**, 427–435. doi:10.1038/nri2990.

Retzepi, M., Tonetti, M. & Donos, N. (2007a) Comparison of gingival blood flow during healing of simplified papilla preservation and modified Widman flap surgery: a clinical trial using laser Doppler flowmetry. *Journal of Clinical Periodontology* **34**, 903–911. doi:10.1111/j.1600-051X.2007.01119.x.

Retzepi, M., Tonetti, M. & Donos, N. (2007b) Gingival blood flow changes following periodontal access flap surgery using laser Doppler flowmetry. *Journal of Clinical Periodontology* **34**, 437–443. doi:10.1111/j.1600-051X.2007.01062.x.

Rimondini, L., Bruschi, G. B., Scipioni, A., Carrassi, A., Nicoli-Aldini, N., Giavaresi, G., Fini, M., Mortellaro, C. & Giardino, R. (2005) Tissue healing in implants immediately placed into postextraction sockets: a pilot study in a mini-pig model. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **100**, e43–e50. doi:10.1016/j.tripleo.2005.05.058.

Rossi, S., Tirri, T., Paldan, H., Kuntsi-Vaattovaara, H., Tulamo, R. & Narhi, T. (2008) Peri-implant tissue response to TiO2 surface modified implants. *Clinical Oral Implants Research* **19**, 348–355. doi:10.1111/j.1600-0501.2007.01478.x.

Santagata, M., Guariniello, L., Prisco, R. V., Tartaro, G. & D'Amato, S. (2012) The use of subepithelial connective tissue graft as a biological barrier: a Human Clinical and Histologic Case Report. *Journal of Oral Implantology*. doi:10.1563/AAID-JOI-D-11-00109.

Schapher, M., Wendler, O. & Groschl, M. (2011) Salivary cytokines in cell proliferation and cancer. *Clinica Chimica Acta* **412**, 1740–1748. doi:10.1016/j.cca.2011.06.026.

Schroeder, A., van der Zypen, E., Stich, H. & Sutter, F. (1981) The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. *Journal of Maxillofacial Surgery* **9**, 15–25.

Schupbach, P. & Glauser, R. (2007) The defense architecture of the human periimplant mucosa: a histological study. *Journal of Prosthetic Dentistry* **97**, S15–S25. doi:10.1016/S0022-3913(07)60004-3.

Schwarz, F., Ferrari, D., Herten, M., Mihatovic, I., Wieland, M., Sager, M. & Becker, J. (2007)

Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. *Journal of Periodontology* **78**, 2171–2184. doi:10.1902/jop.2007.070157.

Schwarz, F., Mihatovic, I., Becker, J., Bormann, K. H., Keeve, P. L. & Friedmann, A. (2013) Histological evaluation of different abutments in the posterior maxilla and mandible: an experimental study in humans. *Journal of Clinical Periodontology* **40**, 807–815. doi:10.1111/jcpe.12115.

Schwarz, F., Mihatovic, I., Ferrari, D., Wieland, M. & Becker, J. (2010) Influence of frequent clinical probing during the healing phase on healthy peri-implant soft tissue formed at different titanium implant surfaces: a histomorphometrical study in dogs. *Journal of Clinical Periodontology* **37**, 551–562. doi:10.1111/j.1600-051X.2010.01568.x.

Sciubba, J. J., Waterhouse, J. P. & Meyer, J. (1978) A fine structural comparison of the healing of incisional wounds of mucosa and skin. *Journal of Oral Pathology* **7**, 214–227.

Sculean, A., Nikolidakis, D. & Schwarz, F. (2008) Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials - biological foundation and preclinical evidence: a systematic review. *Journal of Clinical Periodontology* **35**, 106–116. doi:10.1111/j.1600-051X.2008.01263.x.

Serhan, C. N., Chiang, N. & Van Dyke, T. E. (2008) Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews Immunology* **8**, 349–361. doi:10.1038/nri2294.

Shaw, T. J. & Martin, P. (2009) Wound repair at a glance. *Journal of Cell Science* **122**, 3209–3213. doi:10.1242/jcs.031187.

Shi, C. & Pamer, E. G. (2011) Monocyte recruitment during infection and inflammation. *Nature Reviews Immunology* **11**, 762–774. doi:10.1038/nri3070.

Siar, C. H., Toh, C. G., Romanos, G., Swaminathan, D., Ong, A. H., Yaacob, H. & Nentwig, G. H. (2003) Peri-implant soft tissue integration of immediately loaded implants in the posterior macaque mandible: a histomorphometric study. *Journal of Periodontology* **74**, 571–578. doi:10.1902/jop.2003.74.5.571.

Singer, A. J. & Clark, R. A. (1999) Cutaneous wound healing. *New England Journal of Medicine* **341**, 738–746. doi:10.1056/NEJM199909023411006.

Sobral, L. M., Montan, P. F., Zecchin, K. G., Martelli-Junior, H., Vargas, P. A., Graner, E. & Coletta, R. D. (2011) Smad7 blocks transforming growth factor-beta1-induced gingival fibroblast-myofibroblast transition via inhibitory regulation of Smad2 and connective tissue growth factor. *Journal of Periodontology* **82**, 642–651. doi:10.1902/jop.2010.100510.

Staffileno, H., Jr & Levy, S. (1969) Histologic and clinical study of mucosal (gingival) transplants in dogs. *Journal of Periodontology* **40**, 311–319. doi:10.1902/jop.1969.40.6.311.

Staffileno, H., Levy, S. & Gargiulo, A. (1966) Histologic study of cellular mobilization and repair following a periosteal retention operation via split thickness mucogingival flap surgery. *Journal of Periodontology* **37**, 117–131. doi:10.1902/jop.1966.37.2.117.

Stahl, S. S., Weiner, J. M., Benjamin, S. & Yamada, L. (1971) Soft tissue healing following curettage and root planing. *Journal of Periodontology* **42**, 678–684. doi:10.1902/jop.1971.42.11.678.

Stavropoulos, A. & Wikesjo, U. M. (2012) Growth and differentiation factors for periodontal regeneration: a review on factors with clinical testing. *Journal of Periodontal Research* **47**, 545–553. doi:10.1111/j.1600-0765.2012.01478.x.

Sugarman, E. F. (1969) A clinical and histological study of the attachment of grafted tissue to bone and teeth. *Journal of Periodontology* **40**, 381–387. doi:10.1902/jop.1969.40.7.381.

Sullivan, H. C. & Atkins, J. H. (1968) Free autogenous gingival grafts. 3. Utilization of grafts in the treatment of gingival recession. *Periodontics* **6**, 152–160.

Susin, C. & Wikesjo, U. M. (2013) Regenerative periodontal therapy: 30 years of lessons learned and unlearned. *Periodontology* **62**, 232–242. doi:10.1111/prd.12003.

Szpaderska, A. M., Egozi, E. I., Gamelli, R. L. & DiPietro, L. A. (2003a) The effect of thrombocytopenia on dermal wound healing. *Journal of Investigative Dermatology* **120**, 1130–1137.

Szpaderska, A. M., Zuckerman, J. D. & DiPietro, L. A. (2003b) Differential injury responses in oral mucosal and cutaneous wounds. *Journal of Dental Research* **82**, 621–626.

Tete, S., Mastrangelo, F., Bianchi, A., Zizzari, V. & Scarano, A. (2009) Collagen fiber orientation around machined titanium and zirconia dental implant necks: an animal study. *International Journal of Oral and Maxillofacial Implants* **24**, 52–58.

Thoma, D. S., Hammerle, C. H., Cochran, D. L., Jones, A. A., Gorlach, C., Uebersax, L., Mathes, S., Graf-Hausner, U. & Jung, R. E. (2011) Soft tissue volume augmentation by the use of collagen-based matrices in the dog mandible – a histological analysis. *Journal of Clinical Periodontology* **38**, 1063–1070. doi:10.1111/j.1600-051X.2011.01786.x.

Thomsen, P. & Ericson, L. E. (1985) Light and transmission electron microscopy used to study the tissue morphology close to implants. *Biomaterials* **6**, 421–424.

Tomasek, J. J., Haaksma, C. J., Schwartz, R. J. & Howard, E. W. (2013) Whole animal knockout of smooth muscle alpha-actin does not alter excisional wound healing or the fibroblast-to-myofibroblast transition. *Wound Repair Regeneration* **21**, 166–176. doi:10.1111/wrr.12001.

Tomasi, C., Tessarolo, F., Caola, I., Wennström, J., Nollo, G. & Berglundh, T. (2013) Morphogenesis of peri-implant mucosa revisited: an experimental study in humans. *Clinical Oral Implants Research*. doi:10.1111/clr.12223.

Tonetti, M. S., Gerber, L. & Lang, N. P. (1994) Vascular adhesion molecules and initial development of inflammation in clinically healthy human keratinized mucosa around teeth and osseointegrated implants. *Journal of Periodontal Research* **29**, 386–392.

Vignoletti, F., de Sanctis, M., Berglundh, T., Abrahamsson, I. & Sanz, M. (2009) Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. III: soft tissue findings. *Journal of Clinical Periodontology* **36**, 1059–1066. doi:10.1111/j.1600-051X.2009.01489.x.

Waerhaug, J. (1978) Healing of the dento-epithelial junction following subgingival plaque control. I. As observed in human biopsy material. *Journal of Periodontology* **49**, 1–8. doi:10.1902/jop.1978.49.1.1.

Watzak, G., Zechner, W., Tangl, S., Vasak, C., Donath, K. & Watzek, G. (2006) Soft tissue around three different implant types after 1.5 years of functional loading without oral hygiene: a preliminary study in baboons. *Clinical Oral Implants Research* **17**, 229–236. doi:10.1111/j.1600-0501.2005.01217.x.

Weber, C. E., Li, N. Y., Wai, P. Y. & Kuo, P. C. (2012) Epithelial-mesenchymal transition, TGF-beta, and osteopontin in wound healing and tissue remodeling after injury. *Journal of Burn Care and Research* **33**, 311–318. doi:10.1097/BCR.0b013e318240541e.

Weber, H. P., Buser, D., Donath, K., Fiorellini, J. P., Doppalapudi, V., Paquette, D. W. & Williams, R. C. (1996) Comparison of healed tissues adjacent to submerged and non-submerged unloaded titanium dental implants. A histometric study in beagle dogs. *Clinical Oral Implants Research* **7**, 11–19.

Welander, M., Abrahamsson, I. & Berglundh, T. (2008) The mucosal barrier at implant abutments of different materials. *Clinical Oral Implants Research* **19**, 635–641. doi:10.1111/j.1600-0501.2008.01543.x.

Wennerberg, A., Sennerby, L., Kultje, C. & Lekholm, U. (2003) Some soft tissue characteristics at implant abutments with different surface topography. A study in humans. *Journal of Clinical Periodontology* **30**, 88–94.

Wennström, J. (1983) Regeneration of gingiva following surgical excision. A clinical study. *Journal of Clinical Periodontology* **10**, 287–297.

Wennström, J. & Lindhe, J. (1983) Role of attached gingiva for maintenance of periodontal health. Healing following excisional and grafting procedures in dogs. *Journal of Clinical Periodontology* **10**, 206–221.

Wennström, J., Lindhe, J. & Nyman, S. (1981) Role of keratinized gingiva for gingival health. Clinical and histologic study of normal and regenerated gingival tissue in dogs. *Journal of Clinical Periodontology* **8**, 311–328.

Wikesjö, U. M., Crigger, M., Nilveus, R. & Selvig, K. A. (1991) Early healing events at the dentin-connective tissue interface. Light and transmission electron microscopy observations. *Journal of Periodontology* **62**, 5–14. doi:10.1902/jop.1991.62.1.5.

Wikesjö, U. M. & Selvig, K. A. (1999) Periodontal wound healing and regeneration. *Periodontology* **2000** (19), 21–39.

Wilson, T. G., Jr, Carnio, J., Schenk, R. & Myers, G. (2008) Absence of histologic signs of chronic inflammation following closed subgingival scaling and root planing using the dental endoscope: human biopsies - a pilot study. *Journal of Periodontology* **79**, 2036–2041. doi:10.1902/jop.2008.080190.

Wong, J. W., Gallant-Behm, C., Wiebe, C., Mak, K., Hart, D. A., Larjava, H. & Hakkinen, L. (2009) Wound healing in oral mucosa results in reduced scar formation as compared with skin: evidence from the red Duroc pig model and humans. *Wound Repair Regeneration* **17**, 717–729. doi:10.1111/j.1524-475X.2009.00531.x.

Wynn, T. A. & Ramalingam, T. R. (2012) Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nature Medicine* **18**, 1028–1040. doi:10.1038/nm.2807.

Yamano, S., Al-Sowygh, Z. H., Gallucci, G. O., Wada, K., Weber, H. P. & Sukotjo, C. (2011) Early peri-implant tissue reactions on different titanium surface topographies. *Clinical Oral Implants Research* **22**, 815–819. doi:10.1111/j.1600-0501.2010.02059.x.

Yoneda, N., Yasue, A., Watanabe, T. & Tanaka, E. (2013) Down-regulation of Smad3 accelerates palatal wound repair. *Journal of Dental Research* **92**, 716–720. doi:10.1177/0022034513491575.

You, T. M., Choi, B. H., Li, J., Xuan, F., Jeong, S. M. & Jang, S. O. (2009) Morphogenesis of the peri-implant mucosa: a comparison between flap and flapless procedures in the canine mandible. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **107**, 66–70. doi:10.1016/j.tripleo.2008.05.045.

Zelles, T., Purushotham, K. R., Macauley, S. P., Oxford, G. E. & Humphreys-Beher, M. G. (1995) Saliva and growth factors: the fountain of youth resides in us all. *Journal of Dental Research* **74**, 1826–1832.

Zhang, C., Tan, C. K., McFarlane, C., Sharma, M., Tan, N. S. & Kambadur, R. (2012) Myostatin-null mice exhibit delayed skin wound healing through the blockade of transforming growth factor-beta signaling by decorin. *American Journal of Physiology. Cell Physiology* **302**, C1213–C1225. doi:10.1152/ajpcell.0.0179.2011.

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Clinical relevance

Passive flap adaptation and suturing together with infection control play a critical role for optimizing oral wound healing, and maintaining integrity of the maturing wound. Connective tissue grafts with or without epithelium are valuable options for increasing the width of keratinized mucosa. The long-term biological and clinical consequences, the conditions that favour and the measures that reduce the formation of a faster growing and eventually longer peri-implant junctional epithelium on dental implants require further investigations.