Histological Changes in Nasolabial Cutaneous Flaps Employed in Intraoral Reconstruction

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Abstract

Introduction Nasolabial cutaneous flaps have been routinely used to reconstruct intraoral defects. The purpose of this study was to study histological changes that may occur in the skin flap as a result of its exposure to a new environment.

Patients and Methods Thirteen patients took part in this study. Fusiform tissue specimens were obtained from the intraoral cutaneous portion of the flap. Biopsy specimens were also taken from the skin of nasolabial region and from the buccal mucosa to serve as control. Thickness of stratum corneum, degree of inflammatory infiltration and number of skin appendages were evaluated. Periodic-acid Schiff (PAS) staining was also performed to identify the presence of hyphae.

Results The characteristic features of the skin are almost always maintained, although the thickness of stratum corneum and the number of skin appendages are often significantly reduced. In two patients the inflammatory infiltration was intense and accompanied by elimination of skin appendages and stratum corneum. These flaps tend to mimic mucosa macroscopically. In four patients fibrosis was histologically demonstrated. These flaps resembled atrophic skin macroscopically. Hyphae could not be identified with PAS staining in this study. Koilocytes, which are indicative of HPV infection, were identified in two flap specimens.

Conclusions Intraorally placed nasolabial flaps may undergo a variety of histological and macroscopical changes. In the majority of cases the intraoral cutaneous flap maintains skin features, except if it becomes heavily infiltrated with inflammatory cells. Inflammation occurs for unknown reasons and results in a mucosa-like microscopic and macroscopic appearance of the intraorally placed flap.

Keywords Cutaneous flaps · Mucosalization · Koilocytes · Intraoral reconstruction

Introduction

Reconstruction of intraoral tumor ablative defects with free tissue flap transfer has numerous functional and aesthetic advantages. However, local and regional pedicled flaps may also be useful, particularly when the age and medical status of the patient do not permit a lengthy surgical procedure or when the patient is not willing to undergo a free flap reconstruction. One of these flaps is the nasolabial flap, which can be used either as a pedicled or as an islanded flap.

Oral mucosa epithelium differs from skin epithelium in many respects. These differences represent exposure to different environment. However, skin flaps or skin grafts are very often transferred intraorally to reconstruct tumor ablative defects. It is still unknown whether the intraorally transferred skin undergoes adaptive alterations to serve its new functional role. Some studies suggest that intraorally placed skin flaps tend to obtain with time a mucosa-like clinical and histological appearance during a process called “mucosalization” [1–3], whereas other studies support that skin characteristics are maintained even after many years [4–6]. Some authors suggest that the process of

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“mucosalization” is more a reactive than an adaptive process, which may be reversible when the responsible factor is removed [7, 8].

This study aims to assess the long-term histologic skin changes that may occur on intraorally placed nasolabial skin flaps, to look for evidence of chronic fungal infection and to examine the relationship between macroscopic and microscopic changes. The hypothesis that adaptive mucosa-like changes occur on intraorally placed skin flaps will also be tested and discussed.

Patients and Methods

The protocol described here has been approved by our Department’s Ethics Committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients enrolled in this study signed an informed consent form. All patients were treated in the Department of Oral and Maxillofacial Surgery of Papanikolaou Hospital or in the Department of Oral and Maxillofacial Surgery of Anticancer Theageneio Hospital and had undergone excision of oral squamous cell carcinomas and reconstruction with pedicled or islanded nasolabial flaps. Over the period analyzed, 49 patients underwent reconstruction of oral cavity defects with nasolabial flaps following tumor ablative procedures. Among 49 patients, 13 were selected based on the following eligibility criteria.

Eligibility Criteria

1. Every patient enrolled in this study should sign an informed consent form.
2. The patient should not suffer from local or regional recurrence at the time of the study.
3. At least 1 year after the end of cancer treatment ie surgery or surgery plus radiotherapy should have passed.
4. No postoperative complications should have occurred following reconstruction of the defect with nasolabial flap.

The clinical appearance of the flap was recorded as “skin-like” when the flap resembled skin macroscopically, “mucosa-like” when the macroscopic appearance of the flap resembled mucosa and “atrophic skin-like” when the flap had a whitish appearance that resembled atrophic skin. The clinical appearance was separately judged by two clinicians to minimize bias.

Fusiform biopsies were performed under local anesthesia from the following sites: (a) center of the intraorally placed skin flap, (b) buccal mucosa c. skin of the nasolabial region. Nasolabial skin and buccal mucosa served as

control. Tissue specimens were submitted for routine haematoxylin and eosin staining (H & E) and periodic acid Schiff staining (PAS).

The following parameters were evaluated:

1. Presence and thickness of stratum corneum under the 40× objective lens. The maximum thickness was calculated both in the flap and the control skin specimen with the aid of a microruler graduated in 0.1 mm units. Decrease of maximum stratum corneum thickness of the flap greater than 30 % compared to control was considered significant. Maximum thickness does not represent the whole specimen. However, it can be used as a reference point.
2. Degree of inflammation both in the dermis and the epidermis under the 40× objective lens. The degree of inflammation was assessed based on the following scale: grade 0 no inflammation, grade 1 mild inflammation (scattered inflammatory cells or small aggregates), grade 2 moderate inflammation (large aggregates of inflammatory cells), grade 3 diffuse inflammatory reaction.
3. Presence and density of skin appendages (hair follicles, sebaceous glands) by square mm. Any decrease of skin appendages in the flap specimen that exceeded 30 % compared to the skin specimen was considered significant.
4. Presence of fibrosis, mucomyxoid degeneration, basal cell pigmentation and koilocytes were recorded as positive (+) or negative (–).
5. PAS staining for evaluating presence of *Candida albicans* infection.

Results

Thirteen patients (9 males and 4 females) were enrolled in this study. The age of the patients ranged from 42 to 70 years (mean 63). Eleven patients had a history of tobacco use and alcohol consumption. All patients underwent excision of stages I and II squamous cell carcinomas of the following intraoral sites: floor of the mouth (9 patients), buccal mucosa (2 patients), tongue (1 patient), lower alveolar process (1 patient). The intraoral defect was reconstructed with an islanded unilateral nasolabial flap (one-stage procedure) in 11 patients and with pedicled bilateral nasolabial flaps (two-stage procedure) in 2 patients. Seven patients underwent bilateral neck dissection and six patients underwent unilateral neck dissection simultaneously with excision and reconstruction of the primary site. Eight patients were submitted to adjuvant radiotherapy dose ranging from 5400 to 6500 cGy. The rest of the patients did not receive adjuvant therapy. Biopsies were obtained 14–144 months (mean 60) after the day of operation in patients who did not receive radiotherapy and

Table 1 Macroscopic and microscopic features of the flap specimens in relation to demographic and operative data of patients

Sex	Age	SMOK	R/T	Follow-up	Primary site	MACR	SCT	INFL	NSA	F	D	M	K
M	70	+	–	96	FOM	Mucosa	0	++	0	–	+	–	+(+)
M	55	+	+	52	FOM	Skin	→	–	↓	–	+	+	–(–)
M	66	+	–	55	FOM	Atrophic	↓	–	↓	++	–	–	+(+)
M	59	+	+	60	FOM	Atrophic	↓	–	0	++	–	–	–(+)
F	65	+	+	124	Buccal	Skin	→	–	→	–	+	+	–(–)
F	70	–	–	144	Buccal	Skin	→	+	→	–	+	–	–(–)
F	60	–	–	36	LAP	Skin	→	++	→	–	+	–	–(–)
M	75	+	+	45	Tongue	Skin	↓	–	↓	–	–	–	–(–)
M	63	+	+	58	FOM	Skin	↓	–	↓	–	–	–	–(–)
M	68	+	+	26	FOM + T	Mucosa	0	+++	0	–	–	–	–(–)
M	42	+	+	26	FOM	Atrophic	→	–	→	+	–	+	–(–)
F	61	+	+	14	FOM	Skin	↓	+	↓	–	+	–	–(–)
M	65	+	–	46	FOM	Skin	→	+	→	+	–	–	–(–)

Follow-up is expressed in months

The column “age” refers to the age of the patients at the time of oncologic surgery and the column “follow up” refers to the months that have passed from that day. The maximum thickness of stratum corneum of the flap specimen is compared to the thickness of the same layer of the control skin specimen. The symbol right arrow represents no significant change in thickness and the symbol down arrow represents decrease in thickness greater than 30 %. The number 0 represents elimination of stratum corneum. The column «inflammation» refers to the inflammatory infiltration that is observed in the flap specimen. The number of skin appendages of the flap specimen is compared to the number of skin appendages of the control skin specimen. The symbol down arrow represents decrease in number of skin appendages that exceeds 30 %, whereas the symbol right arrow represents no significant change. The number 0 represents elimination of skin appendages. Presence of fibrosis, mucomyxoid degeneration and melanosis in the flap specimen is recorded with + and absence with –. Presence of koilocytes in the flap specimen is recorded with + and absence with –. In parenthesis, presence or absence of koilocytes in the control mucosa specimen is noted

M Male, *F* female, *SMOK* smoking, *R/T* radiotherapy, *FOM* floor of mouth, *T* tongue, *LAP* lower alveolar process, *MACR* macroscopically, *SCT* stratum corneum thickness, *NSA* number of skin appendages, *INFL* inflammation, *F* fibrosis, *D* degeneration muco-myxoid, *M* melanosis, *K* presence of koilocytes

after the end of radiotherapy in patients who received adjuvant radiotherapy.

As 3 biopsies were performed for each patient, there were 39 histological specimens for the entire study. Results are presented in Table 1 in association with various other parameters namely age, smoking, radiotherapy, location of the primary site and time of follow-up.

In the majority of cases (11/13) stratum corneum was present in the flap specimen, although in five specimens the thickness of this layer was markedly reduced. No or mild inflammation was detected in the vast majority of these specimens (10/11). In every specimen that presented a reduced thickness of stratum corneum, a reduced number of skin appendages by square mm in comparison to control specimen was also observed (5/5) (Fig. 1). Four of these five patients had received adjuvant radiotherapy in the past.

Microscopical skin features were maintained in the majority of patients many years following treatment (maximum follow up in this study 144 months). Except from the histological features, these flaps also maintained color and texture of normal skin (Fig. 2). In four patients the skin had a whitish, fibrotic appearance that correlated well with presence of fibrosis and absence of inflammatory infiltration in the histologic examination of the flap

Fig. 1 Section of the flap 46 months following cancer treatment (surgery no radiotherapy). The horny layer is present and there is mild dermis inflammation (hematoxylin-eosin, ×100)

specimen. These fibrotic changes were seen both in irradiated and in non-irradiated flaps (Fig. 3). In three specimens that maintained skin histological features, pigmentation of basal layer that did not exist in control skin specimen was detected (Fig. 4).

Fig. 2 **a** Section of the flap 58 months following cancer treatment (surgery plus radiotherapy). In this case the thickness of horny layer is decreased and there are fewer skin appendages (only one sebaceous

gland can be recognized) (hematoxylin-eosin, $\times 40$). **b** Macroscopic appearance of the flap that maintains color and texture of normal skin

Fig. 3 Clinical appearance of flap that mimics atrophic, fibrotic skin. Histologically reduced thickness of keratin layer and reduced number of skin appendages were observed together with absence of inflammation and dermis fibrosis

The stratum corneum disappeared in two flap specimens. In both cases there was a heavy inflammatory infiltrate both in the epithelium and the dermis of the flap. Macroscopically the flap had a pinkish mucosa-like appearance (Fig. 5). These two patients were both older than 60 years. The primary tumor was located on the floor of the mouth in both cases. However, only one of them had received adjuvant radiotherapy and only one of them had a long period of follow up (96 vs 26 months).

Koilocytes were detected in two flap specimens and in the control mucosa specimens (Fig. 5). Koilocytes are

Fig. 4 Section of the flap 52 months after cancer treatment (surgery plus radiotherapy). **a** The horny layer is easily identified and basal layer pigmentation is also observed (hematoxylin-eosin, $\times 400$). **b** Clinical appearance of the same flap that maintains color and texture of normal skin

Fig. 5 Section of the flap 96 months after cancer treatment (surgery no radiotherapy). **a** Heavy inflammatory dermis infiltration is demonstrated (hematoxylin-eosin $\times 100$). **b** Absence of horny layer, presence of koilocytes and intense inflammatory infiltration are observed ($\times 400$). **c** Macroscopic appearance of the flap that tends to obtain a mucosa-like appearance

often indicative of HPV infection, although in order to verify this assumption, polymerase chain reaction (PCR) methods are needed. PAS staining was negative in all specimens.

Discussion

Many authors have attempted to describe the histologic changes that occur in the intraorally placed skin. Most studies have been performed on free radial forearm flaps [3, 7, 9–11], some studies have been performed on pectoralis major pedicled flaps [1, 2] and some on skin grafts [5, 12]. The final histologic appearance of the skin flap depends on the degree of inflammation, although the factors that induce this inflammation are not yet identified. Flaps with no or very mild inflammatory infiltrate usually maintain clinical and histological skin features [4, 7, 10]. However, some histologic changes are still present such as reduced thickness of horny layer [7, 9, 10], orthokeratosis [11], mild acanthosis [11], normal or reduced number of skin appendages [2, 7, 9, 10], decrease of secretory activity of sweat glands [9], basal keratinocyte pigmentation [9, 10] and elastosis with thicker, tortuous and randomly oriented elastic fibers [8, 10]. In this study, maintenance of skin histological features in flap specimens with no or mild inflammation was confirmed. However, a number of flap specimens with mild or no inflammation demonstrated reduced thickness of stratum corneum and reduced number of skin appendages. Other histological changes observed in these specimens were fibrosis, basal keratinocyte pigmentation and muco-myxoid degeneration. Flaps that histologically presented fibrosis had the clinical appearance of whitish, atrophic skin. Although fibrotic changes could be attributed to radiotherapy, half of these patients did not receive radiotherapy. Fibrosis could also be attributed to other factors, idiosyncratic or environmental, like smoking or eating hard food.

On the other hand, flaps with intense inflammatory infiltrate tend to resemble to oral mucosa. Histologically, epidermal hyperplasia is constantly present with thickness reduction or even disappearance of stratum corneum. Parakeratosis, loss of keratohyalin granules, decreased thickness of stratum granulosum, elongation of epidermal rete ridges, highly undulating dermal-epidermal surface and inflammatory infiltration of both dermis and epidermis may also exist [4, 8, 11]. However, it is still unknown which factor initiates this inflammatory process. Radiotherapy affects oral mucosa and skin but no correlation has been found between irradiated and non-irradiated patients regarding inflammatory infiltration [4, 9]. One study that was performed only in non-irradiated patients found only mild inflammation and maintenance of skin architecture of

skin flaps [9]. Badran et al. [8] state that after radiotherapy the gross appearance of the skin flap changes to a more mucosa-like appearance but these changes are reversible in the majority of cases. In this study two among three patients who presented with significant inflammation had not received radiotherapy. Tobacco smoking, poor oral hygiene, chewing trauma and alcohol consumption may also be factors that promote inflammatory changes.

Fungal infection of the skin flap is the only factor that has been correlated to epithelial changes and inflammatory infiltration. Skin flaps are indeed transferred from a dry environment to the intraoral wet environment. This continuous saliva emersion may predispose the flap to fungal infection. Many authors have proved the presence of *Candida* hyphae in the epidermis of intraorally placed skin flaps using periodic acid Schiff (PAS) staining and support that chronic candidiasis may promote epithelial changes, which in fact represent an attempt to eliminate fungi [10, 11]. The majority of specimens in which fungal hyphae were isolated, were obtained from leukoplacic or erythematous lesions that appeared on the skin flap [9, 10]. We and other authors were unable to prove the presence of fungi in any of our cases and this may be due to the fact that biopsies were performed from clinically normal skin areas [2, 4, 6]. However, candidiasis may be present even when hyphae are not histologically detectable [13]. It is possible that the presence of parakeratosis, increased epithelial proliferation and intense dermis inflammation are indications of *Candida* infection even when the hyphae themselves cannot be isolated [4].

The importance of *Candida* infection is not adequately delineated. Nearly half of dysplastic oral lesions have been related to *Candida* infection, whereas it has been observed that *Candida albicans* is detected more frequently in the biofilm of oral squamous cell carcinomas than in control areas [13]. Although an aetiological relationship between fungal infection and squamous cell carcinoma (SCC) has not been proved, these findings suggest that fungal infection may play a role in promoting epithelial dysplastic changes. Woolgar and Triantafyllou report a rather high percentage of dysplastic changes in skin flaps used intraorally. They report focal dysplastic changes in 25 % of specimens, that were obtained from abnormal areas of intraoral skin flaps [10]. In another study that focused on clinical apparent lesions that existed on the intraorally placed skin, the authors report that dysplasia was detected in 4 out of 10 specimens and that dysplastic changes extended to pilosebaceous units and eccrine ducts [9]. Interestingly, dysplastic changes were not located to the center of the flap but they were always contiguous to mucosal dysplastic lesions. This finding suggests that dysplastic changes detected on skin flaps are probably the result of exposure to a genetically altered intraoral

environment. Genetically altered mucosal keratinocytes with a growth advantage may creep to the adjacent skin epithelium or a horizontal gene transportation to basal epithelial cells of the skin may take place that subsequently transforms them to cancer stem cells [14]. However, true malignant transformation rarely occurs on the skin flap despite the high proliferative activity that is often depicted, probably because the underlying connective tissue of the skin inhibits tumor development in contrast to the underlying mucosal stroma that promotes abnormal epithelial differentiation through recruitment of myofibroblasts [15].

When the skin sloughs or when it is surgically deepithelialized, healing occurs without keratin cover and the final histologic appearance of this part of the flap is basically identical to the histologic appearance of normal mucosa without inflammatory infiltration [6]. Therefore removal of epithelial portion or use of a myofascial flap would seem a better solution for intraoral reconstruction. However, muscle flaps that are transferred into the mouth lose 50–75 % of their volume possibly due to deinnervation or lack of epithelial protection and become hard due to cicatricial transformation of muscle fibers [16]. Moreover, healing occurs by second intention and may delay initiation of irradiation. For these reasons, flaps with skin component are still the most popular flaps for intraoral reconstruction despite the fact that a different epithelium is transferred into the mouth.

Immunohistochemical markers have also been studied in order to answer to the question whether skin epithelium is genetically altered to a more mucosa-like epithelium or these clinical and histological changes that sometimes make the skin flap to resemble mucosa reflect only an inflammatory and not an adaptive process. Rubino et al. [9] studied CK (cytokeratin) expression and found that skin flaps express the same pattern of CK found to be expressed by normal skin and conclude that skin preserves its characteristics. CK expression pattern was also studied by Katou et al. The authors found alterations of CK expression in skin flaps that were infected by *Candida albicans*. These changes suggest that skin epithelium with fungal infection becomes more immature and less differentiated. Moreover, in these specimens there was a 10-fold increase in the expression of Ki-67, which is a marker of mitotic activity, and a weak expression of desmosomal proteins. These changes reflect the proliferative and desquamative epithelial activity that aims to fungal elimination [17]. However, desquamative activity may increase even when no fungi are identified, because the wet environment of the oral cavity induces increased activity of proteolytic enzymes that digest intercellular, desmosomal proteins. From that point of view desquamative activity may reflect an adaptive process to enhance functionality of the reconstructed area. Another study found that SPR3 was expressed in

transplanted skin flaps that had lost stratum corneum. This marker is expressed only by mucosa, either keratinized or non-keratinized, and never by skin not even in inflammatory skin diseases. This fact proves that oral environment induces some epithelial changes to the transplanted skin, which are not only reactive to an inflammatory agent such as *Candida* infection but are also adaptive [17]. In this study no fungi were detected.

Many authors have also studied not only the intensity but also the consistency of inflammatory infiltrate [4, 11]. Cell-mediated immunity was found to dominate. Immunoglobulin-containing cells were found only in specimens with diffuse inflammatory infiltration. Predominance of lymphocytes is indicative of a chronic inflammatory process that leads—through secretion of cytokins—to an increased proliferative epithelial activity and therefore increased epithelial thickness. This process may be reactive to the cause of inflammation or may be adaptive because epithelial thickening and increased turnover provides increased protection against the oral flora.

As it has been mentioned above, the epidermis undergoes mild or gross histologic alterations, when the skin is transferred intraorally. The dermis on the other hand remains largely unchanged and is never replaced by lamina propria. There is always an abrupt junctional zone where dermal appendages are abruptly replaced by minor salivary glands. Skin appendages however may be reduced or even eliminated [2]. Badran et al. [8] found no skin appendages in flaps with a mucosa-like appearance and no hair follicles in macroscopically unchanged irradiated flaps. Other studies, including the current, found no correlation between reduced number or elimination of skin appendages and radiotherapy and confirm the presence of hair follicles even in irradiated patients [2, 7]. This study confirms that the reduction of skin appendages is attributed not only to irradiation but also to inflammatory infiltration of the dermis and may occur both in irradiated and in non-irradiated flaps. Koilocytes were identified in three flap specimens, which demonstrated reduced thickness or elimination of stratum corneum and skin appendages. Koilocytes are usually indicative of HPV infection that may also contribute to mucosa-like changes or to dysplastic changes, although dysplasia was not observed in this study.

Conclusions

There are no constant histological changes that occur on intraorally placed skin flaps. Heavy inflammatory infiltrate of the epithelium is associated with loss of stratum corneum and skin appendages. Inflammation was not related to radiotherapy and no fungi were detected. Inflammation was occasionally associated with koilocytes.

A desquamative activity with reduced thickness of stratum corneum, reduced number of skin appendages and occasionally fibrotic changes has also been detected in flap specimens with no or mild inflammatory infiltrate, especially when the defect was located on the floor of the mouth. This finding suggests that adaptive changes from constant salivary emersion and mastication trauma may occur.

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Compliance with Ethical Standards

Conflict of Interest Lazaridou Maria, Dimitrakopoulos Ioannis, Vaxtsevanos Konstantinos, Iordanidis Fotis, Antoniadis Konstantinos: declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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