Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva

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Abstract. Fourteen sites were chosen in eight subjects where pre-operatively less than 2 mm of keratinised gingiva was present and associated with pocketing of less than 1.0 mm. Three methods were used to provide gingival connective tissue for grafting which facilitated primary closure at the donor site. The pre-operative width of keratinised gingiva was measured by means of a Williams probe from the gingival margin to the muco-gingival junction to the nearest 0.5 mm. This was repeated immediately post-operatively, and at 1, 2, 5, 10, and 12 weeks, and at 6 months. The results showed that a statistically significant increase in width of keratinised attached gingiva could be achieved with this method and the resultant tissue was histologically normal.

It is generally accepted that the presence of an adequate zone of keratinised attached gingiva is important for the maintenance of a healthy dento-gingival junction. The characteristics of attached gingiva enable it to withstand the frictional forces encountered during mastication, and to dissipate the pull of the circum-alveolar musculature. Lang & Löe (1971) have demonstrated that a crevicular exudate can always be detected from a gingival sulcus with less than 2 mm of attached gingiva, and this amount would appear to be essential for gingival health in young adults.

An inadequate width of attached gingiva is commonly found together with a shallow vestibular depth, and various methods have been employed and modified for increasing the width of keratinised gingiva and/or for deepening the vestibular sulcus. (Ariaudo & Tyrell 1957, Bohannan 1962a and b, Robinson & Agnew 1963, Corn 1962, Freedman & Levine 1964, Staffileno et al. 1966).

In recent years the use of free gingival autografts has become popular (Nabers 1966a and b, Haggerty 1966, Becker 1967, Bressman & Chasens 1968, Sullivan & Atkins 1969, Snyder 1969, Hawley & Staffileno 1970) since they can be relied upon to produce more predictable results (Karring et al. 1971).

Free gingival grafts may be defined as "partial thickness" when they consist of epithelium and varying amounts of lamina propria, or "full thickness" when all the lamina propria but no glandular tissue or submucosa is included. Sullivan & Atkins (1968) have outlined the principles of successful grafting; they state that for the first few days the graft vitality is maintained by a plasmatic circulation; and only after the
third day does a normal vascular circulation become re-established.

Their studies also showed that the epithelial cells of a thick graft tend to desquamate, but that this is not important to the final result, as the surface will re-epithelialise from cells deeper in the epithelial ridges.

Oliver et al. (1968) have confirmed these findings in a series of grafts in seven Rhesus monkeys. The histological findings showed that virtually all the epithelium degenerated and desquamated by the fifth day, though some cells deep in the epithelial ridges may have persisted and may have contributed to the re-epithelialisation of the graft. More important however, was the contribution of proliferating epithelial cells from the adjacent tissue, and by the eleventh day epithelial cells covered the entire surface but showed no keratinisation until the twenty-eighth day.

Lange & Bernimoulin (1974) have suggested, on the basis of cytological studies, that the basal epithelial cells of free gingival grafts probably do not degenerate and contribute to the re-epithelialisation of the graft.

In their study, Karring et al. (1971) attempted to determine whether it is some inherent factor in the tissues or whether it is functional adaptation that is responsible for the maintenance of tissue specificity. They performed a series of heterotropic gingival and alveolar mucosal transplants in eight adult monkeys (Cercopithecus). The findings that the transplanted tissues always retained their original structure, even after 1 year, gave no support to the theory of functional adaptation (Ivancie 1957, Bradley et al. 1959, Pfeifer 1963).

Most studies on epithelial mesenchymal interactions favour the concept that histodifferentiation occurs as a response to morphogenetic stimuli from the underlying connective tissue, and that the epithelial cells do not possess any predetermined regional specificity (Cairns & Saunders 1954, McLoughlin 1961, Rawles 1963, Billingham & Silvers 1968, Dodson 1967, Plagmann et al. 1974). It is, however, also possible that histodifferentiation is controlled by mutual interaction of epithelium and the underlying connective tissue (Wessels 1962, Cohen 1965).

The present study was undertaken to determine the predictability of using free gingival connective tissue grafts without epithelium, in an attempt to increase the width of keratinised gingiva.

**Materials and Method**

Fourteen areas were chosen for treatment in eight subjects. The criterion for inclusion in the study was a width of keratinised gingiva of less than 2.0 mm, associated with pocketing of no more than 1.0 mm on the buccal aspect. If more than one tooth was included in an area, then the results for all the teeth were combined, and the mean taken as representative for the total area. The age-range of the subjects was 27–55 years, with a mean age of 39 years. Prior to surgery, scaling and polishing were completed, and oral hygiene instruction was given to ensure good post-operative control.

The width of attached gingiva, measured from the free gingival margin to the mucogingival junction, was recorded pre- and post-operatively (by means of a Williams probe) to the nearest 0.5 mm; this measurement was repeated at 1, 2, 5, 10, 12 weeks, and at 6 months. The recipient site was prepared by making two vertical incisions into the alveolar mucosa, at the buccal line angles of the teeth adjacent to the area to be treated. By sharp dissection, a periosteal bed, which was free of all muscle attachments, and was of sufficient size to take the graft was left covering the underlying
bone. Haemostasis was achieved by applying pressure with a piece of cotton gauze soaked in warm saline. The remaining partial thickness flap was displaced apically, but was not sutured.

**Donor Site**

**Method 1:** The palatal region opposite the molar teeth served as the donor site in six cases. Using a number 15 Swann Morton blade, a primary partial thickness gingival pedicle flap was raised with its base wider than its free end. This primary flap was then displaced by means of a retractor, and a portion of the underlying mucosa (approximately the size of the recipient bed), was dissected out with a fresh number 15 blade. Care was taken not to go too deep in order to avoid the inclusion of any palatal mucosal glands (Fig. 1). If this did occur the graft was trimmed before being placed on the recipient site. The primary flap was replaced and pressure applied for 2 min to prevent any void under the flap. The flap was sutured in the conventional manner with interrupted sutures; these remained in place for 1 week. No dressing was applied.

**Method 2:** The undersurface of a full thickness flap was used as the donor site in six cases. A primary incision was made to the crest of the bone along the axis of the teeth close to the gingival margin. A full thickness flap was raised, and a second incision was made to thin this flap internally. The resultant portion of tissue was trimmed and used as the graft tissue. The palatal flap was then replaced, sutured and left for 1 week (Fig. 2); a dressing was provided if necessary.

**Method 3:** The undersurface of a saddle region between standing teeth was dissected out and used for grafting in two cases. (Fig. 3). In all cases the donor site was left without a dressing as primary closure could always be achieved.

The graft was placed on the prepared recipient bed and anchored to the adjacent tissues with 6.0 atraumatic silk sutures (Ethicon Mersutures®). The principles of
Fig. 2. Diagrammatic illustration of Method 2. Graft material is obtained by the thinning of a full thickness palatal flap. The first incision is made to the crest of the bone and when the flap is reflected a second incision thins the flap internally and provides the connective tissue for grafting.

Fig. 2. Schematische Darstellung der Methode 2. Das Transplantat wird durch das Verdünnen eines palatalen Schleinhautperiostlappens gewonnen. Die erste Inzision erfolgt bis auf den Alveolarknochenkamm. Am zurückgeschobenen Lappen wird durch eine 2. Inzision auf der Lappeninnenseite das Bindegewebe-Transplantat erhalten. Lappenreposition.

Fig. 2. Schéma du procédé chirurgical de la méthode 2. Le matériel à greffer est obtenu en amincissant le lambeau palatin entier. La première incision s’étend jusqu’à la crête osseuse, la seconde crée un lambeau au sein duquel on prélève la greffe conjonctive.

Fig. 3. Diagrammatic illustration of Method 3. A partial thickness flap is raised at the saddle area and graft material is taken from the underlying connective tissue. The flap is then sutured back.

Fig. 3. Schematische Darstellung der Methode 3. Ein Spaltlappen wird im Gebiet des Kieferkammsattels gebildet. Das Transplantat wird dem darunter liegenden Bindegewebe entnommen. Der Spaltlappen wird darauf mit Nähten reponiert.

Fig. 3. Schéma du procédé chirurgical de la méthode 3. Même principe que dans la méthode 1, mais au niveau d’une zone édentée.
Free gingival grafts, as outlined by Sullivan & Atkins (1968), were employed, and the displaced flap was checked to ensure it did not interfere with the graft. A non-eugenol dressing (Coepak®) was applied to the area. The dressing and sutures were removed after 1 week; then the teeth were polished and a dressing was replaced for a further week.

A biopsy specimen was taken of the muco-gingival junction from one area at 6 months; it was fixed in 10% formal saline for 8 h, and processed to wax over a period of 24 h. Five micron sections were cut and stained with haematoxylin and eosin, and Verhoeff's van Gieson stain for elastic fibres.
Results
In all cases the graft area and the donor site healed uneventfully when both methods 2 and 3 were employed (Figs. 4a–d and Figs. 5a–d). In all six cases where method 1 was used the primary flaps degenerated to a varying degree (Figs. 6a and 6b), and were associated with marked discomfort for 7–12 d, as no dressings were applied to protect this region during healing.

Table 1 shows the width of attached gingiva recorded at various intervals during the study. The results were stable at 12 weeks and remained so over a further period of 3 months. At the end of the 6-month period the pocket depths were still no more than 1.0 mm, and when a paired t-test was ap-
### Table 1. Width of keratinised attached gingiva in millimeters

Tabellen 1. Breite der angewachsenen, verhornten Gingiva, mm

Tableau 1. Largeur de la gencive adhérente mm

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**Fig. 6a.** Donor area (Method 1); the flap is sutured back (mirror view).

**Fig. 6a.** Spendergegend bei Methode 1. Der Lappen ist mit Nähten reponiert.

**Fig. 6a.** L'endroit donneur (méthode 1) montrant le lambeau muqueux suturé.

**Fig. 6b.** 1 week post-operatively, showing degeneration of the flap (mirror view).

**Fig. 6b.** 1 Woche nach der Operation. Degeneration des Lappens (Bild im Spiegel).

**Fig. 6b.** 1 semaine après l'opération le lambeau a dégénéré.
plied to the results it showed that a significant increase of attached gingiva had been achieved ($P < 0.001$). The width of graft tissue was recorded immediately prior to the application of the dressing. The mean width of the graft being $5.54 \pm 1.29$ mm (S.D.) with a range of 4.0–9.0 mm. At 6 months the mean width of keratinised gingiva was $4.58 \pm 1.04$ mm, with a range of 3.5–5.0 mm. Therefore, at 6 months the mean contraction was 28% with a range of 0 to 50 per cent.

At 1 week the surface of the graft appeared red and shiny, and was covered in parts with a greyish slough (Fig. 5c). The base of the defect between the graft and the displaced flap was filled with a fibrin clot. At 2 weeks the surface was completely epithelialised; by 4 weeks keratinisation was evident as the graft surface became progressively paler; and by 6 weeks it had blended in normally with the surrounding tissues.

**Histology**

The histological sections stained with haematoxylin and eosin showed a clear demarcation between the keratinised and non-keratinised epithelium at the newly-created muco-gingival junction (Fig. 7). The keratinised epithelium showed well-developed rete pegs in contrast to the non-keratinised alveolar mucosa epithelium. The collagen bundles in the connective tissue were predominantly oriented at right angles to the surface; and the sections stained with van Gieson (Fig. 8) showed that the presence of elastic fibres corresponded to the
region of non-keratinised mucosa, as shown by Lozden & Squier (1969).

Discussion

The results show that a significant increase in attached gingiva can be achieved by grafting gingival connective tissue alone. Clinically, the present study tends to confirm the concept that it is the information in the connective tissue that ultimately determines the character of the surface epithelium. This then raises the question as to whether the thickness of connective tissue is important in achieving this result. The present study can shed no light on this problem. It is a clinical observation, however, that the cases that were associated with the most "creep back" of the mucogingival junction were those where only a very thin graft was used. This may also explain the histological findings of Stambaugh & Gordon (1973) that only 75 per cent of palatal connective tissue grafts produced a keratinised surface; and the cytological investigation of Bernimoulin & Lange (1974), who found only two-thirds of grafts to be covered with keratinised epithelium.

The method has certain advantages over the use of free gingival grafts, providing an adequate donor site exists. In this study, three donor sites were utilised according to availability, and in most cases primary closure could be achieved, which obviated the
need for a periodontal dressing. In those cases where palatal pocketing was present, pocket elimination was performed, and the tissue normally discarded after thinning of the palatal flap was used as grafting material.

The results suggest that the method can be relied upon to produce a predictable increase in the width of keratinised attached gingiva. The average amount of shrinkage that occurred at 6 months was 28% (as compared with 47% quoted by Ward (1974) for free gingival grafts, and 87.5% quoted by Bohannan (1962b) for the perioseal retention operation), and in this study no periostal fenestration was performed.

During the observation period of the present study, no increase in pocket depth occurred, and thus a significant increase in width of attached gingiva was achieved. Histologically the healed tissues showed all the normal characteristics of a fully keratinised tissue in contrast to the results achieved by the perioseal retention procedure, demonstrated by Pennel et al. (1965).

Recently, Fagan & Freeman (1974) have shown that both free gingival grafting and the perioseal retention procedure can produce a predictable increase in the width of attached gingiva, but no mention was made as to the character of the healed tissues. In addition there was a significant difference in the width of attached gingiva created by the two methods, although there was no significant difference in the amount of periostum exposed at the surgical site. This was explained by the inadequate protection of marginal bone afforded by the perioseal retention procedure which caused an apical shift of the gingival margin as a result of marginal bone loss.

The degeneration of the palatal flap when the donor site in Method 1 was used was probably due to the design of the flap. Possibly a thicker, more extended envelope flap would not compromise the blood supply so much, and would enable grafting material to be taken without a full palatal flap procedure. Care was taken to prevent a void forming under the flap by maintaining pressure over the flap for 2 min, and so this is unlikely to have been the cause of the degeneration.

Acknowledgment

The author wishes to express his gratitude to Dr. R. N. Powell for his encouragement during this study, and to Mr. C. R. Day of the Photographic Department of the Dental School for the illustrations.

Zusammenfassung

Klinische Untersuchung der Verbreiterung einer schmalen angewachsenen Gingiva mit freien Bindegewebsimplantaten. Bei acht Personen wurden 14 Stellen mit schmalen (< 2.0 mm) keratinisierten angewachsenen Gingiva und mit Sulci gingivaeh kleiner als 1.0 mm ausgewählt. Mit drei Methoden wurden am Gaumen oder auf dem Kieferkamm Bindegewebe-Transplantate entnommen. Dies ermöglichte den Primärverchluss im Spendergebiet. Die Breite der angewachsenen Gingiva wurde praec- und postoperativ 1, 2, 5, 10, 12 und 24 Wochen mit der Parodontosonde auf 0.5 mm genau gemessen.

Die Ergebnisse zeigen eine statistisch signifikante Verbreiterung der angewachsenen Gingiva, die in Biopsien strukturell wie eine normale Gingiva propria erschien.

Résumé

Evaluation clinique de l'emploi de greffes libres de tissu conjonctif pour augmenter la largeur de la gencive adhérente. Quatorze sites opératoires furent choisis chez huit patients présentant une gencive adhérente de moins de 2 mm de largeur associée à un sillon gingivo-dentaire de moins de 1 mm. Du côté donneur (palais ou crête alvéolaire), les greffons conjonctifs furent prélevés selon trois méthodes différentes permettant une fermeture de la plaie par première intention. La largeur de la gencive adhérente fut mesurée avec une
sonde parodontale au demi millimètre près — et 1, 2, 5, 10, 12 et 24 semaines post-opératoire. Les résultats montrèrent un élargissement gingival statistiquement significatif; les biopsies livrèrent un tissu de structure normale.

References


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