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Evaluation of a collagen–glycosaminoglycan complex as a dressing for gingival wounds

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Protection of gingival surgical sites by second-intention healing using a collagen–glycosaminoglycan complex (Glycagen®) has been reported to be clinically effective. Optical and scanning electron microscopy was used to investigate these reports. Wound healing was observed clinically and histologically, at unprotected sites and at sites protected by the wound dressing, over 90 days in four patients. The results confirm the clinical efficacy of this wound dressing.

1. Introduction

Collagen has been used for its haemostatic properties in odontology since Laudenbach and Scheffer [1] demonstrated its usefulness in the form of powders, compresses and sponges. These haemostatic properties can be optimized by the use of native, unaltered collagen [2, 3], which acts at the parietal level by facilitating platelet aggregation and in plasma by activating factor XIII [4]. Vivier and Rozencweig [5] were the first to use collagen on palatal grafts to ensure haemostasis, and they were able to show that it encouraged wound healing.

Magnuson *et al.* [6] introduced the idea of using collagen membranes in directed tissue regeneration. The variable resorption of collagen avoids a second surgical intervention for removal of the membrane. Although Tanner *et al.* [7] reported disappointing results using this method, others [8, 9] have corroborated the initial findings.

Glycosaminoglycans are the second important group of macromolecules that constitute the extracellular matrix. Yannas and Burcke [10] tested a biological dressing made of collagen and glycosaminoglycans in dermatological surgery and obtained good results in the treatment of burns; they subsequently described this combination as "artificial skin".

In 1987, a similar wound dressing, Glycagen® was commercialized, which consists of a collagen matrix containing heparan sulphate and chondroitin 4-sulphate, in combination with a biological fibrin glue containing 15 mg fibronectin per ml glue [11]. Roussille and Barthet [12] referred to the same material as an "artificial extracellular matrix". Itic and Serfaty [13] obtained interesting results using a combination of a biological glue, Glycagen and natural coral to treat intra-osseous defects. Cafesse *et al.* showed first in animals [14] and then in man [15] that fibronectin favours connective attachment and that its plasma concentration is sufficient to obtain optimal results. We have thus tested a collagen–glycosaminoglycan wound dressing, Glycagen®, using the patient's blood as the only source of fibronectin. Wound healing was observed at unprotected gingival surgical sites and at sites protected by this dressing over 90 days in four patients.

2. Materials and methods

Glycagen was prepared by Bioetica (Lyon, France). This resorbable biological dressing is available as rectangular compressed sponges (80 mm × 15 mm) sterilized by gamma rays and blister-packed (Fig. 1). It is composed of native Type 1 collagen (80%) extracted and purified from calf skin, chondroitin 4-sulphate (13%) from the nasal septa of newborn lambs, and heparan sulphate (6.5%) from ovine placenta.

Eight sites were opened surgically on four volunteers: wounds of 2 cm × 1 cm of partial depth were made on the same side of the oral vestibule. Four of the sites were protected by a Glycagen wound dressing maintained with a suture and the four adjacent sites were left unprotected. Biopsy specimens were taken from the patients according to the schedule shown in Table I.

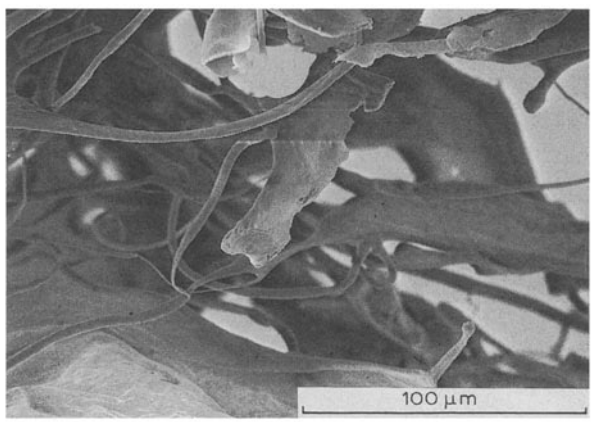

Figure 1 Glycagen sponge structure observed by scanning electron microscopy.

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TABLE I Experimental design for obtaining biopsy specimens

Patient	Days after operation				
	1	3	7	11	90
A			+		+
B	+	+		+	
G	+	+		+	
R			+		+

Specimens for light microscopy were fixed in Boin solution and embedded in paraffin; sections were stained with haematoxylin–eosin–saffron and Masson's trichrome.

Specimens for scanning electron microscopy (SEM) were fixed with glutaraldehyde (05%) treated with alcohol and metallized with an Edwards sputter coater. They were examined on a Cambridge Stereoscan, and foreign particles were identified with an X-ray analyser. Both a microphotographic and a video recording were obtained.

The clinical examination comprised a visual characterization of surface healing and determination of pain after (i) palpation of the wounds by light pressure with a finger and (ii) axial and transverse percussion of the teeth situated under the wounds.

3. Results

3.1. Clinical examination

The clinical results are summarized in Table II. It can be seen that fibrinoleucocytic tissue was present on the protected sites as early as 1 day after operation but only on day 3 on the control sites (Fig. 2). Palpation of the control sites was variably painful between days 3 and 11, whereas palpation of the protected sites was painless in three of the four patients on days 3 and 7 and painless for all patients on day 11. Percussion of the teeth beneath the control sites was perceptible on days 3–7 for two of the four patients, but was painless at all times on the protected sites. On day 90 after operation, no difference was observed between the control and protected sites.

3.2. Histological examination

Examination by optical microscopy gave the results shown in Figs 3–5. On days 1 and 3, the control sites have more or less well-organized clots. The protected sites show organization of blood elements between the collagen fibres of the Glycagen, with a total absence of macrophages (Fig. 3). On day 7, epithelial cells cover the healing tissue in all sections (Fig. 4). The protected sites have a more elaborate organization of cells, however: the basal lamina has a sinusoidal aspect, under pressure from connective papilla erupting

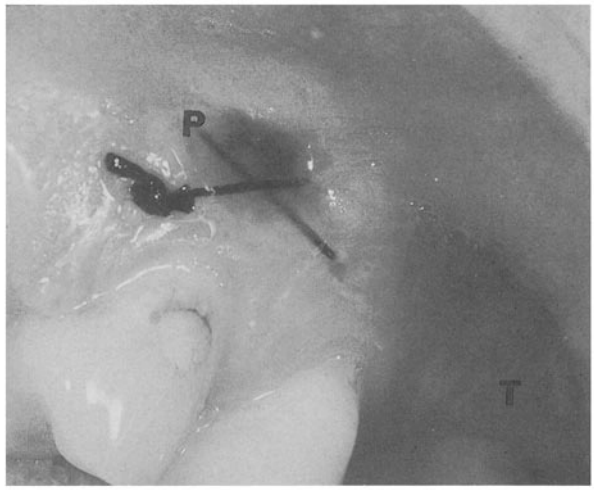

Figure 2 On day 1, the site protected by the dressing is covered with a fibrinoleucocytic tissue, whereas the control site remains uncovered.

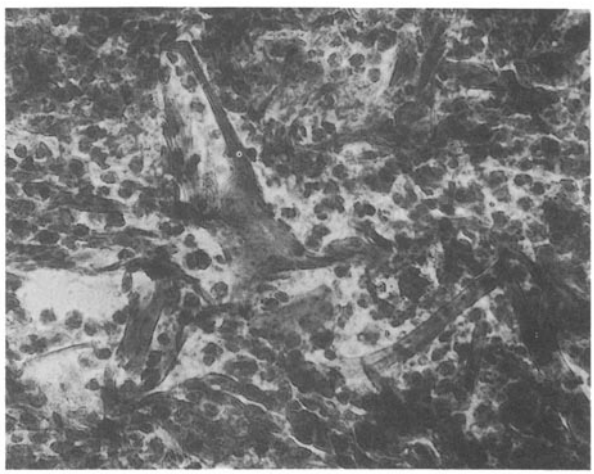

Figure 3 On day 1, note the organization of the blood elements in the fibrin network and the collagen fibres of the dressing. Masson's trichrome: × 500.

TABLE II Comparative clinical analysis of control (CS) and protected sites (PS)

Days after operation	Visual aspect		Palpation		Percussion	
	CS	PS	CS	PS	CS	PS
1	Cruented	Fibrino-leucocytic tissue	Light pain	Painless (3/4)	Painless	Painless
3	Fibrino-leucocytic tissue	Fibrino-leucocytic tissue	Painful	Painless (3/4)	Light pain	Painless
7	Epithelium	Epithelium	Light pain	Painless	Painful	Painless
11	Epithelium	Epithelium	Light pain	Painless	Painless	Painless
90	Epithelium	Epithelium	Painless	Painless	Painless	Painless

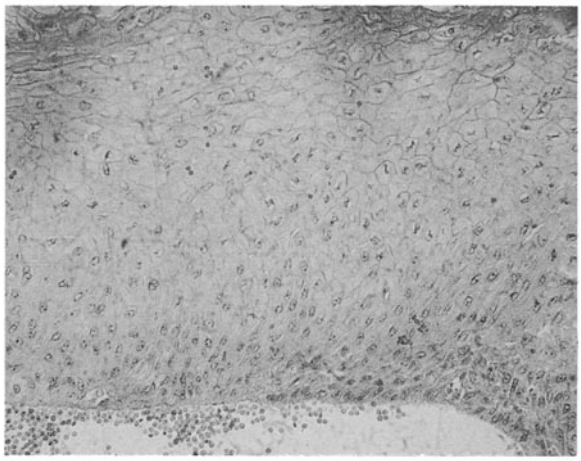

Figure 4 On a control site (day 7), the epithelial tissue covers the connective tissue. Haematoxylin–eosin–saffron (HES): × 200.

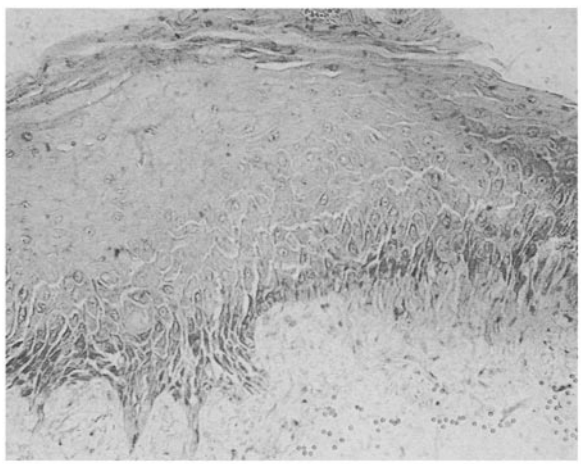

Figure 5 On a protected site (day 7), the epithelial cells seem more natural and it is important to note the sinusoidal aspect of the basal lamina with the eruption of the connective papillae. HES: × 200.

into the epithelium (Fig. 5), whereas the basal lamina in the control sites is more linear (Fig. 4).

The results of SEM examination of the wounds are shown in Figs 6–10. On day 3, the control sites contain a mass of leukocytes, polymorphonuclear leukocytes and fibrin. Specimens from the protected sites show perfect homogeneity between the collagen

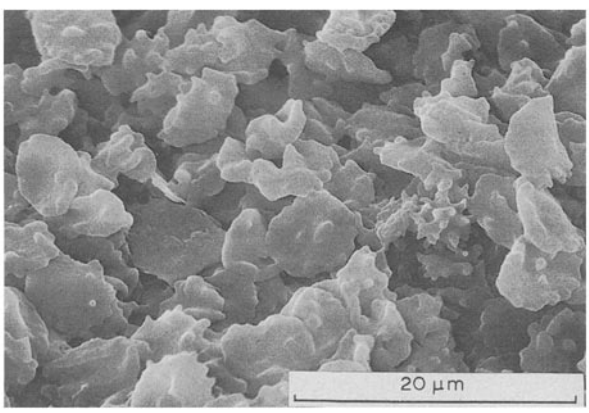

Figure 6 Many blood cells cover the control site on day 3.

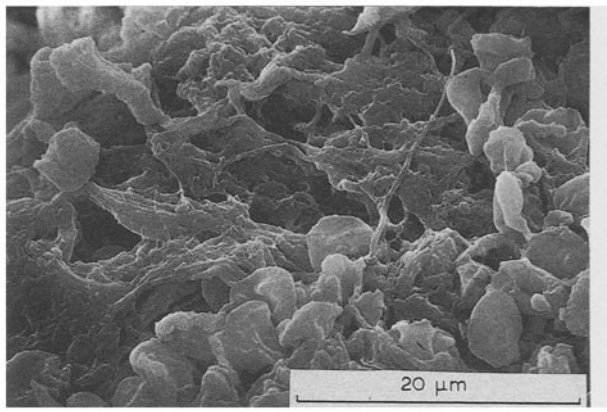

Figure 7 On day 3, the blood cells are visible on the protected site, linked up on the fibrinous tissue and the Glycagen fibres.

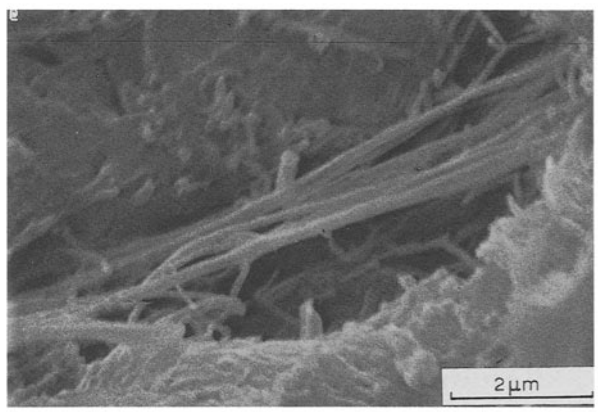

Figure 8 On day 7, note on the protected site the fibrous network of new synthesized collagen, and the absence of inflammatory cells.

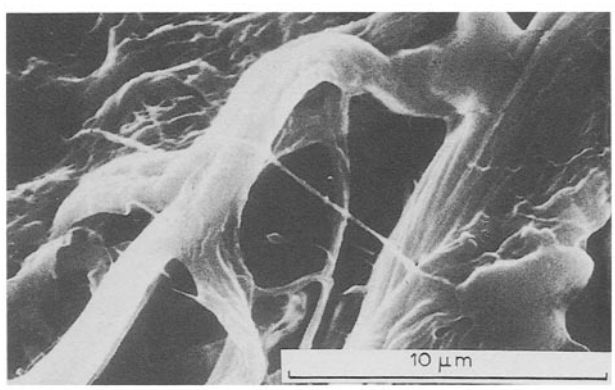

Figure 9 Gingival tissue, day 0.

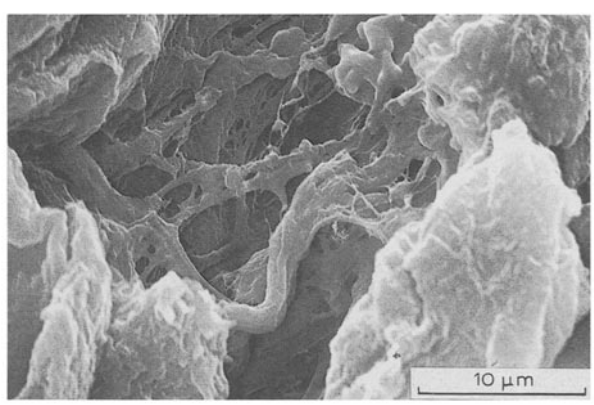

Figure 10 There is no difference between the microscopic structure of the fibres examined on day 0 (Fig. 9) and day 90. Protected site.

fibres and the fibrin network of the blood cells (Figs 6 and 7). Examination on days 7 and 11 was limited to the deep layers in order to compare the structure of the underlying connective tissue. On the protected site (Fig. 8) newly synthesized collagen fibres were observed. On day 90, no structural difference can be seen between the control and protected sites or between the protected sites and the normal gingiva (Figs 9 and 10).

4. Discussion

Our clinical and histological results demonstrate that the Glycagen dressing is active during the first few hours of the tissue-healing process. This dressing acts by combining the constituents of the dressing (collagen and glycosaminoglycans) with the patient's glycoproteins (mostly fibronectin) and plasma transglutaminase. Fibronectin forms covalent bonds with fibrin in the blood clot by reacting with factor XIII and shows chimiotactic action on fibroblasts [16–18]. In the presence of glycosaminoglycans, fibronectin improves the adhesion between cells and between cells and the extra-cellular matrix [19, 20]. Doilon *et al.* [20] observed *in vitro* that a collagen sponge impregnated with fibronectin was colonized by connective tissue cells. Two studies have shown that the interactions between collagen and fibronectin are mediated by heparan sulphate, hyaluronic acid and chondroitin 4-sulphate [19, 21].

The results of these studies demonstrate the importance of the interactions between the constituents of the extracellular matrix and the plasma glycoproteins and explain the rapid healing that occurred at protected sites in our study, with the rapid appearance of fibrinoleucocytic tissue. To understand the importance of this initial healing stage, one must recall how the organism responds to aggression and through a second-intention healing. Physiological inflammation is the first response to aggression [22]. The first stage is the vasculo-exudative period, with four signs (tumour, rubor, colour and dolor) and the formation of fibrinoleukocytic tissue containing the fibrin of the clot, altered polynuclear cells and cell fragments. This tissue becomes the scab, which protects the second stage, the proliferative stage, with endothelial and connective tissue cells and formation of granulation tissue. This tissue is progressively covered by epithelial cells, which multiply on the edges of the wound and move from the periphery to the centre on the surface of the connective buds.

In our study, fibrinoleukocytic tissue appeared as early as the first day on sites protected with Glycagen and optimal organization by the third day. This biological matrix thus acts like a scab, protecting the healing processes in the deep layers; it therefore reduces the inflamation and its consequences (redness and pain) as shown by our clinical results on days 3–11.

The total absence of macrophages indicates the biocompatibility of Glycagen, which is resorbed enzymatically, as well as the degree to which the operative sites are protected. If foreign elements had been present, an additional healing phase, the cleansing phase, would have occurred, with the appearance of macrophages, which triggers inflammation and slows down the healing process. The hermetic nature of this dressing eliminates the cleansing phase and contributes to the post-operative comfort of the patient.

5. Conclusion

In conclusion, these results explain and confirm our previous clinical observations on 120 patients using this dressing. Post-operative use of Glycagen as a dressing on gingival wounds is effective, as shown by:

1. complete absence of macrophages;
2. rapid transformation of the clot to fibrinoleukocytic tissue;
3. decrease in peripheral inflammatory phenomena;
4. acceleration of tissue organization;
5. absence of modifications to the long-term healing process.

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