Evaluation of Free Gingival Grafts as a Treatment for Gingival Involvement of Mucous Membrane Pemphigoid: Periodontal Considerations and a Study of Six Cases
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Abstract
Background: Mucous membrane pemphigoid is a chronic autoimmune blistering disease of unknown etiology. The attached gingiva in dentulous areas is one of the primary affected sites. Oral mucous membrane pemphigoid is often treated with hygiene measures in association with topical corticosteroids and/or immunosuppressive agents. Lesions can be temporarily controlled by medication but there is no definitive cure. Treatment is focused on treating active lesions and alleviating symptoms. Aims and Objectives: To assess free gingival grafts as a novel approach in the treatment of recalcitrant for oral mucous membrane pemphigoid lesions. Materials and Methods: Inflammation was subjectively graded using a visual analog scale preoperatively and three months after the free gingival grafts were performed. Patients were also re-evaluated six months postoperatively. Results: All patients displayed complete, albeit localized, permanent remission of lesions in the treated areas, despite discontinuation of topical treatment. Slight radial improvement around the gingival grafts was noted. Conclusion: Free gingival grafts could be useful in treating refractory gingival lesions due to mucous membrane pemphigoid. These results may help to shed deeper insight on the pathogenesis of this disease and help developing more specific therapies.

Keywords: Desquamative Gingivitis; Free Gingival Graft; Mucous Membrane Pemphigoid; Vesiculobullous Disease.

Introduction
Mucous membrane pemphigoid (MMP) is a chronic autoimmune blistering disease. The main etiology of MMP remains unknown. Most patients are above 60 years of age at the time of diagnosis and the disease is twice more frequent in women. It is characterized by varying degrees of involvement of ocular, oral, genital, oesophageal and laryngeal mucosa, and skin. Oral lesions are often the first sign of disease and represent one of the most debilitating aspects of this chronic condition. Primary pathognomonic signs include desquamative gingivitis and separation of the epithelium from the underlying connective tissue by tangential pressure, known as a positive Nikolsky sign. Bullous lesions may lead to large painful ulcerations. The attached gingiva in dentulous areas is typically affected. The junction of the hard and soft palate is also commonly involved; this area is prone to blistering in the general population, either caused by coughing, sucking or other forms of trauma. Patients affected with oral lesions are susceptible to gingival bleeding, pain and secondary infections. MMP is rarely fatal but death could result from sepsis or iatrogenic complications.

Histopathologic examination shows a sub-epithelial cleft, and direct immunofluorescence demonstrates a continuous linear band of IgG and C3 antibodies along the basement membrane. Studies have shown antigenic recognition by autoantibodies in the basement membrane zone, more precisely affecting hemidesmosomal anchorage complex proteins that assure epithelial integrity. The affected antigens are known as laminin-5 (α3β3), laminin-6 (α6β4), BP-180 (α3β1), BP-230 (α6), and β4 integrins. The α6 integrins are considered as target antigens when patients exclusively present MMP-associated oral lesions. Inflammatory mediators could also be involved in the pathogenesis of this disease. An increase in interleukin (IL) 1, 6, 8, and 10 accompanied by neutrophils, mast cells, macrophages, langerhans cells, and lymphocytes was observed in MMP leukemic sites.

Up until now, there is no available cure for this disease. In four small case-controlled studies complete and long-term remission of
lesions has been achieved with a combination of aggressive systemic therapy (Cyclophosphamide, Dapsone, Intravenous immunoglobulin’s, Methotrexate, Azathioprine, Mycophenolate mofetil, Doxycycline, Sulfasalazine, Tacrolimus, Tetracycline, Triamcinolone acetonide, and/or Rituximab). Furthermore, three of these studies were performed in the same center and remission periods last more than 24 months. The treatment was focused on preventing blisters, treating active lesions and alleviating symptoms. Spontaneous resolution of the lesions does not occur. Moreover, untreated patients or non-compliant patients are more prone to develop acute lesions. As expected, ulcer formation often compromises diet and oral hygiene. Ocular grafting with buccal mucous membrane has been attempted as a treatment for MMP for patients developing scar lesions such as entropions and symblepharons. Although some patients benefited from this technique, many developed postoperative complications most often associated with keratoconjunctivitis sicca. Moreover, only temporary visual improvement was shown after cultivated oral mucosal epithelial sheet transplantation on patients with ocular cicatricial pemphigoid.

In the present study, we attempted to assess free gingival grafts (FFGs) as a novel approach in the treatment of MMP recalcitrant to conventional therapy. This technique has been successfully used to treat gingival lesions caused by other chronic dermatoses, such as lichen planus, although the evidence is only based on case reports. In our practice, we have observed that dental extractions locally eradicate MMP gingival lesions. Removing the sulcular epithelium by exodontia may prevent antigenic stimulation by periodontal microorganisms. These observations led us to the working hypothesis that FFGs could thicken gingival epithelium, thus reducing antigenic response to dental plaque.

Material and Methods
This split-mouth case-controlled prospective clinical trial was conducted on six patients diagnosed with MMP, as confirmed by histopathology and direct immunofluorescence. The Centre Hospitalier de l’Université de Montréal Institutional Review Board approved this study. We aimed to assess if FFGs may be used as an alternative therapy for gingival lesions caused by MMP that do not respond to conventional treatment. The latter included a combination of topical and systemic corticosteroids, topical Tacrolimus, Tetracycline / Niacinamide and, whenever medically feasible, the use of systemic immunomodulators such as Azathioprine.

Inclusion criteria were listed as follows: patients had to be over 18 years of age and present with gingival lesions due to MMP in dentate areas that do not respond to conventional therapy. Exclusion criteria were as follows: tobacco consumption in the month preceding the gingival graft, bone-targeted intravenous therapy, head and neck radiation therapy, uncontrolled diabetes, cyanoacrylate allergy, antplatelet or anticoagulant therapy associated with an International Normalized Ratio greater than 3.0, and concomitant disease of the oral mucosa (e.g. candidiasis). Among our patient database, only six patients were eligible and accepted to participate in this study. Baseline photographs were performed on the first appointment. The intra-oral site to be grafted was randomly selected for each patient. The non-grafted contralateral site was used as an internal control.

Procedure: After local anaesthesia at the recipient and donor sites, teeth were lightly scaled prior to grafting. A partial thickness flap was made with a scalpel at the free gingival margin of the recipient site. The flap was displaced apically, redundant tissues were excised, and residual muscle fibers were scraped off in order to ensure graft bed stillness. A graft was taken from the ipsilateral hard palate. Each graft measured 5 mm in width, at least 1.5 mm in thickness, and its length was adapted to the recipient site. Any adipose tissue was removed on the connective tissue side of the graft, which was positioned and secured at the recipient site. Light pressure was applied on the graft for five minutes, thereby forming a thin fibrin clot between the recipient bed and the graft. In addition, few droplets of n-butyl-2 cyanoacrylate (Periacyrl™, GluStitch Inc., Delta, BC, Canada) were applied to the superior margin. A comparable technique has been used in the past with analogous operative success. An absorbable gelatin compressed sponge (Surgifoam™, Ethicon Inc., Somerville, NJ, USA) with n-butyl-2 cyanoacrylate was placed at the donor site as a surgical dressing.
Data collection, criteria, and statistical analysis: Topical treatment was discontinued for two weeks after the procedure and prior to the three months post-surgical appointment. Patients had a first postoperative appointment one week after the graft procedure. A second follow-up was implemented three months after the procedure; multiple photographs of the grafted sites were taken. Gingival erythema was then evaluated by a single-blind evaluator preoperatively, and three months postoperatively according to a visual analog scale (VAS). The latter was graded as follows: 0: absence of erythema, 1: mild erythema, 2: moderate erythema, 3: severe erythema (associated with bleeding, positive Nikolsky sign and/or ulcerative lesions). All patients were subsequently re-evaluated six months postoperatively. Grading was repeated randomly by the single-blind evaluator with different photographic views of the same patients in order to ensure scoring reproducibility. A Wilcoxon paired signed-ranks test was applied to the VAS results and was performed with the R 3.0.2 software.

Results
A median difference of 3 (p=0.0313) on the VAS was observed at the FGG recipient site comparing preoperative and postoperative scores. (Figures 1 and 2) A median difference of 1 (p=0.0313) was observed radially around FGGs. There was no difference (median score difference=0) between preoperative and three months postoperative scores on the untreated control side; however, this is not statistically significant. These results confirm the research hypothesis suggesting that FGGs can reduce gingival MMP lesions that do not respond to conventional therapy. Grafted sites still showed no sign of active disease six months after the surgery. The surgery was well tolerated by all patients, and no adverse effects were reported.

Discussion
Based on our results, all patients displayed complete and permanent remission of MMP lesions in the treated areas. Furthermore, slight radial improvement around the FGGs was noted in all patients. To our knowledge, FGGs have never been used to treat gingival manifestation of MMP. We can only hypothesize as to how and why FGGs have successfully, albeit focally, eliminated gingival lesions due to MMP. Important clues may be found by examining the clinical behaviour of MMP. Indeed, the latter affects far more frequently the gingiva in dentulous areas. Edentulous areas are seldom affected; in fact, in our practice, we have yet to see MMP lesions on edentulous ridges. Therefore, we can either assume that there is a relationship between periodontal tissues (i.e. gingival sulcus, periodontal ligament) and the development of MMP lesions, or we can postulate that dental extractions eliminate the accumulation of MMP lesions, thus ending potential antigenic stimulation of the gingiva. 

Figure 1: Preoperative gingival lesions associated with mucous membrane pemphigoid Photograph of mandibular teeth showing desquamative gingivitis, ulcerations, and gingival bleeding on the attached gingiva. The tooth marked with a ‘*’ is associated with the FGG recipient site.

Figure 2: Three months postoperative control following free gingival grafting photograph showing elimination of lesions at the grafted site, despite the presence of plaque and tartar accumulation. Elsewhere, gingival lesions are otherwise stable. Radial diminishment of inflammation is also observed around the FGG. The tooth marked with a ‘**’ is associated with the FGG recipient site.
It has been demonstrated that improving oral hygiene leads to clinical improvement of lesions and symptoms due to MMP. Arduino et al., (2012)38 have demonstrated that periodontal status is worse in MMP patients when compared with healthy controls. Non-surgical periodontal therapy such as scaling, dental prophylaxis, and antiseptic mouthwashes improves the clinical presentation of MMP. These oral hygiene measures are successful in reducing clinical gingival inflammation, thereby complementing conventional therapies of oral MMP lesions.38 Such conventional treatments include topical or systemic corticosteroids, topical or systemic immunosuppressive agents, sulfones (dapsone), intravenous immunoglobulins11, rituximab, and tumor necrosis factor alpha inhibitors.42

Among possible mechanisms of action, FGGs may lead to diminishment of bacterial overload, modulation of the diseased epithelium or the epithelial/connective tissue interactions. Previous studies have shown that gingival connective tissue grafts induce morphologic changes in the overlying epithelium, despite the fact that the latter is not disturbed in such procedures.43 It would be therefore interesting to compare the effectiveness of FGGs and connective tissue grafts in treating gingival lesions due to MMP. Immunologic changes have also been studied in the pathogenesis of MMP. More specifically, higher serum levels of IL-1 were observed in patients with active disease compared with treated candidates.25 These findings may explain why tooth removal and hygiene measures that help decrease inflammatory mediators also lead to an alleviation of MMP gingival lesions.

In parallel, Graziano, et al., (2013)29 showed that baseline levels of IL-1β are higher in the gingival crevicular fluid of grafted sites. However, control sites exhibit a more pronounced increase in the levels of IL-1β upon plaque accumulation. Thus, grafted areas develop less inflammation when compared with healthy gingiva, which may be due to a lower increase in IL-1β levels in the treated gingival areas.29 This could explain why grafted areas do not develop any MMP lesions. However, immunology and histology research on gingival graft is lacking. It is not known whether FGGs lead to a modulation in the distribution and/or concentration of etiologic antigens linked to MMP.

The present study is limited by the fact that we are unaware of the histopathologic and physiologic changes occurring after a FGG. Such crucial data is unavailable in the literature, despite the overwhelming number of clinical reports of outcome on FGGs in the general population. The small number of recruited patients and the relatively short duration of follow-up represent additional limits to our study.

Conclusions
Patients treated with FGGs demonstrate significant and positive responses. FGGs represent a well-tolerated, albeit localized, treatment for refractory gingival lesions due to MMP. We do not advocate the use of gingival grafts as primary treatment for MMP. On the other hand, when all conventional treatment options fail, FGGs may be considered in recalcitrant gingival lesions. We believe that if one can understand the biologic and physiologic mechanisms by which FGGs eliminate gingival manifestations of MMP, one may be able to shed deeper insight on the pathogenesis of this disease and help develop new and more specific therapies. Hopefully, future therapeutic options will focus on prevention and a cure rather than temporary relief.

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