ORIGINAL ARTICLE

Plasma Fibrin/Fibrinogen as Tissucol Adhesive Material in Periodontal Surgery: Part 2: pocket reduction and attachment regeneration.

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ABSTRACT

The regeneration of a periodontal tissues based on the viability of the productive cells, Essential paradigm in periodontal heath keeping and cure after surgical therapy is the regeneration of the attachment apparatus. Predictable periodontal regeneration following periodontal surgery is a major goal of therapy. Surgery aimed to create a new attachment or re-attachment of their apparatus. The aim of this study is to test the benefits of tissucol and stem cell activation to restore the periodontal apparatus with the application of the innovative surgical procedure that deal with the application of the biological glue in addition to the activation of the local stem cell. published as part1 of study in 2019. Forty-two patients have been incorporated in this study, twenty-one females and twenty on male, aged from 16 years old up to 55 years, subdivided in two groups. They were examined clinically and radiologically. All have chronic periodontitis, moderate or severe, with pocket depth ranged from 4 to 10 mm, the surgical procedure applied activates the local stem cells with assistance of tissucol as adhesive and hemostats. results showed, probing depth showed a significant decrease in depth, the probing depth and attachment level showed a significant reduction ($P<0.05$). Statistical compares in variance between data of first group and second showed that the baseline records were significantly indifferent, while the postoperative records showed a significant ($P<0.05$) variations in privilege to the first group (tissucol). In conclusion; Our results could be related, firstly, to a reasonable surgical procedure, the enhancement of tissue regeneration, endosteal cell activation and a rapid hemostasis with thin blood clot formation that could impeach an epithelial invasion.

Keywords: surgery, innovative technique, biologic glue, tissucol, regeneration, pocket reduction, periodontal pockets.

INTRODUCTION

Periodontal regeneration is defined as the reproduction or reconstruction of lost or injured tissue to restore the histologic, anatomic features with an acceptable functional condition. Essential paradigm in periodontal heath keeping and cure after surgical therapy is the regeneration of the attachment apparatus. Experimental studies have shown that the potential of periodontal regeneration seems to be limited by the regenerative capacity of the cells involved [(1).]

The regeneration of damaged periodontal tissues is mediated by various periodontal cells and is regulated by a vast array of extracellular matrix informational molecules that induce both selective and nonselective responses in different cell lineages and their precursors [(2).] Conventional periodontal therapies, such as open flap debridement (OFD), provide critical access to evaluate and detoxify root surfaces and establish improved periodontal form and architecture. Therefore, the disease process is arrested and conditions are created that favors tissue regrowth [(3)] However, periodontal defects, if left empty after Open Flap Debridement, will fill with the first cells to reach the area, that is the epithelial cells [(4).] After cell proliferation, which generates a core of fibro-epithelial tissues that attach to the root surface. Unfortunately, the attachment does not allow time for the bone and periodontal ligament (PDL) cells to refill the pocket, so the defect persists [(5)]. This traditional healing process, known as periodontal 'repair', ultimately prevents orderly and sequential regeneration of true hybrid periodontal tissues [(6).] The poor innate ability of damaged periodontal tissues to regenerate and the constant microbial challenge in the oral cavity demonstrate the need for developing clinically effective procedures to regenerate healthy periodontal tissues [(7).] There is a broad range of treatment options that are available, such as barrier membranes [(8),] auto grafts [(9)], demineralized freeze-dried bone allografts [(10),] bovine-derived xenografts [(11)], and combinations of membranes and fillers [(12),] enamel matrix derivatives [(13)]

Activation of local stem cell is an auto activator with the activation of regional blood stream [(14, 15)]. Favorable periodontal healing is not to create a mixed core of epithelial-fibrous connective tissue, its half-life is too short, needs a lot of maintenance and home care and perfect control of systemic condition. The essential way to create a permanent, sold and resistant attachment is to get a fibrous collagenous attachment, either new attachment or re-attachment [(16, 17).]

Platelet derived growth factor along with bone morphogenetic proteins are among the most researched growth factors in periodontal regeneration [(18).] Platelet rich fibrin; showed significant improvement in clinical periodontal parameter as well as in radiograph when compared with open flap debridement alone [(19).] Demineralized freeze dried bone allograft (DFDBA) have been used in the treatment of infrabony defects [(8).] Local stem cell activation; has been used successfully in periodontal and gingival esthetic and therapeutic surgery [(15,46).] Platelet-rich fibrin has shown significant results for periodontal regeneration [(18)] Fibrin/fibrinogen/lyophilized dried platelets, have been used as a biologic glue to compensate the ordinary suture in internal and cardiovascular surgery and as a hemostatic agent as well, since the eightieth of last century as tissucol or tisseel ™ [(19).]

The facts that the epithelial tissue grow up faster than the fibrous, most cases of bio membrane, first generation, usage in periodontal pocket surgery were failed cause of a micro spaces left in between root surface and membrane allowed the epithelial cell to grow and impeaches the fibrous attachment [20]. Recent researches showed that the precursors of embryonic stem cells are available in the gingival and periodontal healthy tissues especially among the cancellous alveolar bone, able to be activated to proliferate and create a new periodontal tissues [21]. Tissucol able to fill the void of lost tissues and provide a plasmatic coagulant clot which provides a living environment for stem cell to grow and act.

The aim of this study is to test the benefits of tissucol and stem cell activation to restore the periodontal apparatus.

MATERIAL AND METHODS

Forty-two patients have been incorporated in this study, twenty-one females and twenty one male, aged from 16 years old up to 55 years, subdivided in two groups. They were examined clinically and radiologically. All have chronic periodontitis, moderate or severe, with pocket depth ranged from 4 to 10 mm in depth. Collected clinical data included age, sex, general health, GI scores [22], Bleeding on probing BOP, probing pocket depth PPD [23] clinical attachment level (CAL) [24] and systemic bleeding time and clotting time. Surgical site and number of involved teeth were also recorded. Surgical site has been selected according to the clinically deepest pocket depth. Clinical attachment level and probing pocket depth taken immediately before surgery were recorded as a base line record and then first, second and third month later successfully. Mechanical (manual) granulation tissue debridement and root planing have been performed with Gracy curates instrument (HU- Freidy, immunity USA and GC American USA), probing pocket depth and clinical attachment level were performed with WHO CPTN probe. According to the traditional flap surgery protocol.

A- first group:

1- Patients; Twenty-one patient, 10 men and 11 women, aged from 16 years up to 55 were incorporated in this group. Tissucol components were prepared using water path at 37^{oC}, slow solidification applied to gain time for flap adaptation.

2- Compound preparation: Two components, fibrin sealants, heat treated, prepared according to the instruction manual. The lyophilized powder that after reconstitution gives 1 ml of solution contains:
Glottable protein 75-115, thereof fibrinogen 70-110 ^{mg}, plasma fibronectin (CIG) 2-9 ^{mg}, factor VIII 10-50 ^{u}, plasmogen 40-120^{mg}, aprotinin solution (bovine) 300 $^{kiu/ml}$, thrombin (bovine) slow solidification 4 iu/ml, and calcium chloride solution 40 $^{mmol/L}$. lyophilized tissucol mixed with aprotinin solution to get tissucol solution (first component), the lyophilized thrombin mixed with sodium chloride to get thrombin solution (second component), these two solution prepared separately in water bath at 37^{oC}.

3- Application: Simultaneous application by a Duploject (provided) applicator to cover the entire wound site, inner flap surface, flap borders, fill the pocket space, planned roots, embrasures, curated alveolar bone surfaces, all must have done within one minute, replace the flap and adapted to the cemento enamel junction or as required according to the type of flap surgery, bring the flap borders close to the fixed one.

B- Second group; twenty-one patients, 11 men and 10 women, aged 22- 51-year-old, involved in this group. After soft tissue debridement by mechanical root planing, flaps have been turned to the required place and adapted close to the underneath structures. Sutured with 3/0 black silk, semicircular round tip needle (Ethicon), continuous interdental technic used. Several washes with warm normal saline were performed. Entire surgical procedures in both groups have been done by the other in both groups. All patients in both groups showed a normal blood picture. Statistical analyses were based on T test and student Fisher test [25].

C- Surgical technique: The surgical procedure applied activates the local stem cells with assistance of tissucol as adhesive and hemostats [46].

RESULT

Forty-two patient participated in this study of average age from 16 years to 55-year-old, average age 30.88+/-4.7. First group average age was 30.0+/-4.3, and that of second group was 30.95+/-3.26. Neither a significant variable found with age average nor with bleeding and clotting times. Baseline GI was insignificantly deviated in inter the first and second groups, no significant variations found in GI when comparing the data of the first, second and third month follow-up records (table1).

1- First group (tissucol); table 1
Probing depth showed a significant decrease in depth, one month as well as 6 month postoperative records when compared to baseline (table 2, 5). About 3.7^{mm} gain of pocket depth was obtained at the first month, which decreased to 3.5^{mm}at the sixth month, this loss in gain (5.1%) was insignificant (P>0.05).
A significant difference was also obtained in attachment level first and sixth postoperative month when compared to the baseline records (P<0.05). About 3.63^{mm} was gained at the first month, then decreased to 3.28^{mm} at the sixth month postoperative (Table 5). this loss in gain was insignificant (9%).
The postoperative records were significantly indifferent when data compered to each other of GI, BOP, and AL. (Tables 2, 3, 4, and 5) these results could suggest adequate attachment stability.
All patients didn't show neither immediate nor retarded allergic reactions.

2- Second group (sutures): The probing depth and attachment level showed a significant reduction (P<0.05) when compare the baseline to the first and sixth month postoperative records. The gain in pocket depth 3.5^{mm} at the first postoperative month, decreased to 2.94^{mm}at the sixth month, this loss in gain was significant (16%) (P<0.05). the gain in attachment level was 2.68^{mm} at the first month postoperative, then decreased to 2.32 at the sixth

month, this loss in gain was also significant (P<0.05) (tables 2,3,4 and 6).

No significant differences were recorded in GI, PPD and AL, records when compares their postoperative data with each other (first month with second, second with third, first with third month record).

Statistical compares in variance [25] between data of first group and second showed that the baseline records were significantly indifferent, while the postoperative records showed a significant (P<0.05) variations in privilege to the first group (tissucol).

DISCUSSION

Several line of evidences suggested that the initial events in periodontal wound healing were of a critical importance in determining whether healing by connective tissues regeneration is likely to occur or not [26]. Predictable periodontal regeneration following periodontal surgery is a major goal of therapy [27]. Surgery aimed to create a new or re-attachment of periodontal apparatus [28], the eventual debridement of infected tissues with a chemical or mechanical root planning could perform the basic conditions for tissue regeneration [29]. Attachment regeneration requires an interaction between fibroblasts and dentinal root surface in an attempt to confer a selective advantages upon fibroblasts over epithelial cell in order to overcome the epithelial growth during healing events and to enhance the connective tissue regeneration [3].the surgical procedure applied recently[46]which activates the local stem cells with assistance of tissucol as adhesive and hemostats and by its innovative technique showed a successful regeneration and pocket reduction with a long-term clinical stability. A guided tissue regeneration (GTR) [30] procedure with collagen membrane either absorbable or not has been introduced to periodontal surgery in order to obtain a regenerative connective tissue attachment and to impeach the epithelial invasion [31, 32]. GTR has shown a promising result, especially when associated with an absorbable membrane, its action based on the ability of collagen membrane to attract fibroblasts and to facilitate clotting by aggregation of platelets to act as hemostat, so it is effective in inhibiting epithelial migration on the planed dentinal root surface thus promoting a connective tissue grow out and attach to the planed root surface [33]. GTR still associated with surgical complications such as exposure and secondary infection in addition to technical difficulties [34]. The regenerative process, didn't limited to fibroblast activation, but also includes osteoblast and Cementoblasts activations [35]. Cementoblasts engineering [36] confirms the selective behavior of periodontal cell as having a stem cell ability, and supports the role of Cementoblasts [37]. Tissucol able to form a ground substrate for cellular growth of fibroblasts, osteoblasts and Cementoblasts [38]. Plasma factors stimulates fibroblast adhesion and growth, fibronectin covalently linked to fibrin and collagen by factor XIII, thrombin able to convert fibrinogen into fibrin and factor XIII to its active form during the final stage of blood coagulation and to stimulate fibroblasts growth and collagen syntheses.

A cellular dermal matrix [39], and enamel matrix [12], have been experienced in last decade, showed an increase in gingival margin thickness and increased keratinization with a significant gain in attachment level. Their use is limited to only a gum recession cases [40]. Bio-absorbable material, other than collagen, [41] like polydioxanan and polylectide asetyltributyl citrate have been used in infrabony pockets in association with GTR, these synthetic chemical materials showed gain 81% in bone level with stability for 5 years without acting on fibrous attachment [42].

Synthetic oligo-peptide have been used as cell activator of periodontal ligament, showed the mimicking cell binding domain of fibronectin might be a potential tool for arranging a biological attracting environment for periodontal ligament cells which would enhance regeneration efficacy [43]. A selective adhesion and proliferation of ligament's cells been essential to obtain a predictable regeneration [44].

Tissucol, as it is liquid, able to cover the entire surface area with the meticulous lodges, sticky enough to adhere firmly to the underneath tissue and to form a rapid ground substrate blood clot. Besides, its natural constituents able to activate fibroblasts, osteoblasts and cementoblasts and perform a cellular adhesion to a connective tissue network [35]. Rapid coagulation and rapid closure of wound opening helps in forming an attentive and perfect seal of flap borders that could prevent epithelial cells migration [45]. Tissucol gives a uniformly distributed blood clot, all over the wound (X2) while stiches may give irregularly thickened clot, depending upon the pattern of tissue loss and flap adaptation. More, the looseness of stiches on gingival margins leads a loss in flap adhesion which could leads to some complication like a retarded healing. The gain in pocket depth and attachment levels with tissucol application was significantly superior to that followed traditional suturing, this result suggests that tissucol gave a successful pocket reduction and attachment.

After Six-month monitoring, there were only 5% loss in pocket depth gain (TG), while with traditional suture, the loss in pocket depth gain was 16%. Which appeared significant (<0.05). Attachment level, showed that the loss in gained attachment reduced 9% during the same period of monitoring in TG, and 13% in sutured pockets (SG). These results suggest a gradual loss in stability during 6 months that could be due to inevitable tissue shrinkage during fibrous organization and maturation. Indeed, this difference appeared significant between TG and SG. (P<0.05).

The advantages of tissucol in obtaining a significant gain in pocket depth and attachment level which have been maintained for six months with adequate oral hygiene control. The stability of attachment depending positively on maintenance procedure and oral hygiene performance. Indeed, the gingival disease initiated as a result of plaque accumulation, the recurrence of the disease could occur due to the same cause. Even though, any gain in pocket depth could be maintained as far as the oral hygiene kept perfect, taking in consideration the connective tissue attachment is more resistant to the bacterial challenge than epithelial or mixed attachments.

Our results could be related, firstly, to a reasonable surgical procedure, the enhancement of tissue regeneration, local stem cell differentiation, endosteal cell activation and a rapid hemostasis with thin blood clot formation that could impeach an epithelial invasion.

Table 1: Gingival Index means; insignificant differences.

	Baseline	First month	2nd month	3rdmonth	6th month
TG	0.59	0.48	0.51	0.65	0.71
SG	0.65	0.61	0.65	0.70	0.82

Table 2: the gain in probing pocket depth in mm.

	First month	2nd month	3rd month	6th month
TG	3.69	3.54	3.55	3.50
SG	3.5	3.28	3.14	2.94

Table 3: the gain in attachment level in mm.

	First month	2nd month	3rd month	6th month
TG	3.63	3.58	3.38	3.28
SG	2.68	2.58	2.45	2.32

Table4: percent of loss in pocket depth and attachment level between First VS 6th month.

	Pockets depth	Attachments level
TG	0.19mm / 5.1%	0.35mm / 9%
SG	0.56mm / 16%	0.36mm 13%

Table5: TG group; changes in pocket depth and attachment level (patient's means). Intragroup difference between baseline and first month records was significant (P<0.05) so between baseline and six month records as well (P<0.05)

Probing pocket depth					Attachment level				
Base line	1month	2month	3month	6month	Base line	1month	2month	3month	6month
6.43+/- 0.76	2.74+/- 0.33	2.9+/- 0.33	2.89+/- 0.32	2.93+/- 0.34	7.18+/- 1.6	3.55+/- 0.56	3.6+/- 0.58	3.8+/- 0.06	3.9+/- 0.57

Table6: SG group; changes in pocket depth and attachment level (patient's means).). Intragroup difference between baseline and first month records was significant (P<0.05), as well between baseline and six month records (P<0.05)

Probing pocket depth					Attachment level				
Baseline	1month	2month	3month	6month	Baseline	1month	2month	3month	6 month
7.07+/- 0.73	3.57+/- 0.5	3.79+/- 0.49	3.93+/- 0.5	4.13+/- 0.49	7.61+/- 2.1	4.93+/- 0.9	5.3+/- 0.88	5.16+/- 0.86	5.29+/- 0.9

Pictures representing some of cases incorporated in this study.

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