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Interposition of a connective tissue graft or a collagen matrix to enhance wound stability - an experimental study in dogs

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Abstract

Aim: To evaluate the role of a connective tissue graft (CTG) or a collagen matrix (CM) interpositioned between flaps and non-shedding hard surfaces on wound stability.

Material and Methods: 60 bone dehiscence defects were prepared in 5 Beagle dogs. Three treatments were performed in 12 sites per dog: 1) repositioned flaps were sutured onto instrumented dentin surfaces (control), 2) repositioning of flaps with an interpositioned CTG, and 3) repositioning of flaps with the application of a CM. To allow postoperative healing with n=5 for 1, 3, 7 and 14 days before evaluation, the sutures were removed, incision lines retraced and tensile forces applied to the flaps. The minimum magnitude of forces required to detach the flaps from the wound bed was recorded.

Results: After one week of healing, 6 N had to be applied to disrupt flaps from their wound bed in the CTG group. In the control group, a similar magnitude of resistance was achieved after two weeks (6.1 N). Flap resistance to tearing was highest in the CTG group (maximum 9.1 N) two weeks postoperatively. On the third postoperative day, the mean tearing forces of all groups differed significantly, displaying a 50% lower resistance to tearing in the CM compared to the CTG group. In comparison, flap resistance to tearing forces established earlier and in higher magnitude in sites with an interpositioned CTG than in flaps repositioned on dentin or CM.

Conclusions: Application of a CTG, sutured to a non-shedding hard surface, significantly increased flap resistance to tearing when applying disrupting forces compared to controls. A less pronounced effect was achieved by interpositioning of a CM.

Clinical Relevance

Scientific Rationale for Study

Mucosal flaps, repositioned on root surfaces, are prone to disruption when tearing forces are applied. The interposition of a connective tissue graft (CTG), until now limited to recession coverage, stabilizes the flap in its coronal position.

The present animal study tested the hypothesis if a CTG or a collagen matrix (CM) can improve wound stability.

Principal Findings

An interpositioned CTG significantly increased the resistance of a flap to tearing when disrupting forces were applied. Similarly, the CM stabilized the wound but to a lesser degree.

Practical implications

The use of CTG or CM may become a basic concept for wound stabilization.

Introduction

Soft tissue healing following flap adaptation on teeth or implants is conceptually a more complex process than in most other sites of the oral cavity, as the connective tissue of the flap opposes an avascular, solid, non-shedding surface instead of another vascularized wound bed.

The healing at the interface between the flap and the debrided root surface depends on early organization and stabilization of the intervening blood clot and the establishment of an attachment resistant to mechanical tearing (Wikesjö & Nilvéus 1990; Wikesjö et al. 1991 a). Depending on the stability and integrity of the blood clot, increased tensile forces are required to jeopardize the adhering blood clot resulting in a rupture between the flap and the wound bed (Wikesjö et al. 1991 b).

In clinical settings, a less hostile wound bed might be encountered, when flaps are replaced on denuded root surfaces to cover mucosal recessions. Several systematic reviews summarize the results, which may vary regarding complete root coverage (CRC), depending on the surgical technique applied (Roccuzzo et al. 2002; Oates et al. 2003; Clauser et al. 2003; Pagliaro et al. 2003; Cairo et al. 2008; Chambrone et al. 2009; Chambrone et al. 2012; Cairo et al. 2014). The best results regarding CRC have been reported for the combination of a coronally advanced flap with an underlying connective tissue graft (CTG) (Cortellini et al. 2009). It may be speculated that the CTG, firmly sutured to the neighbouring periosteum or gingiva, may positively influence the mechanical properties of the wound and stabilize the mucosal flap by improving blood clot adhesion and maturation on the avascular dentinal surface. Without a CTG, the residual flap tension may directly affect the stability of the blood clot and the attachment to the root surface, even if it is very low, and result in a higher risk for a rupture between the root surface and the flap.

Commonly, the connective tissue graft is harvested from the palatal area and hence, a second surgical site has to be accessed. A recent study on self-reported pain perception of patients after mucosal graft harvesting in the palatal area shed light on the causative factors of patient morbidity (Burkhardt et al. 2015). The thickness of the residual soft tissue layer in the donor

site and not the wound surface area has been shown to be the main determinant of perceived pain. Irrespective of these findings, replacement of CTGs by biomaterials in order to reduce patient morbidity has become popular, and the results regarding CRC after application of collagen matrices were similar to those with autogenous tissues (McGuire & Scheyer 2010; Cardaropoli et al. 2012).

It is the aim of the present study to test the hypothesis if an interpositoned CTG or a collagen matrix (CM) mechanically improved flap stability following mucosal coverage of bone dehiscence defects. The stability is measured by the resistance of the flap to tearing, when tensile forces are applied at different postoperative time points.

Material and Methods

Animals

Five male Beagle dogs (age < 1year, weight ranging from 12.5 kg to 16.5 kg) were selected for the present study. The experimental protocol was submitted to and approved by the Local Ethic Committee for Animal Research (Cáceres, Spain) and the study was subsequently performed according to the ARRIVE guidelines (Kilkenny et al. 2011) at the Jesus Usón Minimally Invasive Surgery Center (Cáceres, Spain).

Starting two weeks prior to the surgeries, the teeth of the dogs were carefully cleaned with a toothbrush and dentifrice once per day, five days per week. At the day of surgery, the following procedures were performed in all animals.

After preoxygenation for five minutes, all animals received intravenous induction with propofol 6.5 mg/kg (Propofol®, Hospira Inc., Lake Forest, IL, USA). The trachea was intubated using flexible metallic cuffed endotracheal tubes (UnoFlex™, Unomedical, Sungai Petani, Malaysia) and the lungs mechanically ventilated to maintain normocapnia by a closed circuit anaesthetic breathing system (Leon, Heinen & Löwenstein, Bad Ems, Germany). For

anaesthetic maintenance, all animals received sevoflurane (Sevorane®, AbbVie GmbH, Vienna, Austria) at 1.2 minimum alveolar concentration in oxygen (fresh gas flow 0.5 litres/minute). To provide adequate multimodal analgesia during maintenance, 1 mg/kg ketorolac (Toradol®, Hoffmann-La Roche, Bâle, Switzerland) combined with 2 mg/kg tramadol (Adolonta®, Grünenthal Pharma AG, Mitlödi, Switzerland), both of them 5 minutes after induction, and 0.01 mg/kg buprenorphine (Buprex®, RB Pharmaceuticals Limited, Madrid, Spain) 10 minutes after induction, were administered intravenously before starting the surgery. Fluidtherapy was supported with intravenous Ringer lactate (LacRinger®, B. Braun, Sempach, Switzerland) at 2 ml/kg/hour in all animals.

During the anaesthesia, cardiac, haemodynamic and respiratory parameters were monitored (GE Dash™ 4000, General Electric, Milwaukee, WI, USA). Once the anaesthesia was finished, as determined by the time, the vaporizer was switched off and spontaneous breathing and reflexes progressively recovered in all animals, allowing all dogs to stand and walk.

During the experimental period, the animals were kept in individual cages on hard soil runs and had free access to chow (soft diet) and tap water.

Surgical procedure

A total of 12 sites per dog were chosen for defect preparation. The sites provided a suitable dimension for flaps to be completely replaced on dentin. Vertical incisions of a length of 15 mm were placed in the maxillary and mandibular C, ${}_3{}^3P^3{}_3$ and ${}_4{}^4P^4{}_4$ areas, using a double bladed scalpel with blades mounted 8 mm apart. Full-thickness flaps were elevated beyond the mucogingival junction and bone dehiscence defects of 8x8 mm dimension were created by removing the buccal bone and by scaling the root surfaces (Fig. 1).

Subsequently in each dog, one connective tissue graft (CTG) was harvested from the palatal area as a free masticatory composite mucosal graft from the zone between the bilateral $_3P_3$. After harvesting, the epithelial layer of the graft was removed extraorally with a sharp blade, and the graft was thinned to a thickness of less than one millimeter by cutting the inner part of the graft. The piece of connective tissue was further trimmed into four single grafts of 8x8 mm corresponding to the dimensions of the bone dehiscences.

To reduce morbidity and avoid bleeding, the palatal wounds were covered with a collagen fleece (TissueFleece® E, Baxter, Unterschleissheim, Germany), firmly sutured to the denunded donor area.

In the control sites (one aspect per quadrant), the flaps were elevated and the bone dehiscence defects created. After scaling of the root surfaces, the flaps were closed immediately (control group) and sutured to the neighbouring non-mobilized gingiva with interrupted sutures (Fig. 2) using non-absorbable 7-0 threads (Mopylen®, Resorba Medical GmbH, Nürnberg, Germany).

The remaining 8 sites in each dog were allocated to either test group (CTG or CM). In the former, a CTG was placed on the dehiscence defect (Fig. 3) and firmly secured to the wound bed with crossed mattress sutures (Glycolon®, Resorba Medical GmbH, Nürnberg, Germany). In the CM group, the same procedure was performed using a collagen matrix (Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland) (Fig. 4). Subsequently, after periosteal incisions in both groups, the flaps were slighty coronally advanced to completely cover the underlying devices and to be passively secured in their original positions with 7-0 interrupted and sling sutures (Mopylen®, Resorba Medical GmbH, Nürnberg, Germany). In each surgical site, light pressure was applied with a cotton swab for two minutes to ensure proper flap adaptation.

Each dog thus provided four flaps replaced on debrided dentin (control group) and four flaps each replaced on either the interpositioned CTG or the interpositioned CM, respectively. The study design balanced time point and location between available sites and dogs. For each evaluation time and treatment modality an n=5 was available.

Clinical measurements

On days 1, 3, 7 and 14 postsurgically, the animals were anaesthetized, the sutures were removed, and the releasing incisions retraced in the visible incision lines or scar formations with a surgical blade No 15 (Swann Morton™, Sheffield, UK). To avoid flap tearing, two instead of one prefabricated hooks connected to 4-0 threads were fixed in the marginal gingiva, three millimeters apically to the mucosal margin. To measure the adherence strength of the flaps to the underlying wound beds, a force directed in the long axis of the teeth, was applied to the flaps. The force was measured with a microprocessor force gauge (AFG, Mecmesin, West Sussex, UK) which was set to record and automatically save the highest reading. Progressive force was applied until the flaps were completely separated from the underlying wound beds. The adherence or tensile strengths of the flaps to their wound beds were characterized by the applied disruption forces, divided by the surface area of the dehiscence-type bony defects (64 mm^2).

Thus, each of the five dogs provided one control and two test sites (CTG and CM) for each time point of evaluation.

Statistical methodology

Flap stability was measured by the resistance of the flap to tearing, when tensile forces were applied at different postoperative time points. The initial sample consisted of 60 force

measurements from 12 sites per dog in five dogs. Each dog provided 12 sites, 4 per time point (days 1, 3, 7 and 14) and 3 per treatment (Control, CTG, CM).

The analysis was composed of two parts, namely groups and evaluation points:

1. For each evaluation point, differences in adherence forces among groups were tested for significance (Control, CTG, CM).
2. For each treatment, differences between adherence forces for various time points were tested for significance (day 1, 3, 7, 14).

A bivariante analysis was performed. To assess normality, the Shapiro-Wilk test was applied. Depending on the distribution pattern of the data (normally distributed or not), parametric or non-parametric tests were applied:

- One-way ANOVA for independent samples, if the mean of a parameter was normally distributed
- Kruskal-Wallis test for independent samples, if the distribution of the parameter was not normally distributed,
- Mann-Whitney test for two independent samples, if the distribution of a parameter was not normally distributed over time. The level of significance used in the bivariate analyses was set at 5% ($\alpha \leq 0.05$).

Results

Comparison of adherence forces by evaluation points (time points)

The one-way ANOVA (p-value $0.000<0.05$) revealed significant differences between the mean forces applied at days 3, 7 and 14 (Fig. 5). Between day 3 and day 7, the mean forces increased threefold and doubled between the last two evaluation points (day 7 and day 14). When looking at the median forces for flap disruption for each treatment within the course of the healing time (days 1, 3, 7 and 14) (Fig. 6) (mean values and standard deviations are listed

in Tab. 1), a different pattern with regard to tearing resistance of the flaps could be observed between the treatments. While a similar increase of resistance to tearing forces was noted for all the three treatments between the postoperative days 1 and 3, the sites belonging to the CTG group gained the highest amount of wound strength between days 3 and 7 (Fig. 6). In contrast, the flap resistance to tearing in the control and the CM group slightly, but significantly increased between days 3 and 7. A marked gain in the second week of healing was found in all three treatment groups as well. For comparison, on day 7, the median flap disruption force in the CTG group reached 6.03 N. This value was not reached in the control group before two weeks of healing. In comparison, at the end of the experiment, the median force required for flap disruption in the CM group yielded 8.2 N.

Between the first two evaluation points, only minor, but significant increases in median disruption force could be detected for the CTG and CM group. All longitudinal increases in all treatment groups reached statistical significance with the exception the control group between days 1 and 3 (Table 2). The forces applied for flap disruption were highest in the CTG group at all evaluation points

Comparison of applied forces between the treatments

Mann-Whitney tests for each pair of treatments yielded statistically significantly higher forces in the CTG group compared to the controls, whereas the forces in the former did not differ statistically from those of the CM group.

The average forces that were applied to provoke rupture of the flaps by treatments and time points of evaluation, are listed in Table 1.

On the first postoperative day, no significant differences in the magnitude of applied forces could be detected between the three groups (Table 3). After an initial healing phase of three days, the forces for flap disruption in the CTG group were significantly higher than those of

the control group. A similar relationship could not be detected for the other two comparisons (control versus CM group; CTG group versus CM group).

On the seventh postoperative day, the forces in all three groups differed significantly from each other (Table 3). The highest value was in the CTG group (6.03 N), followed by the CM group (3.26 N) and the control group (2.31 N) (Fig. 6).

It is important to point out the pronounced differences in forces that had to be applied for flap disruption in the three groups after one week of healing. Compared to the control group, the interpositioned grafts in the CTG group increased the required forces for flap disruption by almost threefold. The corresponding comparison with the CM group still revealed a twofold higher resistance to flap tearing (Fig. 6).

Comparing the treatments two weeks after the surgical interventions, the distribution pattern of the results was similar to that of day 3, the differences reaching statistical significance between the control group and the CTG group (Table 3).

Discussion

The results of the present study have clearly demonstrated that the interface between a debrided, non-shedding surface and the mucoperiosteal flap was less resistant to tensile forces than a comparative interface between a flap and a connective tissue layer or a collagen matrix firmly affixed to the denuded root surface. Obviously, the interpositioning of a CTG or a CM improved wound strength.

The tensile strength of a mucogingival flap-tooth interface as a measure of the structural integrity of the wound has previously been investigated in animals (Hiatt et al. 1968; Werfully et al. 2002).

Mucoperiosteal flaps with full gingival retention have been raised in Mongrel dogs (Hiatt et al. 1968) and healing was histologically observed at 2 and 3 days, 1, 2 and 3 weeks and 1, 4

and 6 months postoperatively. The authors concluded that, in early wound healing stages, flap resistance to tearing forces was mainly influenced by the epithelial attachment and not by the fibrin clot. On the contrary, healing seemed to be delayed by large accumulations of fibrin, and repair was accelerated when flaps have been tightly adapted to the roots. By two weeks, the applied force of 1'700 g (17 N) pulled the suture through the gingival margin while the flaps were only partially separated from the teeth and remnants of epithelial cells remained on the root surfaces (Hiatt et al. 1968).

Similarly to the design of that to the present study, rectangular flaps were repositoned on either a denuded root surface or on bone (Werfully et al. 2002). Subsequently, the sutures were removed one week after the surgical intervention, and the resistance to disrupting forces of the flaps was assessed. At all evaluation points, the mean tensile strengths were markedly weaker for the dentinal-flap than for the bone-flap interfaces. On day 7 after the surgery, flap adhesion to bone was almost three times as strong as that to dentin. After day 14 and up to day 28, the difference between the mean forces in the two treatments had decreased. In some specimens, in which adherence forces beyond 10.0 N were recorded, the attachment hooks at the marginal gingiva were pulled out and rendered a measurement impossible.

The coincidence in applied wound rupturing forces with the study mentioned above was limited to the measurements in the first postoperative week. At two weeks postsurgically, the mean force for wound rupture yielded a value of 12.9 N, while the corresponding value in the present experiment was 50% less with a mean force of 6.34 N. Such differences are difficult to explain. One factor influencing the tensile strength may be the time point of suture removal. While the sutures were removed before retracing the insicion lines in the present study, all sutures in the study mentioned, were removed on the seventh day postoperatively.

Early suture removal appears to increase the tensile strength of the wounds as demonstrated in several *in vitro* and *in vivo* experimental studies (Myers et al. 1969; Morin et al. 1989; Burgess et al. 1990).

The fact that closing a flap under tension resulting in a more stable wound may be disconcerting to most surgeons, because it contradicts the tenet of passive wound closure. It must be pointed out that these results are *not* in conflict with fundamental principles of surgery (Burkhardt & Lang 2014). Wounds closed under tension are still more likely to disrupt during the early periods of healing (Burkhardt & Lang 2010), especially those comprising a delicate wound bed such as denuded dentin.

In a clinical study on the role of flap tension in primary wound closure of mucoperiosteal flaps (Burkhardt & Lang 2010) it was shown that forces excercised to the flap margins during wound closure affected the occurrence of wound dehiscences after a one-week healing period. Once a threshold of 0.1 N was exceeded, a substantial increase in the frequency of wound dehiscences was observed. Surprisingly in some sites, wound dehiscences did not occur despite the fact that higher closing forces were applied. It has to be realized that these outcomes were observed in conjunction with the placement of a CTG indirectly supporting the hypothesis that an interpositioned CTG may improve wound stability.

In periodontal plastic surgery, mucosal and mucoperiosteal flaps often have to be advanced for coronal repositioning. As a consequence, the functional forces on the flap increase and are transmitted to the fragile maturing fibrin clot, resulting in a higher risk for wound rupture. Indirect evidence for the susceptibility of the blood clot to be jeopardized by mechanical tensile forces can be drawn from a previous randomized prospective clinical trial (Pini-Prato et al. 2000). The influence of residual tension within flaps before suturing was assessed in patients treated for Miller Class I maxillary recessions (Miller 1985). On one side, coronally

advanced flaps were replaced and sutured under regular residual tension. On the control side, the flaps were further relaxed before suturing. Although the residual tension was only 6.5 g on the test sides, it was as low as 0.4 g on the control sides. After 3 months of healing, the mean root coverage at test sites was 78%, with CRC in 18% of the subjects. However, the mean root coverage on the control side was 87% with 45% of the subjects yielding complete coverage. Although the difference in residual flap tension was minimal (approximately 6 g), the influence on the reduction of recession was significant. This, again, indicates the necessity of a tension-free flap closure providing an undisturbed maturation of the underlying blood clot.

Moreover, in the present experiment, wound tensile strength substantially increased as time increased. This maturation occurred with all three treatments and not only the interpositioning of a CTG, but also the use of a collagen matrix caused an increase of the forces required to disrupt flaps from their wound bed compared to flap replacement without the interpositioning of a tissue layer. The concept of wound stabilization, first described by Wikesjö et al. (1990) and Haney et al. (1993) might explain the fact that an interpositioned connective tissue or collagen layer may absorb tensile forces and prevent transmission of such forces to the underlying delicate fibrin clot. The firm adaptation and tight suturing of the CTG or CM to the dentin surface allowed an undisturbed coagulation cascade and protected the maturing fibrin clot from shear forces.

The results of the present study should be viewed in the light of obvious limitations and shortcomings of the study. In order to ascertain tensile strength values of three treatment modalities at five different postsurgical time points, canines and premolars ($_3{}^3P^3{}_{3,\,4}{}^4P^4{}_4$) have been included as surgical site units. Whereas on canines and $_4{}^4P^4{}_4$ units, flaps could be completey repositioned on dentin, at $_3{}^3P^3{}_3$ premolar sites, the wound bed consisted of dentin,

minor areas of bone in the furcations and the bordering periodontal ligament. Considering this fact in the statistical analysis, results have been stratified in two groups (canines and $_4^4P^4_4$ *versus* $_3^3P^3_3$). No differences in applied disruption forces could be detected between the groups. Hence, the pooling of all surgical sites appeared to be relevant and realistic. Furthermore, the sample sizes after stratification would have been too small to reveal any differences in disruption forces on the two different wound beds.

In the present study, the flaps were detached from either dentin, connective tissue or collagen matrix surfaces. The actual interface of soft to hard tissues was not studied. However, it is reasonable to assume that these specimens parted from the underlying tissues or biomaterials at the junction of the original flap. An interesting observation, however, could be made in the CM group by inspecting the inner aspect of the flaps after tearing. In some specimens, the collagen matrix was breaking within the collagen layer leaving parts of it on the wound bed and other remnants on the flap. This, in turn, means, that the CM was the weakest link and hence, it may be hypothesized that technical improvement of such biomaterials may help to increase wound strength in the early critical phases of healing.

Another important aspect, which may be of clinical relevance relates to the composition of the CTG. The varying composition of the palatal mucosa in humans leads to the assumption that the harvesting site may have an impact on graft structure and composition as well as on the behaviour in the recipient wound bed (for review see Zuhr et al. 2014).

In summary, the present study clearly demonstrated that the interposition of a CTG between flaps and debrided root surfaces substantially increased the wound strength as measured by the resistance of the flaps to tearing when tensile forces were applied. A similar effect, but to a smaller extent, could be achieved by the placement of a CM. The significant differences in wound strength depending on the treatment documented the necessity to define appropriate evaluation points for suture removal.

Histological studies have documented a protective effect of flap stabilization on fibrin clot formation and adhesion to non-shedding surfaces (Wikesjö et al. 1991 b, 1991 c, 1991 d). This, in turn, has a positive impact on subsequent tissue maturation and remodeling. In conclusion, the use of a CTG or a CM as an alternative, until now limited to the coverage of root recessions (Chao 2012), might become a basic principle for wound stabilization in periodontal and peri-implant surgical procedures.

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Figure Legends:

Figure 1: Buccal aspect of an upper canine. The bone has been removed and the root surfaced carefully scaled to create a bone dehiscence defect of 8x8 millimeters. The marginal gingiva appears healthy due to the preoperative tooth brushings.

Figure 2: Wound closure in a control site (no interpositioned graft nor collagen matrix). The flap is passively replaced on the scaled root surface and secured with interrupted sutures, using non-absorbable 7-0 threads.

Figure 3: Connective tissue graft, positioned on bone dehiscence defect of a lower canine, before suturing to neighbouring tissues.

Figure 4: Collagen matrix (Mucograft®), positioned on bone dehiscence defect of a lower premolar, before suturing to neighbouring tissues.

Figure 5: Mean forces (N) applied for all 60 tested sites by each time point of evaluation (days 1, 3, 7, 14 postsurgically).

Figure 6: Median forces (N) applied, by each treatment and each time point of evaluation.

Table 1. **Forces (N) applied to detach flaps from the wound beds, listed by groups (Control, CTG, CM) and time points of evaluation (days)**

	Control group *Flap alone*				***CTG group*** *Flap + CTG*				***CM group*** *Flap + collagen*			
	N	**Mean**	**SD**	**Median**	**N**	**Mean**	**SD**	**Median**	**N**	**Mean**	**SD**	**Median**
		Forces measured in N				*Forces measured in N*				*Forces measured in N*		
Day 1	**5**	**0.69**	**0.53**	**0.45**	**5**	**1.12**	**0.32**	**1.13**	**5**	**0.76**	**0.28**	**0.89**
Day 3	**5**	**0.79**	**0.30**	**0.83**	**5**	**1.87**	**0.39**	**1.68**	**5**	**1.35**	**0.37**	**1.41**
Day 7	**5**	**2.27**	**0.48**	**2.31**	**5**	**6.64**	**1.03**	**6.03**	**5**	**3.23**	**0.53**	**3.26**
Day 14	**5**	**6.34**	**1.39**	**6.12**	**5**	**10.21**	**1.88**	**9.10**	**5**	**8.00**	**0.88**	**8.22**

Table 2. **Comparison of forces between the groups for all time points of evaluation (Kruskal-Wallis test) and the comparisons by paired moments (Mann-Whitney test);**

significant p-values (*)

Group	***p-value*** *Kruskal-Wallis*	***Moments***	***p-value*** *Mann-Whitney*
Control Group *flap alone*	**0.001***	Day 1 vs. Day 3	**0.690**
		Day 1 vs. Day 7	**0.008***
		Day 1 vs. Day 14	**0.008***
		Day 3 vs. Day 7	**0.008***
		Day 3 vs. Day 14	**0.008***
		Day 7 vs. Day 14	**0.008***
CTG Group *flap+CTG*	**0.000***	Day 1 vs. Day 3	**0.008***
		Day 1 vs. Day 7	**0.008***
		Day 1 vs. Day 14	**0.008***
		Day 3 vs. Day 7	**0.008***
		Day 3 vs. Day 14	**0.008***
		Day 7 vs. Day 14	**0.008***
CM Group *flap+collagen*	**0.001***	Day 1 vs. Day 3	**0.056**
		Day 1 vs. Day 7	**0.008***
		Day 1 vs. Day 14	**0.008***
		Day 3 vs. Day 7	**0.008***
		Day 3 vs. Day 14	**0.008***
		Day 7 vs. Day 14	**0.008***

Table 3. **Comparison of forces between groups for each time point of evaluation (Kruskal-Wallis test) and the comparisons by paired groups (Mann-Whitney test);**

significant p-values (*)

Moments	***p-value*** *Kruskal-Wallis*	***Groups***	***p-value*** *Mann-Whitney*
Day 1	**0.224**	control group / CTG group	**------**
		control group / CM group	**------**
		CTG group 1 / CM group	**------**
Day 3	**0.010***	control group / CTGgroup	**0.008***
		control group / CM group	**0.056**
		CTG group / CM group	**0.095**
Day 7	**0.003***	control group / CTG group	**0.008***
		control group / CM group	**0.032***
		CTG group / CM group	**0.008***
Day 14	**0.010***	control group / CTG group	**0.008***
		control group / CM group	**0.095**
		CTG group 1 / CM group	**0.056**