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Connective Tissue Grafts and Soft Tissue Substitute for Multiple Gingival Recessions - Review, Clinical and Histology

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Abstract:

Aim: This study aims to conduct a review on the efficacy of the use of a modified coronally advanced flap (MCAF) with a connective tissue graft (CTG) compared to MCAF and Mucograft® (MG) in terms of complete root coverage (CRC), after a minimal 6-month follow-up in patients with maxillary multiple gingival recessions (MGR), and post-operative pain in patients that underwent CTG harvesting will be determined. CTG obtained from patients and also a soft tissue substitute (MG) implanted in mice at 15 and 30 days will also be histologically characterized.

Material and Methods: A bibliographic review was conducted through an electronic and hand search. Eligibility of the resulting articles was assessed through title and abstract analysis and subsequently full-text-analysis by two independent reviewers. Primary (CRC) and secondary outcomes (recession reduction (RecRed) and keratinized tissue(KT) gain) were evaluated.

Two patients with maxillary Cl I or II MGR on adjacent teeth that needed root coverage were included in this study. One underwent MCAF/CTG while the other MCAF/MG. Post-operative pain questionnaires were handed out to 6 patients that experienced CTG harvesting. Primary (CRC) and secondary (mean of root coverage (MRC) and post-operative pain) outcomes were evaluated at 6-months post-operative.

Palatal biopsies of the CTG donor site were obtained for posterior histomorphometric evaluation, and MG was implanted subcutaneously in mice with subsequent histological evaluation at 15 and 30-days post-implantation.

Results:

After an extensive search, only 3 studies were included, in which 1 reported the use of MCAF/MG. The studies were only comparable at 12-months, where MCAF/CTG obtained a mean CRC value of 73.7% while MCAF/MG attained 88.1%.

The patients that underwent MCAF/CTG and MCAF/MG responded well to the surgical treatment. Healing was uneventful and the 1-week post-operative pain was low in both approaches. At the 6-month evaluation, CRC was obtained in 75% of the treated sites with MCAF/CTG.

Patients who underwent CTG harvesting, regardless of the surgical technique reported a low pain intensity that subsided by the fourth day after surgery. Five in six patients referred that both the donor and the receptor site hurt equally.

Two palatal biopsies revealed tissue with a highly dense connective tissue, the lamina propria (LP), and tissue also dense in connective tissue but with a greater presence of adipose tissue, the submucosa (SM). The histologic evaluation of MG in mice showed a

well-integrated membrane with increasing remodulating and formation of new vascular structures from 15 to 30 days.

Conclusion:

More studies with standardized outcomes and follow-ups are needed to determine which approach, MCAF/CTG or MCAF/MG, is more efficacious after 6-months post-operative. It can also not be assessed whether MCAF/MG will have the same tendency for a coronal shift of the gingival margin that MCAF/CTG has over time.

A larger number of included patients with the same follow-up period would be necessary to draw conclusions about the CRC at 6-months post-operative. Low pain levels were reported and although the donor site may not necessarily be the cause of more pain, more investigation is needed with a larger amount of standardized patients.

The palatal biopsies confirmed that the LP had dense connective tissue and enough thickness for its use as a CTG, while the SM had more adipose tissue, even if some variability was observed. The implanted MG revealed optimal integration, its bilayered structure acted as barrier for preferential tissue ingrowth.

Key words: Multiple gingival recessions, Modified coronally advanced flap, Connective tissue graft, Mucograft, Histology, Complete root coverage, Morbidity.

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1. Introduction

1.1. Gingival Recessions

A gingival recession (GR) is defined as the displacement of the soft tissue margin apical to the cemento-enamel junction (CEJ)(2). They are a frequent reality ranging from 40 - 88% of the population that present some sort of recession(3-5). There is a tendency for cases to increase with age and are more common in periodontaly affected subjects and smokers, but can also be found in up to 42.7% of the population with good oral hygiene but bad brushing technique when it is commonly located at the buccal surfaces(3, 4, 6).

Gingival recessions have a very vast aetiology. Many anatomical conditions can be the source of GR including frenulum pull, thin gingival biotype, teeth prominences, fenestration or dehiscence of the alveolar bone, aberrant paths of eruption of the tooth and direct trauma from malocclusion. Traumatic tooth brushing due to incorrect brushing and/or flossing is also very common and generally creates multiple recessions. Other traumatic agents like intra-oral body-piercings and iatrogenic factors such as orthodontic movement or the placement of restorations on exposed roots can originate GR. Unsatisfactory plaque control and the associated inflammatory response is also associated with GR(6-11).

The exposure of the root surface to the oral cavity due to the displacement of the gingival margin apical of the CEJ is associated to loss of periodontal connective tissue fibres, tooth cementum and alveolar bone(12). The undesirable aesthetical and functional consequences of GR on patients (hypersensitivity to tactile and thermal stimuli, difficulties in achieving plaque control, cervical root caries/abrasion), can influence them to search for procedures that can help combat and reverse these unwanted effects(6, 11, 13). Aesthetically, patients are easily dissatisfied with the presence of GR since the lengthening of the clinical crown may be visible while smiling or even talking. This is often the primary complaint as aesthetics has an ever increasing value in any dental treatment. Hypersensitivity is often a manifestation, although if no aesthetic complaint is mentioned, a less invasive treatment through the local application of chemical desensitizing agents can be applied. The exposure of the root can lead to cervical root caries or abrasion defects, exacerbating the hypersensitivity and an overall negative prognosis over time and should therefore be treated with either surgical or combined restorative and surgical means(2, 6).

Despite initially being thought that a minimal width of KT would be necessary to maintain periodontal tissues healthy and stable, it was later concluded that the biological significance of a sufficiently wide KT was doubtlessly overrated in the past(14). Although

important, the absence of attached KT is compatible with the maintenance of periodontal health. However, it's absence will decrease the gingival resistance to the presence of inflammation or tooth-brushing trauma and difficult KT tissue or even the narrow nature of the recession is considered an indication for treatment of GR(6, 15).

1.2. Periodontal Plastic Surgery and Root Coverage

Periodontology has suffered many evolutionary steps that have helped advance our knowledge and provide the best possible treatment to patients(16). One of these steps was the evolution of mucogingival surgery to plastic periodontal surgery. Mucogingival surgery was initially presented by Freidman in 1957 and referred to as surgical procedures designed to preserve gingival tissue, remove aberrant frenal or muscle attachments and increase the depth of the vestibule (6). The term periodontal plastic surgery was introduced by Miller in 1993 and was later defined by the American Academy of Periodontology in 1996 as surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease-induced defects of the gingiva, alveolar mucosa or bone(6, 17, 18). Among the various surgical procedures that this entices, root coverage is one of the treatments that may resolve GR.

Root coverage can be approached in a wide manner of ways but the ultimate goal is to obtain complete coverage of the recession defect with a good appearance in comparison to the adjacent soft tissues and minimal probing depth following healing, which should be achieved through regeneration and not repair(2, 6). Recession defects can simply be treated by surgical procedures classified as: A) Pedicle soft-tissue graft procedures (A.1-Rotational Flap Procedures (Lateral Sliding Flap, Double Papilla Flap, Oblique Rotated Flap); A.2-Advanced Flap Procedures (Coronally Repositioned Flap; Semilunar Coronally Repositioned Flap); A.3-Regenerative Procedures (with barrier membrane or application of enamel matrix proteins); B) Free Soft-tissue graft procedures (B.1-Epithelized graft; B.2-Subepithelial connective tissue graft))(19).

The surgical approach chosen to obtain root coverage depends on the defect, the patient and the current literature. The defect holds factors in its size, number of recession defects, the presence/absence, quantity/quality of KT, the width and height of the papillae, the presence of frenum or muscle pull and the vestibule depth(6). The patient on the other hand shall determine the importance of the aesthetic outcome as well as the post-operative discomfort, pain and the overall morbidity they are willing to endure. Moreover, the current

literature available should be reviewed by the clinician in order to select the most predictable approach(6, 20).

One of the major prognostic factors in the treatment of GR with root coverage is the type of recession that is going to be treated according to the Miller classification and being able to determine the prospect of complete root coverage or not. According to Miller *et al,* 1985,(21) Class I and Class II GR have no loss of interproximal periodontal attachment and bone and complete root coverage can be achieved(2, 6). Class III GR have mild to moderate loss of interdental periodontal support and therefore only partial root coverage can be accomplished(6). Finally, Class IV GR has severe loss of interproximal periodontal attachment and no root coverage is viable(2, 6). Other prognostic factors include the location of the recession, whether it's unitary or multiple, its width and depth, the flap thickness and the operator skill(2, 20, 22).

Regarding unitary or single recessions, many studies reveal that the use of a CTG in conjunction with coronally advanced flap (CAF) is the most predictable surgical procedure in terms of root coverage(11, 23, 24). This is due to CAF/CTG being a bilaminar technique that provides the graft with a greater blood supply from the covering flap, resulting in the increased survival of the graft above the avascular root surface and improving the aesthetic outcome(2). CAF allows a coronal shift of soft tissues apical to the exposed root by creating vertical incisions lateral to the recessed area, beginning at the point apical to the papilla tip, extending well into the alveolar mucosa, and a sulcular incision and sharp dissection close to the periosteum, allowing a split-thickness flap elevation that reaches the alveolar mucosa(2). The epithelium is then removed from the papillae adjacent to the recession, the CTG is placed and immobilized with sutures and the flap is coronally positioned and stabilized with sling sutures and simple sutures close the vertical releasing flaps.

However, due to their traumatic nature, GRs are rarely localized to a single tooth(3, 6). Multiple defects present a further challenge as the surgical field is larger and more prone to anatomical variations (prominent roots, shallow vestibules and different defect sizes)(4). Several recessions should be treated in a single surgical session to reduce patient discomfort, all the while maintaining the patient's aesthetic demands(6, 25).

GR are rarely localized to a single tooth, and although no reports are available on the prevalence of single recession defects compared with multiple recession defects, clinical experience indicates a greater incidence of multiple gingival recessions (6).

Far less studies report clinical outcomes, especially long term outcomes, for the treatment of multiple recessions. In a systematic review(4), Graziani *et al.* notes that techniques that are most often evaluated for the treatment of MGR are CAF, the modified

coronally advanced tunnel technique (MCAT) with CTG and MCAF. The use of CAF can be extended to treat these multiple recessions so long as the pedicle flap is broad enough to include all of the defect and the vertical incisions will constitute the mesio-ldistal limits of the flap(2). Interestingly, when comparing CAF, in terms of CRC, to the other 2 techniques, it shows a larger range of results between 23.8% and 77.7% between 6 and 24-month follow-ups , rendering it as an irregular approach in terms of CRC outcomes(4). Meanwhile, MCAT reveals itself as a unique procedure that leaves interdental papillas intact while a CTG is placed into the tunnel created(6). According to Aroca *et al.*(26), this approach rendered a CRC of 85% at 12-months post-operative. It presents benefits due to its maintenance of interdental papilla, absence in vertical releasing incisions, minimally invasive nature and negligible post-operative discomfort but also drawbacks due to its tendency in not covering the graft completely leading to colour mismatch and the need of a microsurgical kit and surgical skill to execute the procedure(6).

Zucchelli & De Sanctis proposed a variation of the CAF technique, MCAF, based on an envelope type flap without vertical releasing incisions as a new method to treat multiple adjacent recessions which has been demonstrated to be a safe, predictable and aesthetic approach(2, 6, 24). By avoiding vertical releasing incisions, MCAF aims to preserve the vascular system and reduce potential scars caused by the vertical incisions. However, it requires the involvement of one extra tooth on each side of the treatment area to allow for sufficient flap mobility(2). This approach involves a split (at the level of the surgical papilla) – full (at the soft tissue apical to root exposure) –split (apical to bone exposure) flap. A long term study in which MCAF was used in MGR reported that at 5-years postoperative their successful outcomes remained stable with a CRC of 85% of the treated sites(27).

The association with CTG with MCAF in multiple defects reveals scarce but favourable data in terms of CRC, recession reduction (RecRed), KT gain aesthetic evaluation and post-operative course(23, 24, 28). Both the traditional CAF and MCAF technique are effective in reducing recession depth but Zucchelli´s envelope type CAF was found to be associated with an increased probability of achieving complete root coverage with a better aesthetic and post-operative course(2, 28). Beside this, Pini-Prato *et al.*(23) revealed that MGR treated with MCAF/CTG were associated with a coronal shift of the gingival margin between the 6-month and 5-year follow up, as opposed to the MCAF treated sites that suffered an apical relapse. This could be due to the thick gingival tissue obtained through the use of a CTG and shows that the use of MCAF/CTG may hold promising results in the long-term. However, this poses the question as to what type of graft should be used in order to obtain better clinical and patient-related results.

1.3. Soft tissue Substitutes

Although the use of CTG is a valuable and versatile technique, it bears noteworthy disadvantages such as the possible lack of available tissue for harvesting, the necessity of two surgical sites, a recipient site and another donor site and a longer surgical time(6, 14, 29). This is aggravated by the fact that the treatment of multiple recessions requires more donor tissue and a longer surgical time. The donor site heals by secondary intention resulting in a rather painful post-operative situation(6). For many patients this is a cause of great apprehension, increasing the burden on the patient and the surgical procedure's morbidity substantially(29). Furthermore, complications may also arise associated to the need of a second surgical site, particularly slow wound healing, bone necrosis with sloughed overlying tissues, copious bleeding during or after surgeries, profound pain and paraesthesia or permanent anaesthesia of the palate(29).

In order to avoid this high morbidity and second surgical site, soft tissue substitutes (STS) have risen in the recent years. Three basic STS of different origin can be distinguished: autogenic (of human origin), xenogenic (from another species) and alloplastic (of artificial origin). An eligible STS must be non-infectious, biocompatible, provide good tissue integration behaviour with tissue conductive characteristics, allow good clinical handling and physical stability and be economically efficient(14).

1.3.1. Mucograft®

Collagen-based materials and matrices have been explored as STS since collagen is the most abundant family of proteins in the human body and is physiologically ubiquitous(30). Apart from its natural origins, it is relatively easy to biodegrade because the neutrophils, monocytes and fibroblasts recruited during wound healing release matrix metalloproteases that result in the collagens enzymatic biodegradation(30). Mucograft® (MG) is a xenogenic collagen matrix of porcine origin that is used for soft tissue augmentation and root coverage procedures. It's obtained by standardised, controlled manufacturing processes and is made up of pure porcine collagen type I and II, extracted and purified without additional cross-linking and sterilized by gamma irradiation(31). Although cross-linking would increase mechanical stability and decrease the rate of collagen degradation (a higher rate limits the time scale over which the membrane still has barrier function), it inhibits the attachment and proliferation of human periodontal ligament fibroblasts and osteoblasts compared to native collagen, and its absence avoids severe foreign body reactions, making the lack of cross-linking an advantageous characteristic(30).

MG is engineered into a bi-layer matrix approximately 2.5 mm thick in which the outer smooth cell occlusive layer (compact layer), derived from the porcine peritoneum, is made up of tightly packed collagen fibres, enhancing elastic properties and facilitating tissue adherence, suturing to the host mucosal margins and wound healing(30, 31). The inner roughened and porous layer (spongy layer), derived from porcine skin, is designed to be placed against the host tissue to provide space for blood clot formation and tissue in-growth(31, 32). The volume fraction of pores in the matrix is of 90% and the size distribution for these pores ranges from 5 to 200 µm, with smaller pores being primarily located on the compact layer (CL)and larger pores in the spongy layer (SL)(30).

MG constitution through scanning electron microscopy imaging has shown, through cross-sections, that the compact layer has four or five floors which are orientated parallel to the surface layer, including an orthogonal direction of collagen fibres between the floors, while the spongy layer consists mainly of randomly aligned and diffusely packed collagen fibres(30, 32). Thus, Kasaj *et al*. 2015 concludes that the matrix exhibits a macrostructure facilitating the mechanical and form stability by means of a framework structure, whereas the spongy microstructure of the membrane is designed to ensure blood coagulum stability and tissue in-growth(32). Ghanaati *et al.* 2011 adds that while the porous layer permits cells to integrate and grow into the centre region of the scaffold, the compact layer inhibits connective tissue in-growth, allowing for preferential cell ingrowth, a vital characteristic of scaffolds designed for soft tissue regeneration(30).

The matrix is processed to remove antigenic cellular components without causing any damage to the tissue structure and therefore preserving the three-dimensional collagen porous matrix that mimics the biological and mechanical characteristics of a native extracellular matrix(32). The three-dimension scaffold design permits the in-growth and the re-population of fibroblasts, blood vessels, culminating in the eventual transformation into KT. Through the process of remodelling, the collagen matrix can integrate into patients' tissue without tissue reactivity and rejection following implantation(32).

Studies have shown that MG is an adequate alternative to autogenous soft tissue grafts that eliminates the need for further surgery(33). In localized GR it has shown to be as predictable and effective when combined with CAF as CTG and CAF, while other studies demonstrated that MG in conjunction with CAF was not superior with regard to root coverage, but enhanced gingival thickness and width of keratinized tissue when compared with CAF alone(7, 34, 35). However, its use in multiple recessions, especially in conjunction with MCAF, reveals very little data(3).

Apart from the clinical outcomes MG shows itself to be efficient in maintaining the marginal tissue health and colour blending but with a significantly lower patient morbidity associated(35). Additionally, it has shown excellent handling properties and allows a significant reduction in surgery time(15).

1.4. Histology

The oral mucosa has important functions as it serves as a barrier, protecting the oral cavity against microorganisms, toxins and various antigens while also having mechanical protection against compressive forces; it is sensitive to changes in temperature, pain, taste and also thirst; it regulates the temperature in the oral cavity; and it also secretes saliva from its salivary glands. Furthermore it is divided into masticatory mucosa, lining mucosa and specialized mucosa(36, 37).

The CTG that is collected comes from the masticatory mucosa that covers the hard palate and gingiva, coating all immobile structures. These tissues are exposed to many compressive forces, abrasion and attrition during mastication. Thus the need for moderately thick epithelium that is frequently orthokeratinized and wide papillae to avert any separation from the connective tissue due to masticatory forces(36).

Regarding the hard palate, it is made up of lining epithelium, LP and SM. The lining epithelium incorporates a thick orthokeratinized or parakeratinized stratified squamous epithelium organized in transversal palatine strings.

The LP contains wide papillae, highly dense and thick collagen tissue and moderate irrigation with a typical appearance of highly packed collagen fibres with sparse fibrocytes. It consists in a network of type I and III collagen and elastin fibres in some regions. The main cells of the LP are the fibroblasts, which are responsible for the production of the fibres as well as the extracellular matrix. The LP has two layers: a papillary and a dense layer. The papillary layer is the superficial layer of the LP composed of loose connective tissue within the connective tissue papillae, along with blood vessels and nerve tissue. The tissue has an equal amount of fibres, cells, and intercellular substance. The dense layer is the deeper layer of the LP with a large amount of fibres. Between the papillary layer and the deeper layers of the LP is a capillary plexus, which provides nutrition for the all layers of the mucosa and sends capillaries into the connective tissue papillae.

The SM is made up of dense collagen tissue adhered to the mucoperiosteum, adipose tissue and minor salivary glands. In the lateral regions of the hard palate, namely the molar area, the SM is intercalated with more significant areas of adipose and glandular tissue that

cushions mechanical forces and protects underlying structures like nerves and blood vessels. However, in the central region of the hard palate, where there isn't any SM, the LP is directly inserted into the mucoperiosteum(36-38).

1.5. Aim

The study had the following aims:

a) Answer to the PICOT question: in patients with multiple maxillary gingival recessions what is the efficacy of MCAF plus CTG compared to MCAF plus Mucograft in terms of CRC, after a minimal 6-month follow-up;
b) Present a clinical case description with MCAF/CTG and MCAF/MG technique to treat MGR;
c) Evaluate the post-operative pain during the first week after harvesting palatal CTG, regardless the surgical technique used;
d) Perform a histological characterization of CTG, obtained from patients, and MG, implanted at mice, at 15 and 30-days post-implantation

2. Material and Methods

2.1. Review

2.1.1. Research Protocol and Eligibility Criteria

The present research protocol was designed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement(39). This review was performed in order to answer to the following PICOT question: In patients with multiple maxillary gingival recessions, what is the efficacy of MCAF plus CTG compared to MCAF plus Mucograft® in terms of CRC, after a minimal 6-month follow-up?

2.1.1.1. Inclusion Criteria:

- Treatment of multiple maxillary gingival recessions in order to obtain root coverage;
- Recession Class I or II(21);
- Use of MCAF technique and connective tissue graft (MCF/CTG);
- Use of MCAF technique and Mucograft® (MCF/MG);
- Human studies;
- Minimum of 10 patients(4);
- Contain clinical outcomes;
- Clinical trials, longitudinal studies or comparative studies;
- Studies with high degree of evidence (meta-analysis, systematic reviews, randomized clinical trials);
- Follow-up: minimum of 6 months;
- Language of study: English or Portuguese.

2.1.1.2. Exclusion Criteria:

- Localized recessions;
- Recessions other than Cl I or II;
- Mandibular GR;
- Use of technique other than MCAF/CTG or MCF/MG;
- Root coverage is not the objective of the treatment;
- Animal studies;

- Histological and other outcomes other than clinical;
- Studies other than clinical trials, longitudinal studies or comparative studies;
- Language other than English or Portuguese;
- Follow-up less than 6m.

All studies that did not meet the inclusion criteria were eliminated.

2.1.2. Electronic Search

An electronic search was conducted for articles published between 2000 and January 2016 in various bibliographic data bases (PubMed/MEDLINE, EBSCO and Cochrane Library) using a combination of MeSH terms and free text words grouped into the intervention and the disease studied in this article as well as the study design.

- Intervention: (("connective tissue graft" (text word) OR "soft tissue graft" (text word) OR "subepithelial connective tissue graft"(text word)) OR “Mucograft” (text word)) AND “coronally advanced flap” (text word))
- Disease: “gingival recession” (MeSH term)
- Study design: “clinical trial” OR “longitudinal study” OR “comparative study”

The following electronic searches were made:

- **PubMed/MEDLINE** (http://www.ncbi.nlm.nih.gov/pubmed): The filters, “Clinical trial”, “Controlled Clinical Trial”, “Meta-Analysis”, “Randomized Controlled Trial”, “Review” and “Systematic Reviews” were applied in order to search for: (((("Connective tissue graft" OR "Soft tissue graft" OR "Subepithelial connective tissue graft") OR "Mucograft") AND "Coronally advanced flap") AND ("Gingival recession" OR Gingival recession[MeSH Terms]). From this search, 49 results were found.
- **EBSCO** (http://search.ebscohost.com): No filters were applied and the following was placed in the search box: ((("Connective tissue graft" OR "Soft tissue graft" OR "Subepithelial connective tissue graft") OR "Mucograft") AND "Coronally advanced flap") AND "Gingival recession" AND (“Clinical trial” OR “Controlled Clinical Trial” OR “Meta-Analysis” OR “Randomized Controlled Trial” OR “Review” OR “Systematic Reviews). This search resulted in 49 bibliographic references.
- **Cochrane Library** (http://www.cochranelibrary.com): No filters were applied and the following was placed in the search box under “Search all text”: ((("Connective tissue graft" OR "Soft tissue graft" OR "Subepithelial connective tissue graft") AND "Coronally advanced flap") OR "Mucograft") AND "Gingival recession" AND (“Clinical

trial” OR “Controlled Clinical Trial” OR “Meta-Analysis” OR “Randomized Controlled Trial” OR “Review” OR “Systematic Reviews). From this search, 1 result was found.

Considering the small amount of studies obtained after evaluating their eligibility, a new electronic search was conducted in the same databases, maintaining the search terms but lowering the level of evidence in order to allow clinical studies and case reports.

- **PubMed/MEDLINE** (http://www.ncbi.nlm.nih.gov/pubmed): The filters for “Case Reports”, “Clinical studies”, “Clinical trial”, “Controlled Clinical Trial”, “Meta-Analysis”, “Randomized Controlled Trial”, “Review” and “Systematic Reviews” were applied and the following was searched for: ((("Connective tissue graft" OR "Soft tissue graft" OR "Subepithelial connective tissue graft") OR "Mucograft") AND "Coronally advanced flap") AND ("Gingival recession" OR Gingival recession[MeSH Terms]). From this search, 49 results were found.

- **EBSCO**: No filters were applied and the following was placed in the search box: ((("connective tissue graft" OR "soft tissue graft" OR "subepithelial connective tissue graft") OR "Mucograft") AND "Coronally advanced flap") AND "gingival recession" AND ("case report" OR "clinical study" OR "clinical trial" OR "controlled clinical trial" OR "meta-analysis" OR "randomized controlled trial" OR "review" OR "systematic review"). This search resulted in 70 bibliographic references.

- **Cochrane Library** No filters were applied and the following was placed in the search box under “Search all text”: ((("connective tissue graft" OR "soft tissue graft" OR "subepithelial connective tissue graft") OR "mucograft") AND "Coronally advanced flap") AND "gingival recession" AND ("case report" OR "clinical study" OR "clinical trial" OR "controlled clinical trial" OR "meta-analysis" OR "randomized controlled trial" OR "review" OR "systematic review"). This search obtained 1 result.

2.1.3. Hand Search

Hand searching was performed by two reviewers (O.M., J.M.) on relevant journals (Journal of Clinical Periodontology and Journal of Periodontology) between September 2000 and to January 2016, and also within bibliographies of articles derived from the electronic search.

2.1.4. Data Collection

Eligibility was assessed through title and abstract analysis and full-text-analysis. Titles and abstracts were initially screened by two reviewers (OM and JM).

Abstracts that did not fulfil the inclusion criteria were excluded. If abstracts provided unclear results, they were included for full-text analysis. After this initial screening, full-text analysis of the included articles was performed by two independent reviewers (OM and JM), according the inclusion criteria. If analysed articles were unclear, authors were contacted directly. Possible disagreement was resolved by discussion between reviewers.

2.1.5. Outcomes

Primary outcome was CRC of all treated gingival recessions.

Secondary outcomes were recession reduction (RecRed) and keratinized tissue gain (KT). Both were expressed as the average difference between baseline and follow-up of the treated sites, in millimetres.

Qualitative patient-centred outcomes were also assessed, when present and when data was screened through the use of standardized scales, through the occurrence of complications, the post-operative pain experienced and the patients' aesthetic satisfaction.

2.2. Clinical Procedures

2.2.1. MCAF/CTG vs. MCAF/MG

2.2.1.1. Patient Inclusion Criteria

- Age > 18 years;
- Periodontaly and systematically healthy;
- Patients from the Periodontology appointment (Dentistry department, FMUC) or in private practise;
- Presence of multiple (≥2) Miller Class I or II gingival recessions on adjacent teeth that need root coverage;
- Plaque index and/or BOP (bleeding on probing) ≤ 20%(19).
- Presence of at least 1mm high KT apical to the root exposure.

2.2.1.2. Patient Exclusion Criteria

- Smoking habits;
- Evidence of parafunctions;
- Presents periodontal disease;
- Inadequate plaque control (plaque index and/or BOP>20%);
- Pregnant women;
- Patients incapable of attending all follow-up appointments,

2.2.1.3. Clinical Outcomes

A group of clinical measurements was taken at baseline and 6-months post-operative. These included:

- Periodontal depth (PD) on the mid-buccal site – distance from the gingival margin to the bottom of the gingival sulcus;
- Recession depth (Rec) on the mid-buccal site – distance between the (CEJ) and the gingival margin;
- KT height – distance between the gingival margin and the muco-gingival junction (MGJ)

  The primary outcome was CRC.

  The secondary outcomes were MRC, pain and /or discomfort during the first week postoperatively (VAS scale).

At the end of the surgery a questionnaire (Appendix B) was handed out to all patients inquiring, through a VAS scale based on Scott *et al,* 1976(40), the level of pain and analgesic intake during the first week along with the patients´ perception of surgical time and main reason for surgery. This questionnaire was handed in at 1-week post-operative.

CRC and MRC were evaluated by the operator at the 6-month follow-up of the MCAF/CTG cases.

Post-operative complications such as pain, bleeding and swelling at the donor and/or the receptor site were evaluated at 1-week post-operative.

Patient distribution was done using two identical envelopes, (one envelope with a piece of paper identified as “Mucograft”, and another envelope with a piece of paper identified as “CTG”) that were randomly chosen after identifying the patient as a candidate for this study. In the case of “CTG”, patients were treated with MCAF/CTG, while cases with “Mucograft” were treated with MCAF/MG.

2.2.1.4. Surgical Procedure

2.2.1.4.1. MCAF/CTG:

Zucchelli & De Sanctis´ (2000) MCAF procedure for multiple recessions(41) was used to treat the recession defects. Initially, the gingival recessions were measured and taken note of in order to make the appropriate incisions. After administrating local anaesthesia, chlorhexidine in a gel form was applied at the surgical site and an intramuscular incision was executed, involving at least one tooth mesial and at least one tooth distal to the teeth with gingival recessions. Oblique submarginal incisions were performed to unite these intrasulcular incisions taking into account the measurements that were previously taken. Hence, the value of the recession on a tooth plus 1mm was transferred to its adjacent papilla and served as a guide as to where to start the oblique submarginal incisions considering it should end at the zenith of the adjacent tooth (Figure 1). This was done to all the teeth involved in the defect, therefore uniting the intrasulcular incisions. After these incisions a split-full-split flap was made (split at the level of the surgical papilla, full at the soft tissue apical to root exposure and split again apical to bone exposure). This approach allows, in the full thickness section, the inclusion of the periosteum and the maximum soft tissue thickness in the central portion of the flap covering the avascular root exposure. Meanwhile, the final split thickness flap permits the coronal advancement of the flap, making sure to eliminate all the muscle insertion present. The anatomical papillas were deepithelialized, leaving the receptor site ready for the CTG.

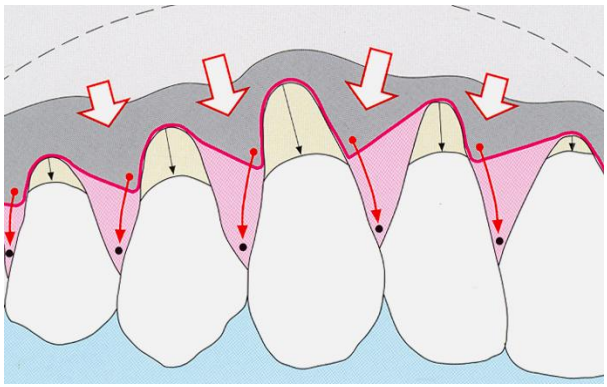

Figure 1 - Schematic representation of MCAF showing the intrasulcular and oblique incisions and the coronal repositioning of the flap. (adapted from: Rateitchack, 2005(1)).

The CTG was collected from the palate at the premolar region by Bruno's modified technique(42), taking care to respect the anatomical conditions in order to avoid the neurovascular bundle. The palatal area mas measured and probed so that the CTG harvested had about 1.5mm in width(43). With the CTG harvested, epithelial and adipose

tissue was removed and any excess tissue was cut so that the CTG fit adequately on the receptor site. The donor site was sutured with sling sutures (silk non-reabsorbable 3/0 sutures) to stabilize the flap created and encourage healing.

The CTG was transferred to receptor site and positioned 1mm apical to the CEJ covering the entire defect and interdental connective tissue bed. It was held in place with simple sutures (absorbable monofilament, PGA ,5/0; Surgilactin; Sutures Ltd; UK) in a way that it covered the defect. The buccal flap was positioned coronally to cover the CTG, surpassing the CEJ by 1mm, taking care to stabilize each surgical papilla over the interdental tissue bed and was secured in position by sling sutures (synthetic non absorbable-monofilament, Polypropylene, 5/0; Premilene; B. Braun; Germany).

2.2.1.4.2. MCAF/MG:

Zucchelli & De Sanctis´ (2000) MCAF procedure for multiple recessions(41) was also used to treat the recession defect in the same manner as previously described.

A second surgical site was avoided, instead the MG was trimmed to the size of the defect and adapted to the receptor site and held in place 1 mm apical to the CEJ with vertical crossing mattress sutures (absorbable monofilament, PGA ,5/0; Surgilactin; Sutures Ltd; UK) that did not pass through the matrix but served to maintain and stabilize the matrix in place (Figure 2). Suture compression was avoided in the grafted material that would later be embedded by blood flow. Similarly as previously described, the flap was advanced coronally, exceeding the CEJ by 1mm, and sutured to cover the underlying material completely using sling sutures that passed through the interdental papilla (synthetic non absorbable-monofilament, Polypropylene, 5/0; Premilene; B. Braun; Germany) (Figure 3).

In all the patients informed consent (Appendix D) was received to use their clinical data as well as the photos taken throughout surgery and in controls.

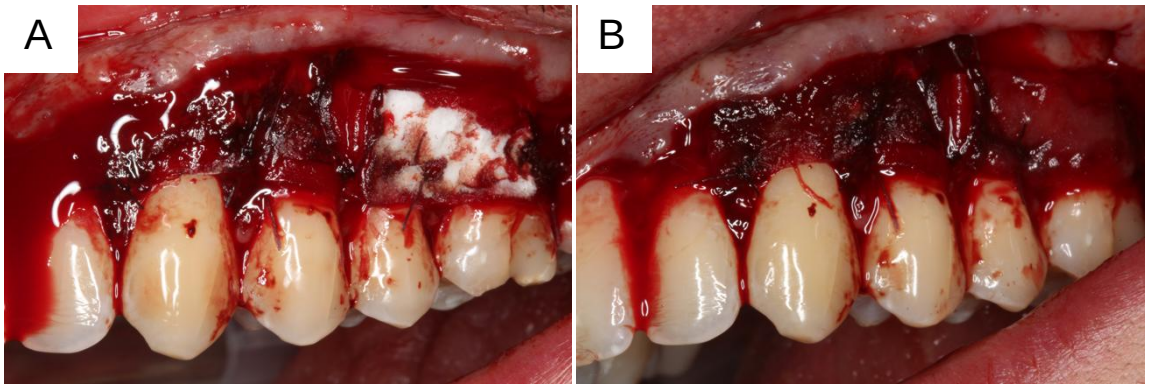

Figure 2 - (A)MG graft in place: after trimming it, half was placed over the defect on teeth 23 and 24 and sutured into place. the second half was placed over the defect of teeth 24, 25 and 26, sutured into place and, contrary to the other half has still not been absorbed by blood. (B)After irrigation with saline solution the portion of MG that has not been absorbed by blood takes on a rosy complexion that imitates that of a CTG.

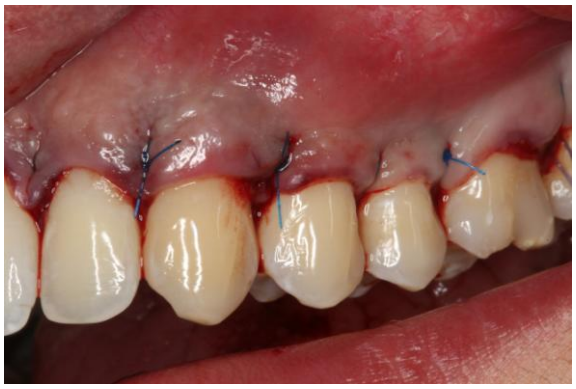

Figure 3 - Clinical appearance imediatly after surgery.

2.2.1.5. Post-surgical Procedure

After the surgery, patients were recommended to: take 600mg of ibuprofen every 12 hours for 3-4 days and accompany it with 1000mg of paracetamol when needed (SOS); apply ice to the side of the face that underwent surgery for small periods of time, alternating between 20 minutes with ice and 15 minutes without; avoid labour, physical activity and extensive talking during the first 24-48 hours; eat cold and soft foods during the first 5 days, and then warm and soft foods for the next 7 days, always chewing on the side opposite of surgery. As for oral hygiene, in order to avoid any mechanical trauma, patients were instructed to not brush their teeth for 5 days (oral hygiene would be maintained through a chlorhexidine mouthwash of 0.12% twice a day) and after these 5 days, to brush all teeth apart from those from the surgical site. At 15-days post-operative, patients could brush the surgical site with non-rotary movements, in a motion from apical do coronal, with an extra soft toothbrush (Elgydium 7/100, Pierre Fabre, France).

Patients were recalled for prophylaxis and reinforcement of motivation and instruction at 1 week, 2 weeks to remove sutures,1-month post-operative and every month during 6 months.

2.2.2. Post-operative Pain Evaluation

2.2.2.1. Patient Inclusion Criteria

- Age > 18 years;
- Periodontaly and systematically healthy;
- Patients from the Periodontology appointment (Dentistry department, FMUC);
- Presence of GR in need of root coverage;

- Plaque index and/or BOP (bleeding on probing) ≤ 20%(19);
- Use of CTG in root coverage procedure;
- Harvesting of CTG using Bruno's modified technique(42).

2.2.2.2. Patient Exclusion Criteria

- Smoking habits;
- Evidence of parafunctions;
- Presents periodontal disease;
- Inadequate plaque control (plaque index and/or BOP>20%);
- Pregnant women.

2.2.2.3. Outcomes

Patients were given, at the end of the surgery post-operative pain questionnaire (Appendix B) that evaluated their level of pain during the first week through a VAS scale{Scott J Fau - Huskisson, #547} and their painkiller intake during that same week. Patients were also asked to answer which surgical site (the donor or receptor site) was associated with greater pain.

2.3. Histological Evaluation

2.3.1. Connective Tissue Evaluation

2.3.1.1. Patient Inclusion Criteria

- Age > 18 years;
- Periodontaly and systematically healthy;
- Patients from the Periodontology appointment (Dentistry department, FMUC);
- Patients needing root coverage procedure with use of a CTG;
- Patients that never had CTG harvesting;
- Plaque index and/or BOP (bleeding on probing) ≤ 20%(19).

2.3.1.1. Patient Exclusion Criteria

- Smoking habits;

- Evidence of parafunctions;
- Presents periodontal disease;
- Inadequate plaque control (plaque index and/or BOP>20%);
- Pregnant women.

2.3.1.1. Sample Collection

In patients that underwent mucogingival surgery with collection of connective tissue, informed consent was obtained to execute a biopsy at the donor site for posterior histologic evaluation (Appendix D). The gingival tissue was attained through a punch biopsy in order to analyse the LP and the SM.

Samples were taken from the palatal area adjacent to the 1st premolar and 1st molar.

2.3.1.2. Sample Preparation

Sample preparation was performed at the Hard Tissues Laboratory (Faculty of Medicine, University of Coimbra) according to an undercalcified technique. Samples were fixated in a 10% phosphate buffered formalin for 24 hours, dehydrated in progressive sequences of ethanol, diaphanized with xylol and impregnated and embedded in paraffin wax. Ultra-fine histological cuts were executed in the order of 5μm. The histological sections were numbered and identified according to the elaborated sequencing and stained with hematoxylin and eosin (H.E).

2.3.1.3. Evaluated Parameters:

The evaluated parameters were:

a) Depth of the LP and SM;
b) Percentage of connective tissue proper present in the LP and SM.

2.3.1.4 Histological Analysis

From each sample, 3 histological sections were randomly chosen and examined under a light microscope (Nikon Eclipse E600, Tokyo, Japan) connected to a high resolution video camera (Nikon Digital Camera DXM-1200C). The histomorphometric evaluation of LP and SM on palatal grafts were done using Bioquant Osteo® 2012 software (Bioquant® -

Image Analysis Corporation, Nashville, EUA). A qualitative evaluation was made to determine the transition from LP to SM. LM started directly adjacent to the epithelium tissue while the SM started when the tissue presented a higher quantity of adipose and glandular tissue.

The depth of the LP, SM and the total depth of the biopsy was measured through linear measurements as shown in Figure 4.

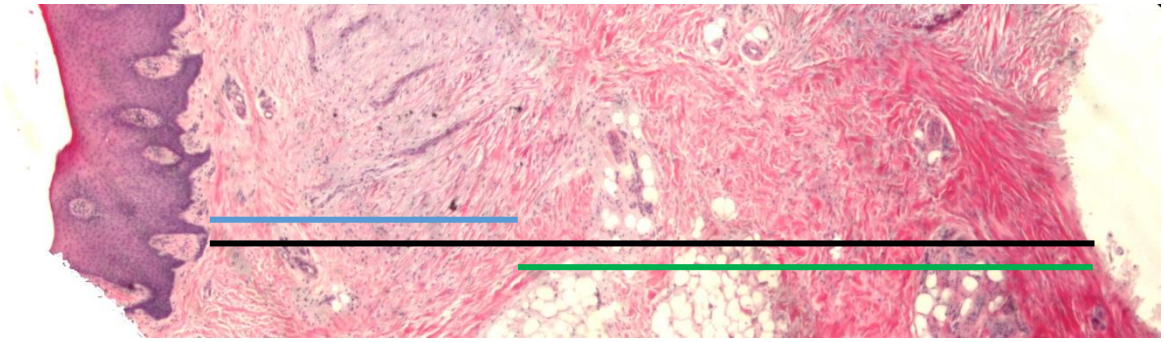

Figure 4 - Histological slide at original magnification (20x) illustrating linear measurements made to determine the depth of the LP (blue), the SM (green) and the total depth (black)

In order to determine the percentage of connective tissue present in the LM and SM, a Region of Interest (ROI) was defined as a rectangle with 1.0159mm/1.3428mm an area of 1.36415052mm (Figure 5). It was defined in two different places in each section, one in the LP, adjacent to the epithelium tissue and another at the beginning of the SM, in perfect continuity of the LP ROI (Figure 6). One observer examined all specimens blinded to group allocation. Samples were analysed under a magnification of X20.

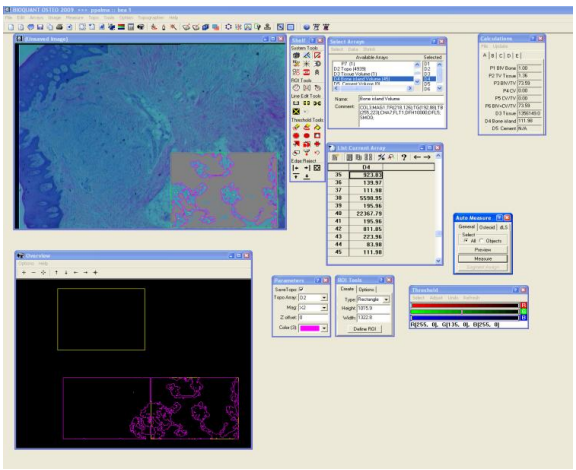

Figure 5 - Representative image of Bioquant® - Image analysis on one palatal graft with the ROI shown in the submucosa.

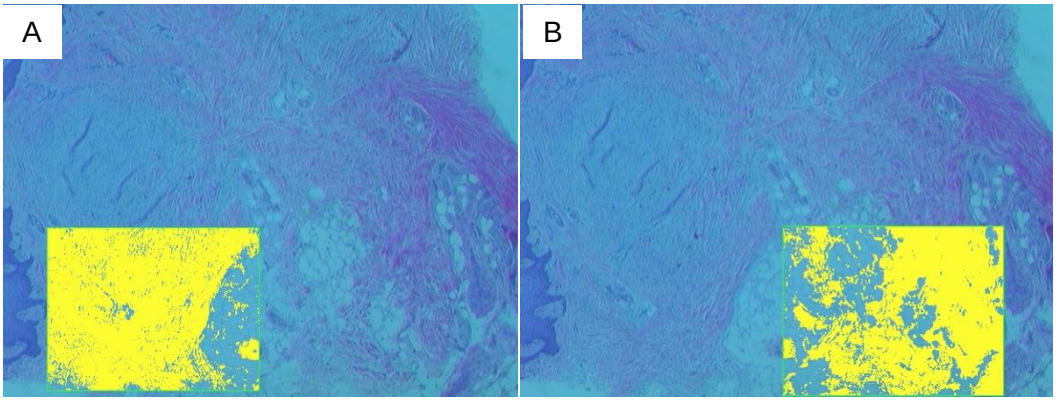

Figure 6 - Histological slide at original magnification (20x) illustrating histomorphometric measurements with the connective tissue in yellow in LP (A) and in SM (B). The spaces not filed correspond mainly to adipose tissue but also some vascular structures present.

2.3.2. Mucograft® Evaluation *In Vivo*

2.3.2.2. Experimental Model

The in vivo biocompatibility and degradability of the xenogenic collagen matrix were examined by implanting the membrane via subcutaneous implantation in the dorsal region of Balb/c mice (adult males, nine weeks old at the beginning of the experiment, and approximately 300g in weight).

For this pilot study only two animals were used and each animal was randomly allocated to the time points evaluated, 15 and 30-days post-implantation.

The study protocol was approved by the Animal Welfare Committee of the General Directorate of Veterinary of Portugal (number 042072011) and complied the International Guiding Principles for Biomedical Research Involving Animals (Geneve, 1985).

2.3.2.3. Surgical Procedure

The surgical procedure was performed at the Institute of Pathological Anatomy at the University of Coimbra. Anaesthesia was administrated intraperitoneally (medetomine at 0.5mg/kg; Medetor; Virbac;, France, in conjunction with Ketamine at 75 mg/kg; Ketalar; Par Pharmaceutical, Inc.; USA) and for better identification, the implantation site (dorsum) was manually trichotomized. Each animal received a small rectangular portion of MG (10mmx7mm) and was implanted subcutaneously (Figure 7). The skin was sutured with simple knots using a resorbable suture (PGA 4/0; Perma Sharp, Hu-Fridey, IL, USA). Reversal of the anaesthesia was performed through an intraperintoneally injection of atipamazole at 1 mg/kg; Revertor; Virbac;, France.

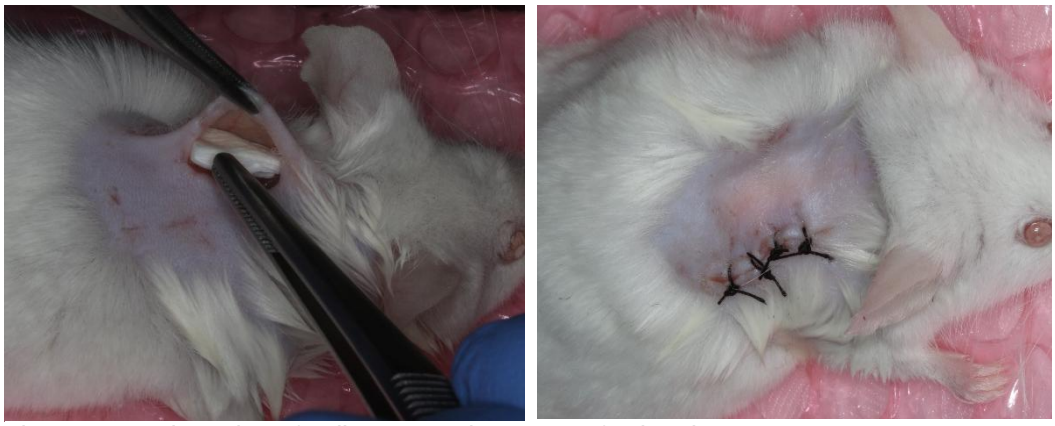

Figure 7 - Implantation of collagen matrix Mucograft® in mice

2.3.2.4. Euthanasia and sample collection

Fifteen and thirty days after implanting subcutaneously the collagen matrix, the animals were euthanized by anaesthetic overdose (pentobarbital at 30 mg/kg intravenously; Butler Company; Columbus, OH) followed by bilateral perfusion with 10% phosphate buffered formalin. The implant was localized at the time of its surgical removal and removed integrally and entirely with a safety margin of surrounding tissue around all its borders. Target organs (lungs, kidney, liver and spleen) were also removed for histological assessment of possible tissue injuries and microscopic debris from the implanted material.

2.3.2.5. Sample Preparation

In the likeness of the previous sample preparation, sample preparation was performed at the Hard Tissues Laboratory (Faculty of Medicine, Unversity of Coimbra) according to an undercalcified technique. Samples were fixated in a 10% phosphate buffered formalin for 24 hours, dehydrated in progressive sequences of ethanol, diaphanized with xylol and impregnated and embedded in paraffin wax. Ultra-fine histological cuts were executed in the order of 5μm. The histological sections were numbered and identified according to the elaborated sequencing and stained with H.E.

2.3.2.6. Evaluated Parameters:

The evaluated parameters were:

a) Tissue integration;

b) Newly formed blood vessels;

c) Encapsulation by fibrous tissues.

2.3.2.7. Histological Analysis

Each sample was histologically examined as an independent sample under a light microscope (Nikon Eclipse E600, Tokyo, Japan) connected to a high resolution video camera (Nikon Digital Camera DXM-1200C). One observer examined all specimens blinded to group allocation.

3. Results

3.1. Review Research Strategy

A total of 84 bibliographic references resulted from this initial electronic search. Of these, 35 were replicas and were therefore removed. 49 titles and abstracts went through an initial screening. The reviewers found a discrepancy in their screening of 4 articles, but upon discussion agreed that 30 were excluded as they did not meet the inclusion criteria. Hence 19 full-text articles were assessed for eligibility. After reading these articles, an additional 18 studies did not meet the inclusion criteria and were also eliminated, leaving a total of 1 article included.

The second electronic search accounted for a total of 112 bibliographic references. Of these, 77 were replicas and eliminated. Then, 35 titles and abstracts went through an initial screening and although reviewers found a discrepancy in their screening of 1 article, upon discussion, both agreed 23 studies being excluded due to not meeting the inclusion criteria, leaving 11 full-text articles to be assess for eligibility. After reading these articles, an additional 10 studies did not meet the inclusion criteria and were also eliminated, leaving a total of 1 article included.

Hand searching identified a total of 3 articles through cross references in bibliographies of articles identified in the searching process. Upon hand searching the Journal of Clinical Periodontology, 4 articles were identified and the Journal of Periodontology identified 9 articles. The 17 were read to access their eligibility and only 1 article met the inclusion criteria.

Tables 12 – 18 are included in the appendix (Appendix C) providing a justification for the exclusion of the studies mentioned above.

Taking into account the combined searches, the following flow chart (Figure 8) presents the combined selection process of the studies.

These 3 studies were inserted into a table (Appendix A) which discerns each of the studies' outcomes, dividing them into clinical outcomes: CRC, Mean Root Coverage (MRC), RecRed, Clinical Attachment Level (CAL), Periodontal Depth (PD), KT Gain, surgical time; patient outcomes: complications, post-operative pain, aesthetic satisfaction; clinician outcomes: colour, contour and contiguity of soft tissues; and histological outcomes.

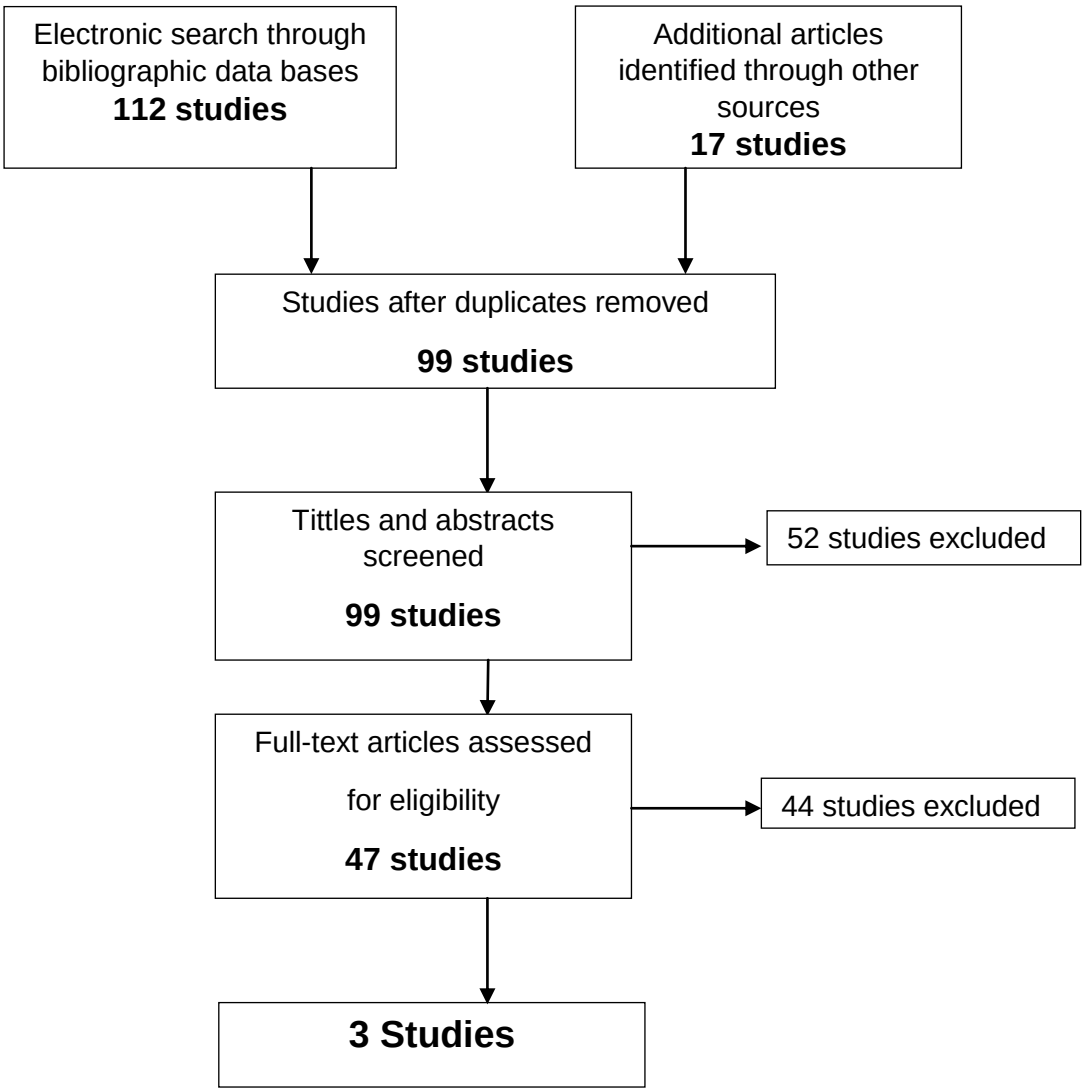

Figure 8 – PRISMA Flow chart demonstrating the identification, screening, eligibility and inclusion of articles.

3.2. Clinical

3.2.1. MCAF/CTG vs. MCAF/MG

A total of 2 patients were included in the clinical aspect of this study. Of these, 1 received MCAF/CTG as treatment and 1 received MCAF/MG. All patients handed in the post-operative pain questionnaire at 1-week post-operative.

	P1	P2
Surgical site	22, 23, 24, 25, 26	23, 24, 25, 26
Intervention	MCAF/CTG	MCAF/MG
Attended all follow-ups	Yes	N/A
Questionnaire	Yes	Yes

Table 1 - Overview of included patients.

The surgical procedures were well tolerated, and the level of pain was described as minimal by all patients. There were no unscheduled appointments or emergencies.

The results of the questionnaire are in the appendix (Appendix B). All patients only referred pain during the day of the surgery and the following two days with values between 0 and 2 and only one report of a 6 on the VAS scale. The mean pain throughout the week for P1 was 1.25 (2.12 standard deviation) while P2 was 0.5 (0.7 standard deviation). The day with most pain was the day of the surgery for both patients (P1:6; P2:2).

Painkillers were only taken by P1 on the day of the surgery and the following two days. P1 patient ingested painkillers at day 6 and 7 due to experiencing a fever during day 5 through 7, however, no clinical anomalies were associated to these complaints.

The clinical results at 6-months post-operative in comparison to the initial collected data are as follows:

P1		23	24	25	26
PD	Initial	2	2	2	3
	6m	1	2	3	3
Rec	Initial	2	2	1	1
	6m	0	1	0	0
KT height	Initial	6	4	4	4
	6m	6	5	6	5
MRC	%	100	50	100	100
CRC	%	100	100	0	100

Table 2 - Initial and 6-month clinical outcomes of P1.

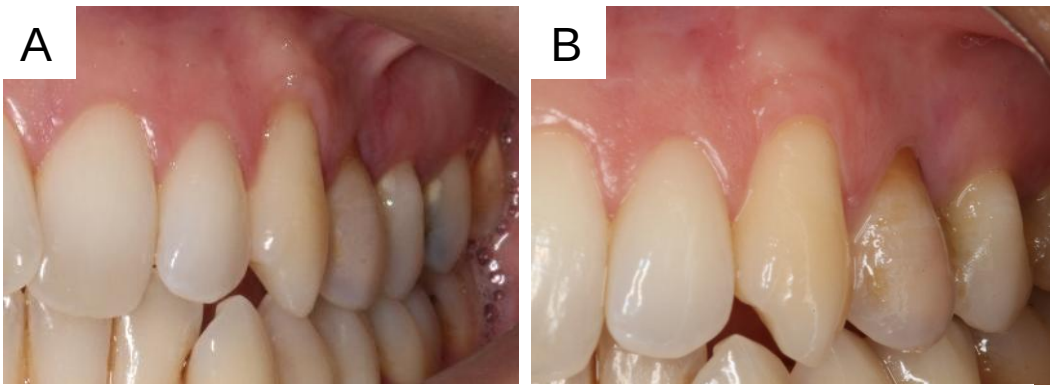

Figure 8 - Initial (A) and 6-months post-operative (B) of P1.

P2		23	24	25	26
PD	Initial	2	2	3	1
Rec	Initial	1	2	2	2
KT height	Initial	6	4	5	4

Table 3 - Initial clinical outcomes of P2.

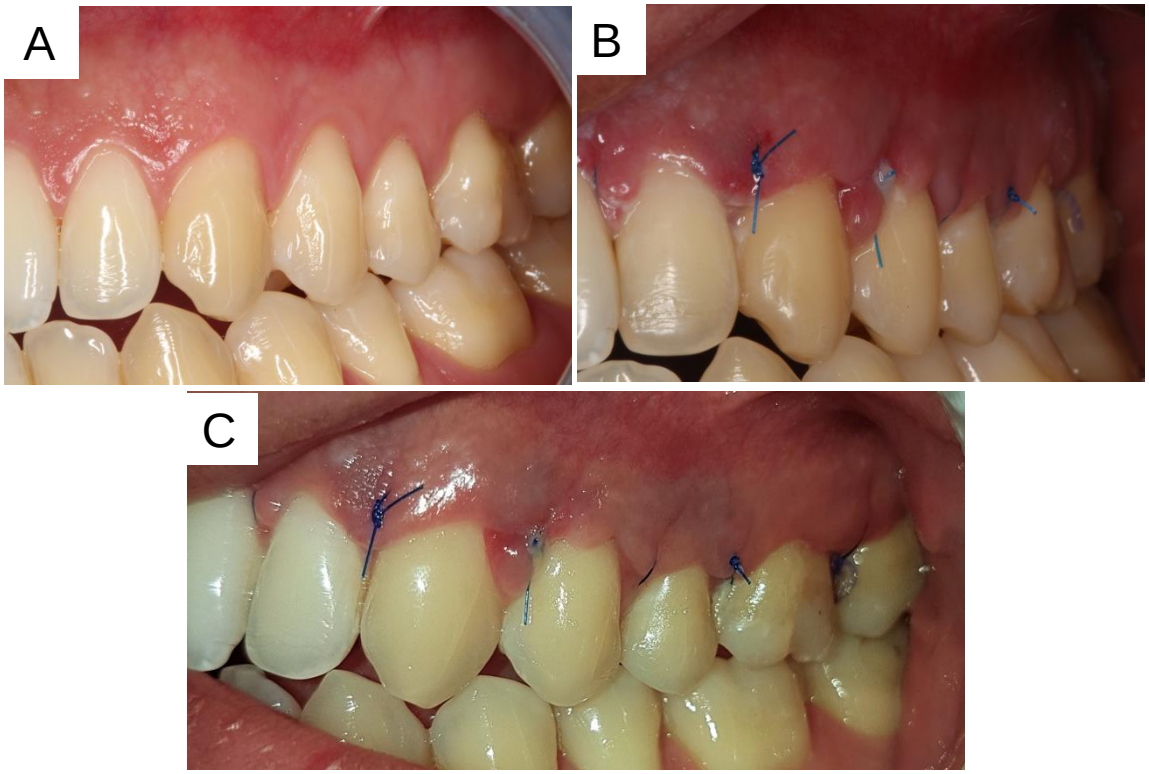

Figure 9 – Initial (A), 1-week (B) and 11 days (C) post-operative of P2.

3.2.2. Post-operative Pain Evaluation

The post-operative pain questionnaire was handed out to 6 patients that were submitted to CTG harvesting and were all collected at one-week post-operative.

The results of the questionnaire are located in the appendix (Appendix B) and show that overall post-operative morbidity was low with pain subsiding on the third day after surgery. The mean pain level of all patients during the entire week was of 0.66 (1.15 standard deviation). The day with most intense pain was the day of the surgery (mean: 2.17, standard deviation: 2.04), followed by the first (mean: 1.5, standard deviation: 1.05), second (mean: 1.33, standard deviation: 0.82) and third day (mean: 0.17, standard deviation: 0.41). The following days, patients reported no pain.

The pain killer ingestion during the entire week was also low with a mean of 0.5 painkillers per day (0.88 standard deviation). All patients but one stopped taking painkiller on

the third day (this patient took painkillers on day 6 and 7 too). The highest intake was observed on the first day after surgery (mean: 1.5, standard deviation: 1.05), followed by the day of the surgery (mean: 1.17, standard deviation: 0.98), the second (mean: 0.67, standard deviation: 0.82), sixth (mean: 0.5, standard deviation: 1.22) and seventh day (mean: 0.17, standard deviation: 0.41).

A total of 5 patients referred to both surgical sites hurting equally, with no greater intensity in either the donor or receptor site, and 1 patient stated that the receptor site hurt more than the donor site.

3.3. Histology

3.3.1. Connective Tissue Evaluation

3.3.1.1. CTG A

This biopsy was obtained from a young female patient, mesially of tooth 14, in a conical shape, containing both superficial (broad end) and deep (narrow end) connective tissue. Histological analysis confirmed the presence of the total thickness of the collected graft with epithelial tissue found in the palatal surface of the graft and periosteum in the opposite end (as shown in Figure 4). As represented in Figure 11A the more incisal portion of the graft is composed by a high density connective tissue with its typical appearance of highly packed collagen fibres with sparse fibrocytes – the LP (Figure 11C). It is also possible to identify the presence of a residual portion of the stratified squamous epithelium of the masticatory mucosa of hard palate. The more apical portion – SM (Figure 11B) – even with a presence of dense connective tissue was primarily composed by adipose tissue and loose connective tissue with great number of vascular structures.

The mean depth of the LP (Table 4) was 1.441 mm (49.3% of the graft). Apical to the LP the mean depth of the SM was 1.480 mm (50.7% of the graft).

The percentage of connective tissue proper present at the LP and the SM was measured as seen in Figures 5 and 6 (Table 5). The LP revealed a mean value of 92.65% of connective tissue proper and the SM showed a mean value of 80.02% of connective tissue.

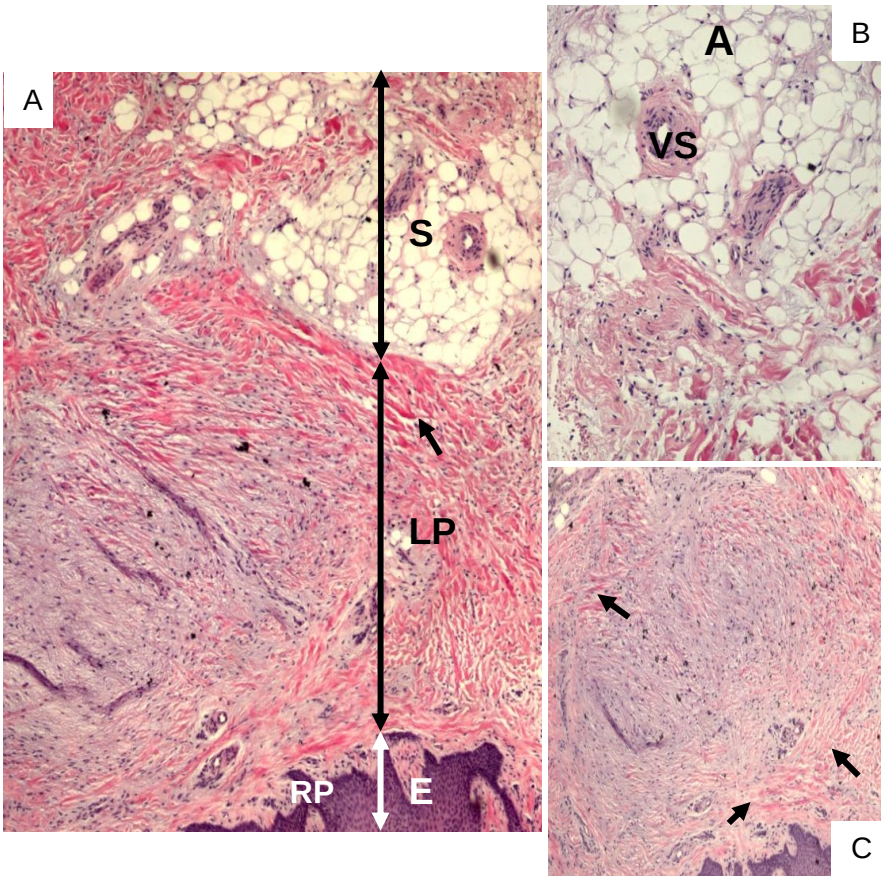

Figure 11 - Histological cross section showing a low magnification of the palatal graft where it is possible to identify a small portion of the ret pegs from the epithelial tissue. The LP (C) is mainly composed by packed thick collagen fibers (examples identified through arrows) while the SM (B) as a higher amount of adipose tissue and vascular structures (A - H.E; 40x. B, C - H.E; x100). EP= epithelial tissue; LP= lamina propria; SM= submucosa; RT= rete pegs; AT= adipose tissue; VS= vascular structure.

	TOTAL DEPTH	LP DEPTH		SM DEPTH	
CTG A1	3.154	1.572	49.8%	1.582	50.2%
CTG A2	2.747	1.376	50.1%	1.371	49.9%
CTG A3	2.863	1.376	48.1%	1.487	51.9%
MEAN	**2.921**	**1.441**	**49.3%**	**1.480**	**50.7%**
STD DEV	**0.209**	**0.113**	**1.07%**	**0.105**	**1.07%**

Table 4 - Depth values (mm) of CTG A with respective percentage of the entire graft and mean values. CTG A1, A2 and A3 correspond to three different sections of the same CTG biopsy.

		TOTAL AREA	CONNECTIVE TISSUE	% CONNECTIVE TISSUE
CTG A1	LP	1.36	1.20	88.87%
	SM	1.36	1.04	76.97%
CTG A2	LP	1.36	1.26	93.17%
	SM	1.36	1.00	73.59%
CTG A3	LP	1.36	1.31	96.70%
	SM	1.36	1.21	89.50%
LP	**Mean**		**1.21**	**92.65%**
	Std Dev		**0.05**	**3.20%**
SM	**Mean**		**1.08**	**80.02%**
	Std Dev		**0.06**	**8.38%**

Table 5 - Percentages of connective tissue proper present in LP and SM from CTGA. CTG A1, A2 and A3 correspond to three different sections of the same CTG biopsy

3.3.1.2. CTG B

This CTG was obtained from a young female patient through a punch biopsy in the area distal of tooth 26. Histological analysis showed that the structure of the graft is essentially composed of a dense network of collagen fibres in the LP but also in the SM. Here, both of the layers have connective tissue as the major component with randomly thin collagen fibrils but also some thicker collagen fibrils could be seen in the SM. A residual portion of the stratified squamous epithelium with its typical rete pegs of the masticatory mucosa of hard palate can also be seen (Figure 12).

The mean depth of the LP (Table 6) was 1.623 mm (51.7% of the graft). Apical to the LP the mean depth of the SM was 1.576 mm (47.6% of the graft).

The percentage of connective tissue proper present at the LP and the SM was measured (Table 7). The LP revealed a mean value of 89.30% of connective tissue proper and the SM showed a mean value of 90.31% of connective tissue proper.

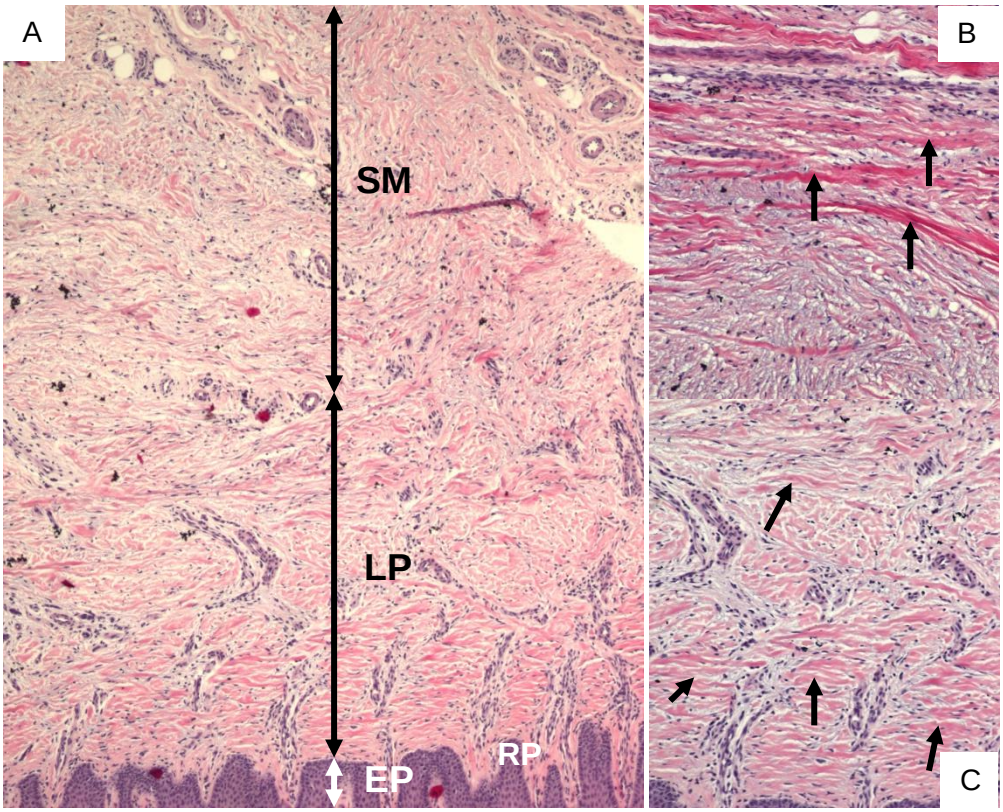

Figure 12 - Low magnification of the palatal graft where it is possible to identify all its thickness as well as a small portion of ret pegs (RP) from the epithelial tissue. The LP and the SM (C and B) are manly composed by packed collagen fibers (evidenced by arrows) with no evident differences between them; (A – H.E; 40x. B, C - H.E; x100). EP= epithelial tissue; LP= lamina propria; SM= submucosa; RT= rete pegs.

	TOTAL DEPTH	LP DEPTH		SM DEPTH	
CTG B1	3.270	1.741	53.2%	1.529	46.8%
CTG B2	3.190	1.793	56.2%	1.449	43.8%
CTG B3	2.911	1.335	45.8%	1.576	54.2%
MEAN	**3.124**	**1.623**	**51.7%**	**1.518**	**47.6%**
STD DEV	**0.188**	**0.251**	**5.35%**	**0.064**	**4.26%**

Table 6 - Depth values (mm) of CTG B with respective percentage of the entire graft and mean values. CTG B1, B2 and B3 correspond to three different sections of the same CTG biopsy.

		TOTAL AREA	CONNECTIVE TISSUE	% CONNECTIVE TISSUE
CTG B1	LP	1.36	1.29	95.26%
	SM	1.36	1.22	89.74%
CTG B2	LP	1.36	1.24	91.18%
	SM	1.36	1.20	88.59%
CTG B3	LP	1.36	1.10	81.46%
	SM	1.36	1.26	92.61%
LP	**Mean**		**1.26**	**89.30%**
	Std Dev		**0.09**	**7.09%**
SM	**Mean**		**1.22**	**90.31%**
	Std Dev		**0.03**	**2.07%**

Table 7 - Percentages of connective tissue proper present in LP and SM from CTG C. CTG B1, B2 and B3 correspond to three different sections of the same CTG biopsy.

3.3.2. Mucograft® Evaluation *In Vivo*

Macroscopic examination revealed that wound healing was good in all animals. No signs of inflammatory process, haemorrhage or necrosis as well as abscess formation were identified around the implants.

Representative micrographs of histologic results are shown in Figures 13 through 18 illustrating the connective tissue-collagen membrane interface retrieved at 15 and 30 days after implantation. Briefly, tissue reaction such as inflammatory and vascular response, infiltration of mast cells, presence of fibrosis capsule and biomaterial degradation were searched in the histological samples.

3.3.2.1. 15 days

At 15 days the thickness of the membrane was almost the same as its initial thickness. The histologic structure of the MG perfectly shows the presence of a thick cell-occlusive superior layer and the thicker, porous bottom layer (Figure 13). The degradation and simultaneous replacement by endogenous collagen fibres started in the porous layer (Figure 14C) while the thin compact layer kept almost all of its primary structure.

This material induced a soft tissue response with a very limited inflammatory cell infiltrate and also lead to new blood vessel formation present predominantly at the periphery of the MG as well as facing the occlusive layer (Figures 14 and 15). Some fibroblast like cells were also detected.

As seen in Figure 14 the more porous layer was rapidly infiltrated by a few host mesenchymal cells while the barrier layer shows almost none and when present, more densely packed cells. The tissue reaction was clinically well tolerated and the histological study showed signs of a well-accepted biomaterial although some multinucleated giant cells were observed (Figure 15).

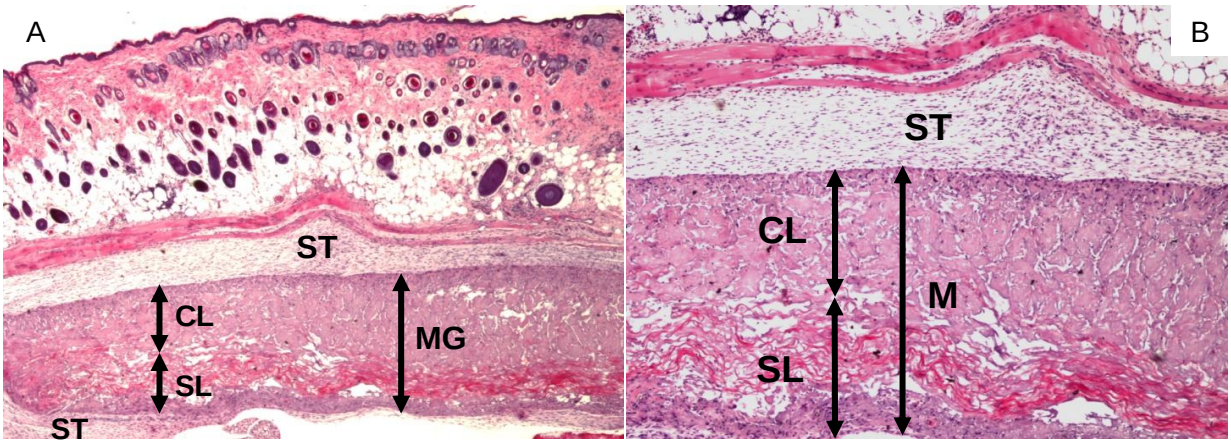

Figure 13 – Histological section after 15 days of subcutaneous implantation of the collagen scaffold in mice, showing a low magnification cross section (A - HE; 20x. B - HE; 40x). MG= Mucograft®; ST= subcutaneous tissue; CL= compact layer; SL= spongy layer.

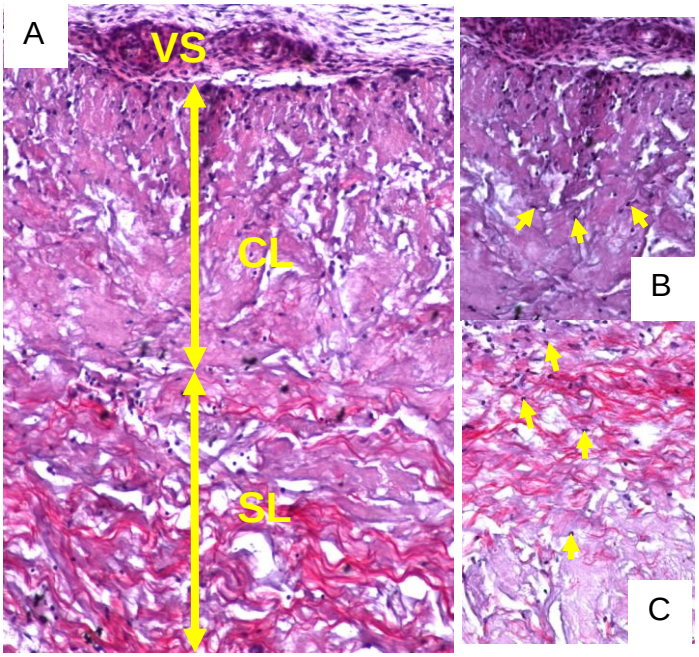

Figure 14 - Higher magnification of the histological structure of the MG. The face of low-porosity or compact layer is shown in the upper part of the graft (B) along with the face of the more porous spongy layer in the in the lower part of the image (C). A higher number of cells (arrows) were seen in the porous spongy layer than in the compact layer. (A - HE, 40x. B - HE, 100x. C - HE, 100x). CL = compact layer and SL = spongy layer; VS= vascular structure.

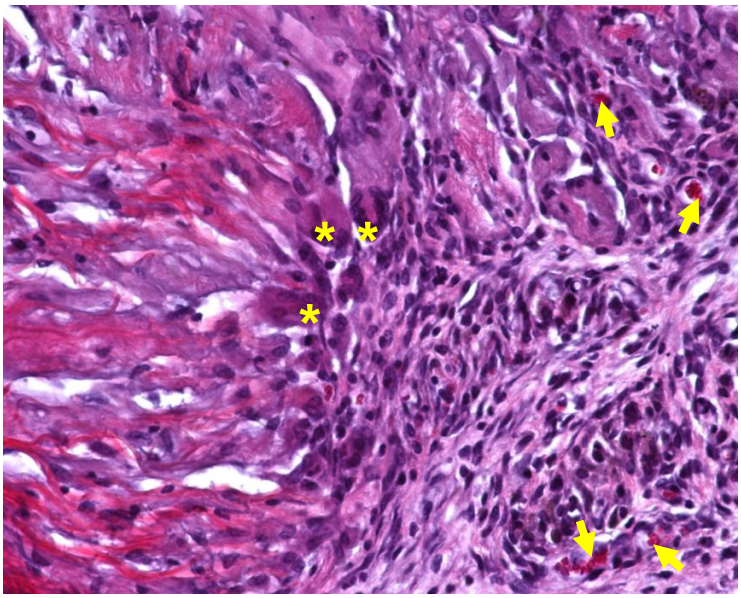

Figure 15 – Note the presence of some giant body cells (*) located at the periphery of the graft as well as a residual number of inflammatory cells together with some small vascular structures (arrows). Although this could be understood as a foreign body reaction no other signs of inflammation could be identified. (HE; 400x).

3.3.2.2. 30 days

The histologic organization of the CM with a thin cell-occlusive superior layer and the thicker porous bottom were not well distinguished at this point of the experimental study.

Thirty-days post-implantation, the successful integration of the membrane with the surrounding tissues was more evident compared to 15 days (Figure 16). In fact, the membrane had kept its original shape and a continuous replacement and integration of the collagen from the membrane by soft connective tissue from the host was clear. A dense network of a well-organized residual collagen matrix including large amounts of newly formed thick collagen fibres was detected (Figure 17). Cellular graft invasion revealed differences in the progression of the cellular infiltration between the samples from 15 days to 30 days. A high number of fibroblast-like cells between the remains of the collagen matrix body as well as the number of blood vessels were significantly higher and were widely spread all over the CM (Figure 18). Hence, some new vascular structures can be seen in the middle of the collagen fibres located in the most deeper parts of the body matrix as well as a higher degree of surrounding vascularity (Figure 17 and 18).

Furthermore, a limited number of inflammatory cells and no peri-graft cellular reaction was seen, although some multinucleated giant cells were present between the collagen fibres from MG (Figure 18B).

It is also obvious the remodulating process and a three-dimensional volume stability over the 30 days. Notably, minimal fibrotic capsule formation was observed surrounding the implant at any time point, which displayed overall scarce amounts of inflammation.

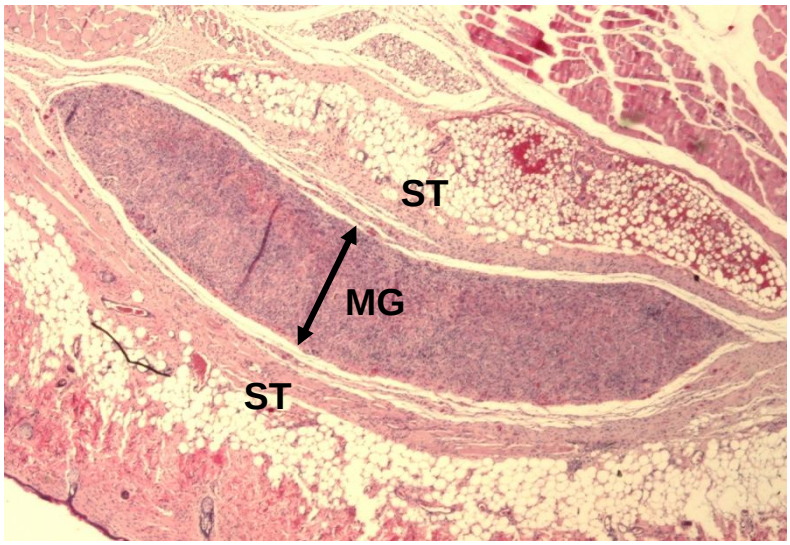

Figure 16 - Histological section after 30 days of subcutaneous implantation of the collagen scaffold in mice, showing a low magnification cross section. It is not possible anymore to identify the presence of a compact and a porous layer. Also a minimal collagen reaction as an encapsulation of the MG is seen (HE; 20x). MG= Mucograft®; ST= subcutaneous tissue.

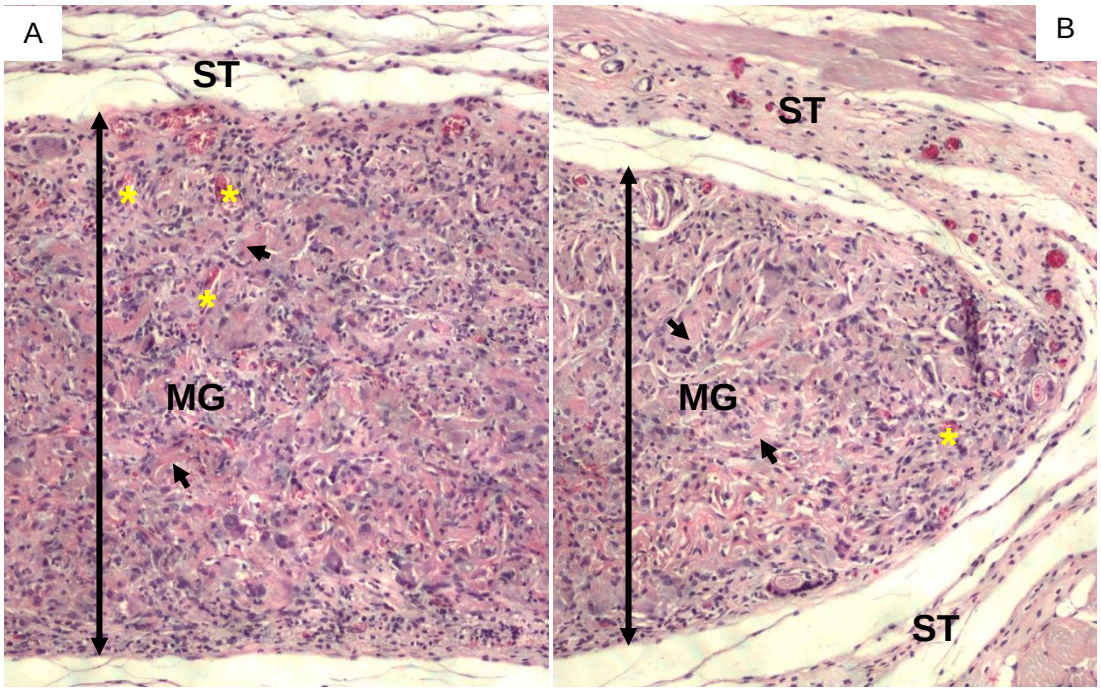

Figure 17 - Histological section after 30 days of subcutaneous implantation of the collagen scaffold (A: middle portion of the membrane; B: extremity of the membrane). It is not possible anymore to identify the presence of a compact and a porous layer. A dense network of residual collagen matrix fibres and newly formed thick collagen fibres (arrows) as well as newly formed blood vessels (*) into deeper portions of the MG is seen (A, B - HE;100x). MG= Mucograft®; ST= subcutaneous tissue.

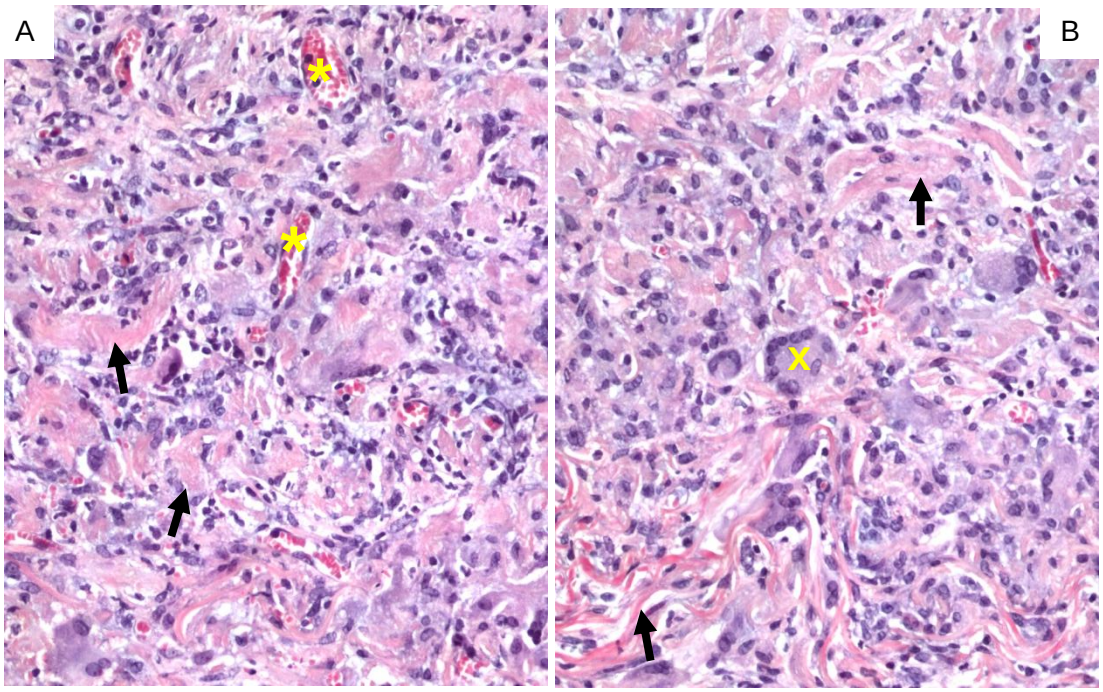

Figure 18 - Higher magnification of the middle portion of the MG after 30 days of subcutaneous implantation. Newly formed blood vessels (*) located between the collagen fibres (arrows) are seen. Although no inflammatory reaction exists some multinucleated giant cells (x) were present between the collagen fibres (A, B - HE; 200x). MG= Mucograft®; ST= subcutaneous tissue.

4. Discussion

4.1. Research

The evidence regarding the comparison of MCF/CTG vs MCAF/MG for maxillary multiple recessions treatment is very limited. There are no studies with a direct comparison of these two techniques and from the included studies only two were randomized controlled clinical trials. The present review included not only high evidence publications but also case series. The inclusion of this lower level of evidence publications was done based on previous publications by another authors that performed reviews on multiple recession defects(9, 44).

Although there is a vast body of evidence as to the treatment of localized recessions with CAF or CAF/CTG(45, 46), the same cannot be said for multiple gingival recessions and this is even more evident when it comes to the use of MCAF. Despite this lack of evidence, according to Zuchelli *et al,* 2009, MCAF was associated with an increased probability of achieving complete root coverage with a better aesthetic and post-operative course(28), however little data address the long-term follow-up of this procedure. In fact, a recent systematic review on multiple recessions defects concluded the very same(4).

Pini-Prato *et al,* 2010 concluded, after a 5-year follow-up, that MCAF treated sites suffered a slight apical shrinkage, while on the other hand, sites treated with MCAF/CTG achieved a slight coronal shift of the gingival margin(23). This creeping attachment effect over time may be due to the thick gingival tissue obtained after positioning the CTG, while the apical shift with MCAF alone may be related to the thinner thickness/amount of keratinized tissue achieved leading to possible apical relapse of the gingival margin during the maintenance phase(23). Zucchelli *et al*, 2014 also concluded that MCAF/CTG treated sites showed a statistically significant higher percentage of sites with CRC compared to sites treated with MCAF alone (47). However, these authors proposed that these long term results could, in part, be ascribed to the decrease in motivation of MCAF treated patients, despite the strict control regiment, rather than true limitations associated with the surgical technique. They further speculated that MCAF/CTG provided greater soft tissue thickness that facilitated long term patient-maintenance alternatively to improving the surgical outcomes compared to MCAF alone(47).

Taking this into account, it is possible to infer that the addition of a graft may be a valid therapeutic approach in terms of long term CRC outcomes. This raises the question as to what type of graft is the most indicated and provides the best short/long term results when treating MGR. The use of CTG provides an autogenic biocompatible solution with low costs and favourable results (47). However, its use is associated with a possible lack of available

tissue for harvesting, the necessity of two surgical sites, a recipient site and another donor site that heals through secondary intention, prolonged surgical time and further post-operative discomfort (6, 14, 29). The use of STS may overcome these disadvantages, namely the use of the xenogeneic collagen matrix MG.

In order to answer the question: “In patients with maxillary multiple gingival recessions, what is the efficacy of CAF plus CTG compared to CAF plus Mucograft® in terms of CRC, after a minimal 6 month follow-up?“ an extensive search was conducted with rigorous inclusion and exclusion criteria leading to only 3 studies with follow-up ranging from 6 to 60 months. Two were clinical trials with a total of 41 patients treated with MCAF/CTG(24, 28), whilst the other, a case series with only 2 patients treated with MCAF/MG(3). This last study was not randomized neither controlled so the level of evidence is very low. However, this was the only publication with MCAF/MG. No study had a direct comparison between MCAF/CTG vs MCF/MG.

The 2 clinical trials had follow-ups of 6(24),12(24, 28) and 60(24) months, while the other and the case report only had a 12-month follow-up(3).

In terms of the primary outcome, in MCAF/CTG treated sites only one study obtained results for 6-months follow-up (89,5%in Zuchelli *et al*, 2014). The 12 months results varied between 86.8%(24) and 89,3%(28). The two controlled, randomized, clinical trials(24, 28) had no major differences most likely due to the fact both studies were performed by the same group of researchers, with the same expertise and technique. Regarding the 60 months evaluation Zucchelli *et al,* 2014 reported 90,8% CRC(24). It is interesting to note, in this last study, that although CRC slightly dropped from 6 to 12 months (89.5% to 86.8%) there was an increase of CRC between the 6 and the 60-months evaluation (86.8% to 90.8%). This increase may be due to the creeping attachment effect and the use of a CTG. In fact, studies state that the inclusion of a CTG will increase the percentage of CRC over time, giving valid and important indications for the addition of this graft to the MCAF technique(23, 24).

For the MCAF/MG group the case report included in the present review had a value of 71,4% of CRC, at a 12-months evaluation(3). Despite the differences between all the studies included the comparison of MCAF/CTG vs MCAF/MG gives a higher absolute value for the MCAF/CTG group (89,3% and 86,8%), however no statistical analysis was done between both groups.

Data from the periodontal literature shows that CAF/CTG (bilaminar technique) is associated with stable results over time. In fact, a comparative clinical trial that addresses multiple maxillary recessions demonstrated an apical relapse of the gingival margin in the

CAF/CTG group when compared to CAF treated sites, over a 5-year period (48). However the bilaminar technique is associated with an additional surgical area, that may cause patient discomfort during and after surgery and patients morbidity mainly if large areas of connective tissue are harvested, additional chair time and specific surgical skills (3, 49). Presently there are alternatives to grafting (i.e. barrier membrane, enamel matrix derivate, acellular dermal matrix, living tissue-engineered human fibroblast-derived dermal substitute, platelet concentrate graft)(50-53) but according to a systematic review none of them had the same effectiveness than CAF+CTG, for single recession(44).

Regarding secondary outcomes, in MCAF/CTG treated sites, RecRed obtained a value of 3.1mm at 6-months(24). At 12-months results varied between 3.0mm(24) and 2.5mm(28) and at 60-months attained 3.1mm(24). These reduction values were all statistically significant when comparing their recession base-line values to those obtained at 6, 12 and 60 months. The MCAF/MG sites obtained a RecRed of 2.4mm at 12-months(3). This value, while similar, is slightly inferior to those obtained by MCAF/CTG at 12 months (3.1mm (24)and 2.5mm(28)), but it should be noted that these patients presented smaller recessions to start off with (2.86mm), compared to Zucchelli *et al*, 2014 (3.15mm) and similar to Zucchelli *et al*. 2009 (2.59mm). Pini-Prato *et al,* 2010 also concluded that the more severe the recession at baseline, the greater the recession reduction will be using MCAF/CTG, further justifying the difference(23).

In terms of KT gain, MCAF/CTG sites obtained 0.4mm at 6-months(24), 1.0mm(24) and 0.7mm(28) at 12-months and 1.7mm at 60 months(24). The gain in KT was statistically significant from baseline to 12-months, 6 to 12-months and 12 to 60 months. Pini-Prato et al(23) attributes an increase in KT Gain to the coronal shift of the gingival margin and determines that the use of CTG will increase KT gain, therefore inducing a coronal shift of the gingival margin over time. The MCAF/MG case report indicated a KT gain of 0.7mm at 12-months(3). This value is comparable to one of the MCAF/CTG studies (28), but inferior to the other(24). It would be interesting to see whether the same creeping attachment effect would be observed if this MCAF/MG study had continued through to 60-months post-operative.

Despite not being secondary outcomes, further clinical outcomes were registered and compared, namely MRC, CAL, PD and surgical time.

MRC in MCAF/CTG sites obtained 96.8% at 6-months(24), 95,9%(28) and 97.3%(24) at 12-months and 97.3% at 60-months(24). On the other hand, MCAF/MG sites achieved 81.3% in MRC(3). These results agree with the CRC results, MCAF/CTG had a

higher absolute value (95.5% and 97.3%) and there is a tendency for a coronal shift of the gingival margin with time.

CAL values were only present in the two randomized controlled studies with MCAF/CTG treated sites(24, 28). One study reported a baseline value of 4.2mm at 12-months and it was 1.2mm and at 60 months, 1.3mm(24). The other stated that the baseline value was 3.8mm and after 12-months it was 1.2(28). All the values at 12 and 60-months were statistically significant in comparison to the base line value. Since the PD values remained stable and un-pathological through-out the entirety of the studies (ranging from 1.1mm to 1.3mm(24, 28)), it can be inferred that the recession reduced significantly, corroborating the RecRed results.

The surgical time was also only evaluated in the two randomized controlled studies(24, 28). The stated values were of 40.2 minutes(24) and 28.7 minutes(28). Considering both these studies have reported results with no major differences thus far, most likely due to the fact both studies were performed by the same group of researchers, with the same expertise and technique, they were expected to achieve similar surgical times. However, upon further comparison, one study defines the surgical time as “the time needed for surgery from the first incision to the last suture”(28), while the other gives no sort of definition. This can lead to ambiguity and the inclusion of steps such as anaesthesia administration and post-operative recommendations that can increase the surgical time, which is the case, the study that does not define its surgical time presents a higher value(24).

Post-operative complications were only described by Zucchelli in the two MCAF/CTG studies(24, 28). While both studies stated healing was uneventful, one study reported 5 patients experienced swelling during the first week after surgery(28). The other study reported bleeding in 1 patient for 2 days at the palatal wound, shrinkage of flap with graft exposure in 2 patients at 6-months, 6 patients at 12-months and 9 patients at 60-months(24). Bleeding at the palatal wound is a complication that may arise when a donor site is used and patients should be made aware of possible complications from the use of a second surgical site in the palatal area(29). The use of STS can prevent these complications from occurring. As for the flap shrinkage, the author speculates that large grafts can impair the vascular exchange between the covering flap and the underlying receiving bed and, thus increase the risk for flap dehiscence and graft exposure with aesthetic consequences associated(24). In fact, studies have suggested that the presence of a CTG should stabilize the flap in a coronal position and therefore serve as an “anchor” for the covering flap during the initial wound healing period and therefore the CTG may be harvested with only a 1mm thickness(14). If for any reason the SCTG failed to anchor the overlying flap and undesired

flap retraction occurred during the early wound healing phase the SCTG might act as a “protector” beyond it and still allow healing by primary intention. Therefore, despite flap shrinkage and graft exposure, the CTG usually does not undergo necrosis and maintains its primary adhesion and lead to successful root coverage(14).

Post-operative pain was measured by standardized scales in all studies. Patients treated with MCAF/CTG answered a VAS scale questionnaire expressed in millimeters on a 100 mm scale (Cortelini *et al,* 2009) and handed them in at 1-week post-operative. All patients reported limited post-operative pain and discomfort(24, 28). One study determined that 8 in 16 patients reported an excellent post-operative course, and 0 reported a less-than-average post-op course. MCAF/MG patients answered a questionnaire asking about the discomfort level, if any, in terms of pain, swelling, bleeding, chewing activity, and quality of daily life perceived during the first 2 weeks. Scores ranged between 0 (no discomfort) and 10 (high discomfort). The results revealed slight discomfort during the first two weeks. Since the same methods were not applied and quantitative values were not supplied, both pain scales are not comparable and therefore it cannot be determined which approach induced a lower morbidity, but it is possible claim that both approaches had a low morbidity. However, it is expected that a second surgical site, which is the case of MCAF/CTG would lead to a higher morbidity level(29).

Both post-operative pain and patient aesthetic satisfaction were once considered as secondary and of less interest but with the patients’ increasing demands and expectancies, the morbidity and aesthetic outcomes of the surgical interventions must be taken ever more into account. All studies measured the patients’ aesthetic satisfaction by standardized scales. MCAF/CTG patients answered a VAS scale questionnaire expressed in millimeters on a 100 mm scale at 12(24, 28) and 60(24) months. One study included 19 incisors, 22 canines and 32 premolars(24), while the other included 10 incisors, 18 canines, and 17 premolars(28). All patients at all evaluated times reported a high aesthetic evaluation with no statistical differences between 12 and 60 months. One study determined that 7 in 16 patients reported excellent aesthetic satisfaction and 1 patient reported average(28). MCAF/MG patients answered a questionnaire with scores ranging between 0 (bad aesthetic outcome) and 10 (optimal aesthetic outcome), including 1 incisor, 2 canines and 4 premolars. Since the same methods were not applied and quantitative values were not supplied, both aesthetic satisfaction scales are not comparable. It cannot be determined which approach achieved a higher patient satisfaction, but both approaches had high patient aesthetic satisfaction.

Clinicians also evaluated the colour contour and contiguity of the soft tissues at 12 (24, 28) and 60(24) months in MCAF/CTG patients. The colour match and contour was rated

with a VAS scale expressed in millimeters on a 100 mm scale (0 indicating very bad, 50 indicating average, and 100 indicating excellent), contiguity was rated as yes (invisible confluence between the treated area and the adjacent soft tissues) or no (visible confluence between the treated area and the adjacent soft tissues)(28). At 12-months colour values ranged from 78.4(24) to 95(28), contour ranged from 89.6(24) to 77.13(28) and only one study determined that continuity was present in all patients(28). At 60-months the colour value was of 73.6 and the contour 87.2(24). While one study referred no patients with keloid formation at 12-months(28), the other reported 11 patients with keloid formation at 60-months(24). This was mainly due to graft exposure as a statistically significant relationship was found between graft exposure and patients with keloid formation, also leading to worse colour match evaluation. The results obtained from these studies are high in incisors, canines and premolars treated. The use of bilaminar technique with the absence of an apical migration of the gingival marginal over a long-term follow-up(23) allows an optimum aesthetic perception by the clinician. MCAF is a technique used to purposefully avoid keloid formation when it avoids the use of vertical releasing incisions, leading to an aesthetic result. From a biologic point of view this design also brings advantages since there isn't any damage to the lateral blood supply to the flap. Another factor that could help the high aesthetic result is the extension of the incision one tooth mesial an distal of the teeth with gingival recessions, as this influences the shape of the soft tissue margin of the neighbouring teeth, resulting in a more harmonious scalloped, knife-edged outline of all teeth in that area(28).

No study presented histological outcomes. In fact, few studies associate clinical outcomes with histology. A noteworthy study that manages to do this is Ghanaati et al(30) that, although not included in this review, histologically evaluated the collagen membrane MG in a murine model at 3, 10, 15, 30 and 60 days and simultaneously clinically applied MG in conjunction with CAF in 11 patients with Cl I and II of Miller GR. Subsequently the authors achieved a human biopsy allowing the analysis of the histological performance of the MG. Although the patient biopsy challenges the ethics of this study, the animal and human studies were able to be compared histologically and added value to the clinical aspect of the study.

4.2. Clinical

4.2.1. MCAF/CTG vs. MCAF/MG

The clinical aspect of this study aims to enrich the conducted research and help in any way possible answer the PICOT question: “In patients with multiple gingival recessions, what is the efficacy of CAF plus CTG compared to CAF plus Mucograft in terms of CRC, after a minimal 6-month follow-up?”. Therefore, only patients that had multiple maxillary Cl I and II recessions that needed root coverage (among other criteria) were included, leading to a total of two patients. This is a very small population and conclusions cannot be drawn from these two patients alone. Furthermore, MG was unavailable for the majority of the study and a 6-month follow-up was not possible to perform. A larger number of included patients with the same follow-up time would have to be included in order to help answer the PICOT question.

Regarding the surgical procedure, both approaches were carried out with Zucchelli’s(41) MCAF technique, bringing advantages to the treatment of multiple gingival recessions. The lack of vertical releasing incisions provides biological and aesthetical attributes, as mentioned above. The split-full-split flap has the benefit of, in the full flap, including the periosteum and a maximum soft tissue thickness in the central portion of the flap covering the avascular root exposure, while the final split thickness flap permits the coronal advancement of the flap, making sure all the muscle insertions present are eliminate (28, 41).

According to Graziani *et al,* 2014, both this MCAF approach and MCAT are most often evaluated for the treatment of MGR(4). However, while MCAT benefits from its maintenance of interdental papilla, absence in vertical releasing incisions, minimally invasive nature and negligible post-operative discomfort, it presents disadvantages due to its tendency in not covering the graft completely leading to colour mismatch and the need of a microsurgical kit and specific surgical skills to execute the procedure(6).

It is interesting to note MG’s behaviour during surgery (Figure 2). While fairly solid before being put into place to ease and accommodate sutures, once in contact with blood the membrane absorbed the blood becoming intensely dark red and slightly compressed. On the other hand, when irrigated with saline solution, the membrane vaguely expands and, in conjunction with underlying blood, takes on a pink hue with a spongey appearance resembling a CTG. Kasaj *et al*, 2015 showed that regardless the rehydration in a saline solution or blood, MG tensile strength is not altered(32) However, unlike a CTG, MG is not a vascularized structure and the success and integration of a graft in root coverage depends

greatly on the vascularization of the graft(14). In the case of MG, the vascularization is provided from the flap and the bed the graft is placed on. Conversely, a significant portion of this bed is made up of an avascular root surface and denuded bone without periosteum since a full thickness flap is carried out in this region, so the only source of vascularization comes from the flap placed over the membrane. Considering the graft only receives a blood source from one side in this region, it is open to speculation whether the extension of the initial split thickness flap would be beneficial to the success and integration of the graft.

After surgery and during the initial follow-ups, healing was uneventful. All the surgical procedures were well tolerated and, in general, considerably little pain was experienced during the first week post-operative.

In terms of CRC at 6-months post-operative, this was only obtained from patients with MCAF/CTG. In patients with MCAF/CTG CRC after 6 months, CRC was 75%, a lower value than the studies in the review previously reported (89,5% at 6-months(24)). MRC also proved to be much lower, 87.5% than the value Zuchelli *et al*, 2014 obtained (96.8%(24)). Nevertheless, only 1 patient is involved to obtain this CRC value, while the conducted review included 41 patients across two randomized controlled studies. It cannot be affirmed that this CRC result obtained is representable of clinical outcomes of the MCAF/CTG technique as the number of patients included in this study is far too little. However, as previously discussed, studies have shown that the use of MCAF with CTG tends to produce a coronal shift of the gingival margin over time, especially from 12 to 60 months(24). Therefore, a longer follow-up would be needed to assure that this creeping attachment effect would occur and the treated sites potentially gain 100% CRC.

The existing recessions reduced, attaining a mean value of 1,25mm in RecRed, a lower value than what Zucchelli *et al,* 2014 obtained at 6 months (3.1mm)(24). However it should, again, be noted that only one patient contributed to this value and their recessions were, in average, smaller (1.5mm) than those Zuchhelli *et al*, 2014 started off with (3.15mm), and since a greater recession is presumed to achieve a greater RecRed, these values are justified(23).

There was also a gain in KT height of 1mm a higher value than Zucchelli *et al,* 2014 achieved (0.4mm) (24). This difference could be due to this study only including one patient while Zucchelli *et al*, 2014 included 25, making it possible to calculate a mean values. Furthermore, the measurements taken were approximated to the nearest millimetre, meaning that if, for example KT was 1.3mm, this would be approximated to 1mm, while a 1.6mm of KT would be approximated to 2mm, potentially explaining the lower value obtained. Nevertheless, an increase in KT height was not the objective of surgery in either

study. Seeing as the increase in KT contributes to the coronal shift of the gingival margin (due to the placement of a CTG) (23), further follow-ups would be needed to evaluate whether this optimistic KT gain would transfer into a greater creeping effect.

Probing depths remained non-pathological during the entirety of the study.

Considering that patients that underwent graft harvesting had an additional surgical site, it would be expected that these present considerably higher complaints of pain. P1 after 1 week reported an average pain level of 1.25 (2.12 standard deviation). Far higher pain levels on the day of the surgery (6 on the VAS scale) were reported and subsided over the next two days (pain levels of 2 on the following two days). This high 6 value on the pain scale is questionable due to its discrepancy in relation to the following values and the painkiller intake did not accompany this high pain value (2 on the day of the surgery and on the first day and 1 on the second day). Additionally, when comparing VAS scores from other patients that underwent CTG harvesting, there was also a discrepancy (the highest level of pain in these patients was 2), although these patients underwent different surgical procedures after the CTG harvesting and therefore a faithful comparison cannot be made. Patient perceived pain is always subjective as some patients are more prone to complaining about experienced pain. This subjectivity should be taken into account when comparing results. The mean painkiller intake of P1 over the week was 1.13 (1.13 standard deviation). This patient also reported experiencing a fever on day 5, 6 and 7, taking painkillers on day 6 and 7, but no clinical anomalies were associated to these complaints. The day most painkillers were taken was day 6 (3 due to fever), followed by the day of the surgery (2) and day 1 (2), day2 (1) and day 7 (1 due to fever). It is difficult to compare these results with those obtained in the conducted review since both are bound to subjectivity and the authors in both MCAF/CTG patients used a different VAS scale. However, it can be said that in the likeliness of the studies included in the review, MCAF/CTG patients had low post-operative pain during the first week.

P2 reported low pain levels (mean: 0.5, standard deviation: 0.7), registering only a value of 2 on the day of the surgery and a 1 on the following two days. No painkillers were taken. Comparison to the case report included in the review is problematic as although the authors evaluated the post-operative pain with a similar VAS scales, it was evaluated during 2 weeks and no quantitative values are supplied. The case report stated a slight discomfort during these 2 weeks(3), which is difficult to translate and compare to the results obtained in P2.

Taking into account the higher pain level in average and the higher intake of painkillers in the MCAF/CTG patient, it may appear that MCAF/CTG was associated with

higher post-operative pain, but due to the subjectivity of this evaluation, and the small number of patients included in each approach it cannot be determined with certainty.

Both patients reported that their main motive for surgery was functional, namely hypersensitivity. After 6-months, P1 reported no more hypersensitivity symptoms. A longer follow-up is needed for P2 to determine if hypersensitivity complaints subside.

The patients' perception of time was also not influenced by the procedure used. In spite of the procedure, both patients supposed that the surgery lasted between 1.5 and 2 hours and thought it was a normal amount of time. An objective measurement of the surgical time would be needed to confirm this. However, although it would be expected the MCAF/CTG surgery to last longer due to the need of CTG harvesting, this may not be the case. The MCAF/MG procedure requires delicate sutures to lay and maintain the MG in place, expending time that may render the surgical time equivalent in both approaches.

4.2.3. Post-operative Pain evaluation

A second surgical site can be a burden on the patient, increasing patient discomfort during and after surgery, surgical chair time and chances of complications. It is also restricted to the patients anatomical limitations, where there may be a lack of available tissue for harvesting, and the clinician must have specific surgical skills to perform the harvesting(3, 6, 14, 29, 49).

In order to evaluate whether the second surgical site, i.e. the donor site, is the main cause of pain, six patients that underwent CTG harvesting, regardless of the posterior surgical procedure applied, answered a post-operative questionnaire and handed them in at 1-week post-operative. The same technique was applied for the CTG harvesting. Patients had a variety of GR, from single to multiple and maxillary to mandibular.

Results revealed a relatively low pain level throughout the week (mean: 2.17; standard deviation:1.15) in which the day with most pain was the day of the surgery, (mean: 217, standard deviation: 2.04). The pain diminished over the following 3 days and by the fourth day after surgery, no pain was reported. These results are also difficult to compare to the studies included in the review that underwent CTG harvesting and described the patients post-operative pain (24, 28) because the authors used a different VAS scale and quantitative values were not supplied.

The mean painkiller intake was 0.5 per day (0.88 standard deviation). Although expected to follow the pain level pattern, the most painkillers were taken on the first day after

surgery (mean: 1.5, standard deviation: 0.88). it can be speculated whether this day was the day patients experienced more swelling, for example, adding to their discomfort and increasing their painkiller intake. The day of the surgery followed as the day with the second highest painkiller intake (mean: 1.17, standard deviation: 0.98) and the second day was the third highest (mean: 0.67, standard deviation: 0.82). One patient referred experiencing a fever on day 5, 6 and 7, taking painkillers on day 6 and 7, but no clinical anomalies were associated to these complaints, as previously referred.

In terms of which site caused more pain, five in six patients referred that both sites hurt equally, not being able to point to one surgical site as the main cause of pain and one patient referred that the receptor site hurt more.

Despite the small number and heterogeneity of patients included, this is an interesting result as allows to infer that a low pain level in described in these patients and when a second surgical site is used, it is not necessarily the donor site that causes the main complaint of pain. Nevertheless, further investigation should be made using the same surgical technique in which the CTG is applied and patients with the type of GR, since a MGR will require a larger CTG and therefore a larger donor site and maxillary and mandibular receptor sites have different clinical outcomes(11), influencing the pain experienced.

4.3. Histology

4.3.1. Connective Tissue Evaluation

The two biopsies collected were intended for histological characterization of the connective tissue grafts through a light microscope, identifying the mean depth of the LP and the submucosa as well as the mean percentage of collagen fibres present in the LP and the submucosa. CTG A was acquired from the pre-molar area, while CTG B was collected from the area adjacent to the first upper molar.

The oral mucosa is composed by a stratified squamous epithelium, which may be keratinized or not, depending on the region. At the hard palate no keratin is seen in the epithelium. The underlying connective tissue is called the LP, and this presents some variations in its density (it may be a loose or dense connective tissue depending on the anatomical region of the oral cavity).

The palatal area between the first upper pre-molar until distal of first upper molar LP is typically made up of highly dense and thick collagen tissue with vastly packed collagen

fibres while the submucosa has relatively rather loose collagen tissue and a greater prevalence of adipose tissue and minor salivary glands. The LP is a more external layer of tissue, and the dense connective tissue serves as a buffer, to all the compressive forces, abrasion and attrition during mastication. Additionally, the SM contains significant amounts of adipose tissue and glandular tissue that also enables a mechanical cushioning and protects underlying structures like nerves and blood vessels(36-38).

Hence, the results were expecting to find a higher percentage of collagen fibres in the LP compared to the SM. In fact, the results corroborate in general these common characteristics. Both CTG A and CTG B show a LP with dense collagen fibres and a higher percentage (92.65% and 89.30% respectively) of connective tissue proper compared with the submucosa that presents adipose tissue and loose connective tissue and also vascular structures. Even though the submucosa presents lower percentages of connective tissue than the LP, the percentage of collagen fibres obtained in the submucosa of CTG A (79.41%) is still a substantial value. Therefore, the connective tissue proper in the submucosa in conjunction with the adipose tissue still enables some type of protection against mechanical forces and also can have a sufficient amount of collagen fibres and blood vessels that makes it suitable for connective tissue graft.

However, CTG B showed a slightly higher value of connective tissue in the submucosa (90.31%) compared with the LP (89.30%). This was not expected and could be explained due to the location where the biopsy was made (distal to tooth 26), although posterior areas such as this one typically present even more adipose and glandular tissue in comparison to the pre-molar area. Another explanation could reside in inter-individual variability or gender related anatomic variations since females typically present thinner palatal masticatory mucosa that increase with age(16). However, both patients were young females, but only the patient with CTG B presented such a high percentage of connective tissue proper.

Clinically speaking, the thickness of the graft and its relative composition are important for the success of the graft in root coverage. The success of CTG implantation and integration depends heavily on the vascularization provided at the receptor site but also the vascularization the CTG has itself(14). According to Reiser *et al*, 1996, CTG should have a minimum of 1.5mm in thickness and studies have shown that it is important to not include submucosa in CTG since the adipose tissue included in the graft could function as a barrier to vascularization from the flap and underlying bed(54, 55). Hence, in theory, the LP is an ideal harvesting tissue due to its low adipose tissue and therefore high quantity of connective tissue and should additionally offer a minimum of 1.5mm in thickness.

On the one hand, this is true in CTG A in which the LP has more connective tissue, the submucosa more adipose tissue and the mean thickness of its LP was 1.441mm (49.3% of the graft) ensuring it as an adequate CTG. On the other hand, CTG B proved to be unexpected in this sense; both the LP and the submucosa had high percentages of connective tissue, proving the entirety of the graft adequate for harvesting and using at the receptor site. The thickness of the LP was appropriate too, with a mean value of 1.623mm (50.7%of the graft) and a total of 3.124mm. Harris *et al*, 2003 reported that the mean depth of the LP was 3.2mm (65.2% of the graft) and the submucosa was 2.0mm (34.8% of the graft)(55). The results in this study reported both thinner proportions of LP and SM and relatively equal percentages. This could be due to both patients being young females, while Harries *et al* included a total of 30 patients, 18 women and 12 men with a mean age of 42.1 years old. Since there were more men and older patients the palatal mucosa is presumed to be thicker(16). Nevertheless, the LP values obtained in this are still considered appropriate for CTG harvesting and use.

4.3.2. Mucograft^{R} Evaluation *In Vivo*

The *in vivo* evaluation of the tissue reaction and collagen membrane integration with the peri-implant tissues were analysed at 15 and 30 days. When assessed in a mouse model, MG was well integrated within the host tissue, persisting in the implantation bed throughout the *in vivo* experimental study. During this time, no signs of inflammatory process, haemorrhage, necrosis or abscess formation were identified around the implants. MG implants were found to go through a biological progression initiated by a transient infiltrate of inflammatory cells, mainly polimorphonuclear neutrophils (PMN neutrophils), followed by mesenchymal cell recruitment and differentiation in fibroblast and vascular structures. The most part of the membrane persisted after 30 days but underwent remodelling and cellular repopulation to form tissue with a morphology similar to the native connective tissue.

At 15 days the membrane had almost maintained its initial thickness and both the thin CL and thick SL were well defined. New blood vessels were present predominantly at the periphery of the MG as well as facing the CL, however the CL remained resistant to infiltration.

On the other hand, at 30 days the membrane had maintained its shape but the two layers were not well distinguished. A continuous replacement and integration of the collagen from the membrane by soft connective tissue from the host was clear. A dense network of a

well-organized residual collagen matrix including large amounts of newly formed thick collagen fibres were detected, indicating that the implants were remodelling during the 30 days, but not entirely replaced with host collagen. More fibroblast-like cells and blood vessels (mainly in the centre of the membrane) were found in comparison to the 15 days, reinforcing the integration of the membrane and proving the membrane more penetrable than it was at 15-days.

At both 15 and 30 days the tissue reaction was clinically well tolerated and the histological study showed signs of a well-accepted biomaterial, although some multinucleated giant cells were observed. Multinucleated giant cells are the dominant early responders to biomaterial implantation and remain at tissue interface s for the lifetime of the device or graft(56). Although they may be typically associated to foreign body reactions it should be noted that they are essential to the mediation of the degradation of bio-resorbable materials through extracellular degradation and phagocytosis(56). Giant cell formation is dependent on the biomaterial surface morphology, requiring the adsorption of a specific spectrum of proteins in order to trigger fusion of adherent mononuclear cells into multinuclear giant cells (30). Although their presence could be understood as a foreign body reaction no other signs of inflammation could be identified and could instead be attributed to the normal biodegradation of the membrane. In fact, all signs point to the membranes biocompatibility, be it through the presence of vascular structures, cellular infiltration, limited inflammatory cells or absence of a fibrotic capsule formation.

The two sides of the membrane had different behaviours, the CL served as a barrier function, preventing cellular infiltration from the surrounding tissue while the SL promoted cell adhesion and tissue integration(30). As seen at 15 days, the SL was rapidly infiltrated by a few host mesenchymal cells while the barrier layer showed almost none and when present, more densely packed cells. A faster turn over and the remodelling process was observed mostly in the porous bottom layer. The degradation and simultaneous replacement by endogenous collagen fibres started in the porous layer while the thin CL kept almost all of its primary structure. The barrier layer prevented unspecific tissue ingrowth, as the scaffold become infiltrated by mesenchymal cells from adjacent tissue into the porous layer. Only a few sparse cells were able to attach to the CL and host tissue integration but at the same time the CL remained impermeable to invading cells for the first 30 days of the study. When the SL was placed next to host tissue, it aided in clot formation and supported cell in-growth and tissue integration. This SL, while allowing cells to infiltrate without much resistance, maintained the membranes structure and became integrated into the host tissue as opposed to being completely degraded. Its porosity endorsed a scaffold behaviour on which peri-implantary cells, connective tissue and blood vessels were able to grow and advance into

the centre of the matrix(30). Taking this into account, this bilayered collagen matrix elicits a favourable tissue reaction showing a great potential as a barrier on the compact layer and a preferential tissue ingrowth on the porous bottom layer.

Similar results were reported in Ghanaati *et al,* 2011(30) in which MG was implanted in mice and these were sacrificed and the membrane histologically analysed at 3, 10, 15, 30 and 60 days. At 15 days the two layers of the membrane were distinctly visible, the CL served as a barrier for cell ingrowth into the core of the matrix, with some cells being diffusely spread through the thick sheets of collagen, while the SL was invaded by peri-implant tissue, with a further increase in matrix production by these host cells. By day 30 the CL no longer maintained its function as a non-penetrable barrier as cells has penetrated the scaffold from both surfaces of the matrix and become more dispersed throughout. However, no multinucleated giant cells were detected throughout the study, indicating that, in their case, macrophages were sufficient for biodegradation of the membrane.

5. Conclusions

More studies with the use of both MCAF/CTG and MCAF/MG are needed, especially with long comparable follow-ups. The initial question “In patients with multiple gingival recessions, what is the efficacy of CAF plus CTG compared to CAF plus Mucograft in terms of CRC, after a minimal 6-month follow-up?” was not satisfactorily answered. Due to the included studies’ limitations, it is not accurate to affirm that MCAF/MG is more efficient than MCAF/CTG at this time.

The clinical portion of this study also presented limitations when comparing MCAF/CTG to MCAF/MG, a larger number of patients with the same follow-up period would be necessary to draw conclusions about the CRC at 6-months post-operative. Nevertheless, it should be noted that the use of MG avoided a second surgical site, overcoming anatomical limitations, avoiding surgical difficulties in acquiring a CTG and possibly reducing post-operative pain.

Patients that underwent CTG harvesting and responded to a post-operative pain questionnaire revealed low post-operative pain and that the donor site is not necessarily the cause of more pain. However, more investigation is needed with a larger amount of standardized patients.

Histologic results are important and should accompany clinical results. The palatal biopsies collected supported existing notions about the composition of CTG, namely that the LP contains a higher density of connective tissue, while the SM has more adipose tissue, although variability between CTG are not uncommon. The LP serves as the better harvesting tissue due to its lack of adipose tissue as long as it is at least 1.5mm thick, which was the case of the palatal biopsies.

When MG was implanted in mice and analysed at 15 and 30 days, the matrix revealed an optimal integration and remodelling process with newly formed collagen fibres and vascular structures.

6. Acknowledgments

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And finally, to my family, my Mother and Brother, my boyfriend and all my friends, thank you for supporting me along this remarkable journey. None of this would be possible without you and you made it all worthwhile.

7. Appendix

A. Review Results

Article	STUDY				RESULTS			
					CLINICAL			
	Study design	n	Recession type/jaw	Comparison / Follow-up (m)	CRC	MRC	RecRed	CAL
Rotundo et al, 2012 *Use of a new collagen matrix (mucograft) for the treatment of multiple gingival recessions: case reports.*	Case report	3 patients with multiple recessions (2 patients treated with MCAF+MG).	Cl I and II/max.	MCAF+Mucograft (2 patients - 7 recessions) and Tunnel technique+Mucograft (1 patient - 4 recession)/3, 6 and 12m.	12m: 71.4%	12m: 81.3%	12m: 2.4.	-
Zucchelli et al, 2014 *Coronally advanced flap with and without connective tissue graft for the treatment of multiple gingival recessions: a comparative short- and long-term controlled randomized clinical trial.*	Comparative short and long-term RCT	50 patients with multiple (≥2) recessions. (25 patients with MCAF+CTG)	Cl I and II / max.	MCAF vs. MCAF+CTG /6m, 12m, 60m.	6m:89.5%; 12m: 86.8%; 60m: 90.8% .	6m: 96.8%; 12m: 95.9%; 60m: 97.1%.	6m: 3.1; 12m: 3.0; 60m: 3.1 ($p<0.01$ in all).	Baseline: 4.2; 6m: -; 12m: 1.2; 60m: 1.3 ($p<0.01$ in all).
Zucchelli et al, 2009. *Coronally advanced flap with and without vertical releasing incisions for the treatment of multiple gingival recessions: a comparative controlled randomized clinical trial.*	Parallel RCT	32 patients with multiple (≥2) recessions. (16 patients with MCAF+CTG)	Cl I and II / max.	MCAF vs. MCAF+CTG /12m	12m: 89.3%	12m: 97.3%	12m: 2.5 ($p<0.01$).	Baseline: 3.8; 12m: 1.2 ($p<0.01$).

Results							
Clinical			PATIENT			CLINICIAN	Histology
PD	KT Gain	Surgical time	Complications	Post-operative pain	Aesthetic satisfaction	Colour, contour and contiguity of soft tissues	
Baseline: 2.00; 12m: 1.00.	12m: 0.7.	-	-	2 weeks: slight discomfort	12m: high.	-	-
Baseline: 1.1; 6m: -; 12m: 1.1; 60m: 1.2.	6m: 0.4; 12m: 1.0 (p<0.01); 60m: 1.7 (p<0.01).	40,2min (p<0.01 compared to MCAF).	Healing: uneventful in all treated cases. Shrinkage of flap with graft exposure: 2 patients in MCAF+CTG group at 6m, 6 patients at 12m, 9 patients at 60m. Bleeding: 1 patient for 2 days at palatal wound	1 week - limited post-op pain/ discomfort	12m: high; 60m: high.	Colour: 12m: 78.4 60m: 73.6. Contour: 12m:89.6; 60m:87.2. Contiguity: - Keloid formation in 11 patients at 60m.	-
Baseline: 1.2; 12m: 1.1.	12m: 0.7 (p<0.01).	28.7min (p<0.01 compared to MCAF).	Healing: uneventful. 5 patients reported swelling	1 week - Limited morbidity: 8 patients reported na excellent post-op course, and 0 reported a less-than-average post-op course	12m: high; 7 patients reported excellent and 1 patient reported average aesthetic satisfaction	12m: Colour: 95. Contour:77.13. Contiguity: present in all patients. No keloid formation	-

Table 8 - Review results

B. Post-operative Questionnaire

Inquérito Dor Pós Operatória

1. Para avaliar a dor pós-operatória, classifique a sua intensidade, diariamente e sensivelmente às mesmas horas, através de uma escala numerada de 0 a 10, em que o zero corresponde à ausência de dor e o 10 à dor máxima. Adicionalmente registe o número de analgésicos ingeridos nos primeiros sete dias pós-cirúrgicos.

Sem Dor	Dor Ligeira			Dor Moderada			Dor Intensa			Dor Máxima
0	1	2	3	4	5	6	7	8	9	10
										
										
Dia 0	Dia 1	Dia 2	Dia 3	Dia 4	Dia 5	Dia 6	Dia 7			
Número de Analgésicos Ingeridos (Paracetamol 1000mg)										

2. No caso de ter dor em que local esta foi mais forte (colocar círculo em torno opção mais correta)?
   2.1 – No local de recolha do enxerto (palato)
   2.3 – No local da colocação do enxerto
   2.3 – Ambos locais

3. O principal motivo que o levou a fazer a cirurgia foi (coloque um círculo à volta da opção mais correta):
   3.1 – Estético
   3.2 – Funcional

4. Quanto tempo demorou a cirurgia (coloque um círculo à volta da opção mais correta)?
   5.1 – Menos de 1h
   5.2 – Entre 1-1,5h
   5.3 – Entre 1.5-2h
   5.4 – Mais de 2h

6- Achou que o tempo de cirurgia foi (coloque um círculo à volta da opção mais correta)?
   6.1 – Muito Rápido
   6.2 – Rápido
   6.3 – Normal

6.4 – Lento
6.5 – Demasiado Lento

A preencher pelo médico: Houve algumas complicações ou necessidade de tratamentos adicionais?

		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
P1 – MCAF/CTG	VAS Scale pain	6	2	2	0	0	0	0	0
	No. Painkillers taken	2	2	1	0	0	0*	3*	1*
	Site with most pain: Both	Main motive for surgery: Functional		Surgery time perceived by patient		1 – 1.5 hours			
						Normal amount of time			
	Pain			**1w Mean: 1.25**			**Std Dev: 2.12**		
	Painkillers			**1w Mean: 1.13**			**Std Dev: 1.13**		

Table 9 - Post-operative pain results of P1 that underwent MCAG/CTG (*patient reported fever).

		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
P2 – MCAF/MG	VAS Scale pain	2	1	1	0	0	0	0	0
	No. Painkillers taken	0	0	0	0	0	0	0	0
	Site with most pain: N/A	Main motive for surgery: Functional		Surgery time perceived by patient		1 – 1.5 hours			
						Normal amount of time			
	Pain			**1w Mean: 0.5**			**Std Dev: 0.7**		
	Painkillers			**1w Mean: 0**			**Std Dev: 0**		

Table 10 - Post-operative pain results of P2 that underwent MCAF/MG.

		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
POST-OPERATIVE PAIN RESULTS	VAS Scale pain	2	1	1	0	0	0	0	0
	No. Painkillers taken	2	1	0	0	0	0	0	0
	Site with most pain: Receptor site	Main motive for surgery: Functional		Surgery time perceived by patient		1.5 – 2 hours			
						Normal amount of time			

POST-OPERATIVE PAIN RESULTS	VAS Scale pain	2	2	1	0	0	0	0	0
	No. Painkillers taken	1	2	1	0	0	0	0	0
	Site with most pain: Both	Main motive for surgery: Functional		Surgery time perceived by patient	1.5 – 2 hours Normal amount of time				
	VAS Scale pain	1	1	2	0	0	0	0	0
	No. Painkillers taken	2	3	2	0	0	0	0	0
	Site with most pain: Both	Main motive for surgery: Functional		Surgery time perceived by patient	1.5 – 2 hours Normal amount of time				
	VAS Scale pain	2	3	2	1	0	0	0	0
	No. Painkillers taken	0	0	0	0	0	0	0	0
	Site with most pain: Both sites	Main motive for surgery: Functional		Surgery time perceived by patient	1 – 1.5 hours Normal amount of time				
	VAS Scale pain	0	0	0	0	0	0	0	0
	No. Painkillers taken	0	1	0	0	0	0	0	0
	Site with most pain: Both sites	Main motive for surgery: Aesthetic		Surgery time perceived by patient	More than 2 hours Normal amount of time				
	VAS Scale pain	6	2	2	0	0	0	0	0
	No. Painkillers taken	2	2	1	0	0	0*	3*	1*
	Site with most pain: Both	Main motive for surgery: Functional		Surgery time perceived by patient	1 – 1.5 hours Normal amount of time				
PAIN	**Mean**	**2.17**	**1.50**	**1.33**	**0.17**	**0**	**0**	**0**	**0**
	Std Dev	**2.04**	**1.05**	**0.82**	**0.41**	**0**	**0**	**0**	**0**
	1w Mean: 0.66			**1w Std Dev: 1.15**					
PAIN KILLERS	**Mean**	**1.17**	**1.50**	**0.67**	**0**	**0**	**0**	**0.50**	**0.17**
	Std Dev	**0.98**	**1.05**	**0.82**	**0**	**0**	**0**	**1.22**	**0,41**
	1w Mean: 0.50			**1w Std Dev:0.88**					

Table 11 - Post-operative pain results of patients that underwent CTG harvesting regardless of the surgical technique (*patient reported fever).

C. Excluded Articles

Exclusion criteria:

1. Localized recessions
2. Recessions other than Cl I or II
3. Mandibular GR
4. Use of technique other than MCAF/CTG or MCF/MG
5. Root coverage is not the objective of the treatment
6. Animal studies
7. Histological and other outcomes other than clinical
8. Studies other than clinical trials, longitudinal studies or comparative studies
9. Language other than English or Portuguese
10. Follow-up less than 6m

☐ Excluded after reading full-text

i. First Electronic Search

PubMed (MEDLINE)

Author/Year	Reason for Exclusion	Author/Year	Reason for Exclusion
Bellyer-fernández et al, 2015	1	Santamaria et al, 2010	1
Femminella et al, 2015	4	Zucchelli et al, 2010	1,10
Cairo et al, 2015	1	Sanz et al, 2009	1,2,5
Lops et al, 2015	1	Núñez et al, 2009	1,2,6
Zucchelli et al, 2014	3	Byun et al, 2009	1
Salhi et al, 2014	1	Abolfazli et al, 2009	1
Zucchelli et al, 2014	1	Cortellini et al, 2009	1
Zucchelli et al, 2014	4	Lafzi et al, 2007	5
Fren et al, 2014	1	Domaniak et al, 2006	1
Zuhr et el, 2014	4	Moses et al, 2006	1
Alkan et al, 2014	3,4	Gapski et al, 2005	8
Aroca et al, 2013	4	Nemcovsky et al, 2004	1
Kuis et al, 2013	1	Zucchelli et al, 2003	1
Roman et al, 2013	1	McGuire et al, 2003	7
Nart et al, 2012	2,3	McGuire et al, 2003	7
Cairo et al, 2012	1	Roccuzzi et al, 2002	8
McGuire et al, 2012	1	Berlucchi et al, 2002	4
Nevins et al, 2011	5	Zucchelli et al, 1998	1
Cardaropoli et al, 2011	1	Wennström et al, 1996	1
Alkan et al, 2011	1,4	Tatakis et al, 2015	8
Cairo et al, 2010	5	Pini-Prato et al, 2014	1
Pini-Prato et al, 2010	2	Cairo et al, 2014	8
McGuire et al, 2010	1	Oliveira et al, 2012	8

Table 12 - Excluded articles from the first electonic search in PubMed (Medline).

EBSCO

Author/Year	Reason for Exclusion
Zucchelli et al, 2010	1

Table 13 - Excluded articles from the first electronic search in EBSCO.

i. Second Electronic Search

PubMed (MEDLINE)

Author/Year	Reason for Exclusion	Author/Year	Reason for Exclusion
Atieh et al, 2015	8	Lukács et el, 2011	1,4,9
Nizam et al, 2014	1	Stimmelmayr et al, 2011	3,4
Cheng et al, 2014	8	Mazzocco et al, 2011	1
McGuire et al, 2014	1	Jaiswal et al, 2010	2,4,5
Zucchelli et al, 2013	1	Roman et al, 2010	7
Buti et al, 2013	8	Henriques et al, 2010	2
Fu et al, 2012	8	Amberkar et al, 2010	1
Landsberg et al, 2012	1	Mcguire et al, 2009	1
Cortellini et al, 2012	8	Mcguire et al, 2009	7
Camelo et al, 2012	7	Cairo et al, 2008	8
Koop et al, 2012	8	Hwang et al, 2006	8
Clauser et al, 2003	8	Iwano et al, 2013	1
Kleinfelder et al, 2002	9		

Table 14 - Excluded articles from the second electronic search in PubMed (MEDLINE).

EBSCO

Author/Year	Reason for Exclusion	Author/Year	Reason for Exclusion
Uraz et al, 2015	4	Matthews et al, 2003	8
Thakare et al, 2015	4	Zucchelli et al, 2015	8
Öncu et al, 2015	9	Waghmare et el, 2013	3
Carvalho et al, 2006	3		

Table 15 - Excluded articles from the second electronic search in EBSCO.

ii. Hand Search

Cross References

Author/Year	Reason for Exclusion	Author/Year	Reason for Exclusion
Cetiner et al, 2004	4	Schlee et al, 2011	3

Table 16 - Excluded articles from hand searching cross references.

Journal of Clinical Periodontology

Author/Year	Reason for Exclusion	Author/Year	Reason for Exclusion
Santamaria et al, 2009	4	Keceli et al, 2008	1
Santamaria et al, 2009	**4**	**Moslemi et al, 2011**	**1**

Table 17 - Excluded acrticles from hand searching in the Journal of Clinical Periodontology.

Journal of Periofdontology

Author/Year	Reason for Exclusion	Author/Year	Reason for Exclusion
Bittencourt et al, 2009	1	Erlwy et al, 2006	1
Bittencourt et al, 2006	1	Hirsch et al, 2005	1,4
Bittencourt et al, 2012	1	Harris et al, 2004	1,4
Carvalho da Silva et al, 2004	1	Henderson et al, 2001	4
Chambrone et al, 2006	3,4	Rosetti et al, 2000	1,4

Table 18 - Excluded acrticles from hand searching in the Journal of Periodontology.

D. Informed Consent

Consentimento Informado

Dou permissão para a utilização dos registos clínicos efetuados no decorrer da minha reabilitação, para fins de publicação científica e investigação, que devem preservar o anonimato e declaro que, para tal, não obtive qualquer tipo de renumeração ou gratificação.

Dou igualmente autorização para que, na consequência de uma cirurgia a que fui submetido(a), utilize parte excedente do meu tecido gengival para análise histológica. Esta tem como finalidade a investigação cientifica e publicação.

Compreendi as explicações que me facultaram numa linguagem clara e simples. Esclareci todas as duvidas. Compreendi que em qualquer momento e sem necessidade de explicação posso revogar o consentimento que agora assumo.

Pelo exposto, manifesto que estou satisfeito(a) com a informação recebida, que compreendi as implicações dos riscos perante tal consinto.

O doente: Fernando Miguel Marques dos Santos

O médico dentista responsável:

Testemunha: Sónia Cabdo

Data: 13-06-2016

Consentimento Informado

Dou permissão para a utilização dos registos clínicos efetuados no decorrer da minha reabilitação, para fins de publicação científica e investigação, que devem preservar o anonimato e declaro que, para tal, não obtive qualquer tipo de renumeração ou gratificação.

Dou igualmente autorização para que, na consequência de uma cirurgia a que fui submetido(a), utilize parte excedente do meu tecido gengival para análise histológica. Esta tem como finalidade a investigação cientifica e publicação.

Compreendi as explicações que me facultaram numa linguagem clara e simples. Esclareci todas as duvidas. Compreendi que em qualquer momento e sem necessidade de explicação posso revogar o consentimento que agora assumo.

Pelo exposto, manifesto que estou satisfeito(a) com a informação recebida, que compreendi as implicações dos riscos perante tal consinto.

O doente:

O médico dentista responsável:

Testemunha: Sónia Cabdo

Data: 15/11/15

Consentimento Informado

Dou permissão para a utilização dos registos clínicos efetuados no decorrer da minha reabilitação, para fins de publicação científica e investigação, que devem preservar o anonimato e declaro que, para tal, não obtive qualquer tipo de renumeração ou gratificação.

Dou igualmente autorização para que, na consequência de uma cirurgia a que fui submetido(a), utilize parte excedente do meu tecido gengival para análise histológica. Esta tem como finalidade a investigação cientifica e publicação.

Compreendi as explicações que me facultaram numa linguagem clara e simples. Esclareci todas as duvidas. Compreendi que em qualquer momento e sem necessidade de explicação posso revogar o consentimento que agora assumo.

Pelo exposto, manifesto que estou satisfeito(a) com a informação recebida, que compreendi as implicações dos riscos perante tal consinto.

O doente: Rita Cruz

O médico dentista responsável:

Testemunha: Sónia Calado

Data: 30/11/15

Consentimento Informado

Dou permissão para a utilização dos registos clínicos efetuados no decorrer da minha reabilitação, para fins de publicação científica e investigação, que devem preservar o anonimato e declaro que, para tal, não obtive qualquer tipo de renumeração ou gratificação.

Dou igualmente autorização para que, na consequência de uma cirurgia a que fui submetido(a), utilize parte excedente do meu tecido gengival para análise histológica. Esta tem como finalidade a investigação cientifica e publicação.

Compreendi as explicações que me facultaram numa linguagem clara e simples. Esclareci todas as duvidas. Compreendi que em qualquer momento e sem necessidade de explicação posso revogar o consentimento que agora assumo.

Pelo exposto, manifesto que estou satisfeito(a) com a informação recebida, que compreendi as implicações dos riscos perante tal consinto.

O doente:

O médico dentista responsável:

Testemunha: Sónia Calado

Data: 22/1/16

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