

ORIGINAL ARTICLE

Yuri Castro, Sixto Grados

Facultad de Odontología, Universidad
Nacional Mayor de San Marcos, Perú.

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Corresponding author: Yuri Alejandro
Castro Rodríguez, Jr. Tomás Catari 463,
Urb El Trebol. Dept. 201. Los Olivos.
Phone: 989836354. Email:
yuricastro_16@hotmail.com.

Porcine collagen matrix for treating gingival recession. Randomized clinical trial.

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Abstract: Achieving root coverage after exposure caused by gingival recession is one of the main goals of reconstructive periodontal surgery. Even though a large variety of techniques and mucogingival grafting procedures are available, their long-term results are not clear yet. Therefore, this study aimed to compare clinical effectiveness of the porcine collagen matrix with subepithelial connective graft for treating Miller class I and II gingival recessions. **Materials and methods:** The randomized clinical trial included twelve patients assigned to two groups. In the first group (experimental), six patients were treated using collagen matrix (mean age, 54.3 ± 5.6 years; mean recession 2.67 ± 1.03 mm). Another group (control) of six patients was treated using connective grafts (mean age, 57.1 ± 2.7 years; mean recession 4.33 ± 1.03 mm). All patients underwent periodontal evaluation and pre-surgical preparation including oral hygiene instruction and supragingival scaling. Gingival recessions were exposed through partial thickness flaps where the grafts and matrices were placed. Patients were assessed periodically until complete healing of tissue. **Results:** Root coverage parameters, amount of keratinized gingiva, gingival biotype and clinical attachment level were evaluated. The root coverage percentage for the group using connective graft was $24.7 \pm 13.5\%$ and $16.6 \pm 26.8\%$ for the one treated with the matrix. The amount of increased keratinized tissue was 4.33 ± 2.06 mm and 4.5 ± 0.83 mm for the control and experimental group respectively. Both groups increased gingival biotypes from thin to thick at 100%. The final clinical attachment level was $4.17 \pm 3.17 \pm 0.4$ mm for the control group and 0.98 mm for the experimental group. There were significant differences between the outcome of gingival recession and clinical attachment. **Conclusion:** Results indicate both techniques, besides being predictable, are useful for improving clinical parameters when treating gingival recessions. Regardless of the fact that better root coverage was obtained with the subepithelial connective graft, both methods are really useful for increasing the thickness of keratinized tissue.

Keywords: Gingival recession, connective tissue, grafts, periodontal disease, collagen matrix.

Matriz de colágeno de origen porcino en el tratamiento de las recesiones gingivales. Ensayo clínico aleatorio.

Resumen: Lograr la cobertura radicular que se expone por las recesiones gingivales es uno de los principales objetivos de las cirugías periodontales reconstructivas. Una gran variedad de técnicas e injertos mucogingivales se encuentran como opciones terapéuticas. Sin embargo, los resultados y efectividad a largo plazo aún no son claros. Este estudio tuvo como objetivo comparar la efectividad clínica de la matriz de colágeno de origen porcino versus el injerto conectivo subepitelial en el tratamiento de las recesiones gingivales Miller clase I y II. **Materiales y métodos:** Ensayo clínico aleatorizado donde participaron 12 pacientes asignados de manera aleatoria en 2 grupos; el primer grupo de 6 pacientes (grupo experimental) fueron tratados con la matriz de colágeno (promedio de edad de $54,3 \pm 5,6$; promedio de las recesiones de $2,67 \pm 1,03$ mm) y el segundo grupo de 6 pacientes (grupo control) fueron tratados con el injerto conectivo (promedio de edad de $57,1 \pm 2,7$; promedio de las recesiones de $4,33 \pm 1,03$ mm). Todos los pacientes recibieron una evaluación periodontal y una preparación pre quirúrgica incluyendo las instrucciones de higiene oral y una raspaje supragingival. Las recesiones gingivales fueron expuestas a través de colgajos de espesor parcial en los cuales se colocaron los injertos y las matrices. Los pacientes fueron evaluados periódicamente hasta completar la cicatrización de los tejidos. **Resultados:** Se evaluaron los parámetros de cobertura radicular, cantidad de encía queratinizada, biotipo gingival y nivel de inserción clínica. El porcentaje de cobertura radicular para el grupo del injerto fue de $24,7 \pm 13,5\%$ y de $16,6 \pm 26,8\%$ para el grupo de la matriz. La cantidad de encía queratinizada aumentada fue de $4,33 \pm 2,06$ mm y de $4,5 \pm 0,83$ mm para el grupo del injerto y la matriz respectivamente. Ambos grupos aumentaron los biotipos gingivales de finos a gruesos en un 100%. El nivel de inserción clínica final para el grupo del injerto fue de $4,17 \pm 0,4$ mm y de $3,17 \pm 0,98$ mm para el grupo de la matriz. Se encontraron diferencias significativas en los parámetros de recesión gingival e inserción clínica. **Conclusión:** Los resultados indican que ambas técnicas son útiles para mejorar los parámetros clínicos al momento de tratar las recesiones gingivales además de ser técnicas predecibles. Sin embargo, la mejor cobertura radicular se obtiene con el injerto conectivo subepitelial siendo ambas técnicas muy útiles para aumentar el grosor de la encía queratinizada.

Palabras clave: Recesión gingival, tejido conectivo, injertos, enfermedad periodontal, matriz de colágeno.

Introduction.

Many forms of gingival and periodontal diseases have affected humans since the beginning¹. Classification of periodontal diseases has changed over recent decades and, though a consensus between various global partnerships has been sought, agreement has not happened.

Instead, new divisions seeking to address the above shortcomings have appeared². The most widely accepted categorization was proposed by the American Academy of Periodontology in 1999³. It includes numerous gingival and periodontal mucogingival diagnoses, several of which must be treated surgically. Thus, periodontal

and implant surgery emerge as sciences contributing rules, protocols, techniques, concepts, etc⁴. These should be carefully studied in order to provide the most appropriate treatment. One of the most prevalent diagnoses in the classification is gingival recession, which, like the others, has the potential to raise complaints from patients about aesthetic and dentin hypersensitivity. Recession is defined as the displacement of the gingival margin apical to the cement enamel-junction with exposure of the root surface to oral environment³. Others define it as the apical migration of the gingival margin along the root surface^{1,2,5}. Carranza said recession is the exposure of the root surface by an apical shift in the position of the gum¹. There are many surgical and nonsurgical procedures involving preventive and corrective methods for dealing with this diagnosis⁶. The first ones attempt to prevent different types of predisposing factors (traumatic brushing, orthodontic treatment, piercings, etc) which can cause or aggravate mucogingival defects. On the contrary, corrective procedures try to reverse the mucogingival defect and are most common for treating gingival recession⁷. The most widely used corrective treatments are surgical, for example, the classical techniques of epithelial and connective grafts and replacement flaps⁸. The epithelial, or free gingival graft, is usually placed where there is absence or reduction of keratinized tissue⁹. For connective subepithelial grafting, tissue is obtained from the palatal fibromucosa. Then, it is placed in the affected area and covered by a coronally repositioned flap¹⁰. There are also more current procedures, like guided tissue regeneration (GTR) and the use of matrices or alloplastic grafts, to reverse mucogingival defect around the tooth. GTR allows connective and bone tissue regeneration by placing a coverage membrane on top of the defect area. Matrices are exogenous or autogenous grafts obtained after rigorous sterilization processes and used to simulate human body tissues¹¹. Using matrices under moved flaps in regenerative procedures seeks to avoid obtaining grafts from a palatal donor site, but their ability to increase keratinized gingiva has been questioned. Also, it is essential to create some space, which is difficult because of the type of bone defect associated with recession¹². The most commonly used matrices are collagen and acellular dermal such as Dinamatriz, AlloDerm and Mucograft¹³.

The current trend is opting for regenerative procedures and avoiding increased patient morbidity. Therefore, new biomaterials for treating periodontal diseases such as gingival recessions are being tested. Acellular dermal matrices based on collagen tissue are being used successfully and becoming a feasible option as well.

This study aimed to compare clinical efficacy of a new biomaterial in periodontics, the collagen matrix of porcine origin versus subepithelial connective graft for treating gingival recessions in twelve patients at the

Faculty of Dentistry of the Universidad Nacional Mayor de San Marcos. We hypothesized the collagen matrix has a similar clinical efficacy as subepithelial connective graft with the advantage of avoiding a second surgery.

Materials and methods.

Design: A parallel randomized clinical trial designed according to the requirements of CONSORT guidelines was carried out¹⁴.

Population: Twelve systemically healthy patients aged 30 to 60 years and treated at the Periodontal Clinic of Dentistry, Faculty of Dentistry, Universidad Nacional Mayor de San Marcos during July and August 2013 were chosen. They were selected with the convenience non-probability sampling method with clinical trial reference. The sample was also in accordance with the type of exploratory study needed to investigate the effect of a new biomaterial in periodontics.

Bioethical Considerations: The study protocol and informed consent were approved by the Ethics Committee of the Faculty of Dentistry of the Universidad Nacional Mayor de San Marcos and were formulated in accordance with the ethical standards of the Declaration of Helsinki¹⁵.

Selection Criteria: All patients were questioned about aesthetic or dentin sensitivity caused by gingival recession. The subjects were non smokers and did not have to present probing depths exceeding 4 mm in the entire oral cavity (classified as ASA I), have tooth mobility and must have shown effective bacterial plaque control (lower than 20% IOH). All selected patients had Miller Class I or II = 3 mm maxillary or mandibular gingival recession areas. For over 5 mm recessions, the ones with the best prognosis for surgical treatment (adjacent pieces with good amount of papillary tissue) were selected. All surgical areas must not have shown periodontal pockets or periodontal inflammation appearances. All patients received instruction on oral hygiene, plaque control and use of soft-bristled toothbrush in order not to injure the gingival margin. All these parameters were monitored throughout the experimental phase.

Exclusion Criteria: Patients with systemic diseases (ASA II, III and IV), pregnant women, smokers, alcoholics, those with periodontitis and/or tooth mobility, who had taken antibiotics within 3 months prior to the study, were using antibiotics prophylactically as well as those who did not show good plaque control were excluded.

Procedure: After signing the informed consent, each patient was randomly assigned to one of the two groups (control and experimental). The first group (control) received treatment with subepithelial

connective graft (SCG) and the other (experimental) with the collagen matrix of porcine origin (CMP). CMP was obtained from the tissue bank of the Institute of Children's Health (INS), processed through cryogenics and lyophilization at the Peruvian Institute of Nuclear Energy (IPEN).

Clinical measurements were done by two previously precalibrated examiners. Inter-observer agreement and reliability was achieved in the pilot study with 0.82 Kappa value for qualitative variables and 0.7 intraclass correlation coefficient for quantitative variables. All measurements were performed with a millimeter probe OMS of 15mm. Gingival recession was measured as the distance between the amelocemental junction (ACJ) to the most apical position of the gingival margin (GM). The probing depth (PD) and clinical attachment level (CAL) were measured from the GM to the most apical portion of the gingival sulcus. The amount of keratinized gingiva (KG) was measured from the GM to the mucogingival junction (MGJ). All measurements were performed at the labial surface level of the tooth. The gingival biotype (GB) was determined at 1 mm from the GM through the perforation gum with the probe as measured from the epithelial surface to the bone level. The probe was perpendicular to the epithelium. Less than 0.5 mm biotypes were considered thin while larger width was estimated as thick.

Intervention: For the surgical procedure, a specialist in periodontics induced local anesthesia. Root surface was properly scraped and carefully smoothed with specific curettes. In both groups we began with a sulcular incision following the gingival recession and extending through the papillae of the adjacent teeth (as described by Bruno¹⁶). A partial thickness flap was raised in the first incision. In the control group (SCG), connective tissue was obtained from the palate area comprised between premolars distancing 3mm from the gingival margin. In the second group (CMP), the matrix was hydrated in saline solution for 10 minutes according to manufacturer instructions.

In both groups, donor tissues were placed on the exposed root surface in order that the flap could cover both tissues coronally. Polyglycolic suture 5/0 was used to hold the two flaps in position using separate continuous suspensory ties to the independent flap. All patients received the same postoperative pharmacological protocol which involved the use of chlorhexidine mouthwash 0.12% every twelve hours for three weeks and a prescription for painkillers. Sutures were removed two weeks after surgery. Postoperative care regarding diet and brushing was indicated. Patients were checked until achieving complete healing.

Case 1: Subepithelial connective tissue graft: Miller's Class I gingival recessions in teeth 14. Partial thickness

flap was opened and subepithelial connective tissue graft was harvested from the donor site and placed in the open flap of the recipient site and sutured. After two months, postoperatively good root coverage and gum increased thickness and width were observed (Figure 1).

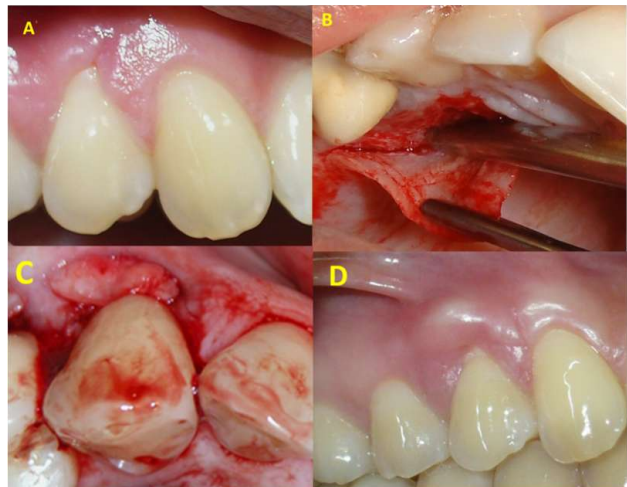


Figure 1. Surgical procedure for the subepithelial connective graft. A: Miller Class I recessions teeth 14. B: Removal of palatal graft. C: Laying subepithelial connective graft in the recipient area. D: Postoperative.

Case 2: Porcine collagen matrix: Miller's Class I gingival recessions in tooth 32, 33, 34 and 35. A partial thickness flap was opened in which the porcine collagen matrix was placed. After two months, increased thickness and width of the keratinized gum was obtained, but root coverage was not extensive (Figure 2).



Figure 2. Collagen matrix of porcine origin intervention. A: Miller Class I recessions tooth 33, 34 and 35. B: Partial thickness flap opened. C: Placement of the collagen matrix in the recipient area. D: Postoperative.

Clinical evaluation: Postoperative data was recorded by an experienced periodontist who ignored each patient's preoperative information. The data evaluated was the same as that assessed at the beginning of treatment (ACJ-GM, PD, CAL, GB, KG and aesthetics).

Data Analysis: Values were entered into a database and the SPSS statistical package v21.0 was used for analyzing data. For the descriptive analysis of quantitative variables, dispersion measures (mean, standard deviation and variance) were used. Percentage of root coverage (RC) was expressed as the ratio between ACJ-GM baseline - ACJ-GM month / ACJ-GM baseline x 100. The Mann-Whitney test was used to compare quantitative data between the experimental and control groups. For qualitative data, Fisher's Exact Test was employed to determine the significance level of clinical change between the two groups considering a $p < 0.05$ for null hypothesis refutation.

Results.

No differences in mean age, follow-up time, or number of men and women who underwent surgery between the two groups were found (Table 1).

The mean baseline gingival recession for the SCG group was 4.33 ± 1.03 mm and 2.67 ± 1.03 mm for the CMP. Postoperative data was 3.17 ± 0.4 mm for the SCG and 2.17 ± 0.98 mm for the CMP group. Root coverage percentage of 24.72 ± 13.56 % was obtained for the first group and 16.67 ± 25.82 % for the second with no significant differences ($p = 0.051$).

The increased amount of postoperative keratinized tissue for the SCG group was 4.33 ± 2.06 mm and 4.5 ± 0.83 mm for the CMP. Better growth was found in the second group in terms of KG; however, there were no significant differences ($p=0.31$) (Table 2).

All patients increased gingival biotype from thin to thick in both groups. PD decreased to 1mm in both groups. The end CAL for the SCG group was 4.17 ± 0.4 mm and 3.17 ± 0.98 mm for the CMP, whereby the latter improved periodontal clinical parameters. Were found significant differences for this parameter ($p=0.045$, Table 3). Regarding the aesthetic component, 83.3% of the SCG group and 66.7% of the CMP showed contouring.

Discussion.

The main objective of this study was to compare clinical efficacy of the porcine collagen matrix versus

| | SCG | CMP |
|-------------------|------------------|------------------|
| Age (years old) | 57.17 ± 2.78 | 54.33 ± 5.68 |
| Male(N) | 2 | 4 |
| Female(N) | 4 | 2 |
| Follow-up (weeks) | 7.5 ± 1.049 | 7.67 ± 0.51 |
| Patients (N) | 6 | 6 |

Table 1. Patient profile.

| | SCG | CMP |
|----------|---------------------|---------------------|
| ACJ-GM | 4.33 ± 1.033 mm | 2.67 ± 1.033 mm |
| PD | 1.5 ± 0.54 mm | 1.67 ± 0.51 mm |
| CAL | 5.83 ± 1.16 mm | 4.33 ± 1.46 mm |
| KG | 3.33 ± 2.16 mm | 2.5 ± 0.83 mm |
| Thin GB | 66.7 % | 83.3 % |
| Thick GB | 33.3 % | 16.7 % |

Table 2. Preoperative clinical parameters.

| | SCG | CMP | Difference | p-value |
|---------------|---------------------|---------------------|------------|---------|
| ACJ-GM | 3.17 ± 0.4 mm | 2.17 ± 0.98 mm | 0.045 | <0.05 |
| PD | 1mm | 1mm | - | - |
| CAL | 4.17 ± 0.4 mm | 3.17 ± 0.98 mm | 0.045 | <0.05 |
| KG | 4.33 ± 2.06 mm | 4.5 ± 0.83 mm | 0.31 | >0.05 |
| Thin biotype | 0 | 0 | - | - |
| Thick biotype | 100% | 100% | - | - |
| Root coverage | 24.72 ± 13.55 % | 16.67 ± 25.82 % | 0.519 | >0.05 |
| Contoured | 83.3% | 66.7% | 0.6 | >0.05 |
| No contouring | 16.7% | 33.3% | - | - |

Table 3. Postoperative clinical results.

the subepithelial connective graft for treating Miller's class I and II gingival recessions.

Results indicate both techniques are effective to improve clinical treatment of gingival recessions. Differences are not significant for several clinical parameters. Improvement in probing depth, keratinized gingiva and clinical attachment level were similar for both groups. Similar findings were reported by Paolantonio *et al.*¹⁷. Root coverage percentage was better with the connective graft (24%) than the collagen matrix (16 %). This may be due to difficulties in maintaining sutures with one patient. In Harris' studies¹⁸, he mentions both techniques achieve broad root coverage and

his long-term studies (2-year follow-up) indicate up to 84% root coverage was obtained. Hirsch¹⁹ coating percentages is up to 96% for the use of acellular dermal matrix and 98% for the use of subepithelial connective graft. This may be due to the short follow-up for patients and the few who underwent surgery in this case. One improvement for both groups was the amount of keratinized gingiva, especially in the short time they were reassessed. The reason why is not clear²⁰. The collagen matrix is not a vital collagen connective tissue but can be seen as an essential component to induce regeneration of the epithelium located under the flap²⁰. However, other authors disagree with this hypothesis²¹. Basically, the collagen matrix acts as a barrier preventing the epithelial cells to invade the tissue held by the matrix, and this is replaced by vital cells²⁰. Whereas only the cells derived from the periodontal ligament and connective tissue are able to induce the development of keratinized epithelium²², the barrier properties of the matrix can influence the rate of colonization of the cells derived from these two tissues. Consequently, connective grafts, as they are completely composed of tissue, are capable of inducing epithelial keratinization.

An interesting finding was there was no difference in the increased gingival biotype for both groups (Table 3). This result suggests the use of the matrix is similar to connective grafts when the goal is to increase the gingival biotype with the advantage of avoiding a second intervention site for removal of donor tissue. This is important because this way it would avoid harming the palatal tissues. However, although both show similar results in terms of gingival biotype, the collagen matrix increases the same way as increased connective grafts²³. Henderson *et al.*²⁴ found similar

increases in gingival biotype. Recall studies indicate thickness of the gum (gingival biotype) is more decisive than the amount of keratinized gingiva to prevent future gum recession²⁴. It is important to highlight that an important factor to increase the risk of future gingival recession is a thin gingival biotype^{25, 26}. It should be emphasized the purpose of a muco-gingival surgery should not only increase the width of keratinized tissue, but also its thickness.

Another result was improvement of aesthetic appearance. It was better for the group with the connective graft than the collagen matrix. Our results indicate complete healing was achieved in a short time for both groups, but the contouring aspect was better with the connective graft. This can be explained by the fact that the porcine collagen matrix and others are non-vital materials which need to be reabsorbed and replaced by host tissue²⁰. This biological process may require additional time which can affect duration of the healing process^{20, 27}.

In conclusion, both techniques are useful to improve the analyzed clinical parameters: KG, CAL, PD and GB. CMP produces similar gingival thicknesses as SCG. CMP has the advantage of avoiding a second surgical site like palatal tissues thus reducing morbidity and surgical risks. In comparing the matrix with the graft, the first produces a slight increase of the keratinized mucosa. However, it should not represent the proper technique indicated for a maximum increase in the amount of keratinized gingival. Future studies are needed to clarify the effects of the collagen matrix of porcine origin. It is important to underline our kind of sampling cannot be used to make generalizations or infer data so further studies are suggested.

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