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# *In Vitro* Evaluation of Two Tissue Substitutes for Gingival Augmentation

**Tulio Cardoso dos Santos[1], Carlos Nelson Elias[2], Alexandre Barboza Lemos[1], Andresa Borges Soares[3], André Antonio Pelegrine[1], Lucas Novaes Teixeira[4], Elizabeth Ferreira Martinez[4]**

[1]Division of Oral Implantology, Faculdade São Leopoldo Mandic, Campinas, SP, Brazil
[2]Department of Materials Science, Military Institute of Engineering (IME), Rio de Janeiro, RJ, Brazil
[3]Division of Oral Pathology, Faculdade São Leopoldo Mandic, Campinas, SP, Brazil
[4]Division of Cell Biology, Faculdade São Leopoldo Mandic, Campinas, SP, Brazil
Email: elizabeth.martinez@slmandic.edu.br

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## Abstract

Three-dimensional collagen matrices of porcine origin are being used as substitutes for soft tissue grafts in periodontal plastic surgery in search of aesthetic and natural results. This *in vitro* study aimed to compare Fibro-Gide® (GeistlichBiomaterials) and Mucoderm® (BotissBiomaterials) matrices during the initial phase of soft tissue formation. For this purpose, samples of 5 × 5 mm were obtained, and then human fibroblasts were plated on them. After 24, 48 and 72 h, cell viability was assessed using an MTT assay, and the secretion of type I collagen, MMP-2, TIMP-1 and TIMP-2 was analyzed by ELISA immunoassay. The control group (C) consisted of cells plated on polystyrene without the matrices. The morphology of the surfaces was also examined using scanning electron microscopy (SEM), as was the average roughness (Ra) of the samples by a profilometer. Topographic analysis revealed that roughness was significantly higher on Mucoderm® than on Fibro-Gide® ($p < 0.05$). Human gingival fibroblasts plated on both matrices showed similar results for cell viability as the cells cultured on C ($p > 0.05$). The synthesis of type I collagen, MMP-2 and TIMP-1 were significantly higher from cells plated on Fibro-Gide® than on Mucoderm®, in all time points ($p < 0.05$). Furthermore, at 24 and 48 h, TIMP-2 secretion was also significantly higher on Fibro-Gide® than on Mucoderm® ($p < 0.05$). Based on these results, it is possible to conclude that even though both matrices demonstrated cell viability, Fibro-Gide® induced an increase in type I collagen, MMP-2 and TIMP-1 and TIMP-2.

**Keywords**

Collagen Matrices, Gingival Recession, Periodontal Regeneration, Matrix Metalloproteinases

## 1. Introduction

There are several approaches to soft tissue reconstruction of the oral cavity focused on restoring aesthetic and natural features [1]. Such procedures are frequently required to promote proper healing following deficiencies caused by tumor excision, fissures, trauma, dental implants, and gingival recessions [2].

Autogenous grafts remain the gold standard for cases that require gingival augmentation surgery, with unmatched success rates [3]. Soft tissue grafts may be harvested from different donor sites, such as the palate, the retromolar region and the edentulous spaces [4] [5] [6]. The disadvantages of harvesting the graft from the retromolar pad and edentulous sites are a limited amount of tissue availability and the recovery of only thinner grafts. As a result, the palate is the preferred site for soft tissue graft harvesting [7], which demands a second surgical site, increasing morbidity in terms of post-operative discomfort and procedure time [5]. Therefore, various options should be considered in order to avoid a second surgical site while also reducing postoperative time and complications, potentially increasing patient acceptance of this type of procedure [8].

Three-dimensional porcine collagen matrix has been introduced as soft tissue graft substitutes in periodontal plastic surgery [8]. Collagen is an important biomaterial for medical applications due to its biological characteristics such as bio and cytocompatibility and biodegradability [9]. Additionally, its hemostatic function enables early wound stabilization, fibroblast attraction and semi-permeability, promoting nutrient exchange [10].

Most matrices are primarily composed of type I and III collagens derived from pigs, cattle, and humans [11], which are commonly used in membrane production [12]. Type I collagen is found in the majority of connective tissues in the body [13], while type III collagen is found primarily in the skin and vessels [14]. Although different in terms of the three-dimensional arrangement of the molecules, both share the same molecular structure, and act together in the formation of fibrillar aggregates [14].

Two of the collagen membranes currently available as soft tissue substitutes are Fibro-Gide® (Geistlich Biomaterials) and Mucoderm® (Botiss Biomaterials). Both are made of porcine collagen and are indicated to replace the subepithelial connective graft. Besides types I and III collagens, Mucoderm® contains elastin, and Fibro-Gide® is a porous, resorbable matrix that is chemically cross-linked to preserve its volume stability.

Although clinical studies have demonstrated excellent tissue regeneration with

both matrices, additional studies that report on cell viability, and the potential to induce connective tissue synthesis are, however, lacking. Therefore, the aim of this study was to assess the biological behavior of both matrices on human gingival fibroblasts, as well as their ability to induce the production of type I collagen, metalloprotease-2 and inhibitors (TIMP-1 and TIMP-2), which are important in the early stages of oral cavity soft tissue formation.

## 2. Materials and Methods

### 2.1. Sample Groups

Two resorbable collagen matrices of animal origin (porcine) were used, Fibro-Gide® (Geistlich Biomaterials) and Mucoderm® (Botiss Biomaterials), at 30 × 40 mm. Square samples at 5 × 5 mm were obtained from the matrices and human gingival fibroblast cells obtained from three different donors were plated on the membranes, after approval by the Ethics Committee of the São Leopoldo Mandic Research, Campinas, Brazil (#3,833,067). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Nuticell®, Campinas SP, Brazil) supplemented with 10% fetal bovine serum (Cultilab®, Campinas SP, Brazil) and 1% antibiotic-antimycotic solution (penicillin-streptomycin) (Sigma, St. Louis, Missouri, USA). This procedure was performed in each membrane separately. The 96-well plates were kept in an incubator at 37˚C.

### 2.2. Topographic Analysis of the Biomaterials

The morphology and average roughness (Ra) of the different surfaces were evaluated. The ultrastructural morphology of the samples was evaluated using a high-resolution scanning electron microscope (Quanta FEG 250; FEI Company, Eindhoven, The Netherlands). Three specimens of each surface were mounted directly on stubs and photographed at 1000× and 20,000× magnification.

For the analysis of the average surface roughness (Ra), 4 fragments of each surface were measured with a tip instrument (profilometer, Mitutoyo Surftest SJ-200, Japan). Four linear measurements were conducted on each sample according to DIN ISO 1302 standards and the arithmetic mean of the surface roughness profile (Ra) was calculated for each sample.

### 2.3. Cell Viability Assay

Cell viability of the cell culture plated on the different membrane surfaces was measured using MTT assay. After 24, 48 and 72 h of cell plating, 10 µL of MTT solution (5 mg/mL, Sigma, USA) diluted in DMEM serum-free medium was added to the treated cultures, which were then incubated for 3 hours at 37˚C. After the incubation, 100 µL of 10% DMSO solution (Dimethylsulfoxide, LGC, São Paulo, Brazil) was added.

After the crystals were solubilized, an ELX800 microplate reader (Epoch Biotek Instruments, Inc.) at 590 nm was used for quantification, and the optical density (OD) measures were obtained.

### 2.4. Enzyme Immunoassay of Collagen Type I, MMP-2 and TIMP-1 and TIMP-2 Inhibitors (ELISA)

After 24, 48 and 72 h, the supernatants from the cell cultures were harvested and centrifuged at 5000 g for 15 min at 4˚C. Aliquots of each sample were assayed by means of ELISA to determine the type I collagen, MMP-2 and inhibitors (TIMP-1 and TIMP-2) levels, according to the manufacturer's recommendations (R&D Systems, USA). Total type I collagen, TIMP-1 and MMP-2 were quantified in picograms per mL (pg/mL). The results were calculated using the standard curves created in each assay. The ELISA assays were performed in a blind fashion in triplicate.

### 2.5. Statistical Analysis

Descriptive and exploratory analyses of all data were performed. They indicated that the data did not meet the assumptions of an analysis of variance (ANOVA). Generalized linear models (GLMs) considering main effects and the interaction between them were then used. Analyses were conducted using the R program with a significance level of 5%.

## 3. Results

### 3.1. Surface Topography

The ultrastrucutral morphology of the surfaces is represented in Figure 1 by scanning electron micrographs. Both matrices exhibited a porous structure on the surface, enabling the ingrowth of cells after grafting. Mucoderm® presented collagen fibers set in a loose mesh-like arrangement, while Fibro-Gide® exhibited a sheet-like amorphous matrix.

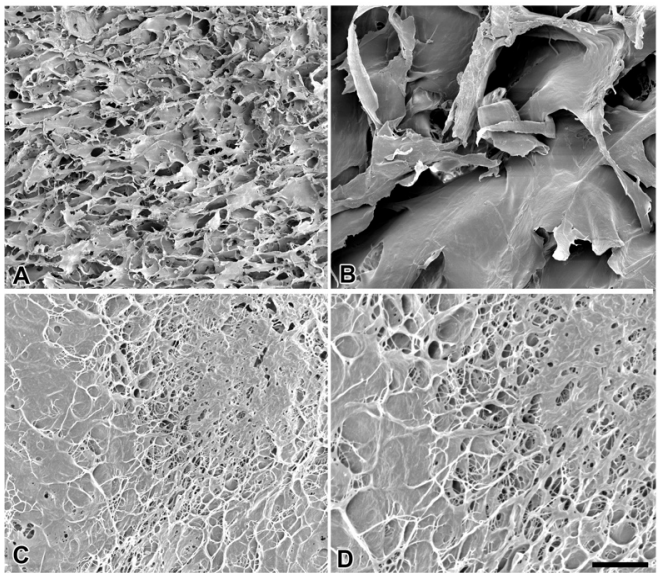


Figure 1. Representative SEM images of Fibro-Gide® ((A), (B)) and Mucoderm® ((C), (D)) membranes. Caption: Bars: (A) = 500 µm, (B) = 50 µm, (C) = 10 µm, (D) = 5 µm.

Regardless of the surface pattern, the roughness mean values for the Mucoderm® were statistically higher (7.87 ± 0.83 μm) than for Fibro-Gide® (6.04 ± 0.36 μm) (Figure 2).

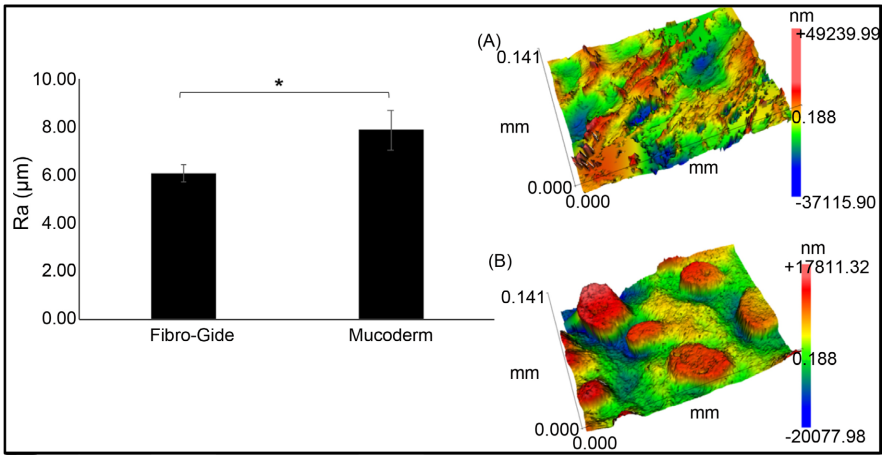


Figure 2. Average surface roughness (standard deviation), in μm, and interferometry of the surfaces of Fibro-Gide® (A) and Mucoderm® (B) membranes. Caption: *representation of the statistical difference between groups ($p < 0.05$).

## 3.2. Cell Viability

Cell viability at the different analysis times for all groups is shown in Table 1. The results of the MTT assay revealed that there was no difference in the viability of cells grown on Fibro-Gide® and Mucoderm® membranes compared with polystyrene (control, C), for each time point ($p > 0.05$). Additionally, no difference was observed regarding the viability of cells for each group at the different times evaluated ($p > 0.05$).

Table 1. Cell viability assay at the different analysis times.

| | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | Mean (standard deviation) | Median (minimum and maximum) | Mean (standard deviation) | Median (minimum and maximum) | Mean (standard deviation) | Median (minimum and maximum) |
| C | 0.58 (0.05) Aa | 0.61 (0.52; 0.62) | 0.74 (0.08) Aa | 0.78 (0.65; 0.81) | 0.77 (0.22) Aa | 0.83 (0.53; 0.95) |
| Fibro-Gide | 0.16 (0.01) Aa | 0.16 (0.15; 0.17) | 0.17 (0.05) Aa | 0.17 (0.11; 0.22) | 0.59 (0.71) Aa | 0.21 (0.15; 1.41) |
| Mucoderm | 0.13 (0.00) Aa | 0.13 (0.12; 0.13) | 0.09 (0.00) Aa | 0.09 (0.09; 0.09) | 0.81 (1.01) Aa | 0.25 (0.19; 1.97) |
| p-value | p (matrix) = 0.0907; p (time) = 0.0573; p (matrix × time) = 0.7054 | | | | | |

Caption: Same letters (upper case horizontally and lower case vertically) indicate that there were no statistically significant differences ($p > 0.05$).

### 3.3. Quantification of Type I Collagen, MMP-2 and TIMP-1 and TIMP-2 Inhibitors

The quantification of the proteins secreted by the fibroblasts plated on the different membranes is shown in Table 2. Lower levels for type I collagen, MMP-2 and TIMP-1 were observed for the cells plated on Mucoderm® than on the C and Fibro-Gide®, at all experimental points evaluated ($p < 0.05$). In relation to TIMP-2, after 24 and 48 h, the secretion levels were lower on Mucoderm® than on Fibro-Gide® and C ($p < 0.05$). After 72 h, no statistical differences were observed ($p > 0.05$).

Table 2. Quantification of collagen I, MMP-2, TIMP-1 and TIMP-2 secreted by fibroblasts plated on the different surfaces. Mean values (standard deviation) expressed in pg/mL.

| | | 24 h | 48 h | 72 h |
|---|---|---|---|---|
| Col I | C | 1025.23 (297.64) Aa | 1265.38 (327.99) Aa | 1135.62 (152.69) Aa |
| | Fibro-Gide | 1379.01 (475.81) Aa | 793.64 (231.39) Aa | 874.88 (119.46) Aa |
| | Mucoderm | 611.91 (125.61) Ab | 439.19 (153.51) Ab | 586.98 (228.7) Ab |
| | p-value | p (matrix) < 0.0001; p (time) = 0.2101; p (matrix × time) = 0.1143 | | |
| MMP-2 | C | 2220.00 (240.00) Ba | 4800.00 (420.00) Aa | 5260.00 (70.00) Aa |
| | Fibro-Gide | 1370.00 (460.00) Bb | 4080.00 (480.00) Aa | 4510.00 (40.00) Aa |
| | Mucoderm | 580.00 (30.00) Ac | 620.00 (30.00) Ab | 620.00 (80.00) Ab |
| | p-value | P (matrix) < 0.0001; p (time) < 0.0001; p (matrix × time) = 0.0041 | | |
| TIMP-1 | C | 2265.61 (179.13) Ba | 3444.83 (338.43) Ba | 4066.92 (107.72) Aa |
| | Fibro-Gide | 2216.45 (42.15) Ba | 3309.11 (168.95) Ba | 4312.74 (225.86) Aa |
| | Mucoderm | 561.77 (129.27) Bb | 501.80 (348.05) Bb | 872.39 (369.86) Ab |
| | p-value | p (matrix) < 0.0001; p (time) = 0.0262; p (matrix × time) = 0.4991 | | |
| TIMP-2 | C | 1748.64 (194.36) Aa | 2212.94 (90.15) Aa | 2572.36 (180.92) Aa |
| | Fibro-Gide | 1625.21 (140.16) Aa | 2349.49 (568.85) Aa | 2066.87 (341.53) Aa |
| | Mucoderm | 277.44 (170.81) Bb | 472.24 (368.31) Bb | 1163.86 (490.17) Aa |
| | p-value | p (matrix) < 0.0001; p (time) = 0.0026; p (matrix × time) = 0.0384 | | |

Caption: Distinct letters (upper case horizontally and lower case vertically) indicate statistically significant differences ($p < 0.05$).

## 4. Discussion

Periodontal plastic surgery is often performed to correct defects of the gingiva, alveolar mucosa and bone. For this purpose, the harvest of autogenous connective tissue graft is a well established treatment. However, autogenous grafting techniques are associated with some level of morbidity, particularly if the donor site is limited. To overcome this issue, new treatment options are being developed, using substitute biomaterials (e.g. xenografts), in order to reduce the number of surgeries as well as intraoral donor sites [15].

The use of xenogenous collagen matrices has increased lately, since they can effectively promote root coverage and tissue thickness gain, with successful rates compared to autogenous connective tissue grafts [15]. Moreover, such substitutes can reduce the morbidity, avoiding the second surgical site (*i.e.* donor area) [16] [17]. Recent findings have shown that collagen matrices are equally effective for both increasing thickness and increasing the band of keratinized mucosa around implants [18] [19].

Mucoderm® and Fibro-Gide® are porous resorbable matrices of porcine dermis origin used as a substitute in cases of loss of the connective tissue structure. These matrices are composed of type I and III collagen with a three-dimensional structure similar to human connective tissue. According to the manufacturer, Mucoderm® is a resorbable matrix of 1.2 to 1.7 mm thickness, with no artificial or chemical cross-linking, constituted by 60% - 96% (w/w) porcine collagen and 4% - 40% (w/w) elastin. On the other hand, Fibro-Gide® is constituted only by collagen exposed to chemical cross-linking to preserve its volume [20]. The surface topographies of both matrices evidenced the roughness and open-porous structure which enables the ingrowth of soft tissue cells and blood vessels. In the present study, it was verified that Mucoderm® presented collagen fibers set in a loose mesh-like arrangement, while Fibro-Gide® exhibited a sheet-like amorphous matrix. Despite the similarity concerning the porous structure, the roughness average of Mucoderm® was slightly higher than that of Fibro-Gide® ($p < 0.05$).

The most favorable conditions for efficient cell adhesion, growth, and proliferation are dependent on cell type. Roughness average higher than 2 μm stimulates the formation of focal adhesion points, which are required for healthy cellular functioning. This context allows a maximum cell-substrate interaction resulting in stable adhesion, and, as a result, the highest cell growth and proliferation [21]. Regardless of the slight differences between both matrices, they are considered to have high roughness, and the viability cell assay revealed no difference between the matrices and the control group. However, concerning the production of extracellular matrix proteins, the results of the present study revealed that fibroblasts plated on the FibroGide® secreted more type I collagen, MMP-2, TIMP-1 and TIMP-2 than Mucoderm®.

Fibro-Gide® is made of reconstituted collagen and receives a cross-linking which prolongs the enzymatic degradation of the product [22], enhancing the subsequent cell repopulation [23] [24]. Despite structural advantages, artificial cross-linked treatment negatively interferes with biocompatibility and may hinder tissue integration as well as vascularization, potentially contributing to increase wound dehiscence and oral exposure [25] [26] [27]. Although the scientific literature suggests that collagen cross-linking has biological disadvantages, some studies suggest that, when compared to matrices without cross-linking, this treatment balances mechanical volume stability with cell compatibility, contributing to cell growth and vascularization [7] [28]. In fact, this data supports

the findings of the present study with higher type I collagen, MMP-2, TIMP-2 and TIMP-2 synthesis when cells were plated on Fibrogide® matrix than on Mucoderm®.

Type I collagen is the main protein of the connective extracellular matrix that provides structural support for a variety of tissues, allowing cell adhesion and migration during wound healing [29]. This may support the clinical studies that demonstrate the regenerative capacity of the matrix in replacing autogenous connective tissue grafts [30] [31].

MMP-2 belongs to the family of matrix metalloproteinases, a group of endopeptidases that participate in the degradation of several extracellular matrix proteins [32], playing an important role in remodeling, osteogenesis, and healing. MMP-2 deregulation can participate in the progression of a variety of physiological and pathological functions such as tissue repair and inflammation [33]. TIMPs are specific endogenous inhibitors of MMP pro-enzyme activity [34] and can inhibit different MMP with varying degrees of efficacy. The MMP/TIMP ratio can influence the specific activities of the metalloproteinases. Furthermore, during inflammation, the expression or activity of MMPs, which are secreted by proinflammatory cells, increases [32].

The results of the study indicate an increase in MMP-2 and TIMP-1 and TIMP-2 on cells plated on Fibro-Gide®, possibly indicating a greater ability of this matrix to stimulate periodontal and peri-implant tissue remodeling when compared with Mucoderm®. However, it's important to state that Mucoderm® can be considered an alternative to autogenous connective tissue graft for root coverage purposes [35]. Further *in vivo* studies using both matrices are required to determine whether the Fibro-Gide® matrix is in fact capable of promoting tissue remodeling in a beneficial way in terms of clinical results, or whether it induces more intense inflammation during healing.

## 5. Conclusion

In conclusion, the results indicated that, despite the fact that both matrices demonstrated cell viability, Fibro-Gide® induced an increase in type I collagen, MMP-2 and TIMP-1 and TIMP-2, which might contribute to periodontal tissue remodeling when compared to Mucoderm®.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References


[1] Vignoletti, F., Nunez, J. and Sanz, M. (2014) Soft Tissue Wound Healing at Teeth, Dental Implants and the Edentulous Ridge When Using Barrier Membranes, Growth and Differentiation Factors and Soft Tissue Substitutes. *Journal of Clinical Periodontology*, **41**, S23-S35. https://doi.org/10.1111/jcpe.12191

[2] Wolff, J., Farré-Guasch, E., Sándor, G.K., Gibbs, S., Jager, D.J. and Forouzanfar, T. (2016) Soft Tissue Augmentation Techniques and Materials Used in the Oral Cavity: An Overview. *Implant Dentistry*, **25**, 427-434. https://doi.org/10.1097/ID.0000000000000385

[3] Kim, D.M. and Neiva, R. (2015) Periodontal Soft Tissue Non-Root Coverage Procedures: A Systematic Review from the AAP Regeneration Workshop. *Journal of Periodontology*, **86**, S56-S72. https://doi.org/10.1902/jop.2015.130684

[4] Köseoğlu, S., Duran, İ., Sağlam, M., Bozkurt, S.B., Kırtıloğlu, O.S. and Hakkı, S.S. (2013) Efficacy of Collagen Membrane Seeded with Autologous Gingival Fibroblasts in Gingival Recession Treatment: A Randomized, Controlled Pilot Study. *Journal of Periodontology*, **84**, 1416-1424. https://doi.org/10.1902/jop.2012.120529

[5] Ramachandra, S.S., Rana, R., Reetika, S. and Jithendra, K.D. (2014) Options to Avoid the Second Surgical Site: A Review of Literature. *Cell and Tissue Banking*, **15**, 297-305. https://doi.org/10.1007/s10561-013-9395-8

[6] Sanz, M. and Simion, M., Working Group 3 of the European Workshop on Periodontology (2014) Surgical Techniques on Periodontal Plastic Surgery and Soft Tissue Regeneration: Consensus Report of Group 3 of the 10th European Workshop on Periodontology. *Journal of Clinical Periodontology*, **41**, S92-S97. https://doi.org/10.1111/jcpe.12215

[7] Thoma, D.S., Naenni, N., Benic, G.I., Hämmerle, C.H. and Jung, R.E. (2017) Soft Tissue Volume Augmentation at Dental Implant Sites Using a Volume Stable Three-Dimensional Collagen Matrix-Histological Outcomes of a Preclinical Study. *Journal of Clinical Periodontology*, **44**, 185-194. https://doi.org/10.1111/jcpe.12635

[8] Kasaj, A. (2016) Gingival Recession Coverage: Do We Still Need Autogenous Grafts? *Quintessence International*, **47**, 775-783. https://doi.org/10.3290/j.qi.a36685

[9] Ortolani, E., Quadrini, F., Bellisario, D., Santo, L., Polimeni, A. and Santarsiero, A. (2015) Mechanical Qualification of Collagen Membranes Used in Dentistry. *Annali dell'Istituto Superiore di Sanità*, **51**, 229-235. https://doi.org/10.4415/ANN_15_03_11

[10] Salamanca, E., Tsai, C.Y., Pan, Y.H., Lin, Y.T., Huang, H.M., Teng, N.C., Lin, C.T., Feng, S.W. and Chang, W.J. (2016) *In Vitro* and *in Vivo* Study of a Novel Porcine Collagen Membrane for Guided Bone Regeneration. *Materials* (*Basel*), **9**, Article 949. https://doi.org/10.3390/ma9110949

[11] Dragan, I.F., Hotlzman, L.P., Karimbux, N.Y., Morin, R.A. and Bassir, S.H. (2017) Clinical Outcomes of Comparing Soft Tissue Alternatives to Free Gingival Graft: A Systematic Review and Meta-Analysis. *Journal of Evidence Based Dental Practice*, **17**, 370-380. https://doi.org/10.1016/j.jebdp.2017.05.008

[12] Lee S.W and Kim S.G. (2014) Membranes for the Guided Bone Regeneration. *Max-*

*illofacial Plastic and Reconstructive Surgery*, **36**, 239-246.
https://doi.org/10.14402/jkamprs.2014.36.6.239

[13] Ricard-Blum, S. and Ruggiero, F. (2005) The Collagen Superfamily: From the Extracelular Matrix to the Cell Membrane. *Pathologie Biologie* (*Paris*), **53**, 430-442.
https://doi.org/10.1016/j.patbio.2004.12.024

[14] Van Der Rest, M. and Garrone, R. (1991) Collagen Family of Proteins. *FASEB Journal*, **5**, 2814-823. https://doi.org/10.1096/fasebj.5.13.1916105

[15] AlSarhan, M.A., Al Jasser, R., Tarish, M.A., AlHuzaimi, A.I. and Alzoman, H. (2019) Xenogeneic Collagen Matrix Versus Connective Tissue Graft for the Treatment of Multiple Gingival Recessions: A Systematic Review and Meta-Analysis. *Clinical and Experimental Dental Research*, **5**, 566-579.
https://doi.org/10.1002/cre2.210

[16] Tonetti, M.S., Cortellini, P., Bonaccini, D., Deng, K., Cairo, F., Allegri, M., Conforti, G., Graziani, F., Guerrero, A., Halben, J., Malet, J., Rasperini, G. and Topoll, H. (2021) Autologous Connective Tissue Graft or Xenogenic Collagen Matrix with Coronally Advanced Flaps for Coverage of Multiple Adjacent Gingival Recession. 36-Month Follow-Up of a Randomized Multicentre Trial. *Journal of Clinical Periodontology*, **48**, 962-969. https://doi.org/10.1111/jcpe.13466

[17] Tonetti, M.S., Cortellini, P., Pellegrini, G., Nieri, M., Bonaccini, D., Allegri, M., Bouchard, P., Cairo, F., Conforti, G., Fourmousis, I., Graziani, F., Guerrero, A., Halben, J., Malet, J., Rasperini, G., Topoll, H., Wachtel, H., Wallkamm, B., Zabalegui, I. and Zuhr, O. (2018) Xenogenic Collagen Matrix or Autologous Connective Tissue Graft as Adjunct to Coronally Advanced Flaps for Coverage of Multiple Adjacent Gingival Recession: Randomized Trial Assessing Non-Inferiority in Root Coverage and Superiority in Oral Health-Related Quality of Life. *Journal of Clinical Periodontology*, **45**, 78-88. https://doi.org/10.1111/jcpe.12834

[18] Lissek, M., Boeker, M. and Happe, A. (2020) How Thick Is the Oral Mucosa around Implants after Augmentation with Different Materials: A Systematic Review of the Effectiveness of Substitute Matrices in Comparison to Connective Tissue Grafts. *International Journal of Molecular Sciences*, **21**, Article 5043.
https://doi.org/10.3390/ijms21145043

[19] Gargallo-Albiol, J., Barootchi, S., Tavelli, L. and Wang, H.L. (2019) Efficacy of Xenogeneic Collagen Matrix to Augment Peri-Implant Soft Tissue Thickness Compared to Autogenous Connective Tissue Graft: A Systematic Review and Meta-Analysis. *The International Journal of Oral & Maxillofacial Implants*, **34**, 1059-1069.
https://doi.org/10.11607/jomi.7497

[20] Vallecillo, C., Toledano-Osorio, M., Vallecillo-Rivas, M., Toledano, M. and Osorio, R. (2021) *In Vitro* Biodegradation Pattern of Collagen Matrices for Soft Tissue Augmentation. *Polymers* (*Basel*), **13**, Article 2633.
https://doi.org/10.3390/polym13162633

[21] Majhy, B., Priyadarshini, P. and Sen, A.K. (2021) Effect of Surface Energy and Roughness on Cell Adhesion and Growth-Facile Surface Modification for Enhanced Cell Culture. *RSC Advances*, **11**, 15467-15476.
https://doi.org/10.1039/D1RA02402G

[22] Sam, G. and Pillai, B.R. (2014) Evolution of Barrier Membranes in Periodontal Regeneration—"Are the Third Generation Membranes Really Here?". *Journal of Clinical and Diagnostic Research*, **8**, ZE14-ZE17.
https://doi.org/10.7860/JCDR/2014/9957.5272

[23] Pabst, A.M., Wagner, W., Kasaj, A., Gebhardt, S., Ackermann, M., Astolfo, A., Ma-

rone, F., Haberthür, D., Enzmann, F. and Konerding, M.A. (2015) Synchrotron-Based X-Ray Tomographic Microscopy for Visualization of Three-Dimensional Collagen Matrices. *Clinical Oral Investigations*, **19**, 561-564. https://doi.org/10.1007/s00784-014-1312-4

[24] Schmitt, C.M., Matta, R.E., Moest, T., Humann, J., Gammel, L., Neukam, F.W. and Schlegel, K.A. (2016) Soft Tissue Volume Alterations after Connective Tissue Grafting at Teeth: The Subepithelial Autologous Connective Tissue Graft versus a Porcine Collagen Matrix—A Pre-Clinical Volumetric Analysis. *Journal of Clinical Periodontology*, **43**, 609-617. https://doi.org/10.1111/jcpe.12547

[25] Rothamel, D., Schwarz, F., Sager, M., Herten, M., Sculean, A. and Becker, J. (2005) Biodegradation of Differently Cross-Linked Collagen Membranes: An Experimental Study in the Rat. *Clinical Oral Implants Research*, **16**, 369-378. https://doi.org/10.1111/j.1600-0501.2005.01108.x

[26] Wessing, B., Lettner, S. and Zechner, W. (2018) Guided Bone Regeneration with Collagen Membranes and Particulate Graft Materials: A Systematic Review and Meta-Analysis. *The International Journal of Oral & Maxillofacial Implants*, **33**, 87-100. https://doi.org/10.11607/jomi.5461

[27] Jiménez Garcia, J., Berghezan, S., Caramês, J.M.M., Dard, M.M. and Marques, D.N.S. (2017) Effect of Cross-Linked vs Non-Cross-Linked Collagen Membranes on Bone: A Systematic Review. *Journal of Periodontal Research*, **52**, 955-964. https://doi.org/10.1111/jre.12470

[28] Naenni, N., Walter, P., Hämmerle, C.H.F., Jung, R.E. and Thoma, D.S. (2021) Augmentation of Soft Tissue Volume at Pontic Sites: A Comparison between a Cross-Linked and a Non-Cross-Linked Collagen Matrix. *Clinical Oral Investigations*, **25**, 1535-1545. https://doi.org/10.1007/s00784-020-03461-8

[29] Rujirachotiwat, A. and Suttamanatwong, S. (2021) Curcumin Promotes Collagen Type I, Keratinocyte Growth Factor-1, and Epidermal Growth Factor Receptor Expressions in the *in Vitro* Wound Healing Model of Human Gingival Fibroblasts. *European Journal of Dentistry*, **15**, 63-70. https://doi.org/10.1055/s-0040-1715781

[30] Thoma, D.S., Gasser, T.J.W., Jung, R.E. and Hämmerle, C.H.F. (2020) Randomized Controlled Clinical Trial Comparing Implant Sites Augmented with a Volume-Stable Collagen Matrix or an Autogenous Connective Ttissue Graft: 3-Year Data after Insertion of Reconstructions. *Journal of Clinical Periodontology*, **47**, 630-639. https://doi.org/10.1111/jcpe.13271

[31] Zeltner, M., Jung, R.E., Hämmerle, C.H., Hüsler, J. and Thoma, D.S. (2017) Randomized Controlled Clinical Study Comparing a Volume-Stable Collagen Matrix to Autogenous Connective Tissue Grafts for Soft Tissue Augmentation at Implant Sites: Linear Volumetric Soft Tissue Changes up to 3 Months. *Journal of Clinical Periodontology*, **44**, 446-453. https://doi.org/10.1111/jcpe.12697

[32] Cui, N., Hu, M. and Khalil, R.A. (2017) Biochemical and Biological Attributes of Matrix Metalloproteinases. In: *Progress in Molecular Biology and Translational Science*, Vol. 147, Elsevier Inc., Amsterdam, 1-73. https://doi.org/10.1016/bs.pmbts.2017.02.005

[33] Mittal, R., Patel, A.P., Debs, L.H., Nguyen, D., Patel, K., Grati, M., Mittal, J., Yan, D., Chapagain, P. and Liu, X.Z. (2016) Intricate Functions of Matrix Metalloproteinases in Physiological and Pathological Conditions. *Journal of Cellular Physiology*, **231**, 2599-2621. https://doi.org/10.1002/jcp.25430

[34] Laronha, H. and Caldeira, J. (2020) Structure and Function of Human Matrix Me-

talloproteinases. *Cells*, **9**, Article 1076. https://doi.org/10.3390/cells9051076

[35] Lakshmi, M.R., Gottumukkala, S.N.V.S., Penmetsa, G.S., Ramesh, K.S.V., Kumar, P.M., Vamsi, E.S. and Mounica, M.B. (2023) Clinical Outcomes of Root Coverage Using Porcine-Derived Collagen Matrix with Modified Coronally Advanced Tunnel Technique (MCAT) in Multiple Gingival Recessions in Smokers—A Randomized Controlled Clinical Trial. *Clinical Oral Investigations*, **27**, 1101-1111. https://doi.org/10.1007/s00784-022-04700-w