Leon Olde Damink*, Ingo Heschel, Hans Leemhuis, Martina Tortorici, Bastian Wessing

**Soft tissue volume augmentation in the oral cavity with a collagen-based 3D matrix with orientated open pore structure**

Abstract: In this study, characteristic features of a new regenerative 3D collagen matrix with an orientated open pore structure are studied in-vitro and in-vivo. The non-crosslinked porcine-based resorbable collagen-elastin matrix is designed to provide support during coverage procedures of localized gingival recessions and for local soft tissue augmentation around teeth and implants and is designed to provide an off-the-shelf alternative to autogenous soft tissue grafts. The in-vitro studies show that the mechanical properties (e.g. suture retention, volume recovery after cyclic compression) and the observed active cell migration into the open porous structure of the matrix fulfil essential design requirements. The in-vivo pig animal study shows that the matrix is well integrated into the surrounding tissue and replaced by newly formed autogenous soft tissue without a significant loss in tissue volume. First clinical case series are being performed to further analyse the new 3D matrix in clinical settings.

**Keywords:** biomaterials, collagen, 3D matrix, soft tissue augmentation, dental implants, guided tissue regeneration.

https://doi.org/10.1515/cdbme-2018-0058

1 Introduction

For implant-supported restoration of failed or failing teeth soft tissue volume augmentation is often required in order to achieve the best functional and esthetic results. Autogenous subepithelial connective tissue grafts are being used for this indication with negative side effects such as requiring a second surgical donor site associated with additional pain and morbidity, and the limited availability of autogenous tissue that can be harvested from the palate.

To avoid the problems associated with the use of autogenous tissues, intensive research and development is being performed to offer off-the-shelf alternatives to autogenous grafts. Such alternatives are based on e.g. decellularized and purified allogeneic or xenogeneic natural tissues or reconstituted scaffolds.

In two recent systematic reviews, soft tissue augmentation techniques in the dental field were assessed [1,2]. However, these reviews also show that there are only few sound studies available describing potential off-the-shelf alternatives to autogenous grafts. Some of the available collagen-based alternatives seem to be too thin to achieve the required volume augmentation without being folded. In addition, encapsulations of certain scaffolds by fibrotic tissue or the formation of epithelial layers on top of the scaffold may have caused suboptimal tissue integration. Furthermore, the observed extensive shrinkage of some off-the-shelf alternatives during their degradation in vivo is a negative side effect.

Therefore, the aim of this study was the development and characterization of a new regenerative 3D collagen matrix with sufficient initial thickness and an oriented open pore structure in vitro & in vivo as a potential alternative to autogenous connective tissue grafts. Essential requirements are listed in Table 1.

**Table 1: Essential scaffold design requirements:**

- bio- and cell-compatibility
- safety
- shall allow trimming to the defect size with scissors & scalpels
- shall adapt to the defect and withstand forces during handling
- shall be easily populated by migrating cells and blood vessels
- shall maintain/regain its volume after compression
- shall be replaced by newly formed soft tissue without healing problems (e.g. no strong inflammations, no dehiscences)
- shall be degraded without toxic/irritating degradation products
- shall provide a long term stable soft tissue augmentation

---

*Corresponding author: Ingo Heschel: Matricel GmbH, Kaiserstr. 100, 52134 Herzogenrath, Germany, heschel@matricel.de
Leon Olde Damink, Hans Leemhuis: Matricel GmbH, Kaiserstr. 100, 52134 Herzogenrath, Germany
Martina Tortorici: Julius Wolff Institut, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
Bastian Wessing: Praxisklinik der Zahnheilkunde am Luisenhospital, Boxgraben 99, 52064 Aachen, Germany

**Open Access. © 2018 Leon Olde Damink et al., published by De Gruyter. This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License.**


## 2 Materials & Methods

The biodegradable 3D matrix (MUC, Mucomaix™, Matricel GmbH) used in this study is composed of porcine collagen and elastin fibers and has a defined open porous structure generated by a patented directional solidification process that is intended to guide migrating cells and blood vessels into the matrix to support the soft tissue regeneration [3, 4].

Before MUC was selected as the optimal final scaffold design, other prototypes were produced to study the influence of several design parameters (e.g. pore size and cross-linking). The results of these verification experiments revealed that optimal wound regeneration results were obtained with non-crosslinked prototypes with a pore size of 80 to 100 µm. Since even a mild crosslinking of the prototypes with EDC resulted in an increased inflammatory reaction [5] (more foreign body giant cells present) the final CE-marked MUC product (certificate Z/18/04257E) is not chemically crosslinked.

Figure 1 shows the microscopic appearance of the MUC matrix. The directional solidification method followed by freeze-drying leads to the vertically orientated open porous scaffold structure with an adjustable pore sizes of 80 to 100 µm.

## 3 Results

In order to assess if the MUC matrix fulfils the design requirements according to Table 1 various studies including the subsequent in-vitro and in-vivo studies were performed.

### 3.1 Mechanical properties

All mechanical tests were performed with a tensile/compression testing machine Z2.5 (Zwick/Roell). Suture retention is a relevant parameter because 3D matrices for soft tissue augmentation are being pulled & stretched in order to position and fixate them within the adjacent tissue. For MUC the measured suture retention forces are shown in Table 2. In addition, handling tests of MUC matrices by selected key experts in periodontology confirmed that the handling properties of MUC comply with their clinical requirements [7].

<table>
<thead>
<tr>
<th>Table 2: Measured suture retention forces (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC scaffold (dry): 2,82 N +/- 0,59 N</td>
</tr>
<tr>
<td>MUC scaffold (wet): 0,90 N +/- 0,29 N</td>
</tr>
</tbody>
</table>

It is essential that a scaffold for soft tissue augmentation withstands certain mechanical forces, regains its volume, and does not collapse below the covering tissue flap in order to provide the space for the formation of new soft tissue volume. In order to assess the mechanical suitability of a scaffold for this indication, a cyclic compression test according to [6] was applied here as well.

Figure 2 shows a typical result of the cyclic compression test with MUC. In brief hydrated MUC samples are subjected to a total of 49 cycles of loading to 12.1 kPa and the respective compression is determined (red bars in Figure 2, compressed thickness/initial scaffold thickness [%]). The regained scaffold thickness after release of the compressive force on MUC is also determined for every cycle (regained/initial scaffold thickness [%], blue bars in Figure 2).
2). The results confirm the previously reported "memory effect" of MUC scaffolds [7]. MUC scaffolds regain almost 100% of their initial volume and the number of cycles applied does not have an influence on the memory effect. So, it can be assumed that MUC scaffolds are not damaged by the maximally applied pressure of 12.1 kPa according to the proposed test [6]. The mean compression of MUC was 30.0 +/-1.0%.

3.2 Cell migration

As mentioned above, the open porous structure of MUC is dedicated to support the migration of soft tissue generating cells and blood vessels into the scaffold. Various previous studies with directionally solidified collagen scaffolds (e.g. [4]) have revealed that the preferential migration of cells into directionally solidified scaffolds is supported by its guiding structure. Here, results on the migration of human dermal fibroblasts (hDF) into the MUC matrix are presented, since these collagen producing cells play an essential role in soft tissue regeneration.

The hDF were first seeded on 2D-TCP. Then, the MUC scaffolds were placed on the cell seeded 2D layer (2*10^5 cells per scaffold) and remained there for up to 5 days. The vertical migration of the cells along the pores was analysed by harvesting samples at day 1, 3 & 5 after seeding. Figure 3 shows an example of the cell migration into the MUC scaffold after five days. The cell skeleton is stained "green" (Phallloidin Alexa 488), the nuclei "blue" (DRAQ5), and the MUC scaffold "white" (second harmonic imaging). In all test samples (n=3), hDF cells migrated actively deep (> 1mm) into the scaffold along the guiding structure against gravity. The area of the well plate where the scaffolds were in contact with the seeded cell layer was almost devoid of cells when the scaffolds were harvested.

3.3 Animal study

The positive in-vitro results justified the use of MUC matrices in a porcine animal implantation study performed by Boekema et al. [5]. This in-vivo study was focussed on dermal wound healing after implantation of a variety of dermal substitute prototypes - including crosslinked scaffolds - besides the non-crosslinked MUC. The scaffolds were applied in surgical full-thickness wounds and were covered with SSG. Wound healing was assessed after 1, 2, 3 and 8 weeks both macro- and microscopically. For the present analysis, only data acquired for MUC on wound healing is extracted and evaluated. For study details, refer to [5].

Table 3: Focus of the animal implantation study with MUC:

1) Macroscopically: volume retention & remodelling
2) Microscopically: tissue integration & inflammation

To investigate whether the augmentation with the degradable soft tissue substitute MUC has a long-term effect on tissue thickness, biopsies of the augmented tissue were taken at different time points and the thickness of the tissue was measured in H&E stained samples. To assess in parallel the degradation behaviour of the implanted MUC, an immunohistochemical staining for elastin (BA-4) was used. Besides collagen, elastin is the second major component of MUC. Since elastin degrades slower than collagen, remnants of elastin can still be found after the collagen is already resorbed. Figure 4 shows a presentation of the results normalized to data for week 1 after implantation (=100%).

It can be seen that the tissue thickness after MUC implantation does not change significantly over 8 weeks after implantation. The implanted MUC matrix is however widely degraded over the 8 weeks implantation time (significant differences from week 1 to weeks 3 & 8 are indicated by * [MWU, P \ 0.05]).

Figure 3: Migration of human dermal fibroblasts (stained blue/green) into MUC collagen matrix (white) along the pores.

Figure 4: Normalized presentation of tissue thickness and remaining matrix over 8 weeks after implantation.
From this data it can be concluded that the implanted MUC matrix is replaced by newly formed autogenous soft tissue without a loss of tissue thickness as hypothesized. Cells migrate into the scaffold and create a vascularized new extracellular matrix within the volume generated by the matrix whilst the biodegradation of the temporary scaffold starts.

The biopsy analyses regarding foreign body giant cells & macrophages showed a good wound healing with only a very mild foreign body reaction for the MUC samples [5].

3.4 Clinical Application

The first clinical applications of the CE-marked MUC matrix were published in [7]. Besides good handling properties of the matrix it was reported that a good wound healing comparable to the use of soft tissue grafts for the same indication was observed. Meanwhile dedicated clinical studies with MUC are being performed but no results are published yet.

The following clinical case shall serve as an example for a combination of immediate & late implant placement, guided bone regeneration, and soft tissue volume augmentation. The patient (61 years, female, smoker) had a hopeless tooth 24 and a missing tooth 25. The surgical solution was to first extract tooth 24 followed by an immediate implant placement (NobelActive®, Nobel Biocare AB) and an augmentation of the remaining gap between implant & bone with a bone graft material (creos xenogain, Nobel Biocare AB). After placement of the second implant (NobelActive®) in regio 25, a split mucosal flap was prepared for the insertion of the MUC matrix. The placement of the trimmed dry MUC matrix below the created mucosal flap can be seen in Figure 5-A. Figure 5-B shows the occlusal view after full insertion of MUC saturated with the patient’s blood to augment the buccal mucosal flap. Below the matrix, one implant at position 24 can be seen; the other implant 25 is covered by patient blood and thus is invisible. Figure 5-C shows the buccal view after wound closure and Figure 5-D the occlusal view of the ridge 4 months after implant placement and soft tissue augmentation before the reentry to start the final restoration. The wide ridge achieved and the thick soft tissue layer on top of the ridge demonstrates the success of the soft tissue volume augmentation. In addition, the primary healing phase was uneventful, the tissue inflammatory reaction within normal limits and the functional and esthetic result of the soft tissue augmentation very satisfactory.

4 Conclusion

To find alternatives to autogenous connective tissue grafts as the current gold standard for soft tissue augmentation in the dental field remains a challenging task. For certain clearly defined indications (e.g. closed healing situations) the first dedicated developments of off-the-shelf alternatives to autogenous tissues become visible. The data presented here supports the conclusion that the investigated MUC matrix might be such an alternative. Especially the finding that the matrix is replaced by newly formed autogenous tissue without volume loss whilst the implanted matrix is degraded is probably attributed to the orientated open porous matrix structure. The animal study results however need to be verified in human clinical studies analysing how the initial volume gain after implantation evolves over long observation periods.

![Figure 5](image-url)

**Figure 5:** Clinical case of a combined implant placement & soft tissue augmentation with MUC (see text for case description).

**Acknowledgments:** The authors acknowledge the assistance of Ansgar Petersen, who provided the method and protocol for the cell migration assay & Bouke Boekema, who shared his original data that was so far only published partially in [5].
Author Statement
Research funding: The research was supported by the Dutch Burns Foundation (#06-203) and the BMBF (#13N12152).
Conflict of interest: Authors 1, 2 & 3 disclose to be employees of Matricel GmbH, the manufacturer of MUC.
Informed consent and ethical approval: Informed consent is not applicable here and the study did not require ethical approvals.

References