Human & Veterinary Medicine
International Journal of the Bioflux Society HVM

OPEN ACCESS
Research Article

First–in-animal experimental pilot study of Suprathel® for oral mucosa and bone regeneration

[1]Sergiu Vacaraş, [1]Grigore Băciuţ, [2]Dan Gheban, [1]Simion Bran, [3]Horaţiu Colosi, [4]Cristian Berce, [5]Winfried Kretschmer, [1]Avram Manea, [1]Gabriel Armencea, [1]Mihaela Băciuţ, [1]Cristian Dinu

[1] Department of Maxillofacial Surgery and Oral Implantology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2] Department of Pathological Anatomy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3] Department of Medical Informatics and Biostatistics, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4] Center for Experimental Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [5] Klinik für Mund-, Kiefer- und Plastische Gesichtschirurgie, Alb Fils Klinikum, Göppingen, Germany.

Abstract. Background: This first-in-animal pilot study was conducted for the purpose of estimating the potential of a split-mouth model implemented in rabbits to be used for investigating the capacity of the polylactide copolymer Suprathel® to promote oral tissue regeneration. Investigation and analysis models have been optimized in order to ensure the reproducibility of measurements and statistical evaluations in a larger-scale future experiment. Materials and Methods: An experimental split-mouth model was developed and tested on two New Zealand rabbits. A clinically validated, widely used material for oral regeneration, Mucoderm®, was selected for comparison with Suprathel®. Comparison was undertaken with regard to mucosal healing (coverage of defects) and bone regeneration (bone healing). Results: Histopathologic analysis of the two rabbits' specimens at 3 months after surgery showed comparatively consistent results for mucosal and bone regeneration in both animals, and adequate tissue formation according to the stage of healing for both materials. Conclusion: The outcomes of this experiment consolidated the comparability and thereby the internal validity of the proposed split-mouth rabbit model, recommending it as a reliable tool for comparative testing of biomaterials. This pilot study also offered a first recommendation regarding the capacity of Suprathel® to promote healing of the oral mucosa and alveolar bone formation.

Key Words: guided tissue regeneration (GTR), animal study, polylactide, resorbable, barrier membrane

Corresponding Author: H. Colosi, email: hcolosi@umfcluj.ro

Introduction

Guided tissue regeneration (GTR) is a technique used with great success in oral and maxillofacial surgery, dentistry and especially implantology. The need to obtain oral keratinized mucosa is a key clinical issue for ensuring oral health conditions. Nevertheless, as bone is the second most transplanted tissue, preceded only by blood, guided bone regeneration has a primary role in regeneration research.

The current concept used in GTR relies on the protected space theory. The principle of GTR consists in applying a barrier (membrane) that serves as a protective layer for the healing area against the intrusion of a competitor, the more rapidly-growing fibrous tissue. Improving the properties of the membranes and especially their local effect, remains a topic of great interest, along with the quest for finding new materials to fulfill this aim. For bone regeneration, the membrane blocks the penetration of non-osteogenic tissue at the site where new bone tissue forms during healing. To this end, the new generations of membranes, in addition to being a physical barrier between tissues, are designed with osteogenetic properties, thus becoming bioactive structures (Turri et al 2016). An important aspect is related to the local inflammation that occurs in the area of bone augmentation, caused by the additional materials but and also by the used membranes, with an obvious influence on the formation of bone tissue. It can be stated that alloplastic materials used in guided tissue regeneration play a role in osteoimmunomodulation and this effect must be fully understood and directed towards a favorable result (Chen et al 2018).

The current pilot study was designed to evaluate the performance of a clinically validated material for skin regeneration - Suprathel® (Polymedics Innovations, Denkendorf, Germany) for a novel use as a barrier membrane in oral tissue regeneration. Suprathel® is a terpolymer, a combination of poly-DL-lactide (70%), trimethylencarbonate and caprolactone, that is designed to be applied to burns after debridement and various other skin defects, and which remains in position until reepithelialization is complete. It has been widely used as a disposable synthetic dressing to treat burns and wounds, with great success for many years, being appreciated by both patients and clinicians. Among the many advantages of this material is that when used on the skin surface, the recovering epithelialization area can be inspected at any time underneath the material, because of its transparency (Highton et al 2013, Keck et al 2012).

These beneficial outcomes in skin healing that were described for Suprathel® represented an incentive for our attempt to evaluate

the capacity of this material to promote healing of the oral mucosa and alveolar bone formation.
The current study compared Suprathel® and Mucoderm® (Botiss Biomaterials GmbH, Zossen, Germany), a material that we have chosen as a reference due to its common and extensive use in GTR (Papi & Pompa 2018).
Mucoderm® consists, according to its producer, of purified collagen fibers (I and III). While being a biological material, it integrates into the host tissue through capillary and fibroblast cells populations, transforming into connective tissue that can no longer be distinguished from the host tissue.
Mucoderm® is an acellular matrix of collagen, offered by manufacturers as an alternative to soft tissue autografts, with multiple uses. This matrix is derived from the porcine dermis and undergoes several stages of preparation in the purification process, a process that removes all non-collagen proteins, all cells and any potential generators of immune reactions, as well as bacteria and viruses. These treatments result in a three-dimensional stable matrix containing type I and type III collagen with preserved extracellular collagenous matrix, a structure very close to that of human connective tissue (Pabst et al 2014).
The present pilot study was conducted for the purpose of perfecting a rabbit-split-mouth model in the evaluation of Suprathel®'s capacity to promote oral tissue regeneration. The present animal model has been established in order to appraise the reproducibility of outcomes that could be feasibly measured in a future, larger, experimental study which could also yield a meaningful comparison concerning the safety and efficacy of the studied membranes.
Given this preliminary purpose, the current study has been performed on only two New Zealand rabbits. Nevertheless, it presents, to the best of our knowledge, the results of the first experimental study of Suprathel® for guided intraoral tissue regeneration.

Materials and methods

For the present pilot study, two New Zealand rabbits raised at the Biobase of the "Iuliu Haţieganu "University of Medicine and Pharmacy in Cluj-Napoca were used, with the approval of the University Research Ethics Commission no. 211, issued on May 4th 2018.
For the evaluation of the epithelial healing capacity of the materials, mucosal defects were planned and created bilaterally under anesthesia, in the buccal sulcus of each rabbit, according to a split-mouth pattern. Closure of the mucosal defects was performed using one of the materials on either side.
For bone healing assessment, the interventions involved bilateral extractions of two maxillary premolars on each side, as well as one molar on one of the sides, followed by alveolar coverage with the investigated materials of both premolar alveolae situated on each of the opposing maxillary sides. The molar alveolar socket was left as control, uncovered for spontaneous healing.
At the beginning of the study, the rabbits were 2 years old and weighed between 3 and 4 kilograms. The surgeries were performed under general anesthesia, for which 10% ketamine (Rotexmedica GmbH Arzneimittelwerk, Trittau, Germany) with 2% xylazine (Bioveta, Ivanovice na Hane, Czech Republic) in a ratio of 2:1 was used. All these procedures were in accordance with the ethical standards comprised in the institutional and National Guide for the care and use of laboratory animals.
In the anterior buccal area of the upper jaw, two split-mouth pattern mucosal defects were created in two distinct buccal areas in order to be closed subsequently by vestibuloplasty. Both defects had a circular shape and a diameter of 1 centimeter. One area was covered with a specially fabricated, double thickness (250 microns thick) Suprathel® membrane patch, while the other area was covered with a similarly constructed Mucoderm® membrane patch. The investigators were not informed about the selection of the material per side of coverage. This way, a blinded protocol was implemented in this first arm of the study (with one of the authors being in charge of strictly controlling the preservation of blinding) (Fig. 1).

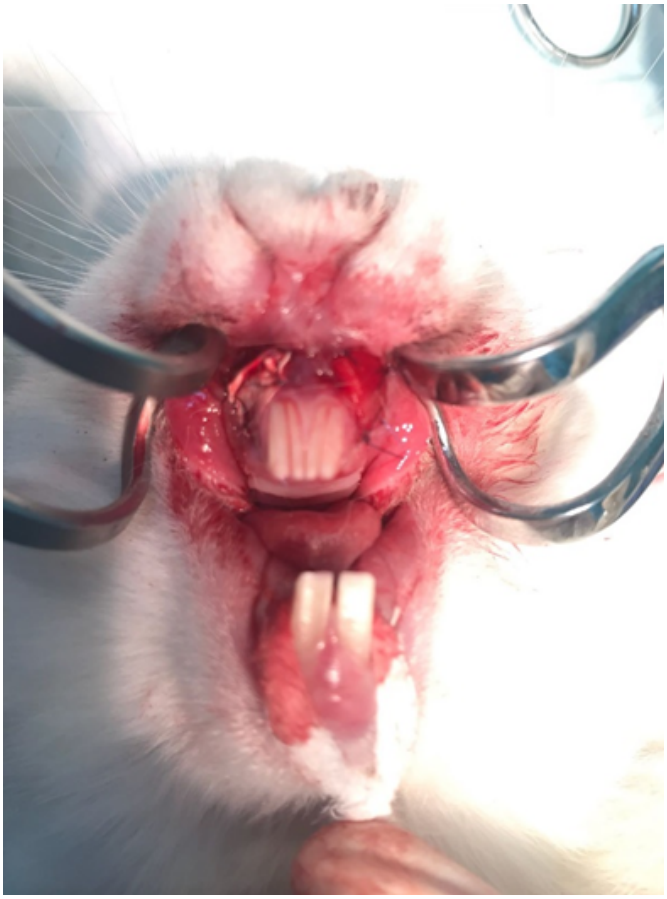

Fig. 1. Coverage of the mucosal defects with the two studied biomaterials. Membranes sutured in position.

In the second arm of the study, during the same surgical operation, five maxillary teeth extractions were performed on each rabbit. The resulting dental sockets (alveoli) were distributed as follows: 2 postextractional alveoli in the premolar region, 2 postextractional alveoli in the contralateral premolar region, and one postextractional control molar alveolus left uncovered for spontaneous healing (Fig. 2). The sockets received blinded random coverage with Suprathel® sutured on one side and Mucoderm® sutured on the contralateral hemimaxilla sockets respectively, according to the split-mouth pattern (Fig. 3). The The correspondence between the alveoli and the coverage material was not made known to the investigators who performed the surgeries nor to those who interpreted the clinical or histological

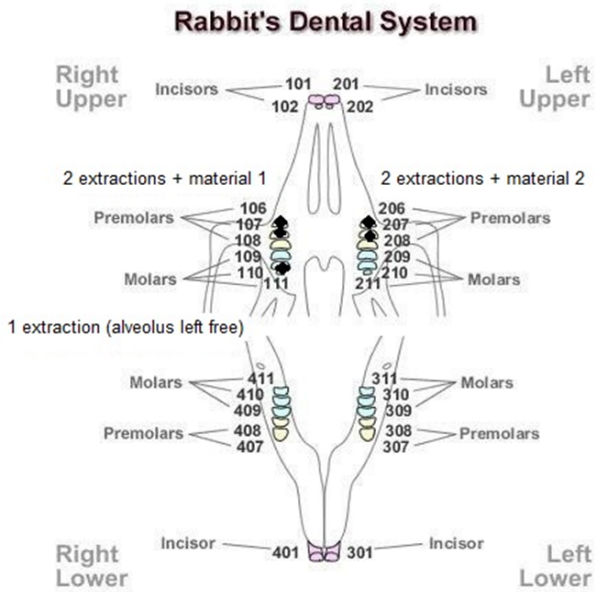

Fig. 2. Schematic representation of the rabbit dental system. The black marked alveoli indicate the performed dental extractions

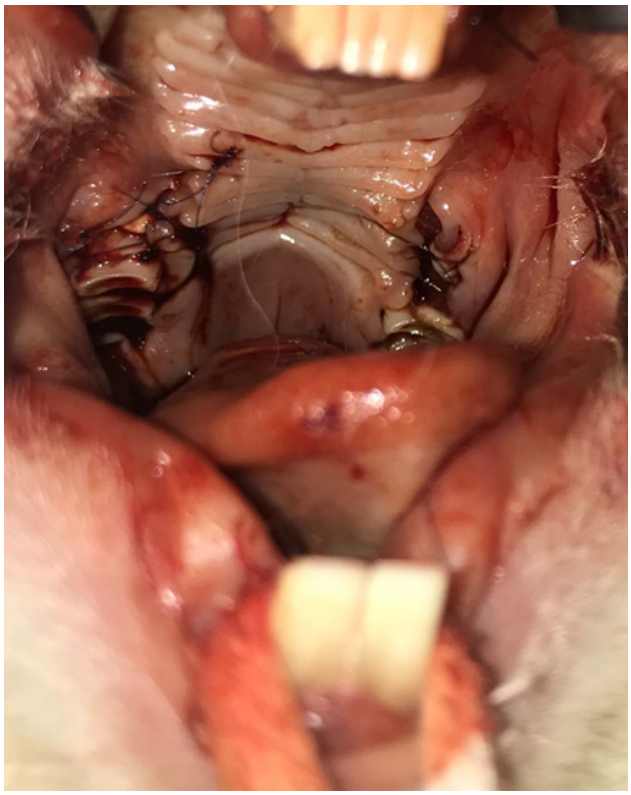

Fig. 3. Closure of bilateral postextraction sockets (alveoli) with the two biomaterials

results, so that the blinding protocol was followed in this second arm of the study as well.

The harvesting of specimens was performed 3 months later and included resection of the restoration sites from the maxillae after euthanasia, to evaluate the mucosal regeneration rates and the bone healing in the alveoli (Fig. 4). Vetased (SC Pasteur Filiala Filipeşti, Filipeştii de Pădure, Romania) in overdose was used for euthanasia.

Histological sections were processed from all operated sites and stained with hematoxylin-eosin, as well as by using Masson's

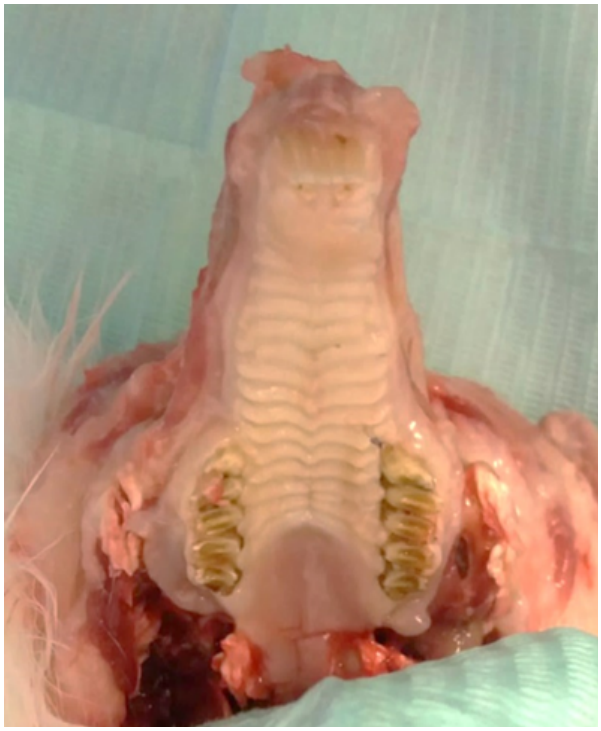

Fig. 4. Specimen – resected maxilla at 3 months postoperatively with healed mucosal and bone sites for GTR evaluation

trichrome technique. The sections were examined by a histopathologist with 25 years of experience in assessing guided oral tissue regeneration.

Healing of the epithelial defects and bone healing were assessed independently. The process of evaluation also served for establishing the array of the most useful criteria for evaluating the newly formed tissue.

After documentation, the results were attributed to each material upon unblinding.

Results

Upon unblinding, the outcomes of mucosal regeneration and bone healing could be confronted with the identified materials (Suprathel® and Mucoderm®).

Healing of the oral mucosa

Following the healing of the vestibuloplasty areas, in the case of Suprathel®, common aspects were observed in both rabbits (Figures 5 – 12). Red, eosinophilic and amorphous areas in hematoxylin-eosin were confirmed as black areas in Masson's trichrome.

The first material, Suprathel® (marked with arrows), appeared in the form of spherical granules of eosinophilic amorphous material (in hematoxylin-eosin). In Masson's trichrome, the black color of the granules disclosed its non-collagenous nature.

The coverage with Suprathel® on a deepithelialized tissue defect generated a reepithelialization of the area with squamous epithelium with pseudocarcinomatous hyperplasia.

There were also some necrotic areas at the margin of the epithelialized area. In those regions the reepithelialization had not yet taken place, and the material remained soaked in the necrotic tissue and surrounded by a purulent inflammatory reaction.

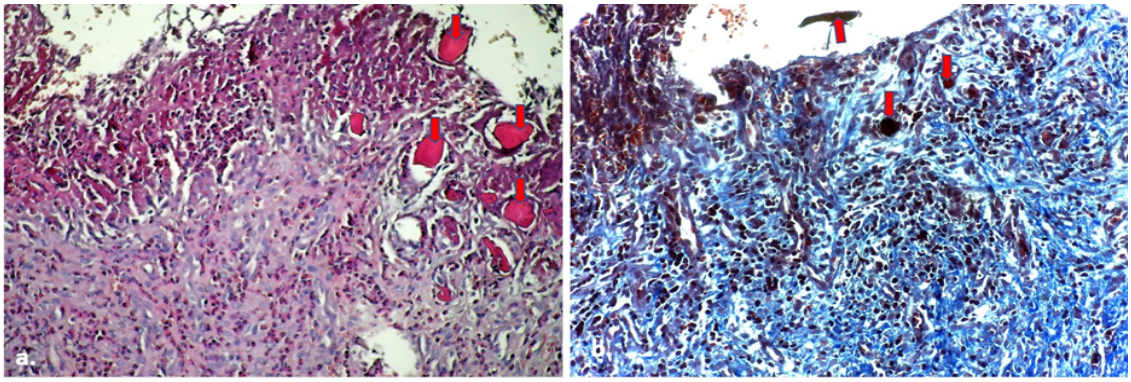

Fig. 5. Suprathel® (red arrows) in hematoxylin-eosin staining (a) and Masson's trichrome staining (b). Surface gingiva-induced defect: an extensive area of ulceration covered by a thick serocellular crust overlying a lamina propria notably infiltrated by many heterophils admixed with plumped fibroblast, blood capillaries (with a vertical or oblique orientation to the superficial surface of the defect) lined by reactive endothelial cells, and a small amount of collagenous extracellular matrix (granulation tissue). Both the serocellular crust and the above-described inflamed lamina propria contained multiple foci of a granular, eosinophilic, well-demarcated, homogeneous foreign material (interpreted as the tested membrane).

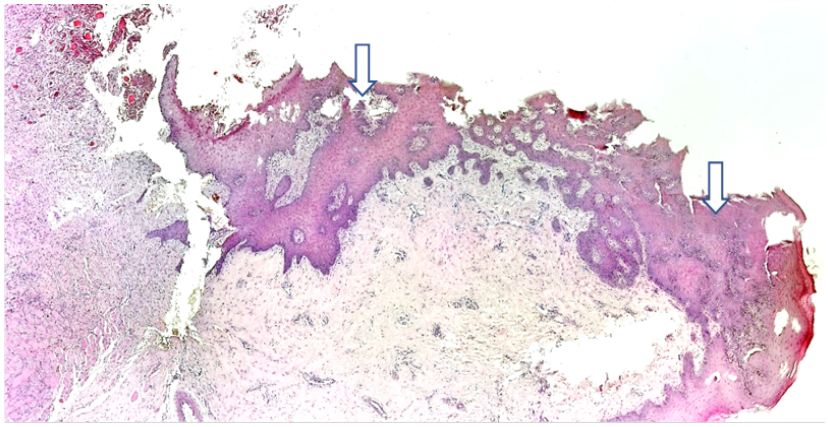

Fig. 6. Reepithelialization area with pseudocarcinomatous hyperplastic reaction. Hyperplasia involved the extension of the basal layer to deeper layers, in the fibromucosa, like roots, at the same time as the thickening of the keratinocyte layer of the epithelium. The area of the induced defect was partially covered by a moderately, focally hyperkeratotic, hyperplastic gingival epithelium, forming small, anastomosing rete ridges within the superficial lamina propria. An area of persistent defect (visible as a focus of ulceration) was also present.

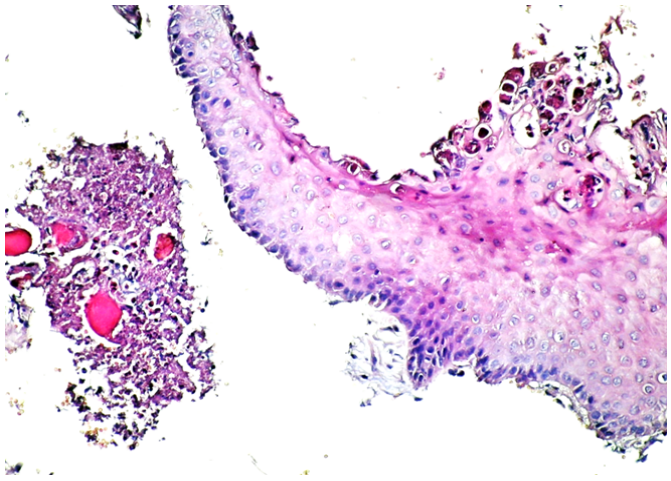

Fig. 7. The regenerated epithelium still contained residues of material on its surface. It is included in the keratinocytes of the newly formed hyperplastic squamous epithelium (arrow). As for the superficial lamina propria, the regenerated, hyperplastic gingival epithelium enclosed superficially a granular, eosinophilic, well-demarcated, homogeneous foreign material (interpreted as the tested membrane). Within the superficial lamina propria, the above-described material was admixed with some necrotic cell debris and granulation tissue.

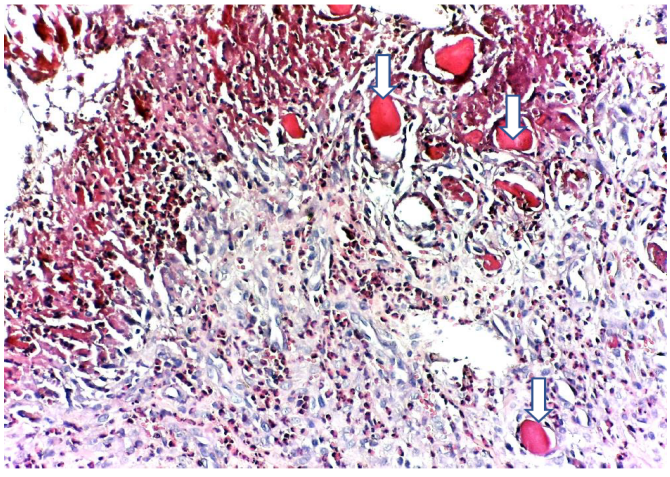

Fig. 8. Eosinophilic amorphous material (arrows) was included in the area of necrosis with purulent reaction at the base. An area of ulceration covered by a thick serocellular crust is visible. The lamina propria was markedly infiltrated by many heterophils blended with granulation tissue. Both the serocellular crust and the inflamed lamina propria contained multifocally a granular, eosinophilic, well-demarcated, homogeneous foreign material (interpreted as the tested membrane).

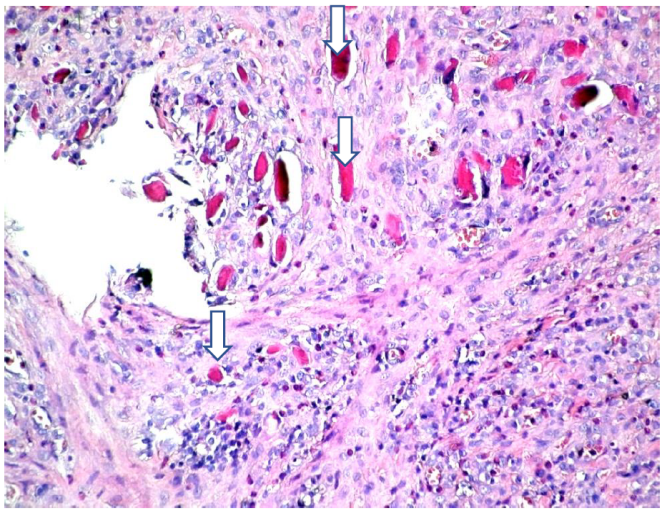

Fig. 9. Fibroblastic tissue with foci infiltrated by polymorphonuclear leukocytes (PMN) with eosinophilic granules (arrows) included. Within the lamina propria, the tested material is embedded in the granulation tissue (markedly infiltrated by many lymphocytes, admixed with fewer macrophages and heterophils) and occasionally surrounded by a few multinucleate giant cells (foreign body type)

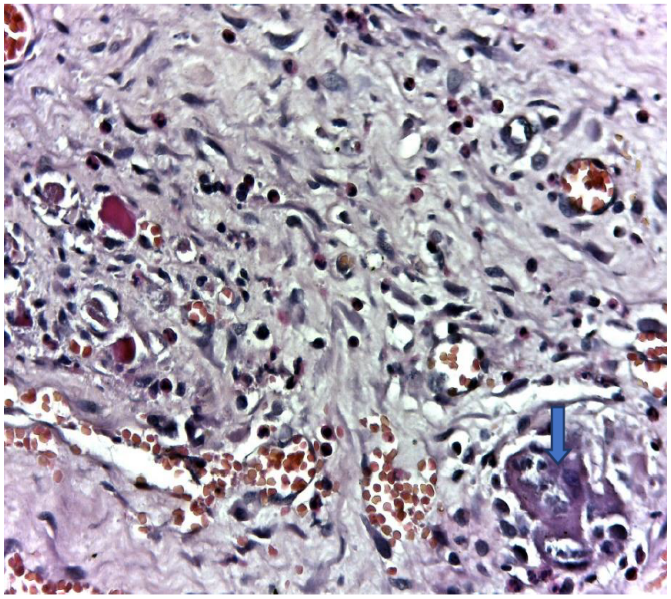

Fig. 10. Foreign body granuloma. Giant foreign body cells (arrow) also appear focally

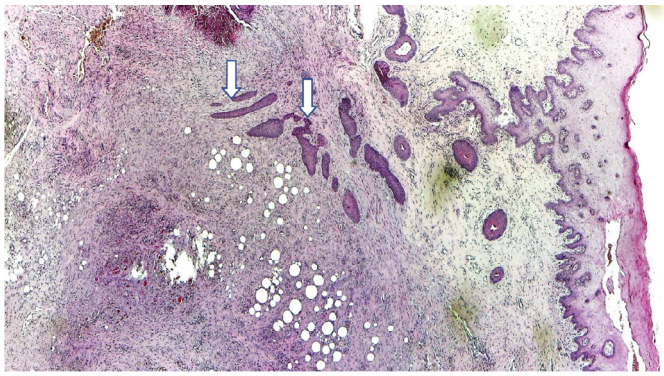

Fig. 11. On the deep penetration path, epithelial islands (arrows) remained trapped in scar tissue, thus fibrosis formed to repair the defect through which the material penetrated

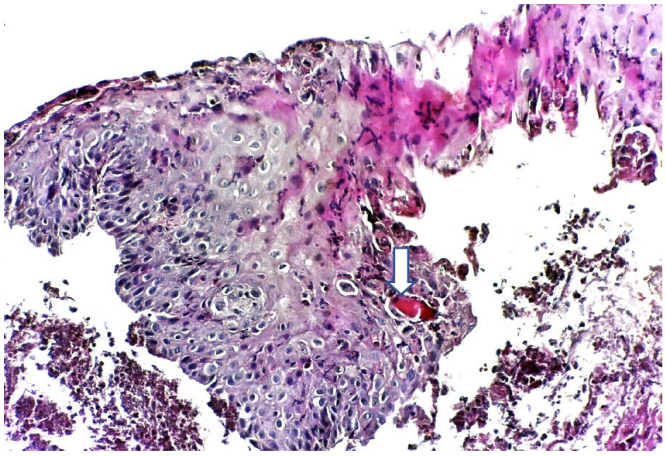

Fig. 12. At the margin of the epithelialization process, in the proximity of the unhealed areas, the eosinophilic material (arrow) may be partially incorporated into the epithelium, which also contains inflammatory cells (PMN). The regenerating, hyperplastic gingival epithelium enclosed the test material superficially. Regular epithelial maturation was present, with many heterophils migrating from the noticeably inflamed lamina propria interspersed between the epithelial cells

This purulent reaction was probably also the cause of the delay in the epithelialization process.

In the deep layers, the material triggered a granulomatous reaction rich in PMN, but also containing giant foreign body cells (arrow in Fig. 10).

The newly-formed epithelium had a basal, spinosum and granular layer, focally depositing small amounts of keratin.

The reepithelialization process began to form by migrating from the side to the center, beneath the material.

The second material (Mucoderm®) (Fig. 13 - 16) showed significant differences in the location of the healing processes. The reepithelialization process was visible above this material, not beneath the material, as in the case of Suprathel®.

Reepithelialization was best seen in Masson trichrome staining, with the reepithelialized area having an abundant granular layer and a poorer spinous layer, standing out by being redder than normal.

The newly formed epithelium was therefore discontinuous, without pseudocarcinomatous hyperplasia, specific to the even more fragile material cell, with poor presence of the spinous layer.

Bone healing

Regarding the healing of the postextractional alveoli, favorable formation of tissue was found at histological examination in both animals, on both sides - in defects covered with each of the studied materials, as well as the socket left for spontaneous healing.

In the first rabbit, on the right hemimaxilla, two alveoli in the premolar region stood out. In both, a bony layer was deposited on the ligamentous apparatus and the cavity was closed at the surface by a squamous epithelial roof (Fig. 17).

Both of these alveoli had been treated using the first material (Suprathel®).

In the case of the second rabbit, the section in the area of tooth 107 showed healing of the epithelial seal. Under the gingival epithelium there was a loose connective tissue proliferation in which a cartilaginous node has developed (Fig. 18 and 19).

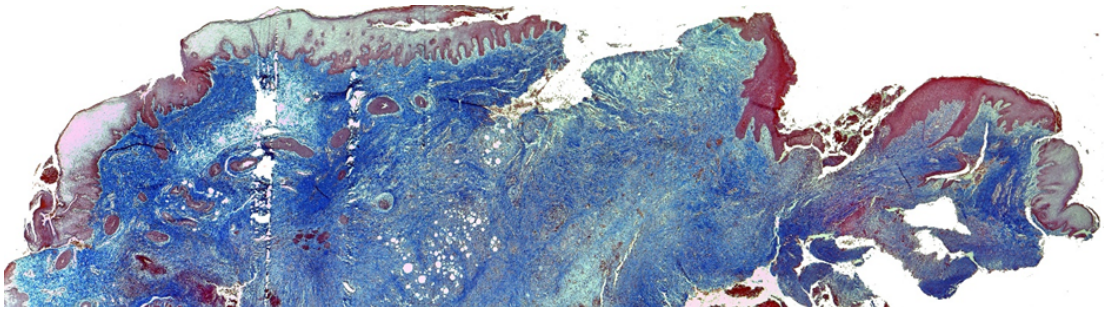

Fig. 13. Reepithelialization with Mucoderm®. Normal area (red arrow) compared to the reepithelialized area (blue arrow). Discontinuity represented by deeper cracks over which the membrane material probably did not reach were also noted. Overview in Masson's trichrome staining for collagen. There was broad epithelization of the induced defect; the lamina propria contained mainly adult-granulation tissue, infiltrated by a few leukocytes. The epithelium was focally and minimally ulcerated, covered by a thick serocellular crust.

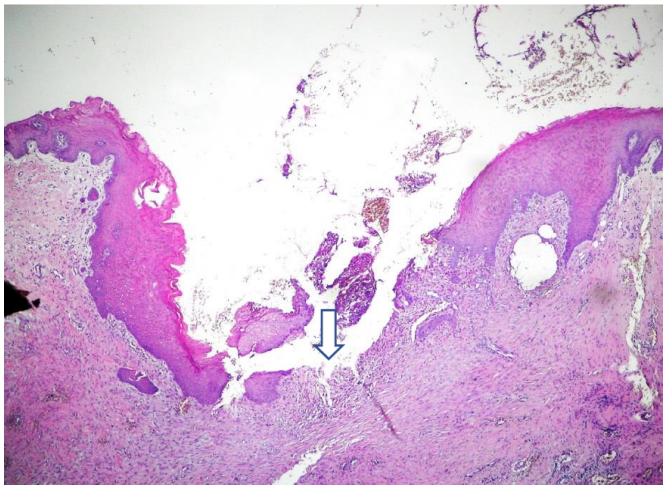

Fig. 14. The aspect in hematoxylin-eosin staining of the reepithelialization area. The epithelium was focally ulcerated (arrow), covered by a thick crust, and the superficial lamina propria infiltrated by a few leukocytes, associated with the above-described ulcerative area. The lamina propria consisted of well-organized, adult connective tissue

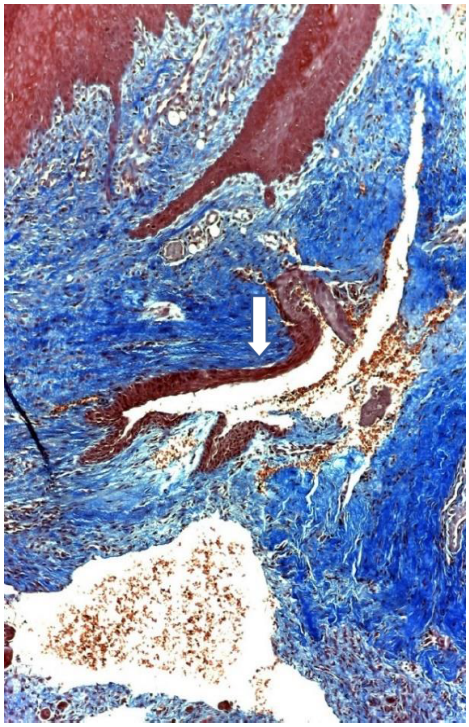

Fig. 15. A discrete but obvious epithelialization (arrow) occurred even in the remaining clefts between the tissue and the collagenous material, resulting in epithelialized cysts similar to the radicular cyst (Trichrome Mason stain)

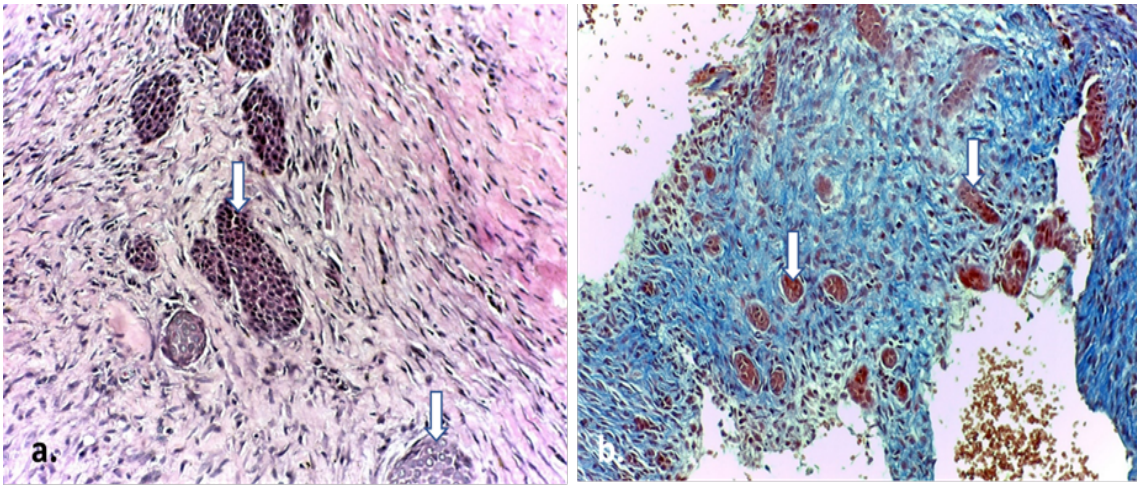

Fig. 16. Deep reepithelialization (arrows) areas described above induced by Mucoderm®, with areas of young fibroblastic tissue, rich in fibroblasts and poor in collagen fibers. These areas contained epithelial buds formed exclusively of basal cells, with proliferation capacity, similar to the Malassez epithelial cell rests. There were multifocal areas of epithelial hyperplasia invading the regenerated lamina propria, presented as well-demarcated islands of the odontogenic epithelium (rests of Malassez). a. HE stain, b. Trichrome Masson stain.

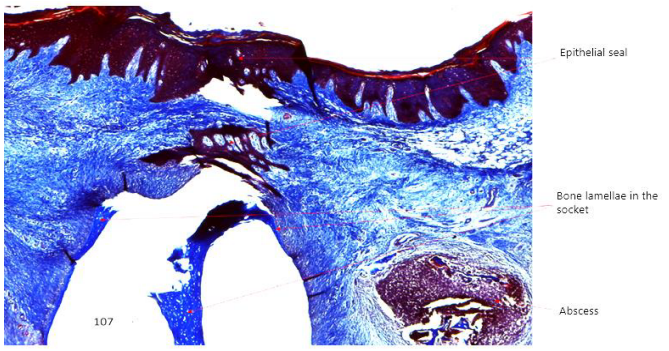

Fig. 17. Postextractional alveoli on the upper right hemiarcade. Masson's trichrome staining (Suprathel®).

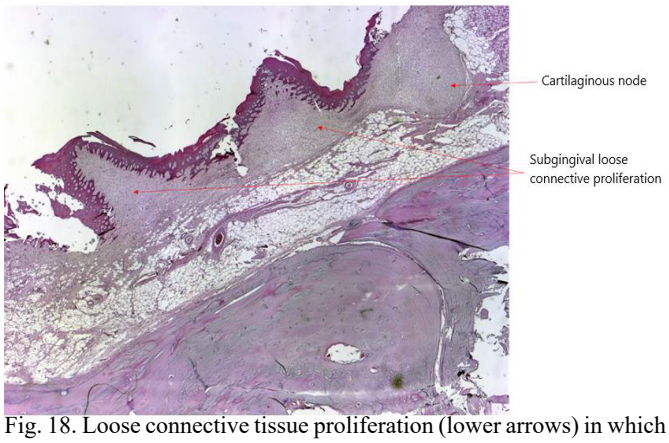

Fig. 18. Loose connective tissue proliferation (lower arrows) in which a cartilaginous node has developed (upper arrow). Hematoxylin-eosin staining (Suprathel®).

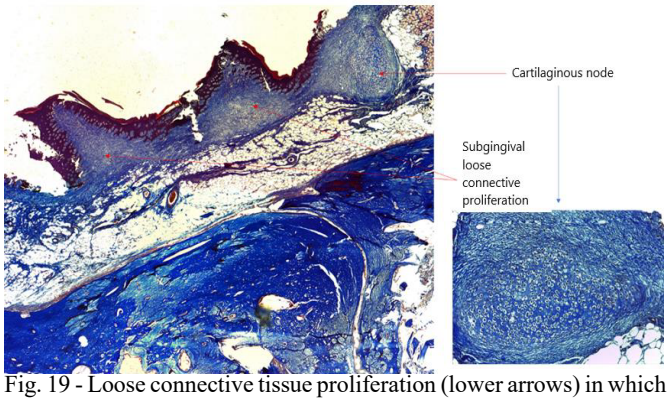

Fig. 19 - Loose connective tissue proliferation (lower arrows) in which a cartilaginous node has developed (upper arrow). Masson's trichrome staining (Suprathel®).

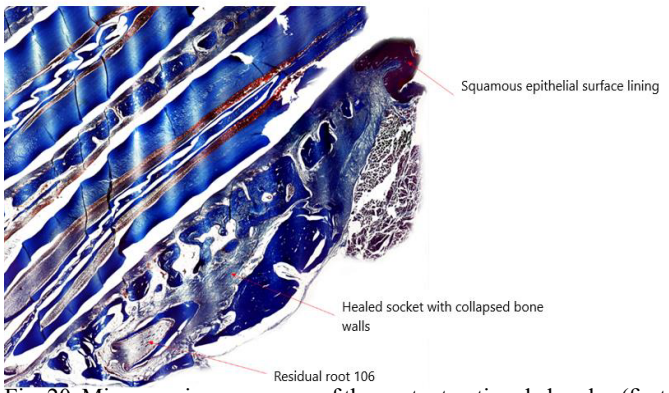

Fig. 20. Microscopic appearance of the postextractional alveolus (first right upper premolar) at 3 months after surgery. Masson's trichrome staining. Upper arrow: squamous epithelial lining on the surface. Middle arrow: healed socket with continuous bone walls. Lower arrow: residual root 106 (Suprathel®).

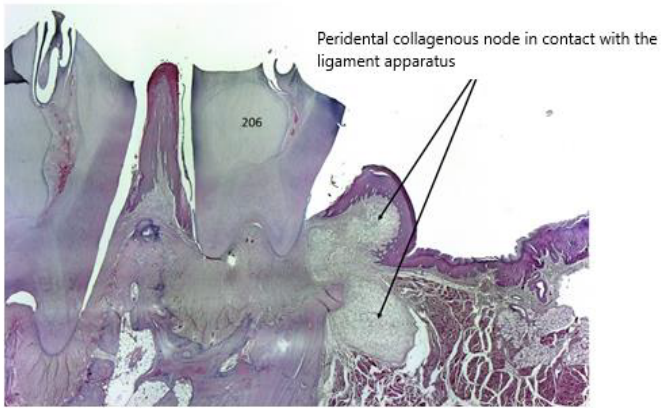

Fig. 21. Lax collagenous node below the intact gingival epithelium anterior to the first left extracted premolar (Mucoderm®).

Favorable healing of the postextractional bone sockets was also observed in Masson's trichrome staining (Fig. 20).

As for the second material (Mucoderm®), in the first rabbit a loose collagenous node was found under the intact gingival epithelium anterior to the first left extracted premolar (Fig. 21).

Discussion

The present rabbit-split-mouth pilot study was designed with two objectives, the first one investigating tissue defect sites for epithelial regeneration (double blind vestibuloplasty, Suprathel® and Mucoderm®) and the second one investigating postextractional alveolar sockets for bone regeneration (double blind socket preservation, comparing the same two materials). Both types of defects were evaluated after three months by histopathological examination.

The favorable outcome of tissue regeneration promoted by Suprathel® stood out for both epithelial and bone healing.

Both materials were evaluated in their role as barrier-membranes. Membranes are generally chosen according to criteria such as: biocompatibility, acceptance / integration into host tissues, ability to maintain isolation between tissues, effect on subjacent and neighboring tissues, ease and predictability of their handling and use.

Over time, several biodegradable synthetic polymers have been used as biomaterials in tissue engineering, including poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactide-co-glycolide (PLGA), poly-vinyl alcohol (PVA) and polycaprolactone (PCL) (Nasalpure et al 2017). Such biodegradable synthetic polymers are valued for their good biocompatibility, chemical versatility and good mechanical properties, favorable immunogenic behavior and absence of disease transmission. These materials are fully biodegradable, with monomers of lactic acid eliminated through the metabolic pathways. At the same time, these compounds promote cell proliferation and differentiation (Nasalpure et al 2017).

All components of the investigated material, Suprathel®, are recognized for their biomedical use. Polylactic acid (PLA) is the main component of several biomaterials of medical use. As a thermoplastic polyester obtained by condensing lactic acid, it is a frequently used material due to its low production costs, using renewable resources. In 2010 it was ranked second in terms of the volume used among all bioplastics worldwide (Material Properties of Polylactic Acid (PLA), Agro Based Polymers |

Polymers Data Sheets (archive.org), Bioplastics - Study: Market, Analysis, Trends | Ceresana (archive.org)).
PLA is the most widely used material in 3D printing. The decomposition of this material is done in 3 ways: by hydrolysis, by thermal decomposition and by photodisintegration. For applications in GTR or other implantable devices, the decomposition method is hydrolysis. The resulting products are not harmful, are not irritating for tissues and are easily excreted by the renal pathway. Thus, due to its biocompatibility, complete decomposition and lack of tissue toxicity, PLA has gained wide applicability in medicine, in areas such as orthopedics, maxillofacial surgery, tissue engineering, urethral stents, drug transport etc.
In maxillofacial surgery, PLA is used in traumatology (resorbable material for osteosynthesis), orthognathic surgery (osteosynthesis, surgical guides for osteotomies, devices for osteodistraction) and especially in implantology (GTR, surgical guides) (Pawar et al 2014).
The second component of Suprathel®, poly(trimethylene carbonate) (PTMC), as well as its copolymers and its derivatives have been studied due to their unique degradation characteristics. Their flexible and hydrophobic nature has driven the application of PTMC-based polymers to soft tissue regeneration and drug delivery (Fukushima 2016).
Boland et al (2006) observed in a comprehensive review on natural and artificial bioresorbable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(caprolactone) (PCL), and the copolymer poly(L-lactide-co-glycolide) (PLGA) their wide use as scaffolds for cell seeding and growth, with progressive resorbtion volume replaced by tissue.
Suprathel® promotes adequate growth conditions for fibroblasts, a condition of paramount importance for the successful healing of deep wounds. In 2020, a study compared the survival and growth characteristics of human juvenile and adult dermal fibroblasts, as well as the murine fibroblast cell line L929 on the synthetic polymer Suprathel®, to a natural polymer using different culture models, and revealed comparably favorable cytocompatibility for both materials (Gögele et al 2020). A recent study has shown that it can also successfully carry amniotic stem cells for burned skin recovery (Kitala et al 2020).
In the first study of Rothamel that described Mucoderm® in 2014, it was demonstrated that its acellular matrix of collagen can serve as a three-dimensional scaffold to facilitate fibroblasts and endothelial cells proliferation, leading to fast revascularization (Rothamel et al 2014, Walters & Steegmann 2014).
Mucoderm® proved to be effective in increasing the thickness of the keratinized mucosa in procedures associated with oral implantology, vestibuloplasty, and other applications in oral and maxillofacial surgery (Papi & Pompa 2018, Nocini et al 2014).
In the current study, with respect to the intraoral epithelial healing, both materials (Suprathel® and Mucoderm®) induced the epithelialization process, creating a solid squamous epithelium, even if initially hyperplastic. Inflammatory reactions produced by the materials were minor, more expressed in Suprathel®, for which few material residues were still visible after the study interval. In both studied rabbits, a deep proliferation of the epithelium has been detected.
In the defects covered with Suprathel®, reepithelialization of the defect area with squamous epithelium with pseudocarcinomatous hyperplasia has been considered a very favorable result.
The reepithelialization process appeared underneath Suprathel®, and above Mucoderm®, respectively. This was best seen in Masson's trichrome staining, the reepithelialized area with abundant granular layer and poor spinous layer standing out as redder than normal.
So far, Suprathel® has been evaluated only in studies that compared the skin epithelialization effect of various clinically validated materials, analyzed according to keratinocyte layer stimulation, and epidermal stratification, along with pain, discomfort during dressing changes and cost (Huging et al 2017, Hundeshagen et al 2018). It has attained high scores of satisfaction in both clinicians and patient groups.
The fabrication with increased thickness ensured the overcoming of problems observed, regarding the mechanical strength and implantation difficulties of such materials (Silva et al 2010).
The effect of Suprathel® on skin healing after wounds and burns was validated clinically and its ability to enable all phases throughout the healing process, i.e. the inflammation, proliferation and epithelialization was proven by various studies, but also by its worldwide successful use for over ten years.
Regarding guided bone regeneration, it can be used to restore a large number of conditions related to the insufficient amount of bone in atrophied maxillary bones. The resorption of the alveolar bone subsequent to the loss of teeth endangers the functional and aesthetic prognostic aspects of the implant-supported prosthetic treatment. This decrease in volume is a consequence of the loss of teeth, since their role in maintaining bone trophicity by functional stimulation disappears. Immediately after the loss of a tooth, resorption begins predominantly horizontally in the alveolar socket, and then continues vertically. Thus, 6 months after extraction, an average loss of 3.8 mm in bone height and 1.2 mm in bone thickness occurs (Tan et al 2012).
In view of these consequences, research has focused on alveolar socket preservation, with an intent to augment the alveolar area for the subsequent insertion of implants. Efforts have been directed to reduce this phenomenon by active guided bone regeneration (GBR), precisely the „socket preservation". In this procedure, a polymeric barrier membrane is applied as cover to the alveolus, so that bone formation can occur in the space protected from fibroblasts ingrowth. This membrane should ideally be resorbable, retentive and demonstrate mechanical strength (Fukushima 2016).
These properties of membranes have been assessed comparatively for a multitude of biomaterials of various compositions and combinations, with a collagen membrane frequently used as the control (Fukushima 2016). Along with these analyses, various markers were used to estimate the bone formation (viability of osteoblast-like cells, the alkaline phosphatase activity level etc.).
Considering this, the debate should include two major issues: the barrier membrane ensuring the protected space and the materials used for tissue growth stimulation or augmentation (Retzepi & Donos 2010).
Autologous bone has long been considered the gold standard, but now, due to severe deficiencies such as morbidity at the donor site, limited amount of available bone, unpredictable resorption etc., it is losing ground to alloplastic materials (Material Properties of Polylactic Acid (PLA), Agro Based Polymers |

Polymers Data Sheets (archive.org), Bioplastics - Study: Market, Analysis, Trends | Ceresana (archive.org)).

Moreover, vertical bone augmentation still poses major problems and, although multiple options have been proposed to achieve it, none of them provide the desired predictable and stable results. GTR stood out in all variants of defects as a successful option with wide addressability. A study conducted by Aghaloo in 2007 showed that GTR increased the success rate of endosseous implants to over 95% (Aghaloo & Moy 2007, Bioplastics - Study: Market, Analysis, Trends | Ceresana (archive.org)).

Certainly, other combinations of methods and materials can also produce satisfactory results, but the aforementioned GTR methods have proven their viability over time, while also being the best documented ones (Tan et al 2012).

Regarding graft resorption, it was found that a coating of an autogenous graft with bovine bone and collagen membrane decreased the resorption at 4 months from 21% to 5.5% (Cordaro et al 2011).

Various strategies can be used for socket preservation, such as: GTR procedures based on graft transplantation, graft plus barrier membrane or barrier membrane alone (Hutmacher et al 1996, Silva et al 2010), autogenous inlay or onlay grafts, free vascularized grafts, osteogenesis by callus elongation (osteodistraction) etc. It is important to note that these techniques, although very different at first glance, follow the same principles of bone physiology. The choice of the augmentation method depends on the amount of bone loss, the shape of the defect, the surgeon's experience and many other objective and subjective factors. The success rate of implants inserted in areas where bone augmentation has been performed is high, but more studies are needed to monitor survival and success rates over longer periods of time and also look for a correlation between the addition method and the stability of results over time (http://www.ceresana.com/en/market-studies/plastics/bioplastics).

The current study set up the choice of a collagen membrane as control material based on all these previously reported findings.

The outcomes in both rabbits showed that in alveolar sockets covered with Suprathel® in GTR for bone preservation purposes, favorable healing could be noted, starting and based on cartilage nodes. The observed tissue structure was in adequate concordance with the stage of healing (John et al 2007).

Tissue regeneration in sockets covered with Mucoderm® displayed very similar features.

The long healing period (3 months), rarely encountered in animal studies due to increased efforts and costs, ensured an increased relevance and stability of outcomes measured by histological evaluation.

To the best of our knowledge, and after thoroughly searching for similar results reported in current literature, this pilot study appears to be the first attempt to demonstrate the effect of Suprathel® in intraoral applications for mucosal regeneration (keratinised mucosa) and socket preservation (alveolar bone).

Despite the very limited number of animals included in the present pilot study, its results were still able to document a favorable effect of Suprathel® on intraoral tissue healing for both epithelium and bone. This expected favorable evolution confirmed an adequate study design. The used protocol proved to be safe and ethically acceptable, ensuring the avoidance of potential adverse effects, thereby supporting the feasibility of a future experiment on a larger scale.

This study also helped in establishing an array of possible outcomes and objective criteria that appeared to be most feasible for a future statistical assessment of the epithelial- and bone healing potential of the investigated membranes. These outcomes and criteria have been recorded and will serve as a basis for collecting and analyzing data to quantify and compare the efficacy of GTR using the investigated membranes, in a trial that will include a larger animal group.

Conclusions

This first-in-animal pilot study on a split-mouth rabbit model demonstrated its feasibility in testing Suprathel® as a membrane in intraoral guided tissue regeneration for keratinized gingiva and bone healing. In this pilot study, Suprathel®, a membrane of polylactic acid, although designed for extraoral application, exhibited a high potential for success in intraoral GTR, owing to its structure and properties (ease of placement, increased thickness and mechanical strength).

The experimental model proved to be highly informative and problem-solving for this testing phase, offering strong support for its continuation in a larger experiment, for more reliable results. A larger trial has therefore been planned based on an optimized protocol, using those outcomes and evaluation criteria that have been found to be most feasible and reproducible during the current study.

The most important reason for planning a second, larger-scale evaluation of the Suprathel® membrane is its high potential for clinical applicability. The integration of results obtained from in vivo studies into producing next-generation materials warrants their improvement and the possibility of an evaluation of their clinical effectiveness by future clinical trials.

References

Aghaloo TL, Moy PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? Int J Oral Maxillofac Implants 2007;22 Suppl:49-70.

Boland ED, Pawlowski KJ, Barnes CP, Simpson DG, Wnek GE et al. Electrospinning of bioresorbable polymers for tissue engineering scaffolds. Polym Nanofibers ACS Symp Ser 2006;918:188–204.

Ceresana. "Bioplastics - Study:Market, Analysis, Trends - Ceresana". www.ceresana.com. Archived from the original on 4 November 2017. Retrieved 9 May 2018.

Chen Z, Chen L, Liu R, Lin Y, Chen S, Lu S, Lin Z, Chen Z, Wu C, Xiao Y. The osteoimmunomodulatory property of a barrier collagen membrane and its manipulation via coating nanometer-sized bioactive glass to improve guided bone regeneration. Biomater Sci 2018 May 1;6(5):1007-1019. doi:10.1039/c7bm00869d. https://pubmed.ncbi.nlm.nih.gov/29485658/

Cordaro L, Torsello F, Morcavallo S, di Torresanto VM. Effect of bovine bone and collagen membranes on healing of mandibular bone blocks:a prospective randomized controlled study. Clin Oral Implants Res 2011;22:1145– 1150.

Fukushima K. Poly(trimethylene carbonate)-based polymers engineered for biodegradable functional biomaterials. (Review Article) Biomater Sci 2016;4:9-24. doi:10.1039/C5BM00123D

Gögele C, Schulze-Tanzil G, Kokozidou M, Gäbel C, Billner M, Reichert B, Bodenschatz KJ. Growth characteristics of human juvenile, adult and murine fibroblasts1:a comparison of polymer wound dressings. J Wound Care. 2020 Oct 2;29(10):572-585. doi:10.12968/jowc.2020.29.10.572.

Highton L, Wallace C, Shah M. Use of Suprathel® for partial thickness burns in children. Burns 2013;39:136–41.

Hüging M, Biedermann T, Sobrio M, Meyer S, Böttcher-Haberzeth S, Manuel E, Horst M, Hynes S, Reichmann E, Schiestl C, Hartmann-Fritsch F. The effect of wound dressings on a bio-engineered human dermo-epidermal skin substitute in a rat model. J Burn Care Res. Nov/Dec 2017;38(6):354-364. doi:10.1097/BCR.0000000000000530.

Hundeshagen G, Collins VN, Wurzer P, Sherman W, Voigt CD, Cambiaso-Daniel J, Nunez Lopez O, Sheaffer J, Herndon DN, Finnerty CC, Branski LK. A prospective, randomized, controlled trial comparing the outpatient treatment of pediatric and adult partial-thickness burns with Suprathel or Mepilex Ag. J Burn Care Res. 2018 Feb 20;39(2):261-267. doi:10.1097/BCR.0000000000000584

Hutmacher D, Hürzeler MB, Schliephake H. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. Int J Oral Maxillofac Implants. Sep-Oct 1996;11(5):667-78.

John V, De Poi R, Blanchard S. Socket preservation as a precursor of future implant placement:review of the literature and case reports. Compend Contin Educ Dent. 2007 Dec;28(12):646-53;quiz 654, 671.

Keck M, Selig HF, Lumenta DB, Kamolz LP, Mittlböck M, Frey M. The use of Suprathel(®) in deep dermal burns:first results of a prospective study. Burns 2012;38:388–95.

Kitala D, Łabuś W, Klama-Baryła A, Kraut M, Maj M, Szapski M. Application of Amniotic Stem Cells on an Acellular Dermal Matrix Scaffold in a Burned Patient:A Case Report. Transplant Proc. 2020 Oct;52(8):2563-2569. doi:10.1016/j.transproceed.2020.01.110. Epub 2020 May 19.

"Material Properties of Polylactic Acid (PLA), Agro Based Polymers". Matbase - Material Properties Database. Archived from the original on 10 February 2012. Retrieved 6 February2012.

Nasalpure AV, Chalannavar RK, Gani RS, Malabadi RB, Kasai DR. Tissue engineering of skin:a review. Trends Biomater Artif Organs 2017;31(2):69-80.

Nocini PF, Castellani R, Zanotti G, Gelpi F, Covani U, Marconcini S, de Santis d. Extensive keratinized tissue augmentation during implant rehabilitation after Le Fort I osteotomy:using a new porcine collagen membrane (Mucoderm). J Craniofac Surg. 2014 May;25(3):799-803. doi:0.1097/SCS.0000000000000524. https://pubmed.ncbi.nlm.nih.gov/24777008/

Pabst AM, Happe A, Callaway A, Ziebart T, Stratul SI, Ackermann M, Konerding MA, Willershausen B, Kasaj A. In vitro and in vivo characterization of porcine acellular dermal matrix for gingival augmentation procedures. J Periodont. Res. 2014;49:371–381.

Papi P, Pompa G. The use of a novel porcine derived acellular dermal matrix (mucoderm) in peri-implant soft tissue augmentation:preliminary results of a prospective pilot cohort study. Biomed Res Int. 2018 Jul 9;2018:6406051. doi:10.1155/2018/6406051. eCollection 2018. https://pubmed.ncbi.nlm.nih.gov/30112412/

Pawara RP, Tekalea SU, Shisodiaa SU, Totrea JT, Domb AJ. Biomedical Applications of Poly(Lactic Acid). Recent Patents on Regenerative Medicine 2014;4:40-51. https://www.researchgate.net/publication/262418499_Biomedical_Applications_of_PolyLactic_Acid

Retzepi M, Donos N. Guided Bone Regeneration:biological principle and therapeutic applications. Clin Oral Implants Res 2010;Jun;21(6):567-76. doi:10.1111/j.1600-0501.2010.01922.x.

Rothamel D, Benner M, Fienitz T, Happe A, Kreppel M, Nickenig HJ, Zöller JE. Biodegradation pattern and tissue integration of native and cross-linked porcine collagen soft tissue augmentation matrices - an experimental study in the rat. Head Face Med. 2014 Mar 27;10:10. doi:10.1186/1746-160X-10-10.

Schwarze H, Küntscher M, Uhlig C, Hierlemann H, Prantl L, Ottomann C et al.:Suprathel, a new skin substitute, in the management of partial-thickness burn wounds. Ann Plast Surg 2008;60:181-185.

Silva SS, Mano JF, Reis RL. Potential applications of natural origin polymer-based systems in soft tissue regeneration. Critical Reviews in Biotechnology 2010;30(3):200 – 221. doi:10.3109/07388551.2010.505561

Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res 2012;23(Suppl):1–21.

Turri A, Elgali I, Vazirisani F, Johansson A, Emanuelsson L, Dahlin C, Thomsen P, Omar O. Guided bone regeneration is promoted by the molecular events in the membrane compartment Biomaterials 2016;84:167-183.

Walters BD, Steegmann JP. Strategies for directing the structure and function of three-dimensional collagen biomaterials across length scales. Acta Biomaterialia 2014;10:1488-1501. doi:https://doi.org/10.1016/j.actbio.2013.08.038

Authors

•Sergiu Vacaraş, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: vacarassergiu@umfcluj.ro

•Grigore Băciuţ, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: gbaciut@umfcluj.ro

•Dan Gheban, MD, Department of Pathological Anatomy, Iuliu Haţieganu University of Medicine and Pharmacy, 3-5 Clinicilor Street, 400006, Cluj-Napoca, Romania, email: dgheban@gmail.com

•Simion Bran, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: simion.bran@umfcluj.ro

•Horaţiu Colosi, Department of Medical Informatics and Biostatistics, Iuliu Haţieganu University of Medicine and Pharmacy, 6 Louis Pasteur Street, 400349, Cluj-Napoca, Romania, email: hcolosi@umfcluj.ro

•Cristian Berce, Center for Experimental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babes Stree, 400012, Cluj-Napoca, Romania; email: cristi.berce@gmail.com

•Winfried Kretschmer, Klinik für Mund-, Kiefer- und Plastische Gesichtschirurgie, Alb Fils Klinikum, Eichertstr. 3, 73035 Göppingen, Germany, email: winfried.kretschmer@af-k.de

•Avram Manea, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: avram.manea@umfcluj.ro

•Gabriel Armencea, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine

and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: garmencea@gmail.com

•Mihaela Băciuţ, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: mbaciut@umfcluj.ro

•Cristian Dinu, Department of Maxillofacial Surgery and Oral Implantology, Iuliu Haţieganu University of Medicine and Pharmacy, 37 Cardinal Iuliu Hossu Street, 400029, Cluj-Napoca, Romania, email: cristian.dinu@umfcluj.ro

Citation	Vacaraş S, Băciuţ G, Gheban D, Bran S, Colosi H, Berce C, Kretschmer W, Manea A, Armencea G, Băciuţ M, Dinu C. Laparoscopic surgical treatment for renal tumors. First–in-animal experimental pilot study of Suprathel® for oral mucosa and bone regeneration. HVM Bioflux 2021;13(2):56-66.
Editor	Antonia Macarie
Received	10 February 2021
Accepted	28 February 2021
Published Online	12 April 2021
Funding	None reported
Conflicts/ Competing Interests	None reported