NEW

Guiding your shift towards synthetic solutions

Easily - Safely - Successfully

R.T.R.+ Membrane

Scientific File

septodont

Agenda

What is R.T.R.+ Membrane?

R.T.R.+ Membrane is a unique resorbable bilayer synthetic dental membrane for guided tissue regeneration in dental periodontology and implant surgery.

R.T.R.+ Membrane is a synthetic membrane made of 100% polymer poly-D,L-lactic/glycolic acid (PLGA). Free from animal derivatives, R.T.R.+ Membrane eliminates the risk of animal-to-human pathogen transmission.

R.T.R.+ Membrane maintains architecture and structural integrity for four to six weeks after implantation and fully resorbs by hydrolysis within four to six months.

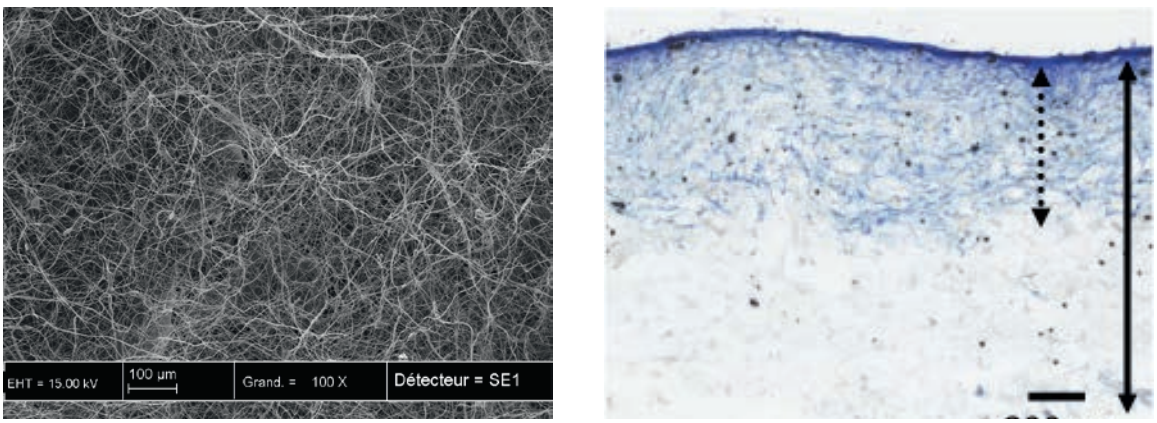
Supple, strong and tear-resistant for tacking and suturing, R.T.R.+ Membrane is easy to handle and cut to size, whether wet or dry, offering optimal membrane shaping and placement to fit defect anatomies.

Microfibre Jet - patented spraying technology

Developed using the very latest advances in tissue engineering, Septodont's proprietary production process generates 3D matrix microfibre membranes for various resorption times. Second-stage surgery for membrane removal is not required, therefore avoiding further wound trauma.

Intricate, non-woven synthetic microfibres imitate the structure of collagen and serve as a 3D matrix for early cell colonisation and vascularisation.

Septodont's unique synthetic polymer fibre shaping technology provides an alternative to collagen-based tissue regeneration devices. This breakthrough technology allows the perfect mimicry of collagen fibres using fully biocompatible, 100% synthetic biodegradable polymers. These biomimetic fibre scaffolds promote tissue repair by acting as stakes for cells. Once tissue regeneration is complete, the scaffolds degrade in a perfectly controlled manner.



Jet-sprayed polymer fibres

Technical characteristics

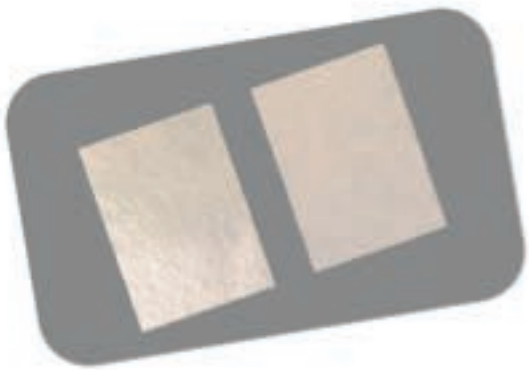
Bilayer structure

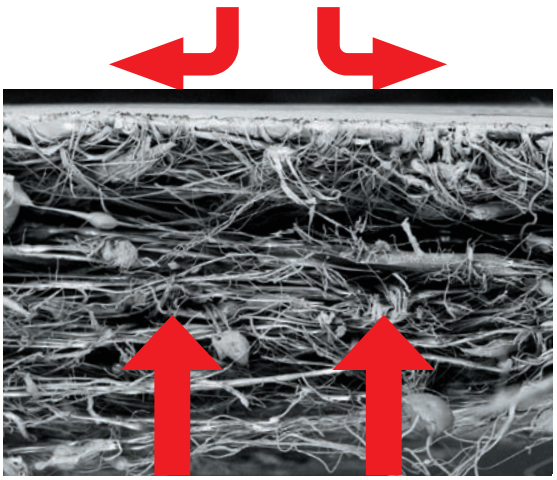
R.T.R.+ Membrane is composed of two distinct layers: a micro-fibrous mat layer and a dense glossy layer.

The dense layer prevents gingival fibroblast ingrowth, while the micro-fibrous layer supports colonization by osteogenic cells and promotes bone regeneration.

The two layers are clearly visible on the cross section observed by SEM.
The micro-fibrous layer exhibits a highly porous structure with interlaced non-woven fibres of diameters ranging from 0.2 to 2 µm.

The thin and dense film appears in contact with a thick and porous layer made of microfibres.
The overall thickness of the membrane is 450 ± 100 µm with a flat, smooth, non-porous dense surface of 25 ± 5 µm.





Mechanical properties

The mechanical properties of the PLGA membrane were measured to ensure both its resistance to surgical handling and its compatibility with suturing.
The ultimate stress and strain were determined as 6.7 ± 2.6 N and 7.6 ± 4%, respectively.
The maximum resistance to initial tear is 47 ± 5cN.
The maximum resistance to suture traction is 2.83 ± 1.09 N.

Biological Properties

Cytotoxicity evaluation

(NF EN ISO 10993-5) (Namsa)

This *in vitro* study was conducted to evaluate the potential cytotoxic effects of R.T.R.+ Membrane following the guidelines if ISO 10993-5, Biological Evaluation of Medical Devices, Part 5: Tests for In Vitro Cytotoxicity.

Materials and Methods: R.T.R.+ Membrane was extracted in single Eagle Minimum Essential Medium (EMEM10) at 37°C for 24 hours. A negative control, reagent control, and positive control were similarly prepared. Following extraction, triplicate monolayers of L-929 mouse fibroblast cells were dosed with the full-strength extracts (100%) and incubated at 37°C in the presence of 5% CO2 for 24-26 hours. Following incubation, 20 µL of the MTS-PMS solution, prepared just before use, was dispensed in each well and incubated during 120-135 minutes at 37°C in 5% CO2.

Cytotoxicity is assessed using a colorimetric test that quantitatively measures cell viability after exposure to the test extracts. A decrease in the number of living cells results in a decrease in the metabolic activity in the sample and correlates in the amount of brown formazan formed, as monitored by the optical density at 492 nm. If the cell viability is reduced to less than 70% of the control blank, a cytotoxic potential is evidenced.

Results: Percent viability of L-929 cells was measured at 89.3% after exposure to undiluted (100%) R.T.R.+ Membrane extract. The full-strength EMEM10 R.T.R.+ Membrane extract showed no cytotoxic potential to L-929 mouse fibroblast cells.

Conclusion: R.T.R.+ Membrane is not cytotoxic.

Hypersensitivity

(NF EN ISO 10993-10) (Namsa)

A guinea pig maximisation test was performed on R.T.R.+ Membrane to evaluate the potential for delayed dermal contact sensitisation.

Materials and Methods: R.T.R.+ Membrane was extracted in both 0.9% NaCl and sesame oil. Each extract was intradermally injected and topically applied to 10 guinea pigs (per extract) to induce delayed sensitisation. Following a recovery period, all the test and control animals received a challenge patch of the appropriate R.T.R.+ Membrane extract and for the 0.9% NaCl group only, a challenge intradermal injection was performed. All sites were scored at 24 and 48 hours after patch removal and after injections.`

Results: The topical application of 0.9% NaCl extract evaluated at a concentration of 100% did not induce delayed sensitisation in the guinea pigs (grade 0). The topical appli-

cation of sesame oil extract evaluated at a concentration of 100% did not induce delayed sensitisation in the guinea pigs (grade 0). The intradermal injection of 0.9% NaCl extract at a concentration of 100% did not induce delayed sensitisation in the guinea pigs (grade 0).

Conclusion: R.T.R.+ Membrane is not considered a sensitiser in the guinea pig maximisation model.

Irritation

(NF EN ISO 10993-10) (Namsa)

An intracutaneous test was performed on R.T.R.+ Membrane to evaluate the potential of the material to produce irritation following intradermal injection in the rabbit.

Materials and Methods: R.T.R.+ Membrane was extracted in both 0.9% NaCl and sesame oil. By intracutaneous route on the left side, three rabbits received five injections of 0.2 mL of the 0.9% NaCl extract and five injections of 0.2 mL of the sesame oil extract. Similarly on the right side, the rabbits received five injections of 0.2 mL of 0.9% NaCl and 0.2 mL of sesame oil, as controls. Immediately after injection, and again at 24, 48 and 72 hours after injection, the sites were examined for gross evidence of tissue reaction, such as erythema and oedema.

Results: Both the 0.9% NaCl and sesame oil extracts of R.T.R.+ Membrane did not induce any erythema and oedema reactions after injection by intracutaneous route in the rabbit. (The difference between the test and the control mean score was 1.0 or less.)

Conclusion: R.T.R.+ Membrane does not trigger irritation in the rabbit intracutaneous model.

Acute systemic toxicity

(NF EN ISO 10993-11) (Namsa)

An acute systemic toxicity test was performed on R.T.R.+ Membrane to determine the systemic toxicity in mice.

Materials and Methods: R.T.R.+ Membrane was extracted in both 0.9% NaCl and sesame oil. A single dose of each extract was injected into five mice per extract by the intraperitoneal route at the dose of 50 mL/kg. Similarly, five mice were injected with each extraction solvent. The animals were observed immediately and at 4, 24, 48 and 72 hours after systemic injection. They were weighed at 24, 48 and 72 hours after injection. The toxicity was evaluated according to a numerical reaction scale ranging from 0 (“no symptoms”) to 4 (“death”).

Results: There was no mortality during the study in mice injected with R.T.R.+ Membrane extracts. All animals appeared clinically normal at the beginning and throughout the study. Body weight data were acceptable and equivalent between the corresponding test and control treatment groups.

Conclusion: R.T.R.+ Membrane shows no evidence of acute systemic toxicity.

Haemolysis

(NF EN ISO 10993-4) (Namsa)

R.T.R.+ Membrane interacts with blood at implantation and plays a major role in the maintenance of the blood clot at the site of the surgery, which promotes healing and progenitor cell recruitment for bone regeneration. Therefore, a blood interaction test was performed to verify that the membrane does not cause haemolysis *in vitro* by direct contact or extraction.

Materials and Methods: Blood was obtained from human participants, then pooled, diluted and added to triplicate tubes with R.T.R.+ Membrane in calcium- and magnesium-free Dulbecco's phosphate buffered saline (CMF-DPBS) and triplicate tubes with the CMF-DPBS R.T.R.+ Membrane extracts. These combinations were evaluated to determine whether direct contact with the R.T.R.+ Membrane or extract of R.T.R.+ Membrane would cause *in vitro* red blood cell haemolysis. Negative and positive controls were prepared in the same manner as the membrane. The tubes were maintained for at least three hours at 37°C with gentle stirring every 30 minutes. Following incubation, suspensions were mixed gently and centrifuged. Total haemoglobin was determined by using a colorimetric method and absorbance reading of the solution at 540 nm. The haemolytic index was calculated by the formula:

Haemolytic index (HI in %) = (free haemoglobin(g/L)/total haemoglobin) x 100

A haemolytic grade was then assigned based on the ASTM F 756-08 scoring system:

- HI 0-2% -- non-haemolytic.
- HI 2-5% -- slightly haemolytic
- HI above 5% -- haemolytic.

Results: The mean haemolytic index for R.T.R.+ Membrane by direct contact with blood was 1.36% and for R.T.R.+ extract was 1.01%. Both the direct contact and the extract of R.T.R.+ Membrane were non-haemolytic.

Conclusion: R.T.R.+ Membrane is considered to be non-haemolytic in vitro.

Implantation in rats

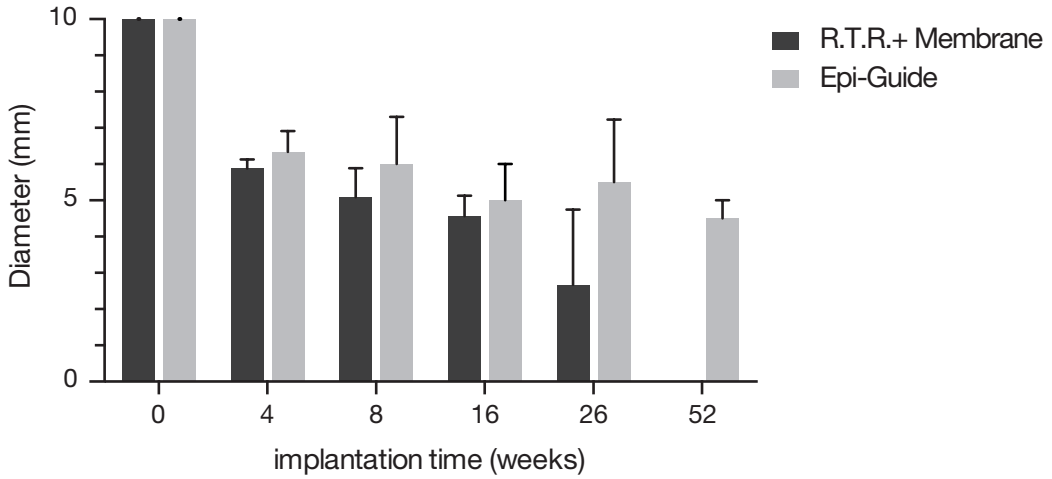
(NF EN ISO 10993-6) (Inserm U457)

The objective of this preclinical GLP study was to evaluate the local tissue effects and resorption time of R.T.R.+ Membrane in comparison with the commercial membrane Epi-Guide after subcutaneous implantation in rats. The local tissue effects were evaluated at 4, 8 and 16 weeks following implantation through histopathological observations while the resorption time was followed up to 52 weeks.

Materials and Methods: 40 Wistar Han rats received a 10 mm diameter PLGA (R.T.R.+ membrane) and PLA (Epi-Guide) discs in two separate subcutis pouches on each side of the vertebral column. After 4, 8, 16, 26 and 52 weeks, the sites were examined and photographed with a graduated ruler to determine the diameter of the remaining implant. After dissection, samples isolating each membrane were fixed in formaldehyde solution

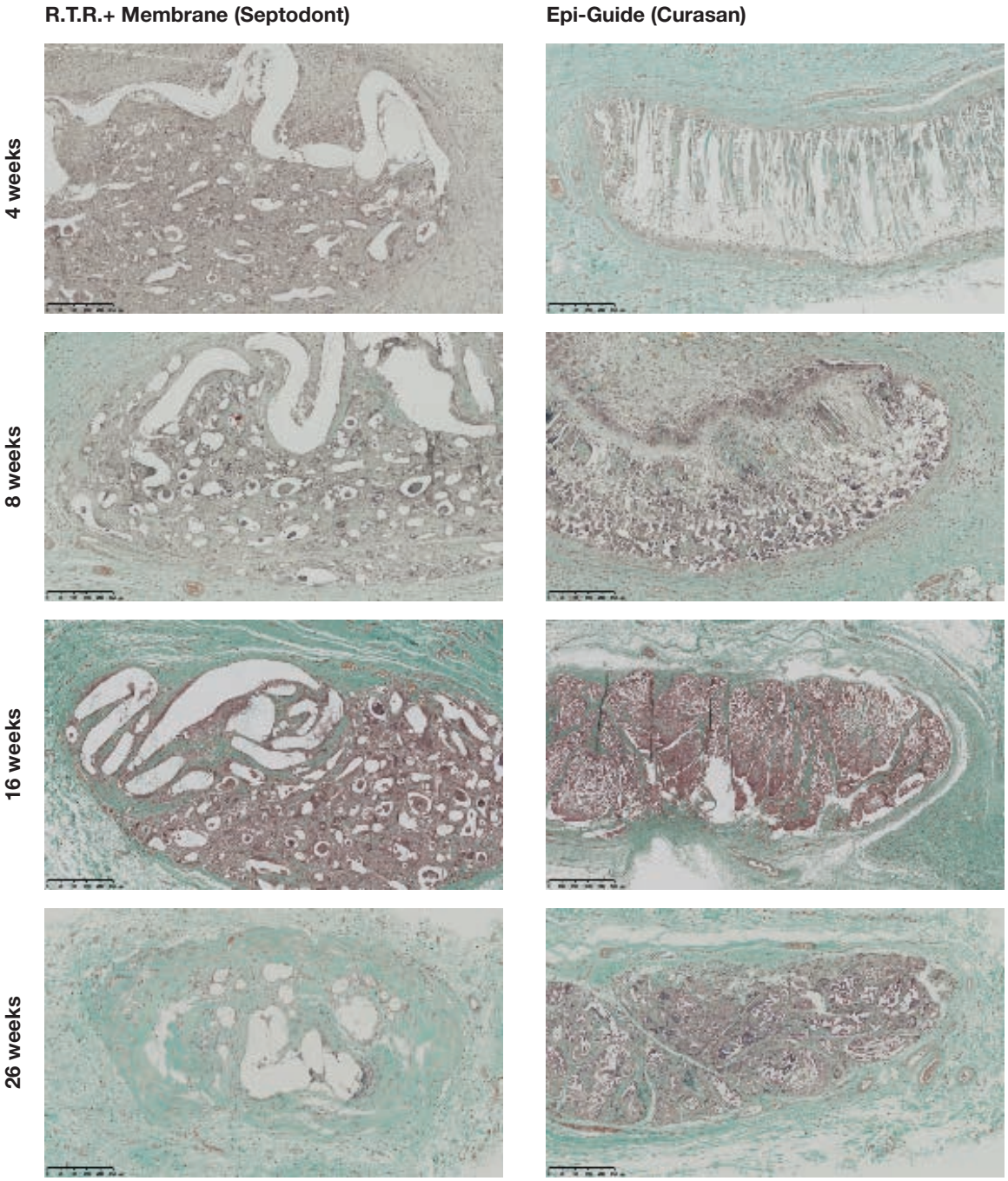
and processed for decalcified paraffin histology. Sections were stained with haematoxylin/eosin or Masson's trichrome. Tissue and cells in the different membranes were identified and scored by a medical doctor specialising in anatomic pathology. Tissue necrosis, neovascularisation, fibrosis and fat tissue infiltration were scored according to ISO 10993-6 standard recommendations. Granulocytes, lymphocytes, plasmacytes, macrophages and giant cells were numbered under virtual microscope (NDP view software; Hamamastu corp.) at a magnification of x 400. A score of 0 was given in the absence of cells and a score of 4 corresponded to a high number of cells gathered in wide-ranging areas.

Results: All rats recovered with no complications and macroscopic examination showed good wound closure without signs of inflammation or infection. After four and eight weeks of subcutis implantation, R.T.R.+ Membrane and Epi-guide were clearly visible and encapsulated within a vascularised fibrous tissue. The reduction in the diameter of both membranes was plotted as a function of the implantation time. Both types of membrane considerably reduced in diameter after four weeks. For Epi-Guide, the diameter decreased gradually over 52 weeks. For R.T.R.+ Membrane, the reduction in diameter was more rapid, and only four out of six implanted PLGA membranes could be retrieved at 26 weeks.



As shown on histology images below, after four weeks, fibrosis was observed around both types of implants. The fibrous capsule was narrow, with a maximum of 1–4-cell layers around both types of membrane. No fat tissue infiltration was observed in either case. Neovascularisation was focalised with a maximum of 4–7 capillaries. In PLA membrane, there was little cell colonisation, particularly after a short implantation time. In contrast, many cells had infiltrated R.T.R.+ Membrane by the fourth week. The dense film (white) was visible up to eight weeks but appeared highly distorted and fragmented. At eight weeks, many macrophage-like cells and multi-nucleated giant cells had infiltrated both types of polymer membrane. Collagen (green) was also present between the R.T.R.+ Membrane micro-fibres. After 16 weeks, the diameter of R.T.R.+ Membrane increased sharply because of hydrolysis and swelling. At 26 weeks, the R.T.R.+ Membrane was almost completely degraded with only a few micro-fibres visible. These remaining fibres were surrounded by macrophages and multi-nucleated giant cells, embedded in collagen fibrous tissue.

Counting of the macrophages and multi-nucleated giant cells indicated less of these cells in contact with R.T.R.+ Membrane than with Epi-Guide membrane (figures 5(B) and (C)). These cellular elements were characteristic of local inflammation due to the presence of a foreign body reaction. Counts of the other cells, such as granulocytes, plasmacytes and lymphocytes, as well as tissue necrosis, neovascularisation and fibrosis always scored less than 2 for both membranes (data not shown).



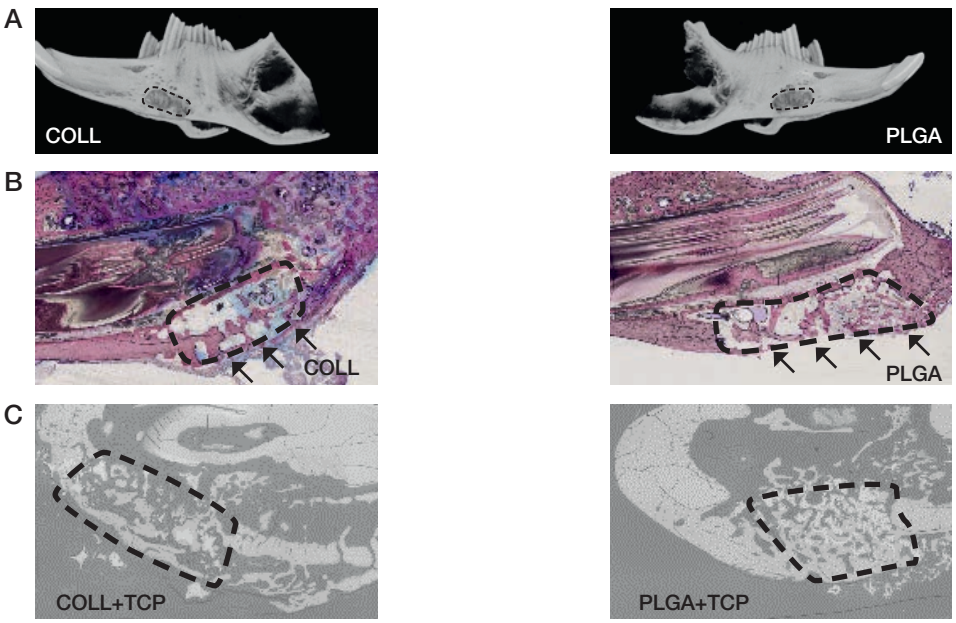
Conclusion: R.T.R.+ Membrane is considered biocompatible.

GBR in rabbit mandibles
(Inserm U747)

This study was conducted to evaluate the GBR potential of the R.T.R.+ Membrane covering a mandibular critical-sized bone defect in comparison with a dental membrane made of porcine collagen. The defect was left empty or filled with TCP granules.

Materials and Methods: 12 New Zealand White rabbits were divided into four groups and implanted bilaterally for eight weeks (sample size of n = 6/group). A bone defect of 10 x 5 x 5 mm was created on both side of the mandible and the defects were filled with TCP granules or not and covered with either R.T.R.+ Membrane or a collagen membrane. After eight weeks, the mandibles were dissected and scanned by micro-computed tomography. Non-decalcified histology with resin (PMMA) embedding was performed to produce 30 µm Toluidine blue/basic fuchsin stained sections for descriptive analyses of the bone defect and blocks for SEM imaging and quantitative measurement of the newly formed mineralised bone.

Results: All the animals recovered from surgery without complications and gained weight normally. After eight weeks, micro-tomographies of the mandibles were taken (A). The sub-mandibular bone defects covered by the membranes were still visible. No signs of osteolysis were observed around the defects. Bone growth was comparable in the defects covered with either type of membrane (B). When the defects were filled with TCP granules and covered with the membranes, more bone growth was observed than without bone filler (C). Histomorphometric analysis corroborated these observations. When TCP granules were used in combination with membranes, newly formed bone was 39.1 ± 3.4% and 39.4 ± 2.7% for the collagen and R.T.R.+ Membrane, respectively.



Conclusion: In this model, R.T.R.+ Membrane formed a physical barrier to maintain the TCP granules, prevented epithelial tissue ingrowth in the bone defects and favoured bone regeneration.

Clinical trial

Observational prospective pilot study of the use of dental membrane R.T.R.+ Membrane for guided tissue regeneration in routine practice.

The purpose of this study was to monitor the post-operative safety and performance of the R.T.R.+ Membrane, a synthetic resorbable dental membrane used in routine practice for the following indications in accordance with the Instructions for Use:

- Alveolar preservation after extraction.
- Alveolar ridge augmentation.
- GTR in immediate or delayed implant placement.
- Periodontal GTR.

This was a non-interventional, multicentre (seven centres), prospective, non-randomised, competitive study based on observation and clinical follow-up of patients and data collection.
R.T.R.+ Membrane was used in accordance with standard practice, within the indications stated in the package insert. The post-operative follow-up of patients was carried out in accordance with standard practice, with no additional visits planned compared to the usual management of patients in the clinical indications considered.

The primary objective was to confirm the safety of R.T.R.+ Membrane in clinical use by monitoring for adverse events and by systematic clinical assessment by the investigator during clinical check-ups.

The secondary objectives were to confirm the performance of R.T.R.+ Membrane in its barrier effect and maintenance of a bone filler in the socket and the ease of clinical use of the membrane through systematic collection of the investigator's opinion.

All patients required dental membrane placement in periodontology or during tooth extraction with socket preservation and/or implant placement with immediate or prior GTR. Patients included were at least 18 years old, were duly informed, and authorised the use of anonymised personal data for IT processing by signing consent.

Patients meeting the following criteria were excluded from the study:
- Uncontrolled diabetes;
- Smoking > 10 cigarettes/day;
- Congenital or acquired immune deficiency;
- A history of neoplastic lesions or having undergone cervico-facial irradiation;
- A disorder of blood flow;
- Undergoing treatment with bisphosphonates;
- Pregnant or breastfeeding women.

The study launched on February 26, 2015 and ended on November 26, 2015. The nine-month period included five months of inclusion and four months of follow-up. 27 patients were included with a premature study withdrawal for one patient, i.e. 26 cases.

The data collected for the 27 patients enrolled in the study demonstrate the safety of R.T.R.+ Membrane in the claimed indications and the number of adverse events (1/27) was not attributable to R.T.R.+ Membrane.

The performance of R.T.R.+ Membrane was confirmed by clinicians with no exudation or leakage of filler and only 11.5% of cases of membrane exposure. The overall average clinician satisfaction rating was 4.5+/- 0.6 on a scale of 0 to 5.

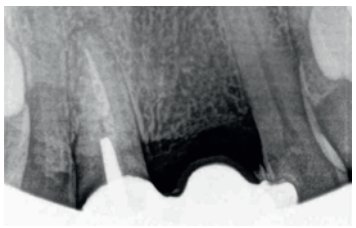
Ergonomics (ease of placement, shaping, site retention, site stability, and stability during flap and suture repositioning) was considered satisfactory by all clinicians with scores ranging from 3.7 to 3.9 +/-1. The analysis of the results in one practice demonstrated the absence of a learning curve for the use of R.T.R.+ Membrane.

Clinical cases

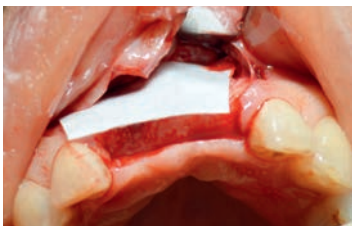
Socket preservation on the day of extraction

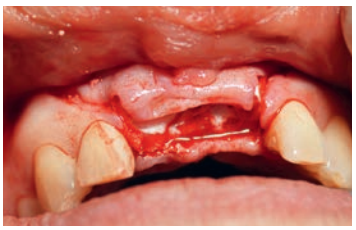
Dr. Hoornaert, Nantes, France

A 51-year-old patient presented with a mobile bridge to replace the upper central incisors on a single support (tooth 11 - upper right 1).

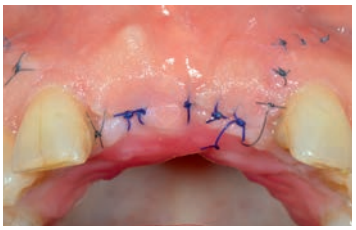


Extraction at T0: upper central incisor is extracted and a temporary prothesis is placed.

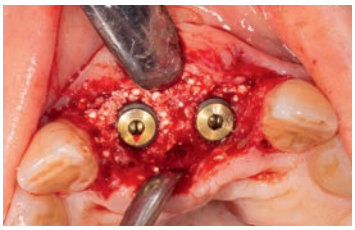




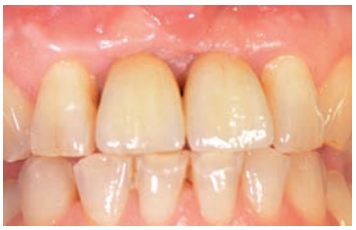
Guided Tissue Regeneration at 6 weeks: placement of the R.T.R.+ Membrane between the flap alveolar wall covering the bone substitute.



Clinical situation at day 10: no sign of inflammation.



Implant placement at 6 months in positions 11 (upper right 1) and 21 (upper left 1).

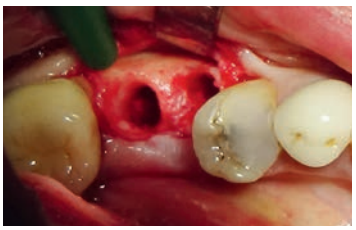


Clinical situation at 14 months with final restoration.

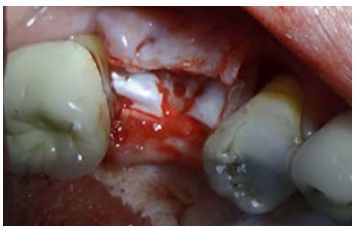
Socket preservation after soft tissue healing at 6 weeks

Dr. Hoornaert, Nantes, France

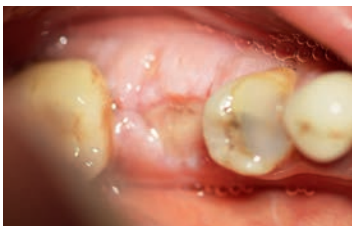
A 55-year-old patient presented with loss of dental crown (tooth 36 - lower left 6) with root still present.



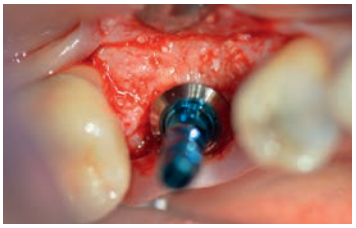
T0: root extraction and socket cleaning.



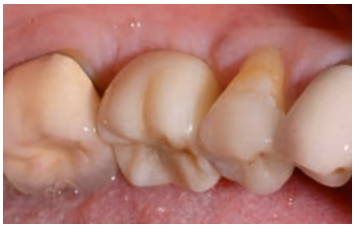
T0: socket preservation using R.T.R.+ Membrane.



T12: a thin layer of fibrin being epithelialised on the membrane.



Implant placement at 6 months.



Final restoration at 8 months.

Conclusion

R.T.R.+ Membrane is a resorbable bilayer synthetic dental membrane for guided tissue regeneration in periodontal and dental implant surgery.

R.T.R.+ Membrane provides high safety and performance, at least equivalent to collagen membranes.

R.T.R.+ Membrane has demonstrated:
- Effective barrier effect and bone graft maintenance;
- Favorable biocompatibility with bone and soft tissue;
- Clinically relevant resorption time;
- Easy handling;
- Guided tissue regeneration.

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Notes

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The Dental Pharmaceutical Company

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