Dual-layered non-resorbable PTFE membrane promotes a bi-directional molecular cross-talk with cells in both soft-tissue and bone defect compartments during GBR

Omar Omar,*1,2 Alberto Turri,1,2 Herman Sahlin,3 Margarita Trobos,1 Peter Thomsen,1 Christer Dahlén1,4
1Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Sweden.
2Department of Oral, Maxillofacial Surgery and Research and Development, NU Hospital Organisation, Trollhättan, Sweden.

Introduction
Recent data show that the membrane plays a biactive role during guided bone regeneration (GBR) (1, 2). However, most of the data been related to collagen membranes, whereas data on the barrier polytetrafluoroethylene (PTFE) is limited. Further, it is not known if different degrees of expansion of the PTFE membrane promote different molecular events compared to dense PTFE. The present study aimed to evaluate and compare the molecular events of GBR using: (i) solid, dense PTFE (d-PTFE) membrane; (ii) dual-textured expanded PTFE (e-PTFE) membrane, versus (iii) untreated sham defect. The study investigated the molecular cascade of inflammation and tissue regeneration in the membrane and in the soft tissue and bone compartments during GBR.

Materials and methods
Membranes: Two types of membranes were used:

- d-PTFE membrane: homopolymer dense PTFE (Cytoplan)™
- e-PTFE membrane: bi-layer membrane (NeoGraft™, Neoss Ltd., UK) with:
  - A top layer (toward soft tissue), bi-axially expanded with dissimilar expansion ratio (semi-closed structure and multidirectional fibers).
  - A bottom layer (toward bone defect), bi-axially expanded with similar expansion ratio (open structure and multidirectional fibers).

Surgical procedure: Trabecular bone defects were prepared in rat femora (A) and treated with either d-PTFE or dual-textured e-PTFE membranes (B) or left untreated as sham site. The sites were saturated (C). Samples were harvested after 6 and 28 days for gene expression analyses in the overlying soft tissue (above the membrane/defect), in the underlying defect, as well as in the membrane compartment itself.

At retrieval, samples were collected as following:
(a) Soft tissue above the membrane/defect using punch.
(b) The membranes by careful dissection & tweezers.
(c) The underlying bone defect using trephine.

The samples were analyzed with respect to the gene expression of inflammatory, regeneration & remodeling factors. Additional samples were retrieved for histology.

Results
Histological evaluations showed that both e-PTFE and d-PTFE promoted defect regeneration and restitution compared to sham.

Gene expression in the underlying bone defect
In the defect compartment, higher expression of pro-inflammatory cytokine (IL-6) was detected in the untreated sham sites compared with the membrane-treated sites (6 days). Otherwise, no major differences in bone formation related genes (e.g. OC), osteogenic growth factor (BMP-2) or remodeling genes (e.g. CTR) were found between the groups.

Gene expression in the overlying soft tissue
Several differences were detected in the overlying soft tissue. Whereas, e-PTFE promoted an early high expression of pro-inflammatory cytokine (TNF-α), both membranes were associated with lower inflammatory activity after 28 days compared to sham. Factors related to fibroblastic differentiation and soft tissue healing and vasculization (VEGF) were mainly promoted in the soft tissue above dual-layered e-PTFE but not the d-PTFE. Anti-fibrosis marker (FOXO-1) was up-regulated in the soft tissue interfacing with the e-PTFE.

Gene expression in the membrane compartment
When analyzing the membrane-associated cells, the dual-layered e-PTFE significantly up-regulated the expression of genes related to osteogenic and soft tissue healing and vasculization (BMP-2, Coll1a1, FGF-2 and VEGF), while down-regulating the expression of pro-inflammatory cytokines (TNF-α & IL-6). Both membranes induced an increased expression of genes related to osteogenic differentiation and bone formation (ALP & OC) between 6 and 28 days.

Correlation of gene expression

| Correlations between genes in the membrane and genes in the underlying bone defect | 15 positive & negative correlations |
| Positive include: BMP-2, TNF-α | BMP-2, TNF-α & IL-6 |
| Negative include: BMP-2 & IL-6 |

| Correlations between genes in the membrane and genes in the soft tissue | 2 correlations |
| Positive: IL-6 and TNF-α | BMP-2 and IL-6 |

| Correlations between genes in the membrane and genes in the bone defect | 2 correlations |
| Positive: BMP-2 & IL-6 | BMP-2 & TNF-α |

Conclusions
The present data provide molecular evidence that barrier non-resorbable PTFE membranes are actively involved in the regenerative processes during GBR. In contrast to untreated sites, the membrane-induced molecular regulations were more pronounced in the surrounding soft tissue. Moreover, both e-PTFE and d-PTFE became populated by cells that express different cytokines, osteoblastic and fibroblastic factors as well as potent growth factors involved in soft and hard tissue healing and regeneration. Dual-layered expanded PTFE promoted an attenuated inflammatory response and an enhanced molecular cascade for soft tissue healing compared with dense PTFE. Important cross-talks between the membrane-associated cells and the cells in both the soft tissue and bone compartments were revealed on the basis of correlation analyses. The enhanced molecular cascade for soft tissue healing in case of dual-layered e-PTFE may be beneficial for soft tissue integration and the clinical outcome during GBR.

References

Acknowledgments
The Osteone Foundation (project grant 15-103), the Swedish Research Council, the BIOMATCELL VINN Excellence Center of Biomaterials and Cell Therapy, the Region Västra Götaland, the Hjalmar Svensson Foundation, the Area of Advance Materials of Chalmers and GU Biomaterials within the Strategic Research Area initiative launched by the Swedish government. HS is employed at Neoss, Gothenburg, Sweden.