Permeability of *P. Gingivalis* or its metabolic products through collagen and dPTFE membranes and their effects on viability of osteoblast-like cells. An in vitro study.

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Background

Guided Bone Regeneration (GBR) is a well predictable and documented bone augmentation procedure.
This technique is based on the employment of a barrier membrane in order to exclude soft tissues from the defect and to allow angiogenic and osteogenic cells to gain bone regeneration. For this purpose both resorbable and non-resorbable membranes are available with proper characteristics and indications. Several past studies showed how flap primary closure above the membrane was a key prognostic factor for GBR procedures, as membrane exposure to oral environment frequently leads to a poor regenerative outcome[1]. Introduction of *dPTFE* (high-density PTFE) membranes, with less than 0,2µm porosity, can lead to a better withstanding of GBR techniques to membrane exposure due to their physical resistance to bacteria penetration. Furthermore, new protocols involving intentional membrane exposure have been introduced, especially for what concerning *Ridge Preservation techniques*[2,3,4].

Purpose

To assess permeability of three different GBR membranes to *P. Gingivalis* or its toxic metabolic products that can affect osteoblast-like cells viability and differentiation capability.

Materials & Methods

Membranes employed in this study were:

- **dPTFE membrane** (Cytoplast™ TXT-200§);
- **type I collagen membrane** (Cytoplast™ RTM Collagen Membrane§);
- **natural porcine pericardium collagen membrane** (Vitala® Collagen Membrane§).

A system leading to filtration of a solution (SOLUTION 1) added with *P. Gingivalis* through the experimental membranes was arranged to assess permeability to bacteria after 24 and 72 hours (*Fig. 1*) by means of cultivation tests on agar plates (*Fig. 2*).
Filtered solution (SOLUTION 2) was then added (30% diluted) to osteoblast-like cells (osteoblasts U2OS) cultures which underwent, after 10 days of incubation, MTT and red alizarin essays looking for toxic effects on viability and differentiation capability.
Membranes were also analysed through Scanning Electron Microscope (SEM) before and after being exposed to *P. Gingivalis*.

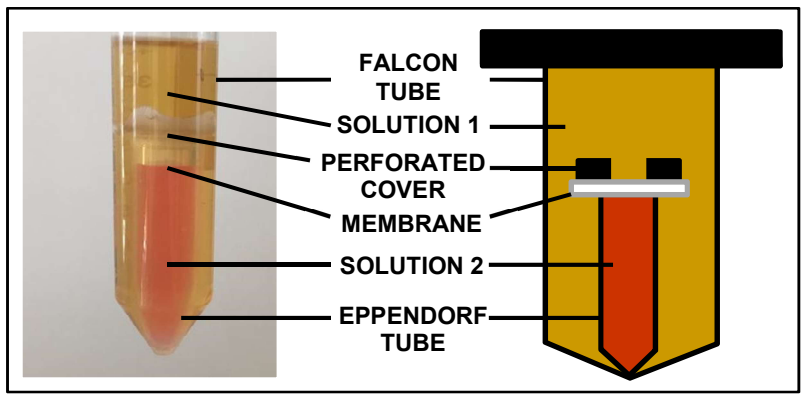

Fig. 1 – Experimental Apparatus

Results

dPTFE membranes showed resistance to bacteria penetration, while both type collagen membranes were crossed by *P. Gingivalis* after 24 hours (*Tab. 1*).
SOLUTION 2, filtered through dPTFE membrane, didn't show any toxicity on U2OS cells as no difference can be observed between test and control groups (p>0,05) (*Fig. 3*).SEM analysis showed bacterial colonization, but no difference on dPTFE surface, while collagen membranes were seriously damaged by *P. Gingivalis* (*Fig. 4*).

Discussion

This study confimed dPTFE resistance to bacterial crossing, as previously assessed by Trobos et al. (2018)[5]. Furthermore, second phase of this study proved no crossing of toxic products of bacterial metabolism for osteoblasts viability, even if indirect toxicity is a possibility that requires further studies.
Both collagen membranes showed no resistance to bacterial penetration. This data agrees with other studies in literature[6].

Table 1

MEMBRANE	*P. Gingivalis**	INCUBATION TIME	*P. Gingivalis* INSIDE SOLUTION 1	*P. Gingivalis* INSIDE SOLUTION 2
CONTAMINATION CONTROL 1	-**	2×10^5 CFU	72 H	+	-
CONTAMINATION CONTROL 2	-**	1×10^6 CFU	72 H	+	-
TXT-200 1	TXT-200	2×10^5 CFU	24 H	+	-
TXT-200 2	TXT-200	2×10^5 CFU	24 H	+	-
TXT-200 3	TXT-200	1×10^6 CFU	24 H	+	-
TXT-200 4	TXT-200	1×10^6 CFU	24 H	+	-
TXT-200 5	TXT-200	2×10^5 CFU	72 H	+	-
TXT-200 6	TXT-200	1×10^6 CFU	72 H	+	-
TXT-200 CONTROL 1	TXT-200	-	24 H	-	-
TXT-200 CONTROL 2	TXT-200	-	72 H	-	-
VITALA 1	VITALA	2×10^5 CFU	24 H	+	+
VITALA 2	VITALA	2×10^5 CFU	24 H	+	+
VITALA 3	VITALA	2×10^5 CFU	72 H	+	+
VITALA 4	VITALA	2×10^5 CFU	72 H	+	+
VITALA CONTROL 1	VITALA	-	24 H	-	-
VITALA CONTROL 2	VITALA	-	72 H	-	-
RTM 1	RTM COLLAGEN	2×10^5 CFU	24 H	+	+
RTM 2	RTM COLLAGEN	2×10^5 CFU	24 H	+	+
RTM 3	RTM COLLAGEN	2×10^5 CFU	72 H	+	+
RTM 4	RTM COLLAGEN	2×10^5 CFU	72 H	+	+
RTM CONTROL 1	RTM COLLAGEN	-	24 H	-	-
RTM CONTROL 2	RTM COLLAGEN	-	72 H	-	-

*number of CFU in 35mL; **intact Eppendorf cover

Tab. 1 – Permeability Experiments

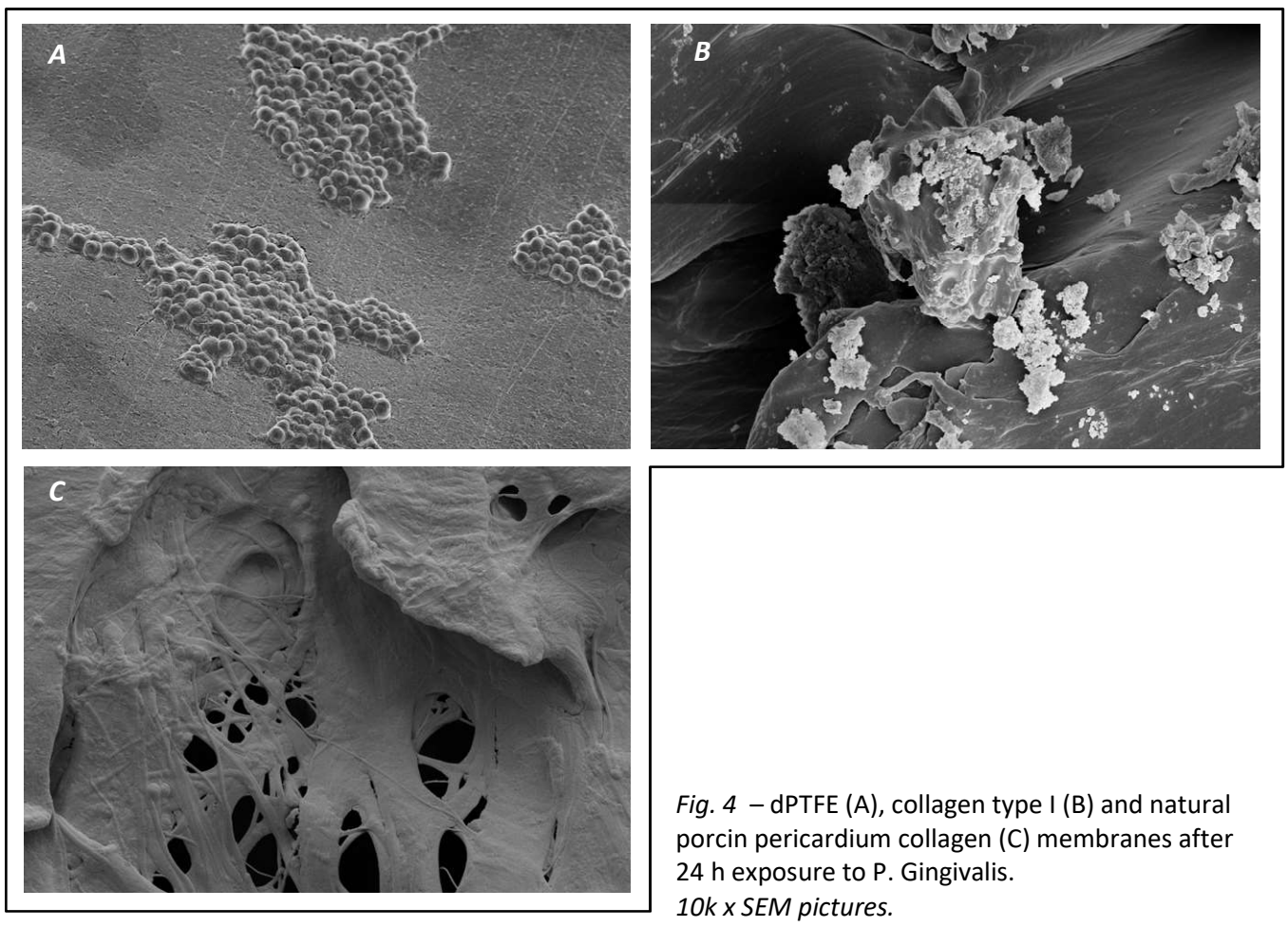

Fig. 4 – dPTFE (A), collagen type I (B) and natural porcin pericardium collagen (C) membranes after 24 h exposure to P. Gingivalis.
10k x SEM pictures.

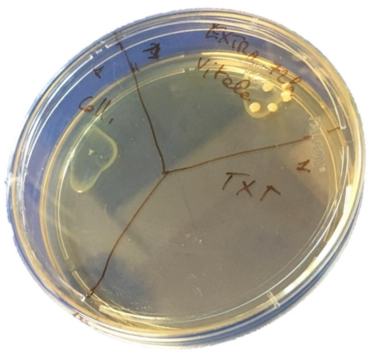

Fig. 2 – P. Gingivalis cultures on agar plate

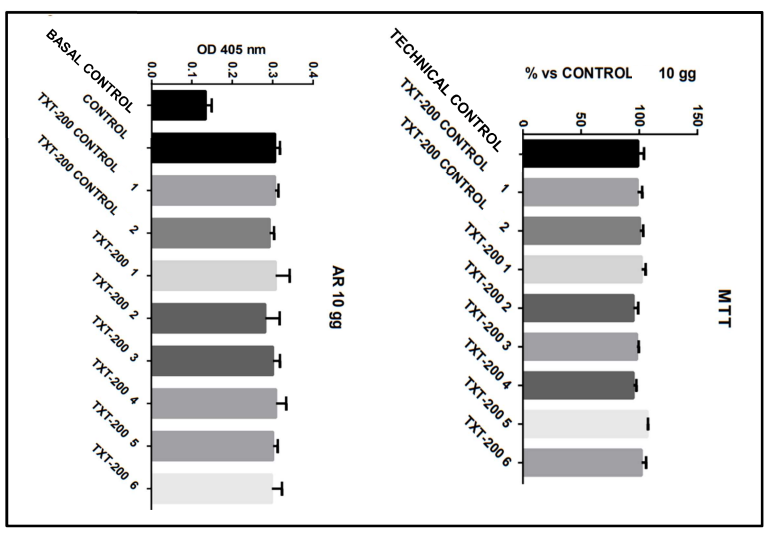

Fig. 3 – MTT and Alizarin Red essays

1. Lim, G, Lin GH, Monje A, Chan HL, Wang HL. Wound Healing Complications Following Guided Bone Regeneration for Ridge Augmentation: A Systematic Review and Meta-Analysis. JOMI 2018; 33(1), 51–50. **2**. Barber HD, Lignelli J, Smith BM, Bartee BK. Using a dense PTFE membrane without primary closure to achieve bone and tissue regeneration. J Oral Maxillofac Surg 2007;65(4):748-52. **3.** Hoffmann O, Bartee BK, Beaumont C, Kasaj A, Deli G, Zafiropoulos GG. Alveolar bone preservation in extraction sockets using non-resorbable dPTFE membranes: a retrospective non-randomized study. J Periodontol 2008 Aug;79(8):1355-69. **4.** Troiano G, Zhurakivska K, Lo Muzio L, Laino L, Cicciù M, Lo Russo L. Combination of Bone Graft and Resorbable Membrane for Alveolar Ridge Preservation: a Systematic Review, Meta-analysis and Trial Sequential Analysis. J Periodontol 2018;89(1):46-57. **5.** Trobos M, Juhlin A, Shah FA, Hoffman M, Sahlin H, Dahlin C. In vitro evaluation of barrier function against oral bacteria of dense and expanded polytetrafluoroethylene (PTFE) membranes for guided bone regeneration. Clin Implant Dent Relat Res 2018;1–11. **6.** Rani S, Chandra RV, Reddy AA, Reddy BH, Nagarajan S, Naveen A. Evaluation of the Antibacterial Effect of Silver Nanoparticles on Guided Tissue Regeneration Membrane Colonization--An in Vitro Studv. J Int Acad Periodontol 2015:17(3):66-76.

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