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In vitro-colonization of Sulfat-Reducing Bacteria (SRB) on resorbable membranes for periodontal regeneration

Quantitative SEM-evaluation

Language: English

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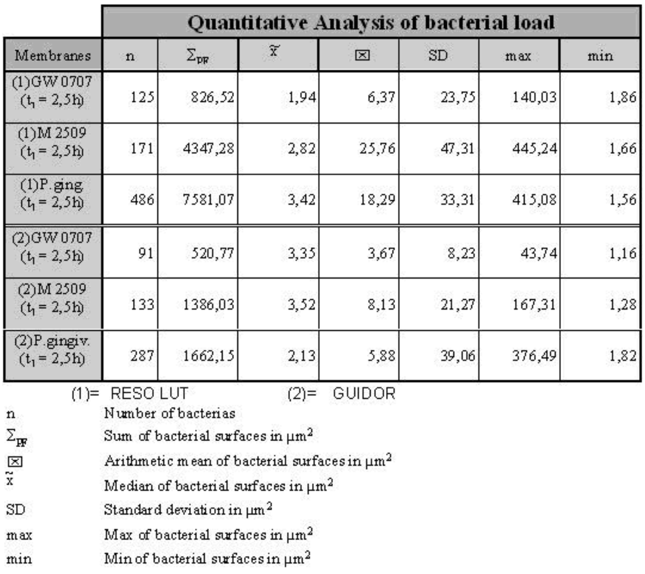
Introduction

A role in the pathogenesis of periodontal disease is played by P. gingivalis and Sulfat-reducing bacteria (SRB). The term sulfate-reducing bacteria (SRB) describes strictly anaerobic microbes that accomplish the dissimilatory reduction of sulfate to hydrogen sulfide. The 16S rDNA sequence showed a high similarity of 99.7% with the 16S rDNA of the proposed species 'Desulfovibrio fairfieldensis'.

Objectives

The aim of this study was to examine in an in vitro assay the colonization of 2 types of resorbable membranes for guided tissue regeneration by strains of SRB and P .gingivalis.



	Quantitative Analysis of bacterial load						
Membranes	n	Σ_{PF}	$\tilde{x}$	☒	SD	max	min
(1)GW 0707 (t_1 = 2,5h)	125	826,52	1,94	6,37	23,75	140,03	1,86
(1)M 2509 (t_1 = 2,5h)	171	4347,28	2,82	25,76	47,31	445,24	1,66
(1)P.ging (t_1 = 2,5h)	486	7581,07	3,42	18,29	33,31	415,08	1,56
(2)GW 0707 (t_1 = 2,5h)	91	520,77	3,35	3,67	8,23	43,74	1,16
(2)M 2509 (t_1 = 2,5h)	133	1386,03	3,52	8,13	21,27	167,31	1,28
(2)P.gingiv. (t_1 = 2,5h)	287	1662,15	2,13	5,88	39,06	376,49	1,82

(1)= RESO LUT (2)= GUIDOR

n	Number of bacterias
Σ_{PF}	Sum of bacterial surfaces in μm^2
☒	Arithmetic mean of bacterial surfaces in μm^2
$\tilde{x}$	Median of bacterial surfaces in μm^2
SD	Standard deviation in μm^2
max	Max of bacterial surfaces in μm^2
min	Min of bacterial surfaces in μm^2

Table 1: Quantitative SEM analyzes of bacterial load on resorbable membranes

In vitro assay

Strains of SRB were isolated from periodontal pockets using enrichment techniques. The strains were preliminary characterized by phylogenetic analysis of 16 S rDNA sequences. Pieces of membrane were submerged in batchcultures of Desulfovibrio spp. and P. gingivalis FDC381 in reduced growth medium specific for SRB or PY medium, and incubated for 1-8 days lagtime. The membranes used were polylactic acid (GUIDOR) and glycolide and lactide copolymer (RESOLUT). The dry weight of membranes was determined after 2, 5 hours and 3, 6, 12, and 24 weeks.

Material and Methods

Measurement of bacterial using the software system Scion Image for Windows

After incubation, membranes were prepared for SEM analysis. Bacterial density was measured quantitatively at 500x magnification for selecting region of interests (ROI). Five 20x25µm fields were randomly selected for each specimen using the software system Scion Image for Windows. In "Threshold Mode" all pixels are equal or greater than a single threshold level. In "Density Slice Mode" all pixels between a lower and upper threshold are highlighted in red.

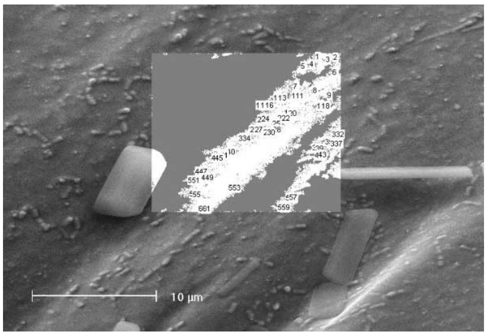


Fig. 4:
Quantitative SEM analyzes of resorbable membranes using SCION Images for **Windows-Analyze particles**

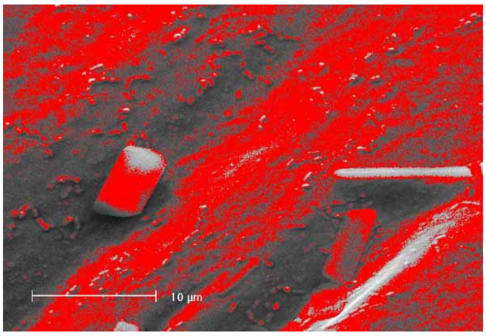


Fig. 5:
Quantitative SEM analyzes of resorbable membranes using SCION Images for **Windows-Density slice mode**

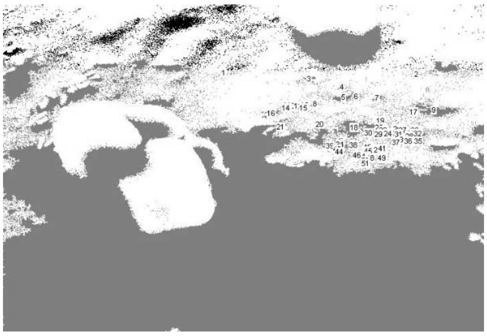


Fig. 6:
Quantitative SEM analyzes of resorbable membranes using SCION Images for **Windows-Threshold Mode**

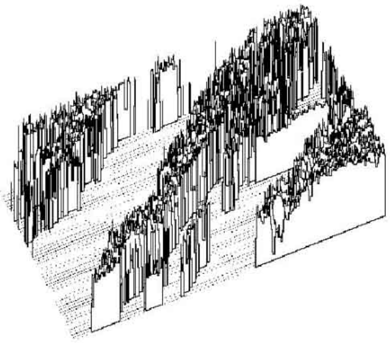


Fig. 7:
Quantitative SEM analyzes of resorbable membranes using SCION Images for **Windows-Threshold Mode-Density Plot Profile**

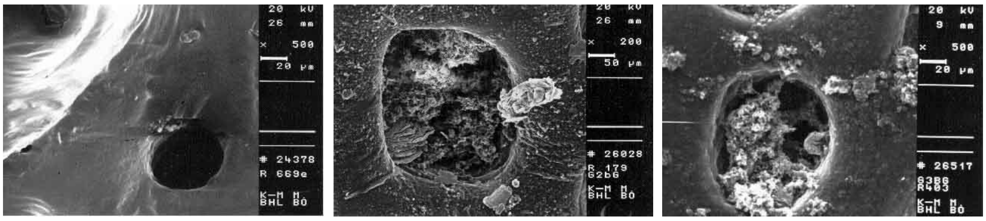


Fig. 8a-c:
Biofilm formation on resorbable membranes (SEM analzes): **Mixed Culture (SRB)**

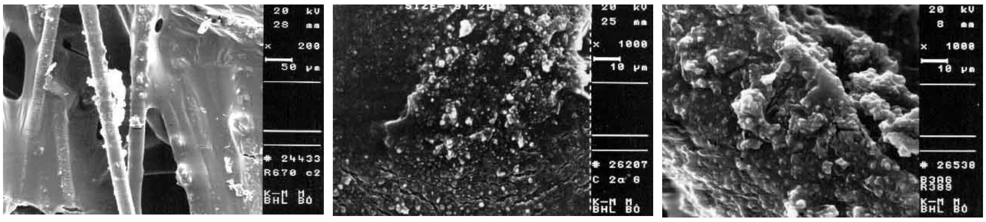


Fig. 8d-f:
Biofilm formation on resorbable membranes (SEM analzes): **GW0706**

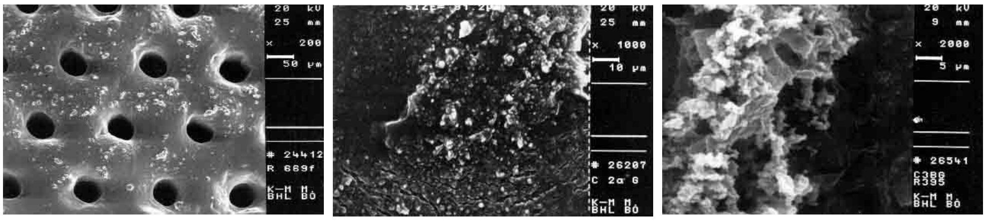


Fig. 8g-i:
Biofilm formation on resorbable membranes (SEM analzes): **M2509**

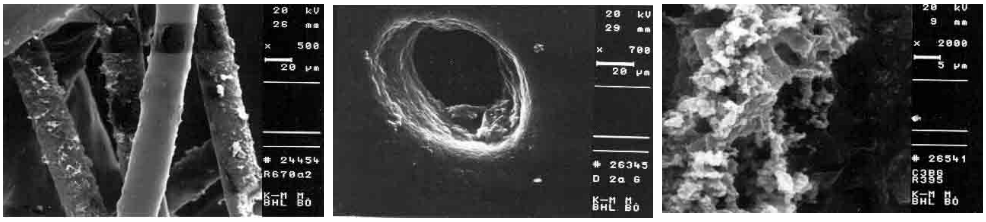


Fig. 8j-l:
Biofilm formation on resorbable membranes (SEM analzes): **P. gingivalis**

Ultramorphological analysis of the surfaces was carried out using a Philips scanning electron microscope (Philips XL 30 FEG) at 20 kV. As detector the secondary electron detector was used. Prior to SEM investigation the membranes were washed with water, fixed in glutaraldehyde, dehydrated in ethanol, critical-point dried sputtered with gold-paladium using a Bal-Tec Scd 050 sputter to achieve a better resolution.

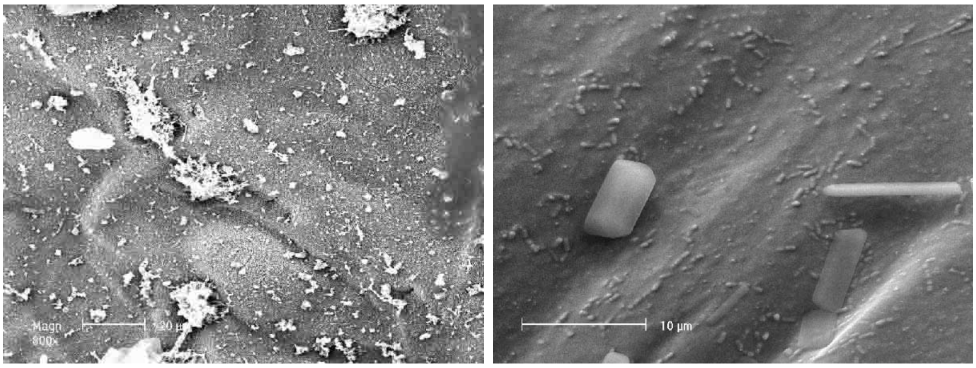


Fig. 1, 2:
SEM analyzes of resorbable membranes

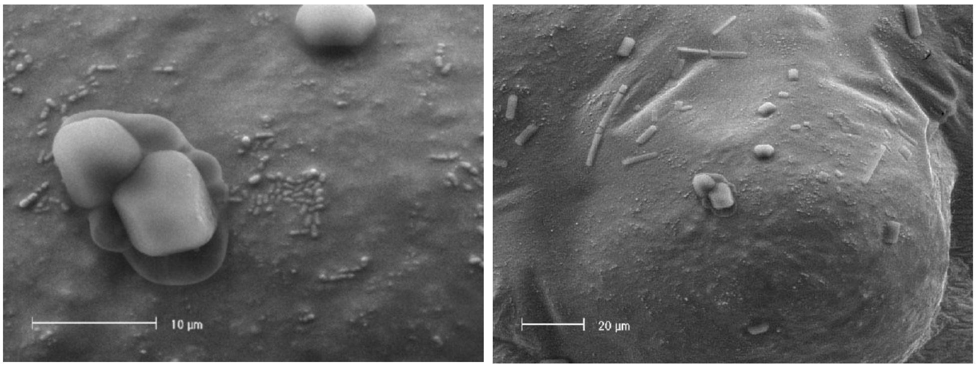
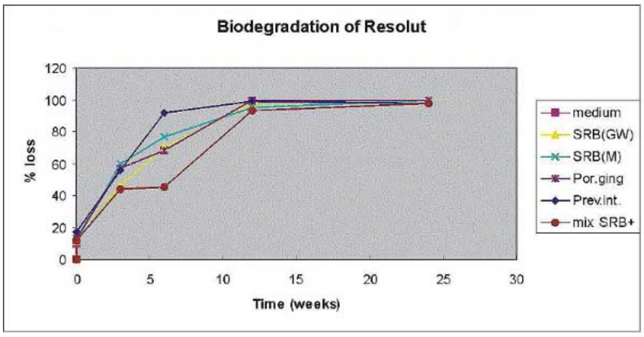


Fig. 3:
SEM analyzes of resorbable membranes

Biodegradation of resorbable membranes

After incubation the hydrolysis of Gore Resolut membranes was much faster than that of Guidor membranes. After 3 weeks the Gore membranes had largely dissolved, while Guidor membranes were still intact. After 6 weeks of incubation Gore Resolut membranes were degraded for the larger part, Guidor membranes remained their mass twice as long, only after 12 weeks their mass had degraded to this extend.



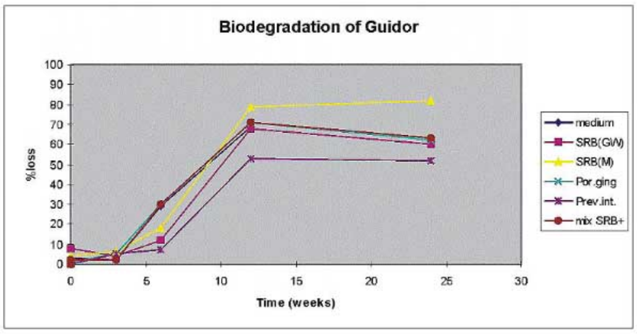


Fig. 9a,b:
Biodegradation of resorbable mebranes

Table 2: Biodegradation of resorbable mebranes

	t=0	2.5h, 0.01w		3 weeks		6 weeks		12 weeks		24 weeks	
		rel. decrease		rel. decrease		rel. decrease		rel. decrease		rel. decrease	
		Resolut	Giudor	Resolut	Giudor	Resolut	Giudor	Resolut	Giudor	Resolut	Giudor
medium	100	10	3	nd	2	68	29	100	69		
GW0706	100	10	8	46	4	70	12	98	68	100	60
M2509	100	12	4	60	6	77	18	95	79	100	82
P.g. 91	100	13	1	57	5	68	30	100	71	100	62
P.i. 655	100	17	0	56	5	92	7	99	53	98	52
mix SRB+	100	12	2	44	2	45	30	93	71	98	63

Results

SEM analysis revealed differences in the accumulation of SRB strains and P. gingivalis on the two investigated membranes using quantitative evaluation of bacterial density. The bacterial cells adhering to the membranes surface were depending on the degree of membranes mass loss over the time interval. SEM analysis: SEM analysis revealed differences in the accumulation of SRB strains and P. gingivalis on the 2 types of investigated membranes. The deep SRB invasion at the 3d week and frequent presence of internal bacteria at the 6th week on the PLA with copolymer membranes underlined the difference obtained quantitative evaluation of bacterial density. The difference between the bacterial layers was statistically significant (Kruskal-Wallis-Test, p=0.05).

Conclusions

- The present investigation examined the relationship between the degrees of PL membrane degradation and the amount of SRB colonization observed after up to 24 weeks undisturbed growth.
- The bacterial cells adhering to the membranes surface was depended on the degree of membranes mass loss over the time interval.
- Interestingly, the results suggest an active role of SRB the degradation of the resorbable PLA membranes.

This Poster was submitted by Univ.-Prof. Dr. med. dent. habil. Wolf-Dieter Grimm.

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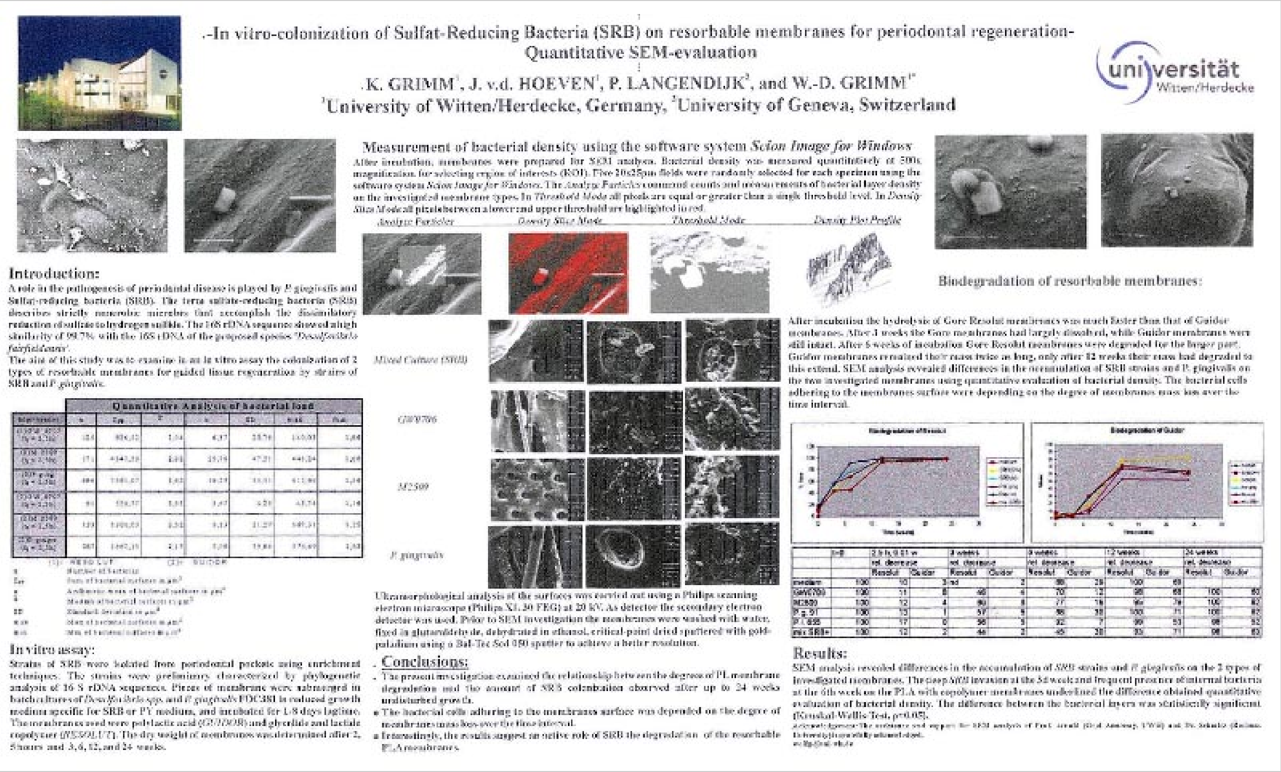
Poster Faksimile:



.-In vitro-colonization of Sulfat-Reducing Bacteria (SRB) on resorbable membranes for periodontal regeneration- Quantitative SEM-evaluation

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[1]University of Witten/Herdecke, Germany, [2]University of Geneva, Switzerland

Introduction:

A role in the pathogenesis of periodontal disease is played by *P. gingivalis* and Sulfat-reducing bacteria (SRB). The term sulfate-reducing bacteria (SRB) describes strictly anaerobic microbes that accomplish the dissimilatory reduction of sulfate to hydrogen sulfide. The 16S rDNA sequence showed a high similarity of 99.7% with the 16S rDNA of the proposed species '*Desulfovibrio fairfieldensis*'.
The aim of this study was to examine in an in vitro assay the colonization of 2 types of resorbable membranes for guided tissue regeneration by strains of SRB and *P. gingivalis*.

Membrane	Quantitative Analysis of bacterial load n	Σμ	x̄	x̃	SD	max	min
(1) GWV [illegible] (g = 2.36)	124	806,12	1,04	4,37	23,76	160,00	1,64
(1) M 2509 (g = 2.96)	171	4347,38	2,01	25,79	47,21	441,24	1,68
(1) P. ging. (g = 2.26)	404	7101,27	1,62	16,23	33,51	412,90	1,14
(2) GWV [illegible] (g = 2.26)	80	326,37	3,81	3,47	4,20	43,74	1,14
(2) M 2509 (g = 1.96)	123	1000,53	2,32	8,13	21,27	149,31	1,15
(2) P. ging. (g = 2.26)	287	1667,15	2,15	5,08	19,86	175,67	1,53

(1): RESOLUT (2): GUIDOR

n	Number of bacteria
Σμ	Sum of bacterial surfaces in μm²
x̄	Arithmetic mean of bacterial surfaces in μm²
x̃	Median of bacterial surfaces in μm²
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In vitro assay:

Strains of SRB were isolated from periodontal pockets using enrichment techniques. The strains were preliminary characterized by phylogenetic analysis of 16 S rDNA sequences. Pieces of membrane were submerged in batch cultures of *Desulfovibrio spp.* and *P. gingivalis* FDC381 in reduced growth medium specific for SRB or PY medium, and incubated for 1-8 days lagtime. The membranes used were polylactic acid (*GUIDOR*) and glycolide and lactide copolymer (*RESOLUT*). The dry weight of membranes was determined after 2, 5 hours and 3, 6, 12, and 24 weeks.

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After incubation, membranes were prepared for SEM analyses. Bacterial density was measured quantitatively at 500x magnification for selecting region of interests (ROI). Five 20x25μm fields were randomly selected for each specimen using the software system *Scion Image for Windows*. The *Analyze Particles* command counts and measurements of bacterial layer density on the investigated membrane types. In *Threshold Mode* all pixels are equal or greater than a single threshold level. In *Density Slice Mode* all pixels between a lower and upper threshold are highlighted in red.

Ultramorphological analysis of the surfaces was carried out using a Philips scanning electron microscope (Philips XL 30 FEG) at 20 kV. As detector the secondary electron detector was used. Prior to SEM investigation the membranes were washed with water, fixed in glutaraldehyde, dehydrated in ethanol, critical-point dried sputtered with gold-paladium using a Bal-Tec Scd 050 sputter to achieve a better resolution.

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	t=0	2 h, 0.01 w		3 weeks		6 weeks		12 weeks		24 weeks	
		rel. decrease		rel. decrease		rel. decrease		rel. decrease		rel. decrease	
		Resolut	Guidor	Resolut	Guidor	Resolut	Guidor	Resolut	Guidor	Resolut	Guidor
medium	100	10	3	nd	2	98	28	100	68		
GWV700	100	11	8	48	4	70	12	98	68	100	80
M2509	100	12	4	90	8	77	16	96	78	100	82
P. g. 91	100	13	1	57	9	98	38	100	71	100	82
P. i. 856	100	17	0	98	5	82	7	99	53	98	52
mix SRB+	100	12	2	46	2	45	28	93	71	98	80

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Acknowledgement: The assistance and support for SEM analysis of Prof. Arnold (Oral Anatomy, UWH) and Dr. Schultz ([illegible]) is [illegible]
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