EVALUATION OF BACTERIAL CONTAMINATION IN RESORBABLE COLLAGEN MEMBRANES AND DECONTAMINATION POTENTIAL OF ANTIMICROBIAL PHOTODYNAMIC THERAPY – *IN VITRO* STUDY

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INTRODUCTION

One of the most common complications in guided tissue regeneration (GTR) procedures is premature membrane exposure and contamination[1,2-4]. Once the membrane is in contact with the oral cavity, the contamination can reduce tissue gain and compromise regeneration treatment outcomes. Studies have shown that the exposure of collagen membranes in GTR procedures is high (up to 50%)[5] and can decrease bone formation (up to 81% reduction)[5]. Considering this context, searching for ways to decontaminate the membranes after exposure in the oral cavity may be an interesting alternative to avoid undesirable clinical results. Thus, the aim of this study was to analyze, *in vitro*, microbial contamination on different resorbable collagen membranes and their potential for decontamination by antimicrobial photodynamic therapy.

METHODS & MATERIAL

This study was approved by the Research Ethics Committee of the Inga University Center (protocol n. 5.108.632). Sample size calculation[6] determined the need for 4 collagen membranes in each experimental group.

Collagen Membranes

- Lumina-Coat® (LC) (Critéria Biomateriais – São Carlos/Brazil)
- Jason® (J) (Straumann – Basileia/Switzerland)
- Bio-Gide® (BG) (Geistlich Biomaterials – Wolhusen/Switzerland)

Thirty-six samples (5x5mm) were obtained from membranes and randomly distributed in the following experimental groups:
- Group S - control (n=12): sterile membranes.
- Group C (n=12): membranes contaminated with oral biofilm.
- Group aPDT (n=12): membranes contaminated with oral biofilm and treated with antimicrobial photodynamic therapy.

Oral biofilm collection and contamination of samples

A healthy 41-year-old male volunteer donated the oral biofilm sample collected with a North Carolina periodontal probe from tooth surfaces near gingival margin. The samples from groups C and aPDT were contaminated *in vitro*. The samples from group S were placed in test tubes containing a sterile culture medium for sterility validation and control (Fig. 1).

Fig. 1 Test tubes with membranes immersed for 7 days, in an incubator at 37°C. Groups C and aPDT (culture medium with oral biofilm); Group S (sterile culture medium).

Decontamination

After 7 days, Group aPDT was decontaminated with antimicrobial photodynamic therapy (Fig. 2, Table 1).
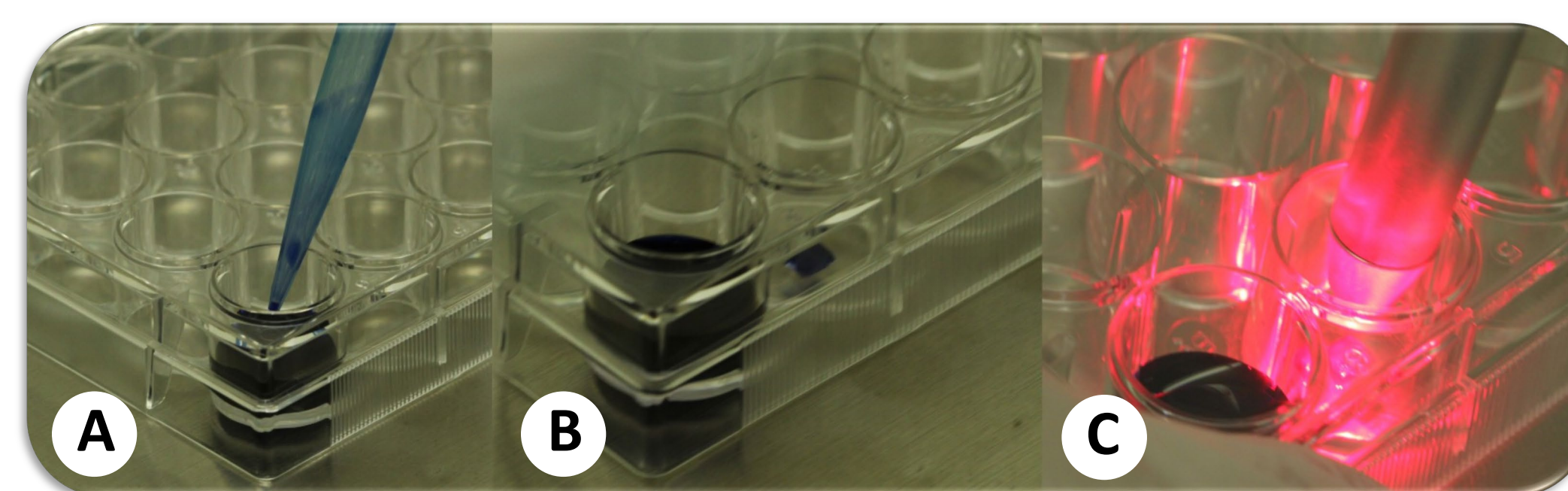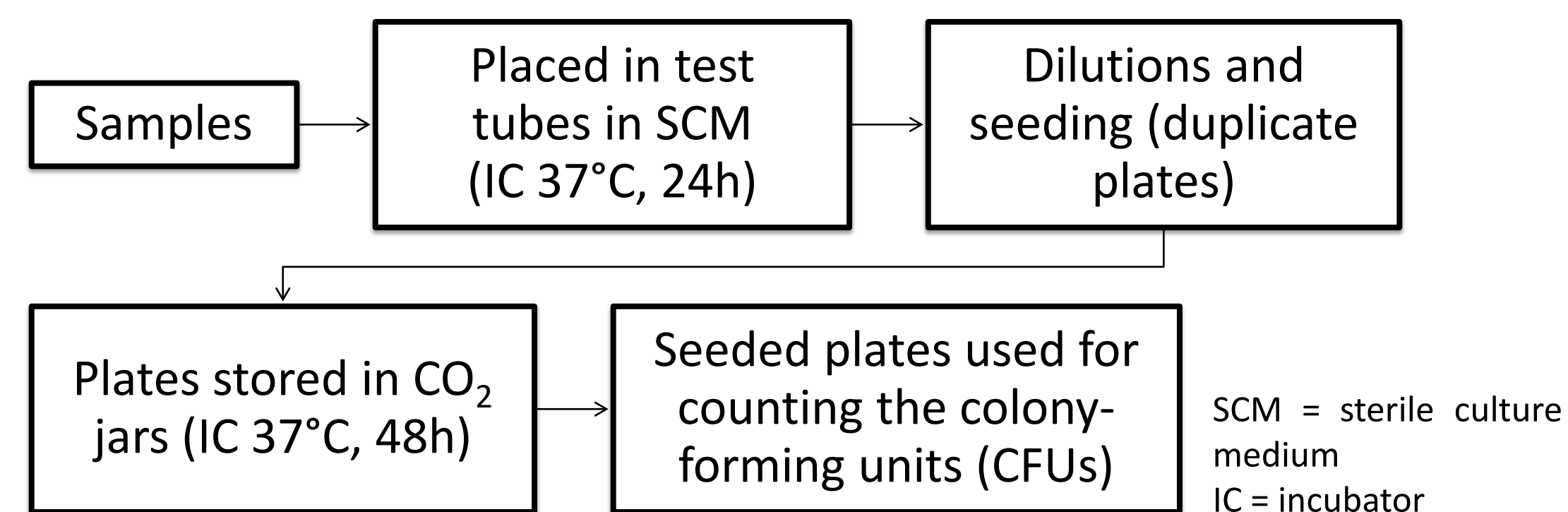

Fig. 2 Group aPDT decontamination. **A)** Pre-irradiation. Sample immersed in 1 mL of toluidine blue O (100 µg/mL), for 1 minute. **B)** Sample placed in a clean well after pre-irradiation period for laser irradiation. **C)** Sample irradiated with Duo red laser (MMO - São Carlos/Brazil).

Table. 1 Laser parameters

Laser	Diode - InGaAlP
Emission mode	Scanning
Wavelength (nm)	660
Power (mW)	100
Time (s)	90
Energy density (J/cm²)	300
Tip area (mm²)	3
Energy (J)	9

Microbiological and Statistical Analysis

Samples → Placed in test tubes in SCM (IC 37°C, 24h) → Dilutions and seeding (duplicate plates) → Plates stored in CO_2 jars (IC 37°C, 48h) → Seeded plates used for counting the colony-forming units (CFUs)

SCM = sterile culture medium
IC = incubator

One-way ANOVA and Tukey's post-test were used to intergroup comparison and repeated measures ANOVA and Tukey's post-test for intragroup comparison. Data were considered significant for $p<0.05$.

RESULTS

Table. 2 Mean values and standard deviations (sd) of the intergroup comparison regarding microbial contamination (groups C) and decontamination with antimicrobial photodynamic therapy (groups aPDT)

Membranes	LC	J	BG	p
Groups	Mean (CFUs)±sd	Mean (CFUs)±sd	Mean (CFUs)±sd	
C	$91.63 \times 10^8 \pm 82.02^a$	$71.63 \times 10^8 \pm 72.87^a$	$111.00 \times 10^8 \pm 61.21^a$	0.564
aPDT	$0.38 \times 10^8 \pm 0.74^a$	$11.25 \times 10^8 \pm 9.30^b$	$10.69 \times 10^8 \pm 11.81^b$	0.034*

*Statistically significant for $p<0.05$. Different letters in the same line mean significant statistical difference

Table. 3 Mean values and standard deviations (sd) of the intragroup comparison in the three types of membrane

Groups	S	C	aPDT	p
Membranes	Mean (CFUs)±sd	Mean (CFUs)±sd	Mean (CFUs)±sd	
LC	$0.00 \times 10^8 \pm 0.00^a$	$91.63 \times 10^8 \pm 82.02^b$	$0.38 \times 10^8 \pm 0.74^a$	0.000*
J	$0.00 \times 10^8 \pm 0.00^a$	$71.63 \times 10^8 \pm 72.87^b$	$11.25 \times 10^8 \pm 9.30^a$	0.006*
BG	$0.00 \times 10^8 \pm 0.00^a$	$111.00 \times 10^8 \pm 61.21^b$	$10.69 \times 10^8 \pm 11.81^a$	0.000*

*Statistically significant for $p<0.05$. Different letters in the same line mean significant statistical difference

CONCLUSION

The resorbable collagen membranes were similarly contaminated in quantity and antimicrobial photodynamic therapy was able to effectively decontaminate the three types of membranes.

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