



Research project

# **Effect of Photodynamic Therapy using B-CURE® laser on an *in vitro* model of dental plaque**

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## **Aims**

To test the disinfection properties of B-CURE® laser as a solo treatment, or as adjunctive with bio-synthesizer Indocyanin green, in a model of pathogenic biofilm.

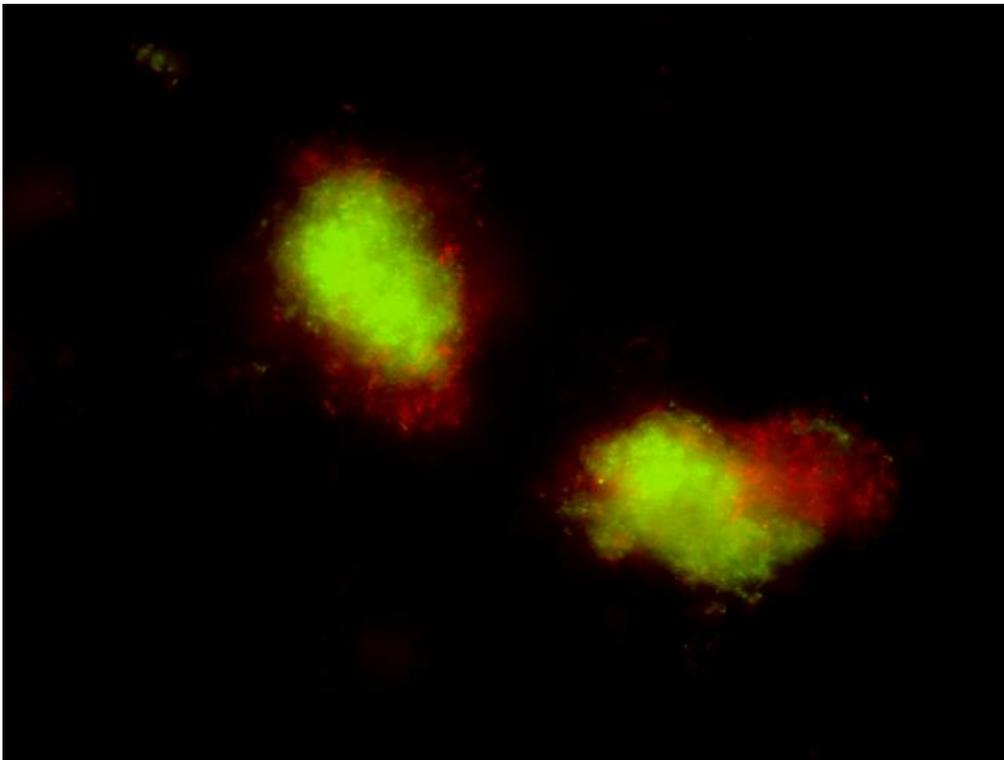
## Materials and methods

In an *in vitro* model, pathogenic biofilm was established using non-pathogenic bacteria – *Streptococcus sanguis* and *Actinomyces naslundii* and pathogenic bacteria – *Porphyromonas gingivalis* and *Fusobacterium nucleatum* on tooth like surfaces (hydroxyapatite discs).

The infected discs were exposed to B-CURE® laser with or without pretreatment with Indocyanin green. Control groups will include not treated biofilm and sham treatment (blank laser machine).

All groups underwent staining for live and dead bacteria using florescent dyes (Figure 1) and analysis was done using a florescent microscopy.

**Figure 1** – pathogenic biofilm staining.  
Green – viable bacteria; red – non-vital bacteria

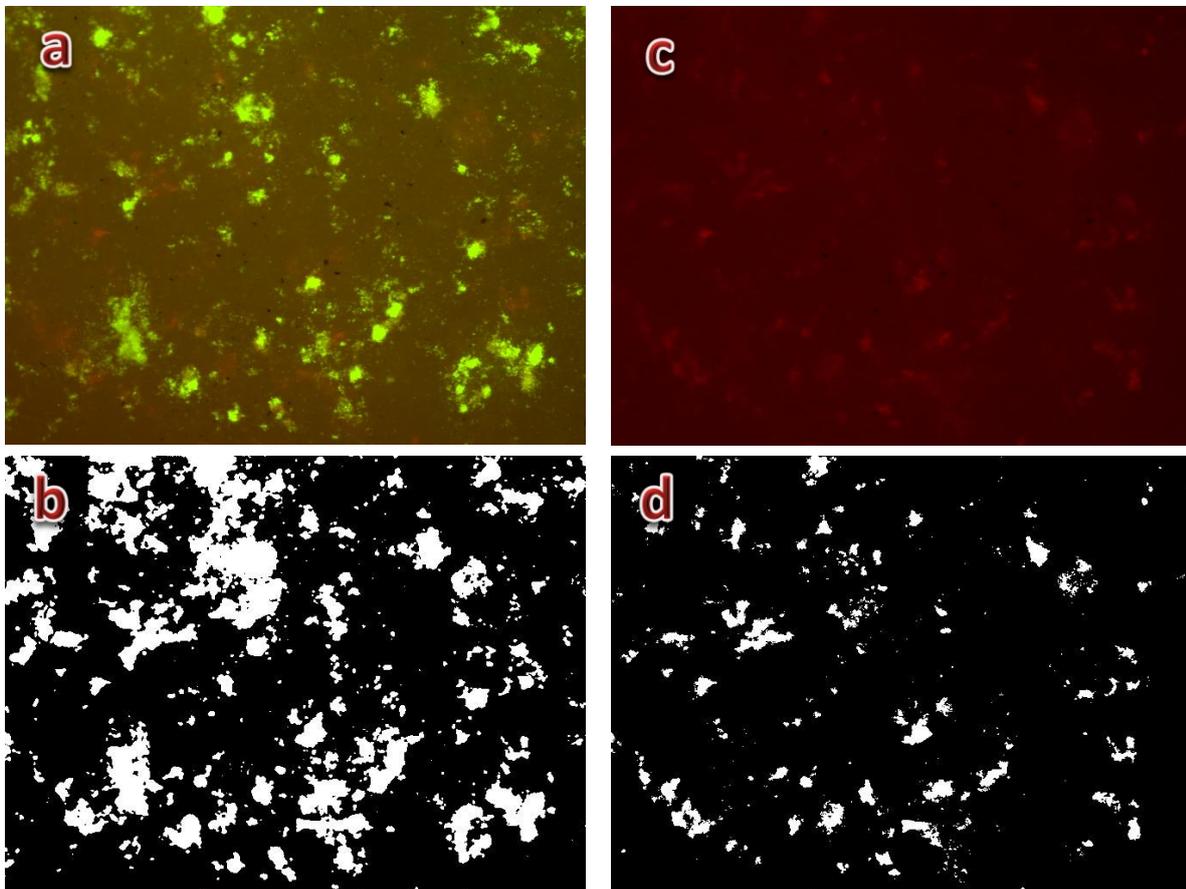


Images were taken at 4 different areas from each group. The images included red & green staining (total biofilm, Figure 2a), and only red staining (dead bacteria, Figure 2c). The images were analyzed using imageJ software (Figures 2b & 2d). Quantitative results are expressed as the dead biofilm area.

All experiments were repeated 3 times.

**Figure 2** – analysis methodology.

**a** - microscope image of viable and non-viable bacteria; **b** – conversion of color image to black and white for quantification of total area of biofilm; **c** – microscope image of non-vital bacteria; **d** - conversion of color image to black and white for quantification of area of dead biofilm



## **Results**

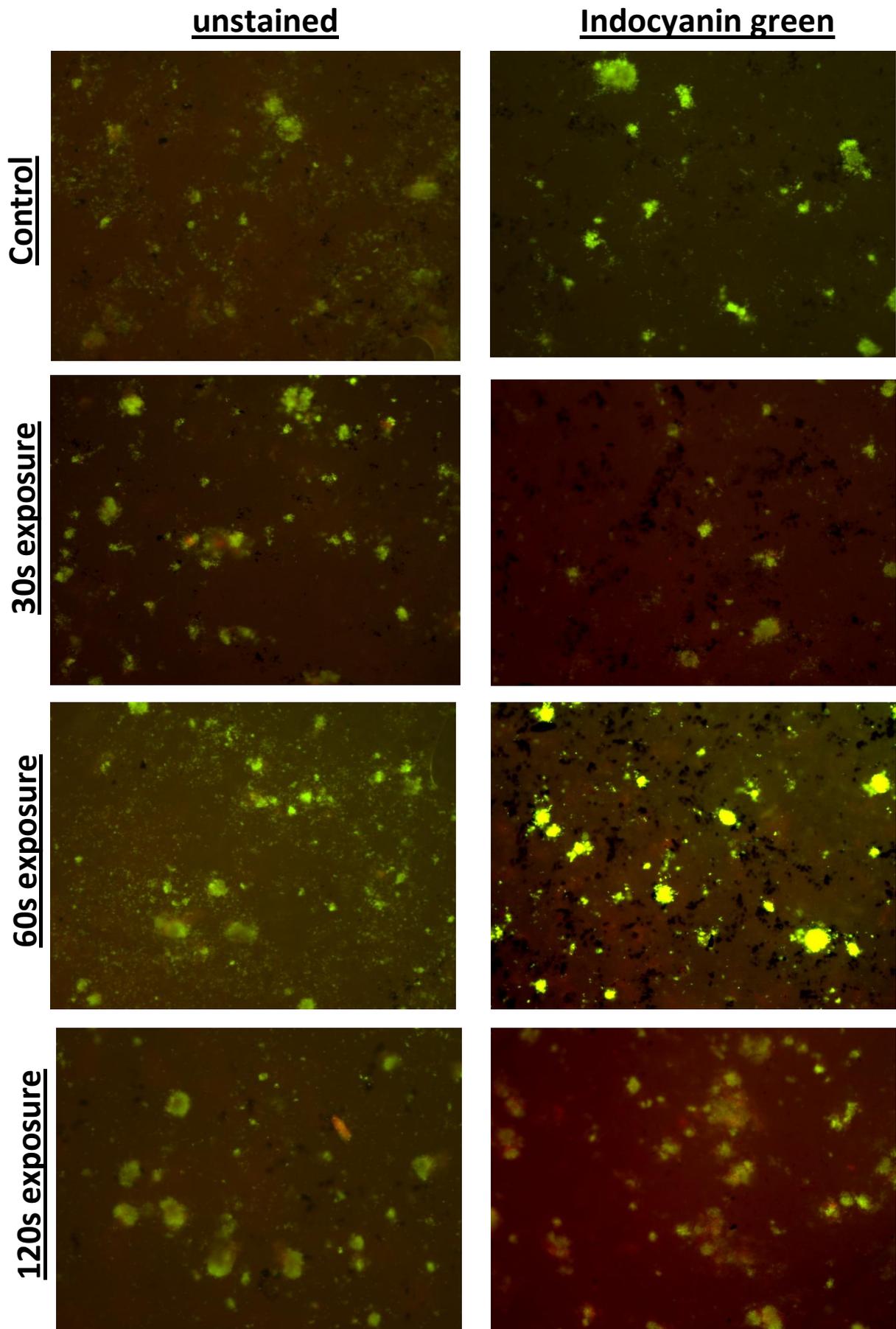
### **1. Part A– Effect of B-cure laser with and without Indocyanin green – kinetics:**

We screened different exposure time intervals of exposure to the B-Cure laser, either alone or in conjunction with indocyanin green pre-treatment. This was done in order to single out the most effective time point for further experiments.

**1a. - Short-term Kinetics:** Time points included 30 seconds, 60 seconds and 120 seconds. Control group was not exposed to B-cure laser at all.

Results of this experiment revealed that the optimum exposure time for visible effect is 120 sec (Figure 3). Also, the ICS group at 120 sec exposure showed significant elevation in bacterial death compared with the other groups.

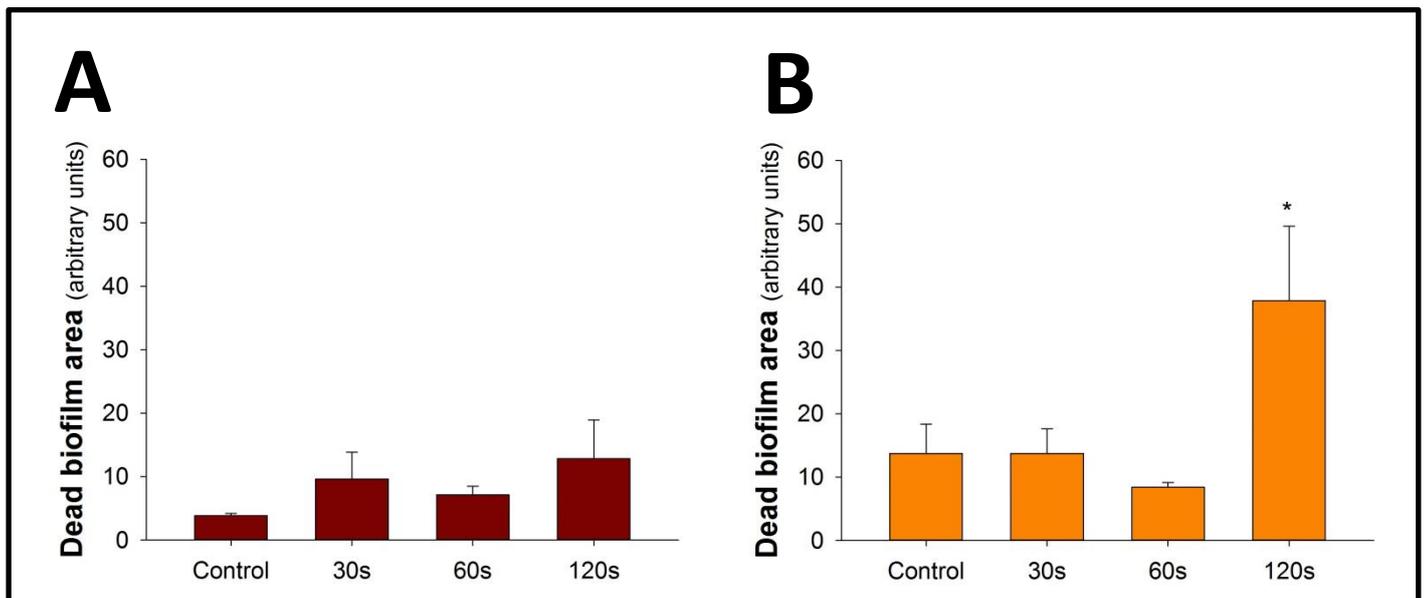
Figure 3 – kinetics exposure experiment.



*Quantitative analysis:* In the unstained groups, quantification of the area of the dead biofilm showed more dead bacteria after exposure to B-Cure laser, in all time intervals tested. This augmentation of the dead biofilm area ranges between 2-4 times the areas in the unexposed group (control, Figure 4a). However, these changes did not reach statistical significant differences.

After exposure of the plaque to indocyanin green, there was a clear increase in the area of the dead biofilm in the following 120 seconds of exposure (Figure 4b). This augmentation of the dead biofilm was ~4 times the area of the control unexposed group. The difference between the 120 seconds group and all other groups was statistically different.

**Figure 4** – quantitative analysis of the kinetics exposure experiment. **A** – Unstained groups, **B** – Indocyanin green groups).



Further statistical analysis was performed in order to compare the adjunctive effect of indocyanin green compared to the effect of the B-Cure laser alone in each time point interval. The results show that indocyanin green alone has some bactericidal effect, but 120 sec exposure to the laser enhanced the bactericidal by ~ 3 –fold.

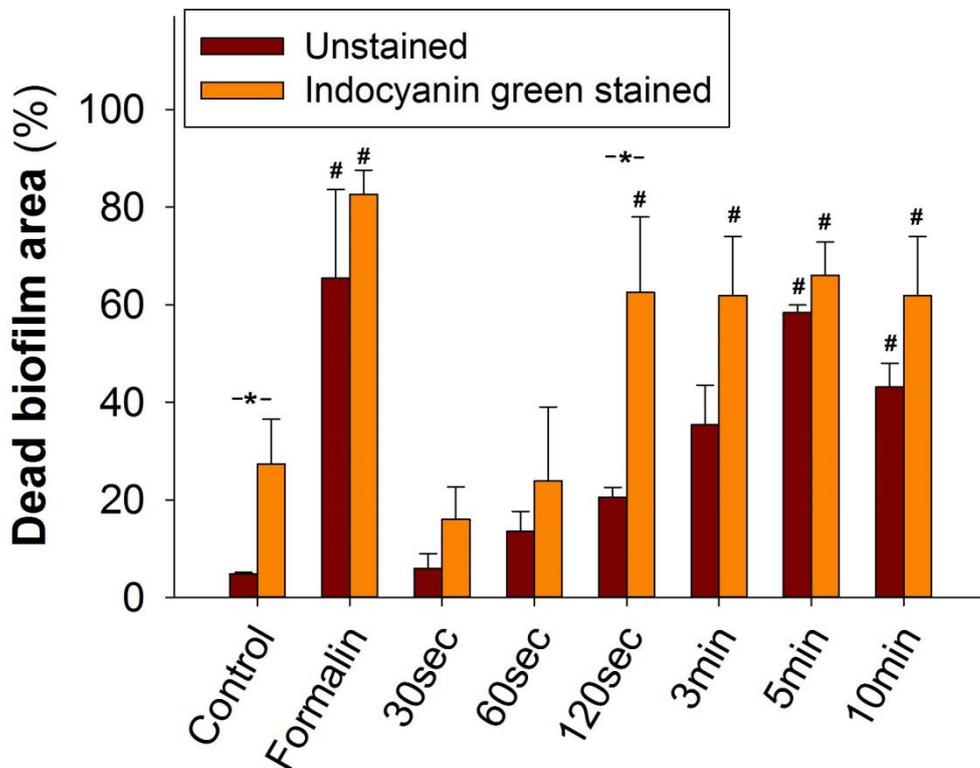
**1b. Long-term Kinetics.** Since the B-cure laser led to augmentation of dead biofilm area, we preformed similar experiments, extending the exposure time up to 10 minutes. We also added a positive control groups (formalin) that causes bacterial death.

Quantitative analysis of the results indicates the almost all biofilm was dead in the positive control groups compared with the negative control groups (Figure 5). Also, exposure duration at 120 sec showed similar pattern as above, indicating augmented bactericidal properties when combined with indocyanin green. However, exposure to the laser beam, with or without indocyanin green, of 5 and 10 min - showed significant death of bacteria in the biofilm, without statistical significant difference between stained and un-stained groups.

**Figure 5** – quantitative analysis of the kinetics exposure experiment up to 10 min.

# - indicates statistical difference from the control group.

\* - indicates statistical difference between unstained and indocyanin groups.



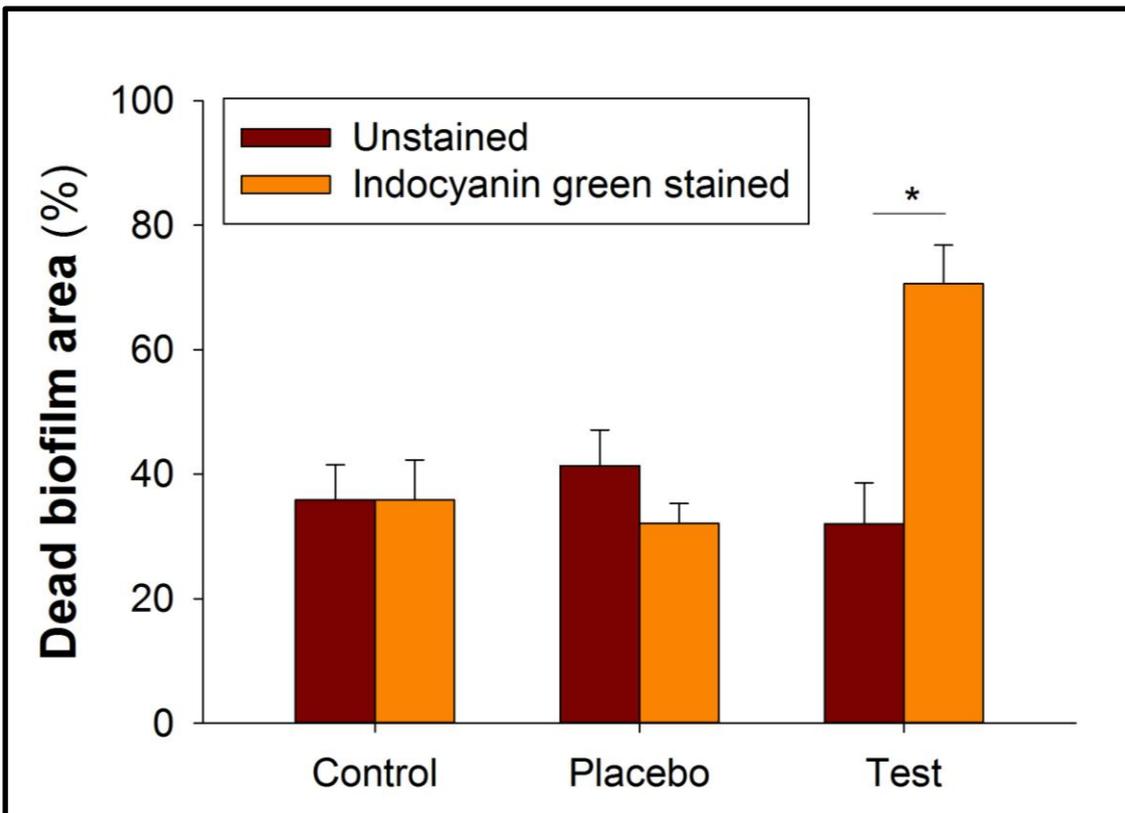
The biofilm thickness was also measured. All groups showed biofilm thickness ranging up to 100µm, with no difference between all the groups (control or test, unstained vs. indocyanin green).

### Part B- Comparing B-cure laser to placebo

For this purpose, we used as placebo a sham laser machine with the same visible light only, but no laser. Exposure time was set as 120 seconds, as was determined for the previous experiments.

The results validate the previous data showing a statistically significant effect of B-cure laser combined with indocyanin green (Figure 6), as compared with placebo irradiation. The placebo group had no effect, confirming the specific effect of B-cure laser light.

**Figure 6** – quantitative analysis of 120sec exposure of B-cure laser vs. placebo laser.



## **Conclusions**

The present study shows that, *in vitro*, the B-cure laser together with indocyanin green, may have a potential to induce death in periodontal-like biofilm. Pre-staining of the plaque with indocyanin green followed by irradiation of the stained plaque with the B-cure laser for at least 2 minutes shows clear and significant bactericidal effect and biofilm death, compared with sham and placebo controls.

In the present experimental conditions, irradiation of the biofilm, with or without indocyanin green, for 5 min and above, induced significant death of bacteria, with no differences between the stained and unstained groups. However, this result should be interpreted with caution, since long exposure to aerobic conditions may affect the viability of the artificial biofilm as well.

The results of this preliminary *in vitro* study indicate for a possibility of the use of B-cure laser in photodynamic therapy, using indocyanin green as the bio-sensitizer. This method may be helpful in clinical situations where dental plaque is the etiologic factor (such as periodontal diseases), but the clinical and the *in vivo* microbial effects need to be first validated in controlled human clinical trials.