# Use of Tryptophan to Prevent *Pseudomonas aeruginosa* Biofilm Growth on Wound Dressings

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**Statement of Purpose:** Wound dressings composed of synthetic and biological materials are routinely used to cover skin wounds, but contamination by *Pseudomonas aeruginosa* may lead to wound infection, biofilm formation, and delayed healing. Our recent work demonstrated that an equal molar ratio of d- and l-isoforms of tryptophan inhibited biofilm formation by *P. aeruginosa* on plastic (1). This effect was not limited to biofilm inhibition as addition of tryptophan to established biofilms caused their disassembly. To move towards determining if tryptophan is of clinical use for chronic skin wounds, we examined whether tryptophan can inhibit *P. aeruginosa* biofilm formation on a biological wound dressing. We also tested equivalent concentrations of tryptophan for *in vitro* cytotoxicity against the HaCaT human keratinocyte cell line.

**Methods:** A commercially available wound dressing (Biobrane, UDL Laboratories, Sugar Land, TX), composed of silicon sheets impregnated with collagen-coated nylon strands, was cut into 8mm discs and incubated in *P. aeruginosa* solutions with or without d-/l-tryptophan (total concentration 10.0mM) at 30°C for 48h. Biofilm formed on the dressing was quantified using two independent and complementary methods. 1) Crystal violet was used to stain the entire bacterial load (cells and biofilm) on each dressing. Bound dye was solubilized with acetic acid and absorbance measured at 595nm. 2) A phosphatase linked lectin (-HHA-), specific for extracellular polysaccharides in *P. aeruginosa* biofilms, was added and incubated for 18h at 4°C. The phosphatase linked to the lectin catalyzed the colorimetric reaction of P-nitrophenylphosphate to P-nitrophenyl that was quantified by measuring absorbance at 405nm. To assess potential cytotoxicity, HaCaT cells were exposed to tryptophan (1.0nM – 10.0mM) in Dulbecco's phosphate buffered saline (DBPS, Thermo Scientific, Logan, UT) for 1h at 37°C and 5% $CO_2$. Cell viability was measured using calcein-AM, which quantifies cellular metabolism as an indicator of cell viability. Fluorescence intensity (Ex:485nm/Em:528nm) was measured and graphed as percentage of control cell viability.

**Results:** *P. aeruginosa* formed robust biofilms on the wound dressing after 48h incubation at 30°C. Figure 1 shows that addition of 10.0mM tryptophan reduced biofilm on the wound dressing to background staining levels ($p<0.05$). Similar results were obtained with the phosphatase linked lectin and crystal violet assays. Figure 2 shows that tryptophan (1.0nM – 10.0mM) did not cause a significant decrease in HaCaT cell metabolism when added to the growth medium.

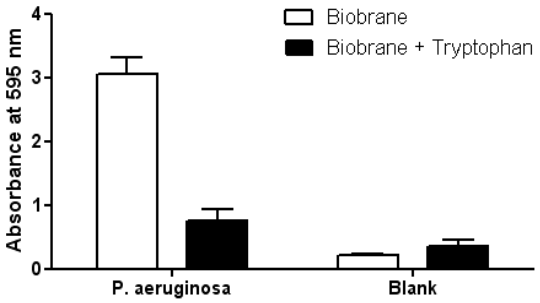


Figure 1: Crystal violet staining showed that 10.0mM d-/l-tryptophan significantly inhibited biofilm growth on the wound dressing.

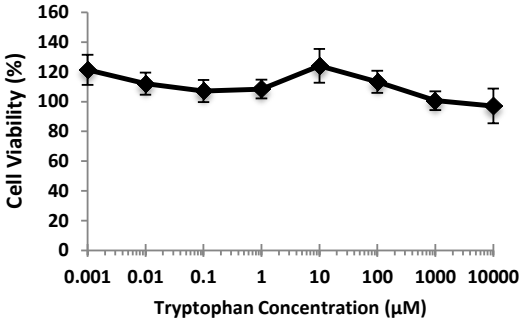


Figure 2: Exposure of HaCaT cells to tryptophan supplemented DPBS did not result in cytotoxicity.

**Conclusions:** The ability of tryptophan to inhibit *P. aeruginosa* biofilm formation on biologic wound dressings and its lack of toxicity against the HaCaT human keratinocyte cell line leads us to further evaluate tryptophan as an antibiofilm agent for topical treatment of chronically infected non-healing skin wounds.

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**Disclaimer:** JFM, NLA, CJM, MJS, AA, and CJC are co-founders of Imbed Biosciences, Inc., a privately held medical device company developing novel and patent-pending technologies for imbedding bioactive molecules in wound dressings and surgical implants.

**References:**

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