USC DENTAL ORAL MICROBIAL BIOFILM DISRUPTION ASSAYS

This data set was produced in the laboratory of Dr. J.W. Costerton in the Biofilm Center at the Dental School at USC. Dr. Costerton performed three experiments on human dental plaque samples using protocols that had been established in his laboratory for anti-biofilm activity screening. The data from these experiments consists of direct observation of anti-biofilm activity through use of advanced imaging technologies. This data set contains primarily SEM images and fluorescent confocal scanning laser microscopy images. The summaries of these assays are presented below.

SALIVARY BIOFILM LIVE/DEAD STAIN ASSAY
In this experiment the effect of exposing a human mixed microbe biofilm derived from human saliva samples to HPBR was studied. Human saliva was first spread onto sterile glass slide coverslips. The coverslips were placed into dishes of growth supporting microbial media and the dishes were maintained in the appropriate incubator environment for 14 days. The media was exchanged once daily. After 14 days the coverslips were washed. Half of them were treated for two minutes with an application of HPBR which was then removed with a water rinse. The other half was simply rinsed again for two minutes in saline as a control sample. After the incubation period all the slides were stained with the Invitrogen Live/Dead Cell Viability Assay System stain. This stain makes live microbes fluoresce green and dead microbes fluoresce red. After treatment the slides of both groups were examined by fluorescent confocal scanning laser microscopy. This technique allows you to visualize discrete “slices” through the biofilm as the plane of focus is adjusted from the top to the bottom of the thickness of the biofilm. The results of this study showed that all of the cells throughout the entire thickness of the control specimen were alive and stained green. However, all of the microbes in the HPBR-treated biofilm were dead from top to bottom. According to the staff of the Biofilm Center this kind of complete killing is very unusual. Typically antiseptics and antibiotics in this assay will kill only the microbes that are on the very top surface of the biofilm layer. This demonstrates that HPBR can effectively desiccate through the full thickness of a typical biofilm layer of mixed human microbes.
HUMAN EX VIVO ROOT CANAL BIOFILM STUDY

In the next set of experiments illustrated below HPBR was evaluated as a root canal cleanser in standard *ex vivo* root canal preparation procedures. Freshly extracted human teeth were clamped to a lab bench apparatus and standard root canal shaping and cleaning protocols were performed on the teeth as if they were still in the patient. Upon completion of the *ex vivo* cleaning procedures, the teeth were fractured along the length of the root canal and split to allow examination of the canal surface by scanning electron microscopy. The SEM pictures immediately below show the root canal surface of a saline-treated control specimen in the upper row and pictures of the surface of a root canal that had been irrigated with HPBR during the *ex vivo* procedure in the lower row. In comparison to the saline-treated control surface, the HPBR-treated surface shows less dentinal debris in general and less of the very fine microbial plaque debris at the openings of the dentinal tubules.

**UNTREATED**

![Untreated SEM Image]

**HPBR TREATED**

![HPBR Treated SEM Image]
In the third experiment, Dr. Costerton exposed cut sections of the root of an extracted tooth from a periodontitis patient with heavy root surface plaque to either a saline rinse or to an HPBR rinse and then performed SEM on the rinsed areas. The pictures below show the root surface that was exposed to saline at levels of increasing magnification. These pictures show thick plaque covering the entire surface of the root section. The long strands of tangled string-like material seen in the SEM picture at the highest magnification in the far right lower panel are created by the SEM sample preparation procedure from shrinkage of the gel-like substances in the plaque matrix. The spheres and rods that are seen clustered around those strands are the bacteria that created the plaque.

**UNTREATED CONTROL - TOOTH ROOT SURFACE PLAQUE**
The SEM pictures in the panel below show the surface of a cut root section from the same tooth that is shown in the pictures above, however, this cut section was exposed to an HPBR rinse before the SEM sample preparation was done. These pictures are also arranged by increasing magnification. In comparison to the pictures of saline-exposed plaque, these pictures show that the HPBR-exposed plaque looks as if it has been dehydrated. At high power in the far right panel the SEM picture shows that the matrix substance and the microbes appear to have been aggregated together into a coagulum by dehydration. The plaque aggregate has cracked and is detaching from the root surface. Clean root surface is visible under the plaque in areas where it has completely detached from the surface.

**TOOTH ROOT SURFACE PLAQUE TREATED WITH HPBR**

THE SIGNIFICANCE OF THE COSTERTON USC DATA
As is well known, Dr. Costerton is considered one of the pioneers of current biofilm technology. He demonstrates here the type of “direct observation” technology that is necessary to evaluate the efficacy of ant-plaque and anti-biofilm agents on the most sensitive levels rather than depending upon secondary clinical indictors and outcomes.