

# **Development and Commercialization Challenges with a Novel Anti-Infective Biofilm Technology**

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[www.hybenx.com](http://www.hybenx.com)

# HYBENX® Technology Development Challenges

- Disruptive Platform
- Simple
- Complex Paradigm: History of microbial disease
  - Clinical SOC for conventional microbiology
  - Antibiotics, disinfectants, cleanliness
  - Product complexity and regulatory expectations
  - Idiopathic disease
  - Global interconnectedness: more virulent and resistant strains
  - **Biofilms**

# **HYBENX<sup>®</sup> Technology Commercialization Challenges**

- Established business knowledge is conservative: we know what works (NIH)
- Financial establishment
  - Industrial
  - Academic
- Reputational risk; personal and institutional
- Regulatory structure
- Legal liability risk

# Lack of Significant Biofilm





# “Normal” Biofilm Diversity





# “Mature” Biofilm Development



# Microbial Biofilm Cycle

## **“Normal”**

Aerobic and facultative anaerobic  
commensal bacteria,  
Diverse, Non-pathogenic,  
Healthy

## **“Abnormal”**

Anaerobic, pathogenic,  
Antibiotic-resistant persister cells  
Inflammatory  
(Periodontal Disease; Chronic Non-Healing  
Wounds, Etc.)

# Current Global View of Oral Microbial Biofilm

**Overwhelming global evidence points to a changing infectious disease model where microbial agents and inflammatory mediators present in oral biofilm contribute to many chronic and acute diseases throughout the human body.**

*Biofilms as Connectors for Oral and Systems Medicine: A New Opportunity for Biomarkers, Molecular Targets, and Bacterial Eradication.* Sintim and Gürsoy, OMICS J Integrative Biology. Vol. 19 Nov 2015 (Epub).

*Biofilm-Based Healthcare-Associated Infections.* Gianfranco Donelli, Ed. Advances in Experimental Medicine, Volumes 830 and 831. Springer 2014.

# Current Therapeutic Approaches are Inadequate

Debridement is incomplete:

- Residual abnormal biofilm bacteria easily repopulate
- Inflammatory components not eliminated

Biofilm macromolecular microenvironment protects microbes and maintains inflammatory state:

- Antibiotic penetration incomplete
- Complex anaerobic biochemistry
- Accelerated horizontal gene transfer
- Persister cells increasing resistance
- Flawed “New” antibiotic development paradigm

*A new molecular cleaning paradigm is needed.*

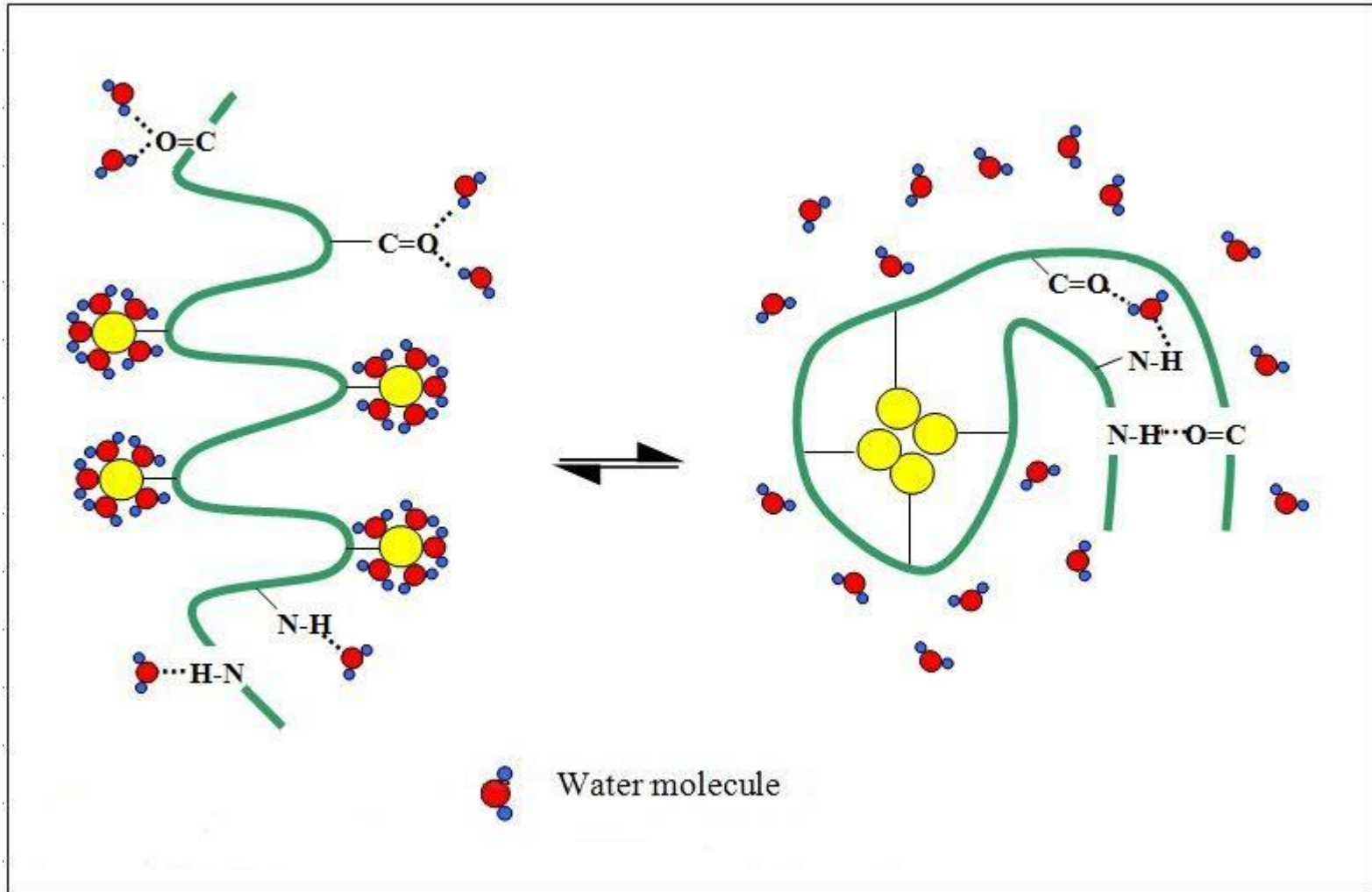


# EPIEN Medical's HYBENX® Technology

A unique blend of common sulfonated phenolic acid constituents that have been safely used in global products as medical astringents, mucolytics, detergents, etc., since the early 1900s.

# HYBENX: Molecular Desiccation

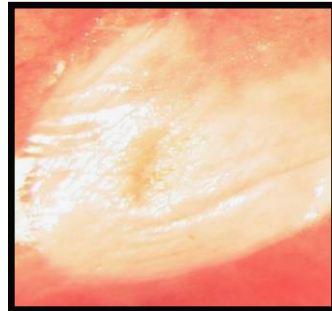
The Sulfate-Water Hydrogen Bonding Effect:  
*Localized Denaturation by Removal of Bound Water*



# Oral Mucosal Ulcers (Aphthae)



**Mucosal  
Ulcer**



**Immediately  
post-HYBENX  
Application  
for 5 seconds**



**Hours post  
HYBENX  
Application**

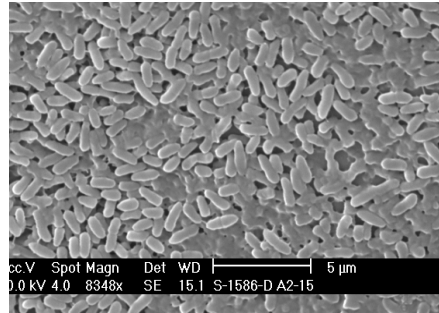


**Week post-  
HYBENX  
Application**

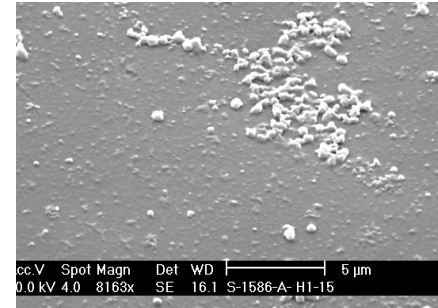


# Microbial Biofilm Eradication Model (Innovotech)

## *PSEUDOMONAS* BIOFILM

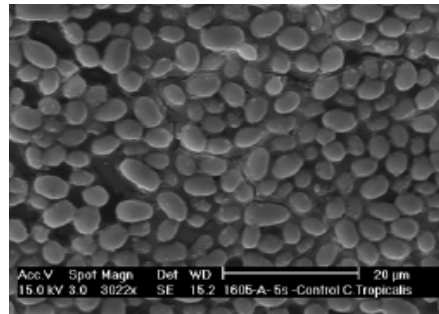


**Untreated Control**

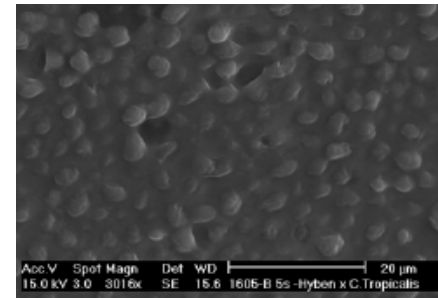


**HYBENX Treated**

## *CANDIDA* BIOFILM

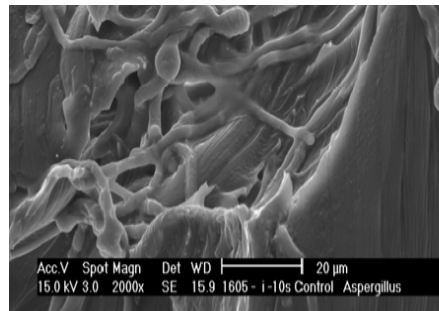


**Untreated Control**

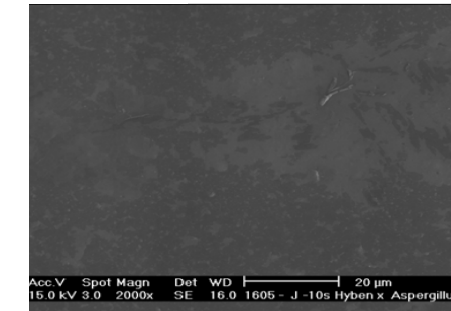


**HYBENX Treated**

## *ASPERGILLUS* BIOFILM



**Untreated Control**



**HYBENX Treated**

# Porcine and Human Skin Microbial Biofilm Eradication Models

University of Miami, Miller School of Medicine (S. Davis)

## Porcine

*Acinetobacter baumannii*

Methicillin-Resistant *Staphylococcus aureus*

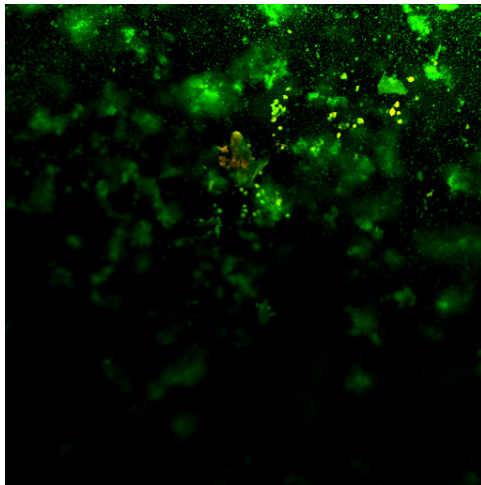
*Pseudomonas aeruginosa*

## Human (cell culture)

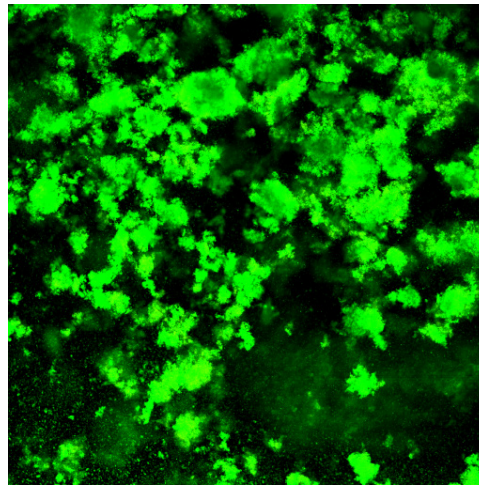
*Trichophyton rubrum*

# Oral Biofilm Evaluations – Live/Dead Confocal Full Depth Microscopy (Costerton – Lawrence)

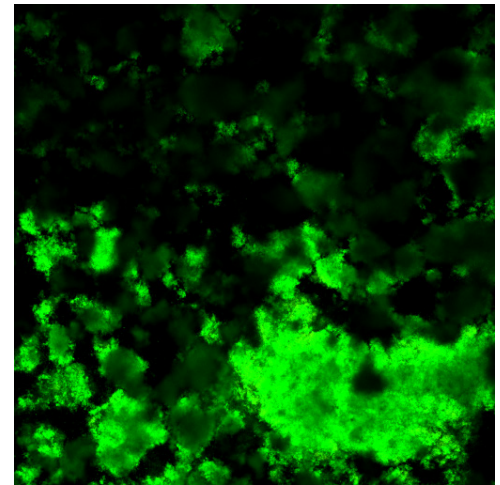
**UNTREATED  
CONTROL**  
Green = Live cells



**SURFACE**

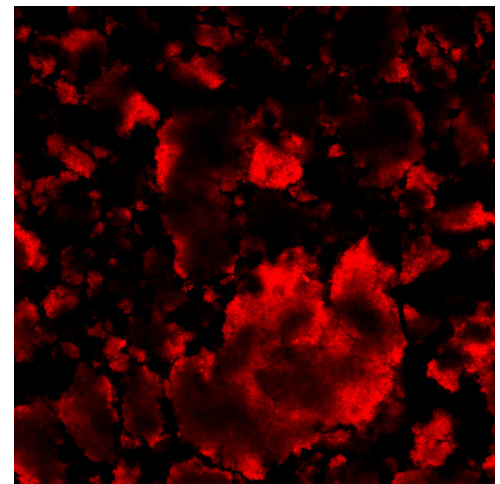
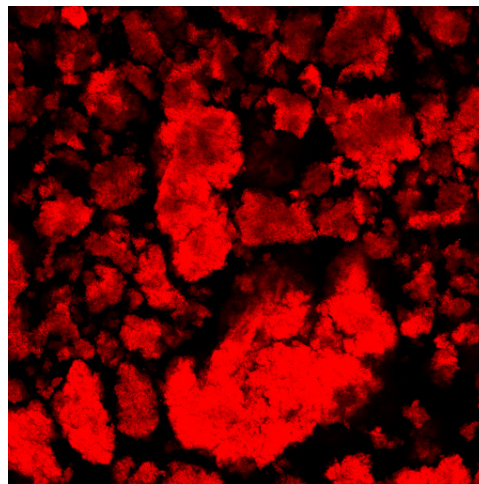
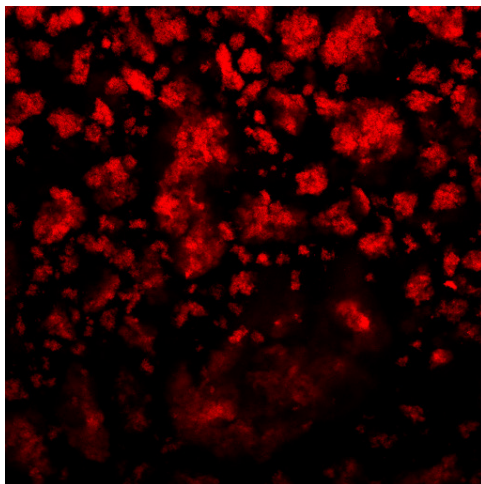


**MIDDLE**



**BASE**

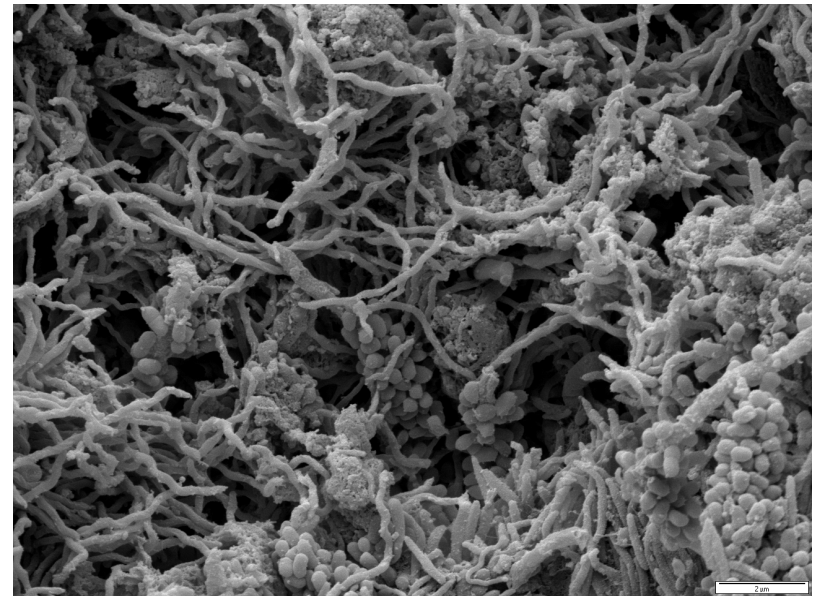
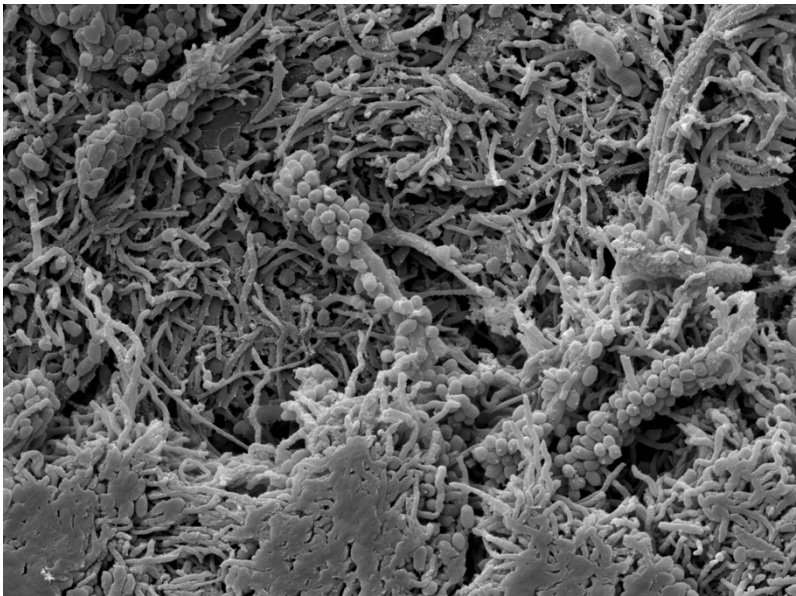
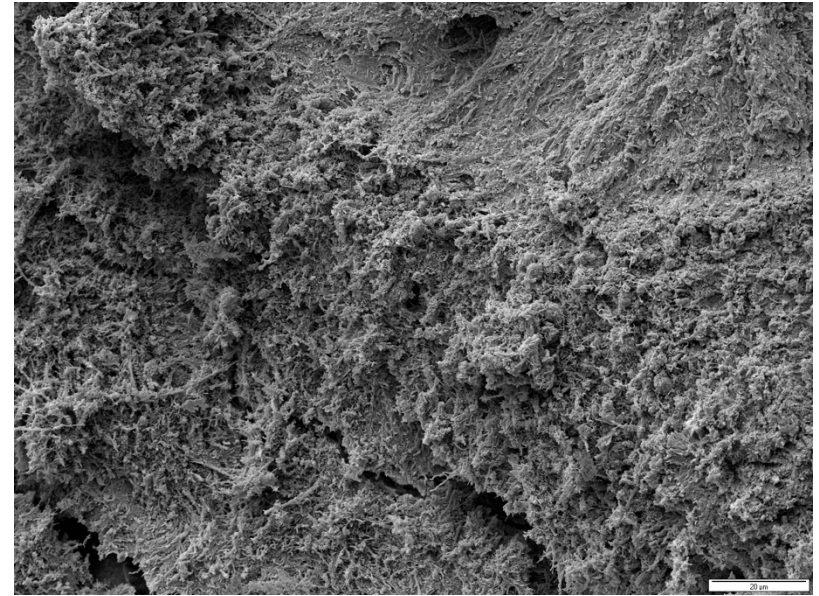
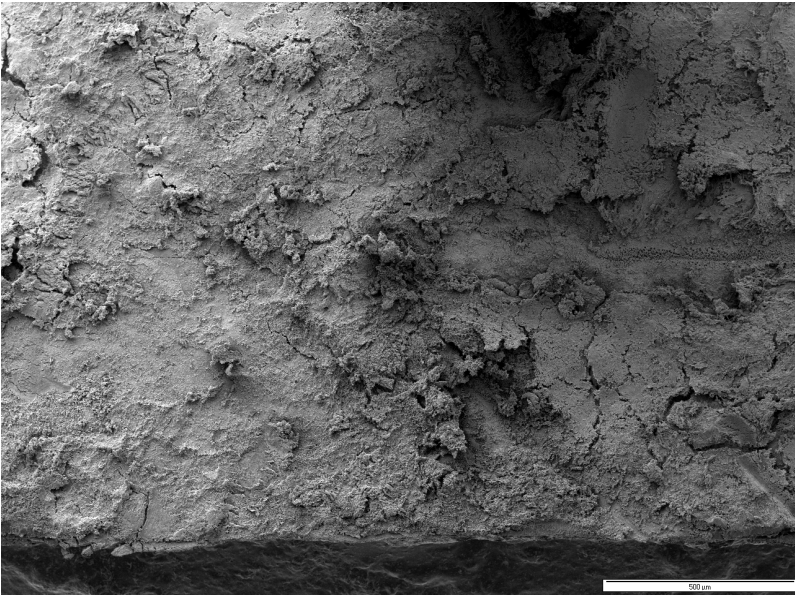
**HYBENX®  
TREATED**  
Red = Dead cells





# USC Dental School Human Oral Biofilm SEM Studies

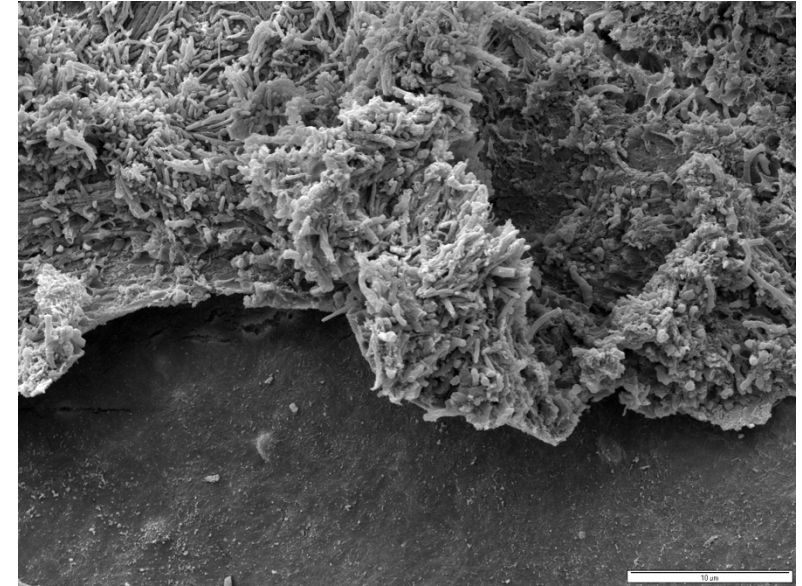
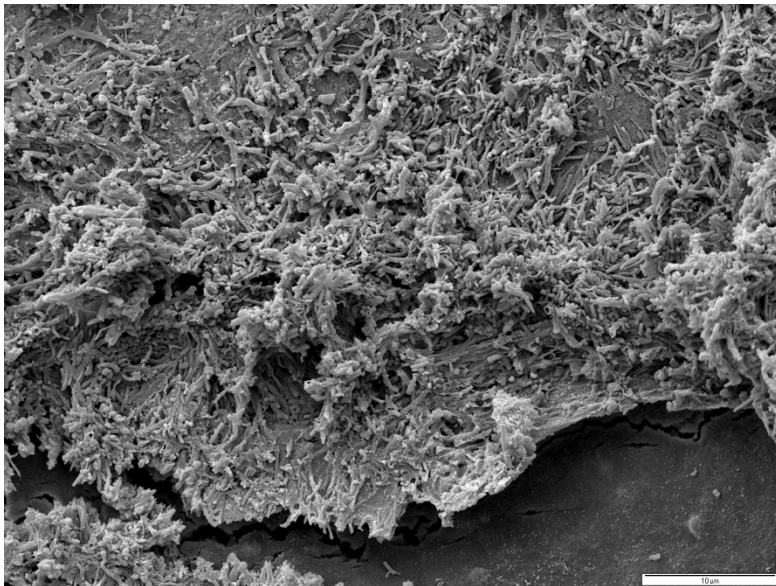
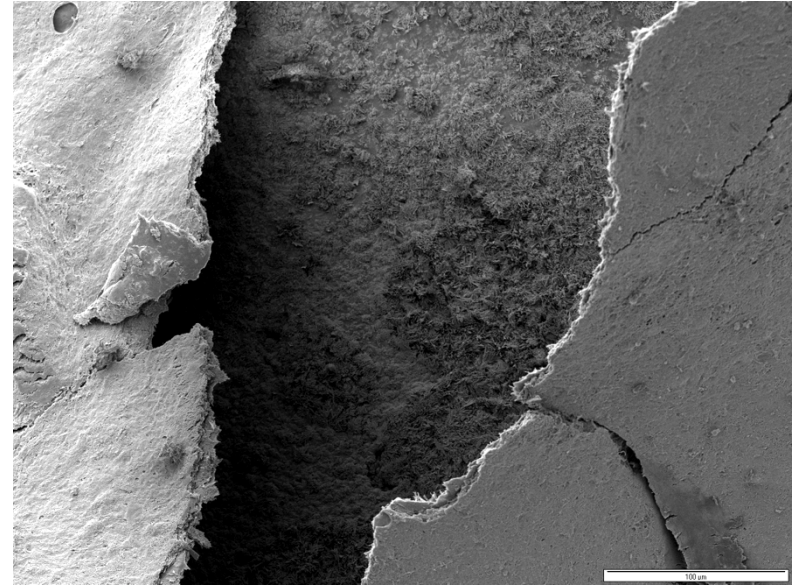
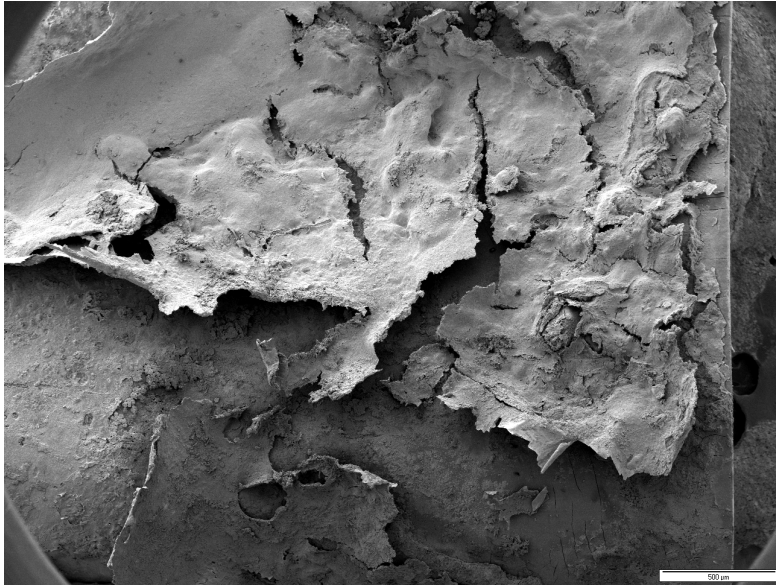
## UNTREATED CONTROL – TOOTH BIOFILM





# USC Dental School Human Oral Biofilm Studies

HYBENX TREATED; Not rinsed







**Giorgio Lombardo,  
MD, “DDS”**

**Professor & Chair,  
Department of  
Periodontology,  
University of Verona**

# **Effect of HYBENX® Oral Tissue Decontaminant on Periodontal Biofilm Microbes - DNA Analyses**

16s PCR rDNA analysis

Samples taken from periodontal pockets before and after HYBENX rinse treatments

Contract laboratory assesses microbial identity and quantity

# Oral Microbial DNA Probe Analyses

## **Higher Risk**

**Aa** = *Aggregatibacter actinomycetemcomitans*

**Pg** = *Porphyromonas gingivalis*

**Tf** = *Tannerella forsythia*

**Td** = *Treponema denticola*

## **Moderate Risk**

**Cr** = *Campylobacter rectus*

**En** = *Eubacterium nodatum*

**Fn** = *Fusobacterium nucleatum/periodonticum*

**Pm** = *Peptostreptococcus (Micromonas) micros*

**Pi** = *Prevotella intermedia*

## **Lower Risk**

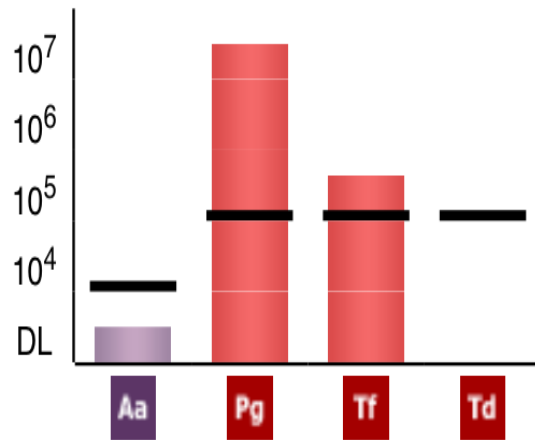
**Cs** = *Capnocytophaga* sp. (*gingivalis*, *ochracea*, *sputigena*)

**Ec** = *Eikenella corrodens*

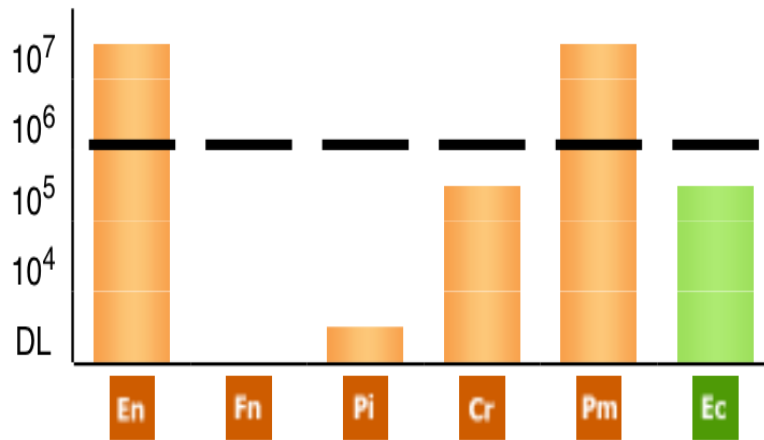


# Baseline: Periodontal Pocket Microbial Composition Before HYBENX Treatment

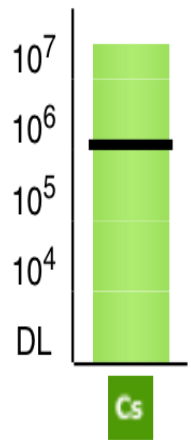
## High Risk Pathogens



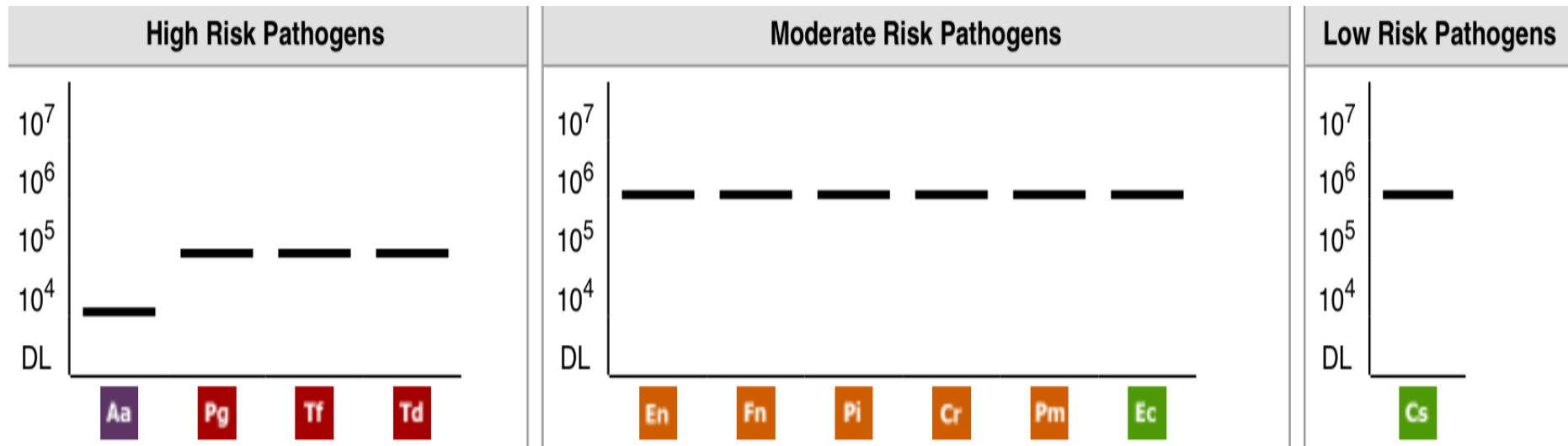
## Moderate Risk Pathogens



## Low Risk Pathogens



# Periodontal Pocket Microbial Composition after SRP and HYBENX Treatment



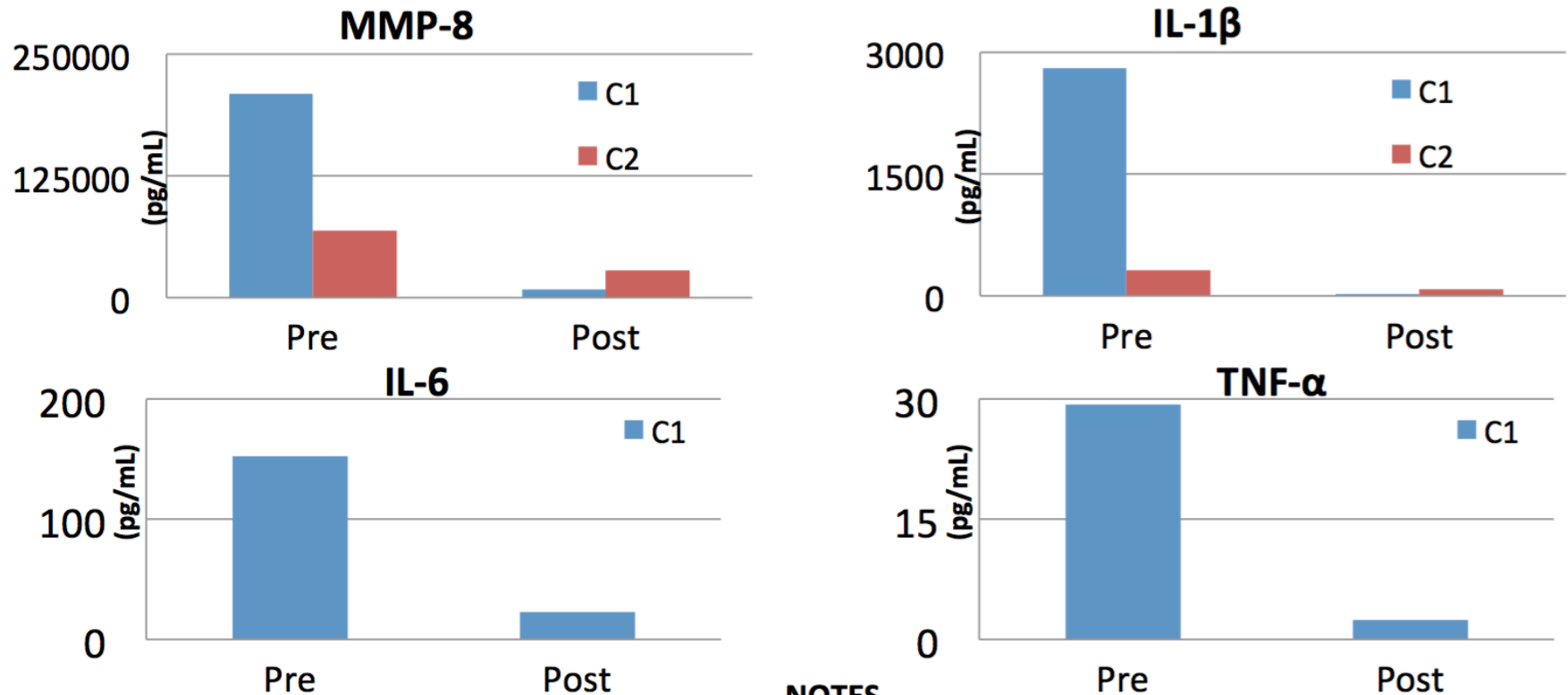
# Effect of HYBENX® Oral Tissue Decontaminant on Periodontal Inflammatory Markers

Inflammatory modulators are molecular surrogate markers of chronic inflammatory disease

Primary periodontal markers appear to be MMP8 along with IL-1 $\beta$ , IL-6, and TNF-alpha

Assayed effect of HYBENX Solution on the levels of these inflammatory markers in patient periodontal quadrants

Figure 2. Evaluation of Biomarkers in Paper Point Samples Collected Pre and Post HYBENX Irrigation Treatment



NOTES

- **Collection Set 1 (C1):** Pooled paper points eluted with 150  $\mu$ L of PBS, assayed at a 1:2 dilution.
- **Collection Set 2 (C2):** Pooled paper points eluted with 50  $\mu$ L of PBS, assayed at a 1:6 dilution (Due to the 1:6 dilution, IL-6 and TNF- $\alpha$  were not detectable.)
- TNF- $\alpha$  Post value presented a result < MDD of 2.40 pg/mL. MDD value graphed for demonstration purpose. (MDD=mean detectable dose)

# A Topical Desiccant Agent In Association With Ultrasonic Debridement In The Initial Treatment Of Chronic Periodontitis: A Clinical And Microbiological Study.

G Lombardo, C Signoretto, G Corrocher, A Pardo, J Pighi, A Rovera, F Caccuri, and PF Nocini. *New Microbiologica* **38**:393-407. 2015

## Summary

This controlled randomized pilot clinical trial demonstrated that debridement accompanied by the use of **HYBENX® Oral Tissue Decontaminant** resulted in clinically superior patient outcomes as assessed with both microbial and clinical parameters.

1<sup>st</sup> Annual Conference

# **ELIMINATION OF BACTERIAL BIOFILM: A NEW APPROACH FOR THE TREATMENT OF PERIODONTITIS AND PERI- IMPLANTITIS: FROM RESEARCH TO CLINICAL PRACTICE**

Florence, Italy

16 May 2015

## **Scientific Coordinator**

Prof. Giovan Paolo Pini Prato

## **Session Coordinators**

Dr. Pierpaolo Cortellini and Dr. Nicola M Sforza

## **Speakers**

James W Bracke, PhD

Prof. Francesco Carinci, MD

Dr. Dorina Lauritano, MD

Dr. Zdenek Jansky, MD

Dr. Stefan Neumeyer, MD

Prof. Giovan Paolo Pini Prato, MD

# General HYBENX Oral Cleaning Regimen

- Apply HYBENX Solution for approximately 10 - 20 seconds; rinse/aspirate—loosens plaque biofilm, reduces subsequent pain and bleeding
- Perform conventional SRP/UD
- Second HYBENX treatment: cleans residual molecular debris and “seals” tissue surface

# Regulatory Challenges

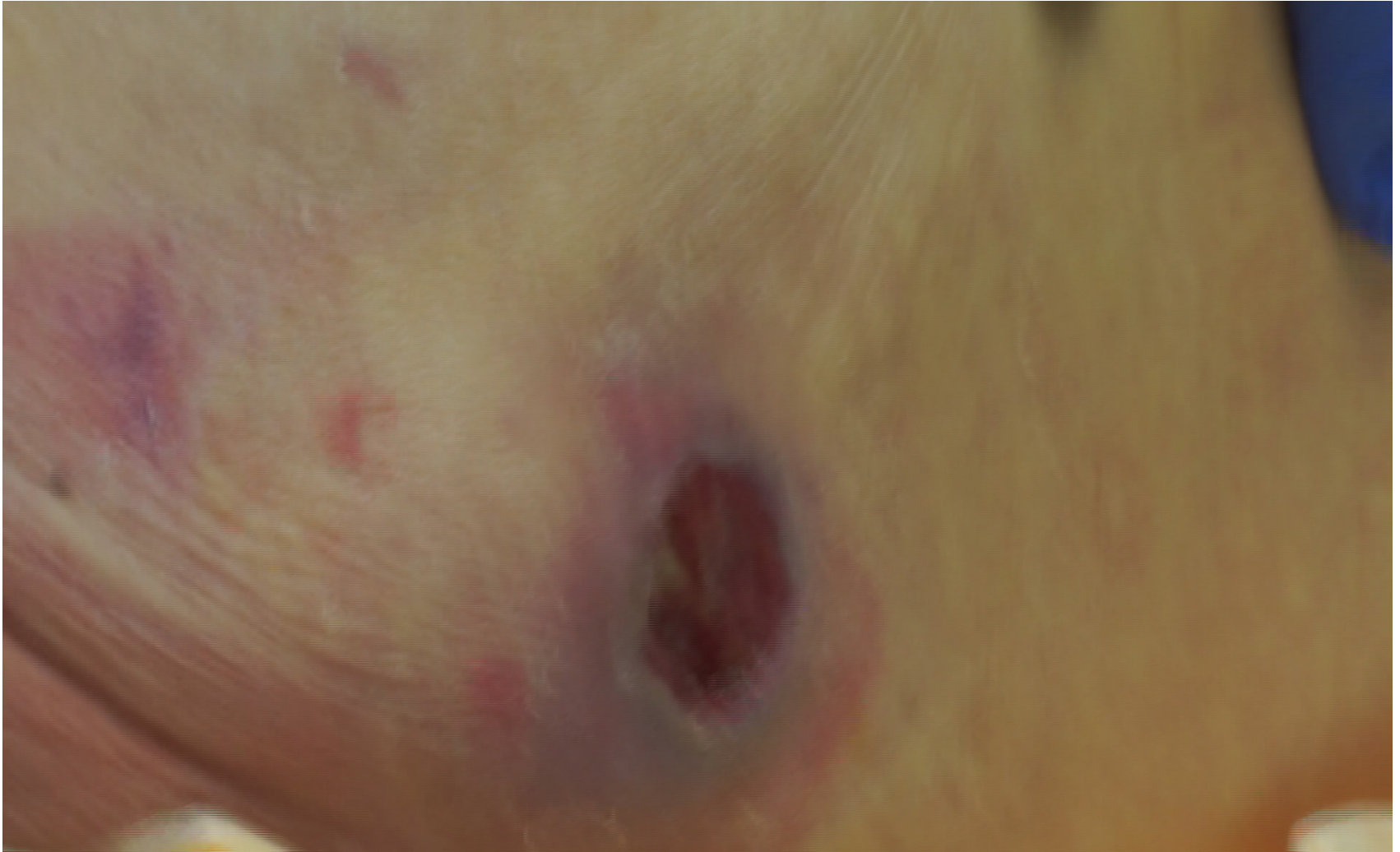
- Old definitions of drug vs. device vs. combination products
- Chemical action vs. metabolic process: desiccation?
- Contact under one minute; Not absorbed; no residue; focal
- Extensive safety file
- 2003: Formal US, Canada, and EU HYBENX Product Registration activity
- 2005: Canada Broad Oral Class I Medical Device
- 2005/6: EU Broad Oral Class I Medical Device
- US: Device section; OCP; device section; legal resolution
- 2013: EPIEN Root Canal Cleanser 510(k) Oral Device
- To date:
  - 8 million oral units sold with 200+ complaints
  - US – Future development activity?



# Future Chronic Disease Clinical Targets: Therapy Beyond the Oral Cavity?

- Non-healing Chronic Wounds
  - Dr. Marcus Gitterle
  - CHRISTUS Hospital System, San Antonio TX USA
  - 50+ patients
    - NHCW = 5-15 months old (Va, Di, De, SSI)
    - Ten-second therapeutic application
    - “Cools” down the hot spot
    - Complete re-granulation and preliminary epithelialization within four weeks
- Additional independent anecdotal clinical reports
- University of Sapienza

# Chronic Venous Stasis Ulcer



# HYBENX® Oral Tissue Decontaminant Solution

[www.hybenx.it](http://www.hybenx.it)



*Simplicity is the ultimate sophistication...*

Leonardo da Vinci

*The irony of the world is that it wants to  
simplify complexity and complicate simplicity...*

Vikrmn