



ENZYMATIC TREATMENT REPLACING PLASMA: BIOCOM- PATIBLE POLYMER SURFACES

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Cell culture plastics are usually made from polymers, most commonly from polystyrene, the most economic material for cell culture tools. This and other polymers have a hydrophobic surface which is not suitable for cell adhesion. Surfaces of polystyrene dishes are usually treated with plasma to improve the attachment of cultured cells. Plasma treatment can oxidize the polymer and will even roughen the surface while penetrating deeply into the polymer. As a consequence we commonly observe not only the generation of toxic compounds but also batch to batch variations, inhomogeneous activation of surfaces. As a consequence toxic effects on cells and the batch to batch variation are a general problem in medical and life science applications.

The Fraunhofer IFAM and the Fraunhofer IZI have now developed an enzymatic alternative for a gentle surface activation. In these proprietary methods we are applying an enzyme not yet recognized for this ability.

Enzyme vs. Plasma Treatment

It is a common experience in medical and biological applications that cell culture dishes are not accepted by all cell types. Besides generally toxic impurities in cheap plastic ware most of the effects are contributed to the activation with different forms of plasma which usually oxidize an about 50 µm thick surface layer and generate an unknown number of organic substances. In addition the plasma treatment is always not reaching all parts of a surface evenly, since distance holders must be applied and the

distance to the plasma emitting electrode is usually variable. In addition the treatment is restricted to surfaces that can be reached by the electrode.

The novel approach applies an enzyme solution which turned out to be very efficient at low concentration in modifying the surface of polystyrene dishes within minutes. Standard plastic can be easily activated using the enzyme in combination with different compounds and substrates to further modify the surfaces. We have tested several cell lines for their compatibility with the method at the Fraunhofer IZI and in collaboration with other researchers. There is similar or even improved growth on polystyrene plates treated with the enzyme solution when comparing them to ordinary plasma activated dishes.

Validation with Cell Lines

Several cell lines have been tested in collaboration with researchers at Fraunhofer IZI (Dr. A. Stolzing) and the Translational Center for Regenerative Medicine (TRM) Leipzig (*Dr. V. Savkovic), and a German hardware company in the last two years. Amongst these were Fibroblasts, MCF7, HaCaT, T47D, Ntera*, Normal Human Epidermal Melanocytes* (NHEM), follicle-cultivated melanocytes* (HM).

Potential Applications

The enzyme solution is working almost identically to normal plasma and can modify also 3D structures like patterned surfaces and even porous materials. Therefore this method is not restricted to life science but can also be used in all other areas where plasma is usually applied as a routine method. Please request more information about these additional application areas.

Potential applications within the life science sector are for example:

- Cell friendly plastics (less toxic)
- Implant modifications
- Surface modifications including attachment of additional molecules
- Print of activated surface areas

This method is not restricted to polystyrene, we have successfully tested:

- CoC (TOPAS®)
- Polypropylene
- Polyethylene
- Polycarbonate

We offer a complete physical, chemical and biological characterization of the enzyme activated surfaces.

Available methods are:

- X-ray Photoelectron Spectroscopy (XPS)
- Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS)
- Atomic Force Microscopy (AFM)
- Scanning Electron Microscopy (SEM)
- Transmission Electron Microscopy (TEM)
- Quartz Crystal Microbalance with Dissipation (QCM-D)
- Surface Plasmon Resonance (SPR)
- Detailed evaluation with primary and established cell lines (FACS, confocal microscopy, biochemical methods)
- Validation in GLP environment
- GMP conform protocol development for medical applications

1 (Front page) NHEM cells growing very well in the small surface area modified previously with a drop of enzyme solution on otherwise normal, untreated polystyrene.

2 (Back page) NHEM cells visualized by fluorescent staining of their nucleus growing on a surgical suture activated just by enzyme treatment.