High Throughput Methods for Testing Hemocompatibility

James N. Cawse[a], Liming Yu[b], Zhengyu Pang[b], Aaron J. Dulgar-Tulloch[b], Anton Beletskii[b]
[a]Combinatorial Chemistry Laboratory, Materials Analysis and Chemical Sciences Global Technology Organization
[b]Molecular and Cellular Biology Laboratory, Biosciences Global Technology Organization
General Electric Company Global Research Center
Niskayuna, NY 12309

Introduction: Development of novel polymers for blood-contact applications requires new evaluation methods able to compare numerous samples with good precision and resolution. We have developed a system for generating large numbers of injection molded polymer samples in a 96-well format with statistically relevant levels of replication. Assays such as leukocyte activation, platelet adhesion, and fibrinogen adsorption have been adapted to this system and used to evaluate samples with appropriate statistical rigor.

Methods: Injection molded polymer samples were produced using a mold that makes a single 8-well strip of a 96-well plate. These strips can then be assembled on a commercial 96-well frame to give a 12-sample x 8-replicate array. After exposure to whole blood or plasma, the polymers were evaluated for hemocompatibility by a battery of methods, e.g.:

1. Leukocyte activation was quantified relative to control materials utilizing flow cytometry for leukkocyte surface markers.
2. Platelet adhesion was measured by two methods: fluorescence labeling followed by image processing and an enzyme based assay.
3. Fibrinogen adsorption was measured by a modified ELISA assay.

Results/Discussion:
1. Quantification of the expression of leucocyte surface markers after material contact demonstrated a clear difference in activation levels between materials. Variation between replicates remained small (CV generally less than 5%), with the capability to significantly differentiate materials separated by ~ 5% expression.
2. Variation of the image processing was very high (σ=μ!) because of an inherent doubly stochastic Poisson process. Enzymatic assay variation was significantly lower, with the capability to differentiate materials separated by <10% expression.
3. Fibrinogen adsorption was capable of differentiating materials separated by < 100 ng/cm^2.

Conclusions:
The high-replicate and high-throughput measurement of hemocompatibility in this effort has allowed examination of numerous formulation parameters in statistically designed experiments. The results have translated into detailed maps of the hemocompatibility of polymeric materials (Figure 1) which will be used as guidance for further material optimization.

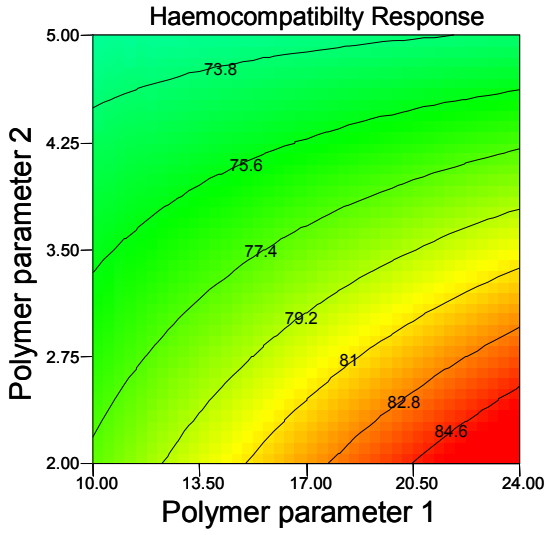

Figure 1.