

Digital Histopathology and Feature Analysis on Consecutive Tissue Sections (FACTS) to Determine Biocompatibility of Biomedical Implants



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OBJECTIVE

Conduct a proof of concept to determine if Feature Analysis on Consecutive Tissue Sections (FACTS*) could be used for quantitative analysis of biocompatibility in post implant histology.

BACKGROUND

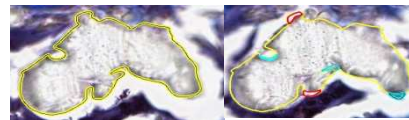
Biomedical implants are being used throughout the body to treat etiologies or replace damage or compromised tissues or organs. Each of these implants must undergo extensive biocompatibility testing prior to their clinical use. Biocompatibility is defined as the "ability of a material to perform with an appropriate response in a specific application" (DF Williams, 1987). The general requirements of biocompatibility testing of implanted devices include: the material must be able to interact with living cells, tissues or systems without being toxic, injurious, or causing immunological reactions while performing or functioning appropriately (JD Bumgardner et al. 2004). Common methods to evaluate implant biocompatibility include the review of post-implant histology. Using digital pathology and subsequent morphometry, biomedical implants can be screened and evaluated on specific characteristics of biocompatibility. Factors to consider while performing histopathology evaluations for biocompatibility include: immune response, inflammation, necrosis, fibroplasia, fibrosis, fatty infiltrate, and angiogenesis. Performing these analyses can be enhanced with the use of appropriate stains to facilitate automated cell counts, determination of the percent area of necrotic or fibrotic tissue, measurements of fibrous capsule, determination of the amount of lipid deposition or infiltration, and accelerate digital pathology and morphometry evaluations when compared to traditional manual methods.

FACTS PROCESS

Feature Analysis on Consecutive Tissue Sections (FACTS) is a histology and image analysis process that allows quantitative multiplexing of histopathology tests. The process is composed of four steps as show below:



- 1. Consecutive tissue sectioning.** 4 μm sections are cut sequentially and stained. A central slide is used as a reference slide, with special stains to assist in automated feature analysis. The serial sections can each have a biomarker for a particular biocompatibility effect.
- 2. Automated feature recognition.** Image analysis is run on the central reference slide to detect regions of interest.
- 3. Image and ROI registration.** Advanced image registration techniques transfer the features identified in the reference slides to the target slides. Image analysis can then be run on the target slides to measure individual biocompatibility effects.
- 4. QC and pathologist review.** An histology technician checks the results and eliminates any poorly annotated regions. The pathologist then reviews the slides and signs off on the biocompatibility report.

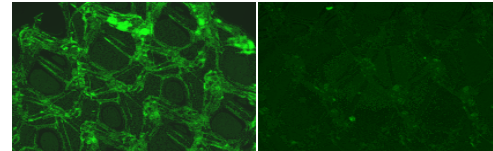


←**Fig.1** mark up image of consecutive images showing a 2.64 overall percent of error between sections. Yellow indicates original mark-up image (2990μm²). Red indicates areas of false positive mark-up (40.6μm²). Blue indicates areas of false negative mark-up (38.5μm²) for an overall percent error of 2.64%.

MATERIALS AND METHODS

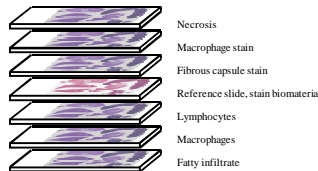
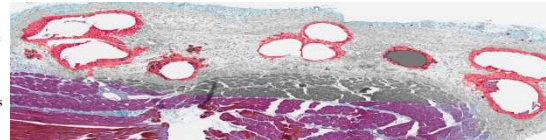
In one study, a human extracellular matrix (hECM) was used as a surface coating on commonly used polypropylene mesh. Coated and uncoated 6mm biopsy punches of polypropylene were terminally sterilized and implanted subcutaneously in the dorsal lumbar position in a murine model. Samples were explanted at 2 and 12 weeks, processed for standard histology and sections stained for Masson's Trichrome. Stained sections were digitally scanned and digital morphometry performed to characterize the resulting fibrous capsule.

By creating high resolution digital scans of histological specimens and differential staining along with pattern recognition software, we are able to train the program to recognize specific colors and textures on the whole slide.



←**Figure 2.** Coating of hECM onto biomedical materials. Anti-fibronectin immunofluorescent staining of hECM-coated (left) and uncoated (right) polypropylene mesh.

Figure.3→ Representative mark-up image of fibrous capsule surrounding polypropylene fibers. Used to determine area of fibrous capsule.



For quantitative biocompatibility analysis with FACTS*, each slide can contain a different marker for a common biocompatibility measurement, as shown for example in the figure at left

FACTS is *Patent Pending

RESULTS

Average Fibrous Capsule Thickness (um)

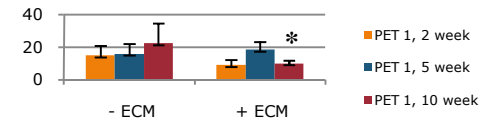


Figure 4. Average Fibrous Capsule. * = p<0.05 PET1 -ECM vs. +ECM @10 wks

Average Fibrous Capsule Thickness (um)

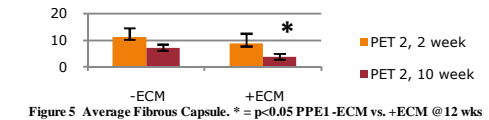


Figure 5 Average Fibrous Capsule. * = p<0.05 PPE1 -ECM vs. +ECM @12 wks

Average Fibrous Capsule Thickness (um)

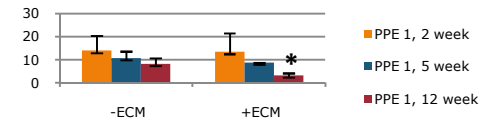


Figure 6 Average Fibrous Capsule. * = p<0.05 PPE12 -ECM vs. +ECM @10 wks

CONCLUSIONS

- The advantages of digital pathology and morphometry over traditional manual methods include:
- 1) Rapid throughput** -- once algorithms have been developed, the analysis can be automated on all samples and performed in a matter of minutes.
 - 2) Consistency of results** -- evaluations can be performed using identical parameters and assumptions on all samples with no inter-investigator bias.
 - 3) Whole slide analysis** -- Using whole slide scanning we are able to generate data from the entire implant specimen instead of selected fields of view through manual methods.

BIBLIOGRAPHY

Williams DF. *Definitions in Biomaterials*. Amsterdam:Elsevier, 1987.
 Bumgardner JD, Vasquez-Lee M, Fulzele KS, Smith DH, Branch KD, Christian SI, Williams DL. *Biocompatibility testing*. *Encyclopedia of Biomaterials and Biomedical Engineering*. 2004