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Staining Protocols for Safety Study of Wireless Fecobionics Device

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SPARC

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Protocols for Hematoxylin and Eosin staining and Immunofluorescence for two SPARC datasets: *Safety Study of Wireless Fecobionics Device* and *Safety testing of predicate device for Fecobionics*. The procedures were approved by Calmi2 Institutional Animal Care and Use Committee (IACUC)

Mongrel dogs were randomly assigned into two groups: the Fecobionics device group, and the THD SensyProbe control group. All animals were anesthetized, the devices were transanally inserted into the rectum, and then inflated at 20 ml. Six minutes later the devices were removed either naturally or manually. The animals in each group were randomly assigned into two subgroups, acute and chronic. The dogs in the acute subgroups were euthanized immediately after the removal of the devices, whereas the dogs in the chronic groups were euthanized two weeks after the device removal. The tissue samples were harvested upon sacrifice.

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Animals

Mongrel hounds - [Oak Hill Genetics](#), Ewing, IL

Device:

Fecobionics device - [California Medical Innovations Institute](#), San Diego, CA

THD SensyProbe - [Balmer Médical SA](#), Concise, Switzerland

H&E Staining

Hematoxylin - [EMS](#) Cat# 26043-06

Eosin - [Fisher Scientific](#) Cat# 314-630

Lithium Carbonate - [Fisher Scientific](#) Cat# L119-500

Immunofluorescence

Macrophages

- Primary Antibody:

Anti-Macrophage Marker Antibody (MAC387): [Santa Cruz Biotechnology](#) Cat# sc-66204, RRID:AB_2184280

- Secondary Antibody:

Alexa Fluor 546 Donkey Anti-Mouse IgG - [Thermo Fisher Scientific](#) Cat# A10036, RRID:AB_2534012

- For mucosa staining:

Wheat Germ Agglutinin, Alexa Fluor 488 Conjugate - [Thermo Fisher Scientific](#) Cat#W11261

- For nuclear counterstain:

Hoechst 33342, Trihydrochloride, Trihydrate - [Thermo Fisher Scientific](#) Cat# H3570

Caspase-3

- Primary Antibody:

Anti-caspase 3 - [Abcam](#) Cat# ab13847, RRID:AB_443014

- Secondary Antibody:

Alexa Fluor 546 Donkey Anti-Rabbit IgG - [Thermo Fisher Scientific](#) Cat# A10040, RRID:AB_2534016

- For mucosa staining:

Wheat Germ Agglutinin, Alexa Fluor 488 Conjugate - [Thermo Fisher Scientific](#) Cat#W11261

- For nuclear counterstain:

Hoechst 33342, Trihydrochloride, Trihydrate - [Thermo Fisher Scientific](#) Cat# H3570

Equipment

Cryostat - Leica CM1850

Inverted Microscope - [Nikon Eclipse Ts2R](#)

Camera - [pco.panda 4.2](#)

Hematoxylin and Eosin Staining

- 1 Fix the tissues with 4% paraformaldehyde (pH 7.4) overnight at 4°C
- 2 Embed the tissues with Optimal Cutting Temperature (OCT) compound and freeze with liquid nitrogen.
- 3 Cut 7 μm sections with cryostat (Leica, CM1850).
- 4 Rinse slides in 1X PBS for 3 minutes.
- 5 Rinse in a gentle stream of tap water until OCT is washed off for 3 minutes.
- 6 Stain in Hematoxylin for 20 minutes.
- 7 Wash in a gentle stream of tap water for 1 minute, then dip in saturated lithium carbonate solution for 45 seconds.
- 8 Blue nuclei in 1X PBS for 20 seconds.
- 9 Wash in a gentle stream of tap water for 1 minute.
- 10 Dip in 70% EtOH for 30 seconds.

- 11 Dip in 95% EtOH for 30 seconds.
- 12 Counterstain in Eosin Y for 10 seconds.
- 13 Dehydrate through 2 changes of 95% EtOH for 15 seconds.
- 14 Dehydrate through 3 changes of 100% EtOH for 15 seconds.
- 15 Clear through 3 changes of Xylene for 1 minute each and coverslip.

Immunofluorescence

- 16 Put the frozen slides at room temperature for 30min.
- 17 Wash slides with PBS 3 times. Each time 5 min.
- 18 1% SDS for 5min, then wash 3 times.
- 19 0.25% triton in 1xPBS for 15min.
- 20 10% Donkey serum in 1xPBS for 1hour.

- 21 Incubate slide with primary antibody diluted in 0.25% triton 2.5% Donkey serum 1xPBS at room temperature for 3 hours.
- 22 Wash slides with PBS 3 times. Each time 5 minutes.
- 23 Incubate slide with secondary antibody (1:100 dilution) for 1 hour.
- 24 Wash in PBS 3 times. Each time 5 minutes.
- 25 Stain with Wheat Germ Agglutinin (1:100 dilution) for 30 minutes.
- 26 Wash slides with PBS 2 times. Each time 5min.
- 27 Stain with Hoechst 33442 (1:100 dilution) for 30 minutes.
- 28 Dry and mount coverslip.