README file

Name of dataset: CFM images for the Living Probiotics-Loaded Wound Matrices

Collection: Farmaatsia andmed

Authors: Oksana Gerulis, Georg-Marten Lanno, Marta Putriņš, Marilin Moor, Beata Niemczyk-Soczynska, Tomasz Kowalczyk, Slawomir Blonski, Tanel Tenson, Piotr M. Korczyk, Karin Kogermann*

*Corresponding author: K. Kogermann, Institute of Pharmacy, University of Tartu, karin.kogermann@ut.ee; ORCID ID: 0000-0002-6813-4828

This dataset contains confocal microscopy (CFM) data collected during experiments at the Univeristy of Tartu, as part of Marilin Moor MSC Thesis project (https://www.etis.ee/portal/mentorships/display/9443d81a-2d4e-4ffd-9fcd-9f8e20f6d718) (Institute of Pharmacy and Institute of Computer Science), Oksana Gerulis PhD Thesis project (at the Institute of Pharmacy).

It is being made public both to act as supplementary data for publications and the PhD thesis of Oksana Gerulis and in order for other researchers to use this data in their own work.

Methods: confocal fluorescence microscopy (CFM)(Zeiss LSM 710; Germany) was conducted on different probiotic-loaded (*L. lactis* IL1403) samples prepared. Samples were stained with green-fluorescent nucleic acid stain SYTO 9, not stained samples were used as controls. The collected fiber matrices containing *L. lactis* IL1403 (pre-stained with FM 4-64 prior electrospinning) loaded agarose microcapsules on microscopy cover glass were put onto the 1% agarose pads consisting of glucose, covered with another cover glass and kept at 30 °C for specified time periods (24 h). These samples were initially measured directly under the CFM (confirmed the red colored bacteria within fibers) and then the cover glass was removed and SYTO 9 stain added (1 µL stain per 1 mL bacterial dispersion). After 30 min these stained samples were again measured under the microscopy. The staining of bacteria within the fibers with SYTO 9 and its efficacy enabled to prove whether the stain can diffuse into the fibers or not. CFM micrographs with a size of 67.48 x 67.48 µm were used for further analysis.

Viability of LAB: The presence, distribution, overall concentration and viability of LAB within the electrospun fibers was investigated using staining with fluorescent nucleic acid stains PI and SYTO 9 and CFM. The electrospun fiber matrices containing LAB-loaded agarose microcapsules were collected on a microscopy cover glass and then were put onto the 1% agarose pads consisting of M17-glucose medium, covered with another cover and analysed immediately or incubated at 30 °C or at 37 °C, for 24 h. For staining 20 µL of 10 µM SYTO 9 and 20 µL of 40 µM PI were added to the agarose pad (opposite side from the fibers). Diffusion of dyes was allowed to take place at least 20 min before imaging. A bacterial mixture consisting of live (overnight liquid LAB bacterial culture) and dead LAB (heat treated at 56 °C for 10 min) in 50:50 ratio was used as a control for imaging.
Zen software was used for the microscopy analysis (Carl Zeiss Microscopy GmbH, Germany).

Names of confocal microscopy (CFM) micrograph files:

PCL_fibers_FM_syto_1.czi (1-10): PCL fibers loaded with probiotics stained with FM and SYTO 9

PCL_PEO_fibers_FM_1.czi (1-4): PCL_PEO fibers loaded with probiotics stained with FM

PCL_PEO_fibers_FM_syto_1.czi (1-10): PCL_PEO fibers loaded with probiotics stained with FM and SYTO 9

------

sack_fibers_NOgrowth_syto_PI_1.czi (1-15) + fibers_NOgrowth_syto_PI_1.czi (1-2) : PCL fibers loaded with probiotics stained with SYTO 9 and PI (starting, no growth)

stack_fibers_24h_growth_syto_PI_1.czi (1-7) + fibers_24h_growth_syto_PI_1.czi: PCL fibers loaded with probiotics stained with SYTO 9 and PI (growth after 24 h)

------

control_50killed_syto_PI_1.czi (1-3): control at 0th hour with PI stained 50% alive, 50% killed bacteria

control_50killed_syto_PI_incub4h_1 (1-3): control after 4 hours with PI stained 50% alive, 50% killed bacteria

control_bac_FM_1.czi (1-4): control of FM-stained bacteria

control_bac_FM_syto_1.czi (1-4): control of FM- and SYTO-stained bacteria

Contact information: K. Kogermann, karin.kogermann@ut.ee ; M. Moor, marilin.moor@ut.ee; O. Gerulis, oksana.gerulis@ut.ee