Electronic Supplementary Material (ESI) for Analytical Methods.

Miniaturized genotoxicity evaluation system for fast biomaterial-related risk assessment

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Table S1. Detailed procedure of genotoxicity assay in microfluidic chip.

Microfluidic chip preparation	1. Fill the chip with agar in the designated wells.
	2. Leave the agar in the chip so as to cool down until agar is solid and at room temperature for 5 minutes.
	3. Pierce the adhesive film with a 1.5 mm diameter biopsy puncher in the position of reservoir inlet, VIs, Vos, and waste outlet.
	4. Stick the adhesive from one end of the chip to the other.
Semi-automated sequence	5a. Pipette 150 µL of bacterial suspension + PBS (in the proportion of Table 1) into the reservoir through the microfluidic chip inlet.
	6a. Load the MCs with 10 µLof sample, negative and positive control through the corresponding VOs using a pipette. Do not let the injected liquid reach the exit of the mixing chamber to enable air circulation until bacterial solution flows in.
	7a. Place A flexible EPDM rubber mat (or alternatively some fitting O-rings) on top of the chip and valves for air-tight connection. Such components are placed using double-sided pressure tapes. The mat and tape should also be pierced into the VIs and VOs positions.
	8a. Place the luer connectors in the inlet and outlet of the chip using pierced double pressure tape.
	9a. Attach the valves to the rubber mat with double-sided pressure tape in the right position. Orientation of the valves is not crucial as they act as a switch (open or close position).
	10a. Connect the valves to the valve controller.
	11a. Connect and empty container into the pressure source and then to the chip using the tubing of choice, as the chip is completely loaded, with no liquid will in circulation through it.
	12a. Connect another empty container as waste to the outlet to enable air circulation without bacterial suspension spilling. Still, the waste chamber is designed to contain all the liquid, whereas it it can be evacuated in the drying steps.
	13a. At the beginning, all the valves are closed, and a pressure of 10mbar is applied. First, the bacterial suspension is pushed individually to each of the mixing chambers until it is full, whereas the liquid has not reached the agar wells. Therefore, the reagents have time to mix.

	14a. Apply a pressure of a pressure of 10 mbar with all valves closed. One at a time, open each valve and push bacterial suspension into each of the MCs until they are full, though the liquid has not reached yet the agar wells. Therefore, the reagents have time to mix.
	15a. Make sure all the valves are in the closed position.
	16a. One at a time, open the valves 110 seconds until the liquid fills completely the three AWs in the circuit line.
	17a. Make sure all the valves are in the closed position again.
	18a. Let the bacteria sediment inside the chip for as long as needed, in this case, 2 hours.
	19a. Open all the valves at the same time and activate the pressure source at the same pressure in order to evacuate all liquid into the waste chamber. If desired, the liquid waste can be safely disposed of by flowing it to the waste container through the outlet.
	20a. Activate the pressure again to help the drying process. A pressure of 200 mbar for 5 min is recommended, at least until no liquid is visible in the agar wells.
	21a. Incubate the chip for 24 hours. In case an incubator is needed, luer connections to the chip and valve connections to the valve controller are removed. The chip attached to the valves can then be transported and placed inside. To ensure safe handling, the inlet and outlet are sealed with adhesive tape.
	22a. Take off the seals and repeat the step 5a for the staining process, this time loading the reservoir with dye.
	23a. Reconnect the pressure source to the inlet and a waste container to the outlet.
	24a. Repeat steps 13a to 18a. Now the MCs are empty; their purpose is to distribute the right volume of liquid for each line, so there is no need to care for mixing.
	25a. Let the dye stain the bacteria and protect from light with aluminium foil as necessary.
	24a. Repeat steps 19a and 20a.
	26a. If washing is needed, fill the container connected to the pressure source (initially empty) with washing solution.
	27a. Repeat steps 19a and 20a, at 30mbar instead, now flowing the washing

	solution, for at least 2 minutes.
	28a. Repeat steps 19a and 20a in order to let them dry. The chip is now ready for observation.
Manual pipetting	5b. Load the MCs with 10 µL of sample, negative and positive control through the corresponding VOs using a pipette.
	6b. Pipette 35 µL of bacterial suspension + PBS into each of the MCs until full; then, mix by pushing back and forth the liquid between the pipette and the well. It is advised to push gently to avoid bubble formation.
	7b. Push each mixture separately into their respective AWs circuit lines by pushing air through the VOs. The liquid should be pushed as a constant and slow stream until all the wells are covered. Bubbles are avoided manually by adjusting speed or slightly backing the liquid front.
	8b. Let the bacteria sediment inside the chip for as long as needed, in this case, 2 hours.
	9b. Extract the liquid from each of the lines using the pipette. Air can be pushed with the pipette in order to dry the agar faster, then letting the chip dry until no liquid can be seen in the agar.
	10b. Incubate the chip for 24 hours. To ensure safe handling, the VOs and outlets are sealed with adhesive tape.
	11b. Remove adhesive tape from the VOs and outlets. Repeat step 7b by now injecting fluorescent dye.
	12b. Let the dye stain the bacteria and protect from light with aluminium foil, if necessary.
	13b. Repeat step 9b.
	14b. If washing is needed, repeat step 7b by now injecting washing solution.
	15b. Wash the wells by injecting fresh washing solution and extracting it consecutively. Gentle pipetting back and forth is recommended. This step is repeating for as many washing steps as recommended in the macroscopic protocol.
	16b. Repeat step 9b. The chip is now ready for observation.

Cytotoxicity test

CellTiter-Glo® Luminescent Cell Viability Assay (Promega, France) was used to confirm the biocompatibility of the resin used for the microfluidic chip elaboration. The experiment was based on ISO 10993-5 guidelines and performed on Balb/3T3 cells seeded in 12-well plates, 10^5 cells per well, and grown for 24 hours at 37°C in High glucose Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin - streptomycin. The resin was cut into small pieces (0.753 +/- 0.039 cm^2) and added to the cells for a direct contact assay. The cells were grown for 24 hours in presence of resin, then CellTiter-Glo® experiment was performed according to manufacturer's instructions. Briefly, the plate containing the cells, DMEM-FBS medium and CellTiter-Glo® reagent were equilibrated at the room temperature, then cell culture medium was replaced by a mixture of DMEM-FBS and CellTiter-Glo® (50/50 v/v), and luminescence was measured using SAFAS spectrofluorometer (Monaco). The results are expressed as percentage of positive control (untreated cells, 100% viability). Each condition was performed in triplicate.

The results show that cell viability in presence of the resin was equal to 80%, indicating that the material is biocompatible, according to ISO 10993-5 (cell viability above 70%). Some decrease in viability is certainly due to the fact that the material slightly damaged the cell layer (mechanical damage).

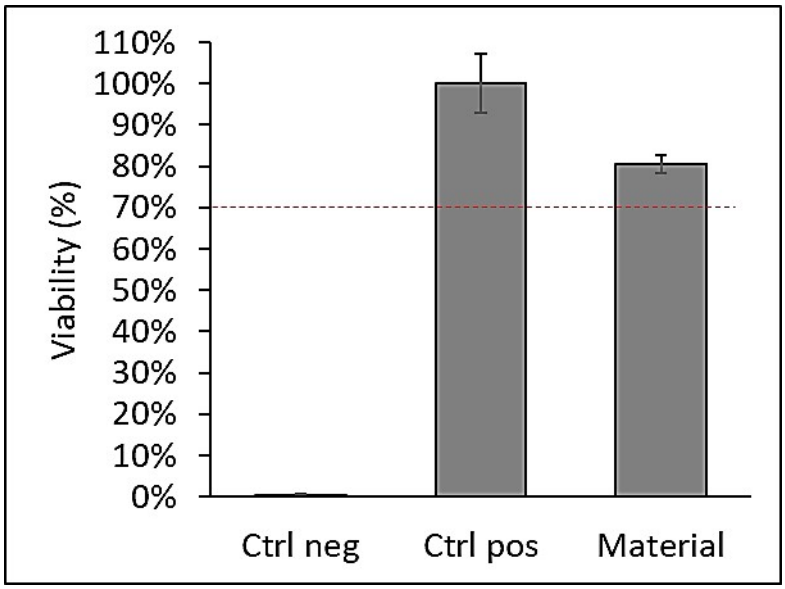

Figure S1. Cell viability measured using CellTiter-Glo® Luminescent Cell Viability Assay on Balb/3T3 cells. Each condition was performed in triplicate, and the values correspond to the averages +/- SD.