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Challenges in nanofiber testing *in vitro*

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Nanofibers provide unique opportunities for drug delivery and tissue engineering. Despite many advantages of nanofibers, they are not yet in clinical use, mainly due to insufficient data about safety. Exploring nanofiber safety starts with in vitro testing, but unlike nanoparticles that have a clearly defined assay cascade by European Nanomedicine Characterisation Laboratory, tests need to be established or modified to account for additional considerations. We identified three main areas of challenges in *in vitro* testing of nanofibers namely pre-processing, cell culture experiment and evaluating the results. Challenges in pre-processing start with preparation of nanofibers (aseptic work) with constant nanofiber mat thickness, and clean nanofiber sample edge. MTS proliferation assay has proven to be sensitive to nanofiber sample orientation, size, and nanofiber collector material. Challenges in evaluating MTS proliferation assay results remain due to cell infiltration in nanofiber mat, formazan dye absorption into nanofibers and absorbance determination. Experimental nanofiber mats were made with an electrospinning device collected on glass or aluminium base. A model *in vitro* test was MTS proliferation assay performed in 96 and 48 well microtiter plates with primary blood derived cells. Experiments were designed using “Design of Experiments” approach to evaluate the influence of individual assay configurations. Most of the challenges can be overcome in the pre-processing phase with optimised nanofiber mat thickness, accurate nanofiber cutting or limiting the assay area with a microtiter insert glued to the nanofiber mat. Formazan dye absorption can be determined with additional experiments to measure the absorption capacity of nanofibers. With optimisation of described experimental parameters we expect more accurate in vitro results.

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