Production of a reusable micromolded microcavity insert to standardize spheroid generation for drug screening

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Drug screening relies mainly on simple *in vitro* and *in vivo* models that even when combined, do not provide a perfect representation of human physiology. Likewise, due to the ethical concerns surrounding the wide use of animals for research, the improvement of *in vitro* models to support the 3R's policy is crucial. Considering the liver's pivotal role in drug metabolism and its high susceptibility to toxicity over time, better 3-Dimensional (3D) liver models are needed for efficient drug screening. We developed an approach based on micromolding to generate a reusable microcavity insert in Polydimethylsiloxane (PDMS), to facilitate spheroid generation.

An acrylic mold with small microcavities was designed and after double-casting, both a negative and a microcavity insert in PDMS were produced. The inserts were attached to the bottom of a 48-well plate, sterilized and coated to prevent cell adherence. HepG2 cells were seeded and after 72h, treatment was initiated. The efficacy of sorafenib was evaluated by assessing the drug's impact on the spheroid size, shape and viability.

The PDMS negative and microcavity insert were successfully developed, presenting the desired topography. The insert was used to seed HepG2 cells and after 48h, 1500 compact spheroids were observed per insert, similar in shape and size. Upon treatment, the spheroids showed reduced viability, loss of shape and size reduction with increasing concentration of the compound.

We were able to develop a reusable micromolded microcavity insert, in which we can generate thousands of homogeneous spheroids, in a simple and fast manner. Compact spheroids are obtained and easily retrieved. Sorafenib treatment was performed, and a dose-dependent effect was observed. The developed microcavity insert is an encouraging platform to screen drugs *in vitro*, on more reliable and physiological relevant models, reducing the need for animal research.

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Conference presentation: this paper was presented at the Fourth Centro 3R Annual Meeting - The role of 3Rs in the age of One Health: where we are and where we're going - 13-15 September 2023, Università degli Studi Milano-Bicocca.

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Biomedical Science and Engineering 2023; 4:209
doi:10.4081/bse.2023.209