
Proceedings Paper:
Mancinelli, E., Miranda, A.S., Picton, H.M. et al. (1 more author) (2024) Structural and biocompatibility challenges for 3D printed microfluidic devices for IVF. In: 2024 46th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2024 46th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 15-19 Jul 2024, Orlando, FL, USA. IEEE ISBN 979-8-3503-7150-5

https://doi.org/10.1109/embc53108.2024.10782247

Structural and biocompatibility challenges for 3D printed microfluidic devices for IVF

Elena Mancinelli
School of Electronic and Electrical Engineering
University of Leeds
Leeds, United Kingdom
elem@leeds.ac.uk

Andreia Santos Miranda
School of Medicine
University of Leeds
Leeds, United Kingdom
um19a2m@leeds.ac.uk

Helen M. Picton
Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine,
University of Leeds,
Leeds, United Kingdom UK

Virginia Pensabene
School of Electronic and Electrical Engineering
University of Leeds
Leeds, United Kingdom
V.Pensabene@leeds.ac.uk

***Abstract*— *In Vitro* Fertilization (IVF) is a widely used treatment for infertility, but success rates for UK women under 35 stand at approximately 32%. Culture conditions significantly affect *in-vitro* embryo development and treatment efficacy. Over 40 years, IVF procedures evolved, with microfluidic platforms emerging to enhance culture conditions. These platforms, designed to mimic a natural environment, show promise without negatively affecting development rates. However, potential impacts on embryo characteristics require further evaluation. The study introduces a microfluidic concept compatible with time-lapse microscopy. Two prototyping methods are compared, being soft lithography in PDMS and 3D printing in HTL resin. Results indicate successful prototype detection and loading efficiency, with the soft lithographic method showing a lower assembly yield. 3D printing facilitates rapid design, in particular for high aspect ratio microfluidic devices. However, viability assessments suggest additional steps are required to exclude material embryo toxicity of the resin.**

Keywords—microfluidic, embryo culture, IVF, rapid prototyping

I. Introduction

Approximately one in six couples have difficulties to naturally conceive, and for many of these assisted reproduction through *in vitro* fertilization (IVF) is the most appropriate treatment. In the United Kingdom, 390,000 babies have been born thanks to fertility treatments involving IVF [1], a number that contributes to the global 12 million births [2]. IVF is practiced across 130 countries and 4,500 IVF centers, presently constitutes 2-3% of all live births. Despite a rising demand for these procedures, success rates have plateaued at around a 33% birth rate for patients under 35, and it significantly decreases for patients aged 43-50 [1]. Embryo culture is one of the steps of IVF cycle and numerous studies show that the culture conditions impact the development of *in vitro* fertilized embryos [3], hence the efficacy of the treatments. Many factors influence the embryo development and health, such as oxygen [4], pH [5], temperature and humidity [6] during the culture. IVF procedures have been revised over the past 40 years to identify the best settings, to avoid detrimental fluctuations and to optimize media volumes and substrates for *in vitro* culture [7]. Microfluidic platforms have been developed to create a more natural environment for embryonic development, which have shown no negative effects on embryo development rates and confirmed enhanced culture success [8]. The major difference between currently used embryo culture systems (e.g. dish) and microfluidic devices is the absence of oil, which facilitates and reduces the handling and washing steps during embryo thawing, retrieval and analysis. Nevertheless, the potential effects of microfluidics on embryo morphology, genetics, epigenetics, and metabolic health and implantation success need to be further evaluated.

In this framework, time-lapse microscopy has been introduced in embryology and adopted in the clinics as a non-invasive method to select the best embryo for transfer, i.e. the embryo with higher quality and higher potential to implant and to lead to a successful pregnancy. Traditionally, the embryologist employs an optical microscope to scrutinize each blastocyst, by examining morphological parameters of development (e.g. the degree of expansion) and quality [9]. This step is facilitated using specific dishes, where individual embryos can be manually placed in distinct wells to maintain their position during the culture [10]. Although morphological assessment provides important insights into embryo quality, it is subjective. Time-lapse microscopy has initially emerged as a method to predict embryo viability, with a rapid adoption that showed positive impact on birth rates [11]. Time-lapse imaging facilitates automated morphological feature identification by machine learning and Artificial Intelligence applications [12].

Combining microfluidic platforms with time-lapse systems would thus enable morphokinetic analysis of the embryo development with important advantages: compared to traditional morphological assessment, it would avoid exposure to environmental changes when moving the dish outside the incubator for inspection under the microscope; in conjunction with time-lapse microscopy, it would reduce repetitive handling and pipetting, facilitate loading and positioning of embryos in the dish and thus favour embryo development.

In this work, we thus proposed a microfluidic device design compatible with time-lapse microscopy; we explored two different approaches for rapid prototyping and compared assembly efficiency, optical transparency, and material toxicity.

II. Methods

A. Soft lithography and 3D printing

In this study, our microfluidic concept for embryo culture [13] was adapted to the footprint of commercially available PrimoVision culture dish (16604) compatible with PrimoVision time-lapse system (Vitrolife, SE). Two approaches were tested.

The polydimethylsiloxane (PDMS, Sylgard® 184, Dow Corning, USA) device was manufactured by assembling two layers of PDMS on a PrimoVision dish (Fig. 1A). The bottom layer consisted of a PDMS layer to cover the uneven surface of the PrimoVision dish (~ 2 mm). The top layer included two single embryo chambers (height×width×length: 0.4×0.4×7 mm^3 for inlet channels, height and Ø: 0.4 mm and 0.65 mm for chambers; height×width×length: 0.1×0.4×10 mm^3 for outlet channels) obtained by casting and curing PDMS on a printed mould in BioMedAmber resin (Formlabs, USA) (Fig. 1B). PDMS was cured in the mould for > 10 h at 65 °C, peeled and cut to fit the size of the dish (Ø: 36 mm). The two chambers were then aligned under an optical microscope with the array of wells (Fig. 1C).

The fully printed devices were instead fabricated by using a MicroArch™ S240 (Boston Micro Fabrication, USA) in HTL Resin (Boston Micro Fabrication, USA). The device was embedded in a 36 mm Petri dish, and presented the two microfluidic channels, with slanted inlets and outlet channels to facilitate loading and aspiration of media from the port (Fig. 2A). The microfluidic chambers were contained in a central block of resin, which was surrounded by a void space. This free volume was included to add water during the culture, to maintain the humidity in the dish and avoid media evaporation, as described in the patented design (Fig. 2B). The central chambers were aligned on top of an array of 9 circles, 0.1 mm deep, that correspond to the arrangement of wells in the Primo Vision dish. Circle array and dish wall indentation facilitated the device placement in microscope holder and software initiation. Device roughness was tuned by printing in two directions: parallel and perpendicular to the bottom of the 0.1 mm deep circle array.

B. *Embryo culture and material toxicity test*

A Mouse Embryo Assay (MEA) was performed to quantify the toxicity of the PDMS and the resin used for the fabrication of the devices. As previously described in [14], in a MEA test, mouse embryos are cultured in contact with a new material or using medium exposed to it; embryo development and viability are then evaluated at different stages, from 1 cell to full blastocyst. In this study cryopreserved 1 cell murine embryos (B6C3F1xB6D2F1 strain, Embryotech, USA) were used to test loading efficiency, optical properties, and compatibility with time-lapse system. Briefly PDMS and HTL resin substrates are overlayed with KSOM medium (Millipore, UK) and oil (BioUltra mineral oil from Sigma Aldrich) for four days. The medium was then used to culture embryos in 5 µL drops (3 drops/ 15 embryos each condition × three replicas), in 35 mm hydrophobic IVF certified dishes (Nunc) in a benchtop MINC™ Mini incubator (Cook, Aus) at 37 °C with humidified 5% CO_2, 5% O_2, 90% N_2. Microdrops of pre-equilibrated KSOM medium were used as control (5 µL drops, five embryos/drop, three replicas). Cleavage, blastocyst, and hatching rate were quantified and expressed as mean ± standard deviation, compared by Student's *t*-test, with significance determined as $p < 0.05$.

C. *Material roughness analysis*

AFM microscopy (Bruker Dimension Fastscan by Bruker, USA with SCOUT 350 HAR silicon probe by NuNano, UK in tapping mode in air) was used to evaluate the roughness and quantify the level the artefacts introduced in the fully printed devices. 900 μm^2 scan images were collected to compare the superficial roughness of the substrates printed with a 0 and 90 angle between the printing planes and the bottom of the 0.1-deep circle array.

D. *Device optical performances assessment*

Polystyrene beads (PPX-800-10, Sphero™, USA), cryopreserved 1 cell murine embryos (B6C3F1xB6D2F1 strain, Embryotech, USA) and freshly IVF derived bovine embryos were used to test loading efficiency, optical properties, compatibility with time-lapse system, inverted microscopes, and fluorescent microscopy. Six embryos were stained for actin filaments and are imaged using an inverted microscope (Nikon ECLIPSE Ti2, Nikon instruments, USA) and LED illumination system (pE-4000 CoolLED, MA, USA) to observe image

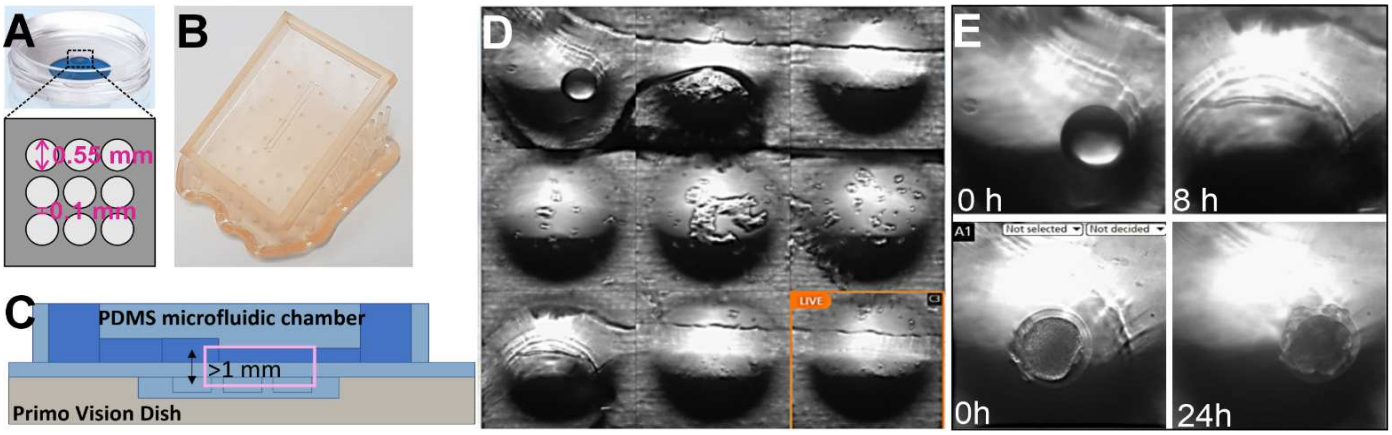

Fig. 1. PDMS device assembly and testing: A) Commerically available PrimoVision dish, presenting an array of wells for accurate positioning of individual embryos and imaging (inset); wells are ~550 µm in diameter with 100 µm distance. B) Mould printed by low resolution stereolithography in BioMed Amber resin. C) cross section of the assembled device showing the microfluidic channels and chamber in dark blue, the PDMS layers in light blue and the PrimoVision dish in polystyren in gary at the bottom; the distance between the bottom of the microfluidic chamber and the well in the dish is 1 mm. D) image acquisition with the PrimoVision microscope when a single bead is loaded in the top left chamber. Defects and roughness of the PDMS surfaces are visible. E) Top row shows two selected images of the same beads at t=0 s and after eight hours, when the microscope lost the focus; the bottom row instead shows two selected images of a 1 cell embryo loaded on in the microfluidic channel, where characteristics features of the embryos, such as the zona pellucida, can be identified; on the last image the same embryo can be detected after 24 hours, when is developed into eight cells.

quality and transparency of the plastic in bright field and FITC channel.

cases impaired by the optical artefacts due to the roughness of the surfaces. In the first iteration, the device was printed with the

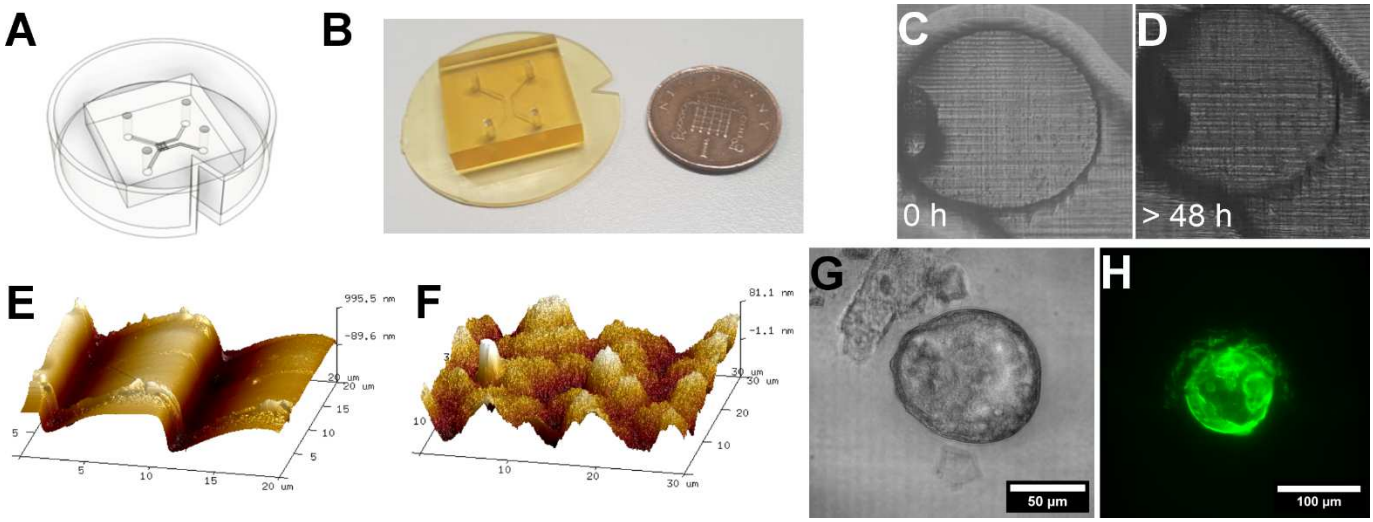

Fig. 2. Fully printed device in HTL resin. A) Schematic of the device, with the indentation in the external dish wall and the two microfludiic chambers with the slanted inlet and outlet channels are visible; B) the fully printed device is shown next to a 1 penny coin as reference. The HTL resin has a typical yellow colour; C-D) Selected images of a timelapse sequence of a bead loaded in the microfluidic chamber, which was kept in focus for more than 48 hours. The images show the traces of the 3D printing which do not allow a clear imaging of the features. E-F) AFM images of the chamber bottom surface printed with 90° and 0° orientation respectively; the 3D image highlight the roughness reduction in the two settings. G) Image of an emrbyo captured in brightfield, confirming the good transparency of the resin and the limited effect of the roughness of the surface (scale bar: 50 µm); H) same embryo, fixed in paraformaldeide and stained for actin filaments in green, to confirm the transparency of the resin in FITC channel (scale bar : 100 µm).

III. Results

A. *Indirect" PDMS devices fabrication and assembly*

The fabrication method required two steps, firstly to cover the array of wells on the dish and secondly to add the microfluidic compartments. The microscope starts the image acquisition when recognizes the array of wells and uses those as areas of interests for the automated time-lapse sequence recording (Fig. 1D). The assembly of the device required a precise alignment of the microfluidic chambers with the wells underneath. This step was completed with a very low yield (<20%). This first manufacturing approach allowed to assemble a fully transparent device, and to maintain the original structures of the Primo Vision dish as reference for the initialization of image acquisition. The microscope effectively recognized these features and thus the region of interest in the X-Y plane. However, the thickness of the PDMS layer and the distance between those wells in the dish and the microfluidic chambers (1 mm) was bigger than the expected range of inspection in Z. The microscope not always identified the bead or the embryos and allowed to maintain the focus for a maximum of 24 hours. Because of the defects introduced on the surface of the mould by the 3D printing and replicated on the PDMS surfaces, the microscopes often focused on the wrong features, lost focus rapidly or encountered an error (Fig. 1E).

B. *"Fully printed" device in resin fabrication and roughness*

The use of a micro-stereolithographic printer for the fabrication of the device significantly simplified the assembly of the device, from three to one step. The fully printed device was easily positioned in the PrimoVision microscope. The microscope identified and focused on the embryos for the entire culture, however the initialization process was in 50% of the printing planes perpendicular to the bottom of the 0.1 mm-deep circles to minimize the bubble formation between top surface of the device and the membrane through which the UV light is projected into the resin for polymerization. While this approach effectively minimized the artefacts caused by the bubbles, the printing layers were visible under bright field microscopy (Fig. 2C) complicating the loading of the embryos. When loaded, the PrimoVision microscope could maintain the focus on the device for more than 48 hours, but printing artefacts did not allow for the recognition of any morphological features (Fig. 2D). A printing orientation parallel to the bottom of the circle array, eliminated these artefacts and reduced the surface roughness from 21 ± 10nm (perpendicular orientation, Fig. 2E) to 10 ± 3 nm (parallel orientation, Fig. 2F). Thanks to these modifications, embryo loading, and visualization of the device improved significantly both with inverted microscopy in bright field (Fig. 2H) and with fluorescent microscopy (Fig. 2G).

C. *Embryo toxicity*

The Mouse Embryo Assay (MEA) is the gold standard test used in reproductive toxicology to assess the potential embryo toxicity and teratogenicity of new materials or substances [14]. A material passes the MEA test if minimum of 80% blastocyst rate is recorded at 72 or 96 hours. Both PDMS and HTL conditioned media did not affect initial cleavage, excluding acute toxicity of the materials. From our cultures, PDMS passed the test, confirming previous data shown in [14,15]. The hatching rate was statistically lower for PDMS compared to the control, suggesting a delayed development. Embryos cultured in medium exposure to HTL resin initially developed but less than 25% reached the blastocyst stage, suggesting potential teratogenic effect of the material or of any compounds leaching from it. After 96 hours, less than 20% blastocysts hatched. As hatching is a critical step for successful implantation of the

embryo into the uterus, a reduced hatching rate suggests a

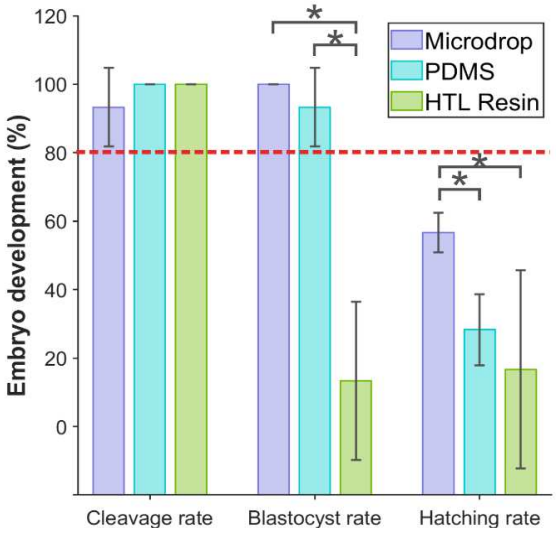

Fig. 3. MEA results: Cleavage rates (% on total embryos) for embryos cultured in microdrops (purple, first column, 93.3 ± 11.5), in PDMS (light blue, second column, 100%) and in HTL Resin (green, third column, 100); Blastocyst rates (% on cleavaged embryos) for embryos cultured in microdrops (purple, first column, 100), in PDMS (light blue, second column, 93.3 ± 11.5) and in HTL Resin (green, third column, 3.3 ± 23.1); Hatching rates (% on blastocysts) for embryos cultured in microdrops (purple, first column, 56.7 ± 5.8, PDMS (light blue, second column, 28.3 ± 10.4) and in HTL Resin (green, third column,16.7 ± 28.9). Values expressed as men ± standard deviation. * for $p<0.05$.

delayed development, and lower implantation potential. This high toxicity of the resin poses a challenge for the use of the material and the prototyping technique for testing new concepts or for the evaluation of new design efficacy.

IV. Conclusions

This work shows a first example of microfluidic device for embryo culture to be used in IVF procedures in combination with time-lapse microscopy.

Embryos culture *in vitro* requires extreme control of environmental settings and exclusion of toxic materials, which can affect the health, development and implantation potential of the embryo. Our results confirm the need for a rapid prototyping method to test new concepts and designs that can support more specific analysis of the embryo during the culture and facilitate handling and manipulation *in vitro*. However, we evidenced the need for high resolution stereolithographic techniques to reduce the roughness of 3D printed parts, such as moulds for soft lithography. Defects introduced on the surface inevitably introduce artefacts in the images which obstacle the automatic image acquisition and limit the resolution. These challenges can be partially overcome by changing orientation during printing. In terms of materials, we confirmed that PDMS remains the best option in terms of optical transparency. It also does not significantly alter the embryo development during the 3-4 days culture; protocols for limiting leaching of toxic chemicals and absorption molecules have been proposed by different groups and can be adopted for the prototyping and initial testing of new devices. Resins and stereolithography presents clear advantages in terms of time for manufacturing and assembly but results toxic as raw material for embryos, as evidenced with other materials such as (PIC100, E-Shell200, and E-Shell300) [16].

Acknowledgment

The work was supported by Medical Research Council (MRC) Confidence in Concept scheme, UKRI Impact Acceleration Accounts and Wellcome Institutional Translational Partnership award. We would like to thank Prof. Valdastri, Dr. Simone Calo', and Mr. Samwise Wilson for access and support with the Boston Microfabrication Printer in the STORM lab of the University of Leeds and Miss Emily Darby for her guidance with the PrimoVision microscope.

References

[1] "Fertility treatment 2021: preliminary trends and figures", https://www.hfea.gov.uk/ June 2023

[2] ESHRE ART fact sheet (2022). Available at: https://www.eshreeu/Europe/Factsheets-and-infographics.

[3] J.E. Swain, G.D. Smith, "Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment", Human Reproduction Update, vol. 17, issue 4, pp. 541–557, July-August 2011.

[4] J.C.M. Dumoulin, C.J.J. Meijers, M. Bras, E. Coonen, J.P.M. Geraedts, J.L.H. Evers, "Effect of oxygen concentration on human in-vitro fertilization and embryo culture", Human Reproduction, vol. 14, issue 2, pp. 465–469, February 1999.

[5] J.E. Swain,"Optimizing the culture environment in the IVF laboratory: impact of pH and buffer capacity on gamete and embryo quality", Reproductive BioMedicine Online, vol. 21, Issue 1, pp. 6-16, 2010.

[6] J.E. Swain, D. Carrell, A.Cobo, M. Meseguer, C. Rubio, G.D. Smith, "Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential", Fertility and Sterility, vol. 105, issue 3, pp.571-587, 2016.

[7] M. Lane, D.K.Gardner, "Embryo culture medium: which is the best?", Best Pract Res Clin Obstet Gynaecol., 21(1):83-100, Feb 2007.

[8] J.E. Swain, D. Lai, S. Takayama, G.D. Smith, "Thinking big by thinking small: application of microfluidic technology to improve ART", Lab Chip, 7;13(7):1213-24, Apr 2013.

[9] Sakkas, Denny, and David K. Gardner. "Evaluation of embryo quality analysis of morphology and physiology." *Textbook of Assisted reproductive techniques*. CRC Press, 2017. 225-242.

[10] S.S. Kang, S. Ofuji, K. Imai, W. Huang, K. Koyama, Y. Yanagawa, Y. Takahashi, M. Nagano, "The efficacy of the well of the well (WOW) culture system on development of bovine embryos in a small group and the effect of number of adjacent embryos on their development", Zygote, 23(3):412-5, Jun 2015.

[11] Y. Guo, Y. Liu, L. Qi, W. Song, H. Jin, "Can Time-Lapse Incubation and Monitoring Be Beneficial to Assisted Reproduction Technology Outcomes? A Randomized Controlled Trial Using Day 3 Double Embryo Transfer", Frontiers in Physiology, vol. 12, 2022.

[12] Y. Sawada, et al., "Evaluation of artificial intelligence using time-lapse images of IVF embryos to predict live birth" Reprod Biomed Online, 43(5):843-85, Nov 2021.

[13] V. Mancini, et al., "Probing morphological, genetic and metabolomic changes of in vitro embryo development in a microfluidic device", Biotechnology Progress, 37, 6, pp. 8756-7938, 2021.

[14] F. Colucci, P. McKeegan, H.M. Picton, V. Pensabene, "Mouse embryo assay to evaluate polydimethylsiloxane (PDMS) embryo-toxicity", Annu Int Conf IEEE Eng Med Biol Soc. 2018, 4484-4487, July 2018.

[15] T. C. Esteves, F. van Rossem, V. Nordhoff, S. Schlatt, M. Boiani, and S. Le Gac, "A microfluidic system supports single mouse embryo culture leading to full-term development," RSC Adv., vol. 3, no. 48, p. 26451, 2013.

[16] de Almeida Monteiro Melo Ferraz, Marcia et al. “Potential Health and Environmental Risks of Three-Dimensional Engineered Polymers.” Environmental science & technology letters vol. 5,2, 80-85, 2018.