PRINTING HUMAN TISSUE

Artificially produced skin, livers, and intestines emerge as an alternatives for cosmetic testing on animals

Sarah Schmidt

This chip partially simulates the human organism for testing the efficacy and safety of cosmetics

From above, this device looks like an old cassette tape. The difference is that instead of having two holes, it can have three or four, each of which holds reconstructed human tissues: skin, intestines, and liver. A fluid with nutrients and oxygen circulates between the orifices to simulate the blood circulation, causing the tissues function as interconnected mini-organs.

Cosmetics and pharmaceutical companies in other countries have used these devices, known as human-on-a-chip or body-on-a-chip (BoC), to assess the toxicity of their products under development, and these devices are now gaining ground in Brazil. The 3D printing technique (*see* Pesquisa FAPESP *issue no. 276*) employed to prepare skin and intestinal tissues has also been applied experimentally for other purposes (*see table on Page 57*), while liver tissue is still produced manually.

"We apply the test-ready ingredient to reconstituted skin and evaluate its toxicity, simulating human body function," explains biologist Juliana Lago, a researcher in preclinical assessment for cosmetics giant Natura, which adopted this technology in the first half of 2023.

Imported from a German corporation, BoC joins other techniques that have been used since 2006 for safety and efficacy testing of beauty, personal hygiene, and perfume products as substitutes for animals, as animal testing was prohibited in March 2023 by the Brazilian Animal Experimentation Control Board (CONCEA) of the Ministry of Science, Technology, and Innovation (MCTI) (*see* Pesquisa FAPESP *issue no. 245*).

In addition to indicating that they experience harm caused by the external agents, the

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tissues filling the chip cavities replicate certain functions of the organs themselves. "The mini-liver produces bile [a yellow–green fluid that facilitates the absorption of fats and vitamins] and carries out all the processes of detoxification [outer layer] and release of mucus [a white or yellowish fluid that facilitates the elimination of feces]," describes biologist Ana Carolina Figueira of the Brazilian Biosciences National Laboratory (LNBio), an arm of the Brazilian Center for Research in Energy and Materials (CNPEM) in Campinas, São Paulo State. Figueira coordinated the project, integrating the use of the chip with other tissues, in collaboration with Natura.

In 2023, CNPEM licensed the production technology and sale rights of the liver and intestinal tissues for the startup 3D Biotechnology Solutions (3DBS), which is also based in Campinas. In exchange, in addition to paying royalties, the company helped refine the process for intestinal tissue production via 3D bioprinting and shared the human skin production method.

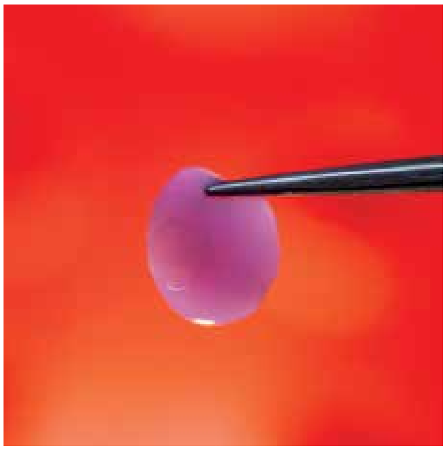
PRODUCTION OF ARTIFICIAL TISSUES

At 3DBS, the intestinal tissue is made from cells purchased from the Rio de Janeiro Cell Bank (BCRJ), and the skin is produced from human cells isolated from tissues after phimosis surgery (removal of excess foreskin from the penis) performed on children at a hospital in Santa Bárbara D'Oeste in the interior of São Paulo State. "Cells discarded after surgeries on children rapidly produce type-I collagen, a protein that we need to give the skin strength and elasticity," explains Ana Luiza Millás, the company's research director.

A solution with different types of cells is the raw material used in the so-called bioprinters, which create three-dimensional structures composed of live cells, molecules, and biocompatible materials. In this case, instead of the plastic material that is injected by conventional 3D printers to create an object, a syringe can, for example, discharge cells mixed with a collagen solution onto a transparent tray with internal compartments, such as those used to make ice in the freezer. A computer sends information regarding the layer-by-layer dimensions and format of the tissue to be constructed to the device.

Reconstructed intestinal tissue forms a circular layer 12 millimeters (mm) in diameter inside the plate compartments and is then kept in an incubator at 37 degrees Celsius (°C) for 21 days. During this time, the cells differentiate to form the intestinal lining, which absorbs nutrients and produces mucus. When ready, the tissues can be used within up to one week.

As soon as it leaves the bioprinter, the skin cell mixture needs 10 days in the incubator to adopt its final form, which is small, pinkish gelatinous discs approximately 6 mm in diameter. "In this period, the cells form five layers in the dermo-epidermal model, known as full human skin. Another simpler model, known as recon-



Manually reconstructed liver tissue (*left*) and human skin produced by bioprinting

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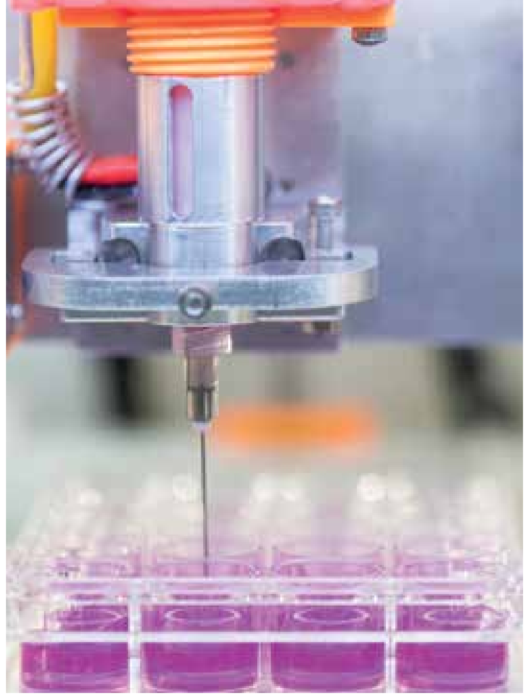

Ana Luiza Millás of 3DBS operates a bioprinter; in a close-up, the syringe deposits the cell solution into the compartments of a tray

structed human epidermis (RHE), has only the epidermal layer and is used for cosmetics safety and efficacy testing," says Millás, who has been researching human tissue reconstruction since 2010 with the initial aim of creating skin for regenerative medicine. During her doctorate studies at the University of Campinas (UNICAMP), supported by FAPESP, she worked with a substance extracted from the diesel tree (*Copaifera langsdorffii*), native to Brazil, which, when incorporated into ultrafine fibers, can be used as a three-dimensional cutaneous substitute (*see* Pesquisa FAPESP *issue no. 226*).

New directions in this research led to skin production via bioprinting, which was developed with specialists from the University of São Paulo (USP) and Natura and described in a March 2019 article in the publication *International Journal of Advances in Medical Biotechnology*.

"Initially, we were producing larger skin masses with twice the current diameter, but corporations and research centers prefer smaller tissues in lower quantities at a lower cost for toxicology testing," says biologist °C Gabriela Gastaldi, a researcher at 3DBS.

The liver tissues are still produced manually using cells imported from overseas and from the Rio de Janeiro bank, which are steeped in an agarose gel and placed in molds with 81 orifices. After five days in the incubator, the cells are bound together to form circular cell aggregates known as spheroids, which were approximately 300 micrometers (µm) in diameter and visible to the naked eye.

These tissues have been sold by the company since 2022, with 80% of the turnover coming from the bioprinters and electrospinning equipment, which have been produced since 2018 at the 3DBS workshop in São Paulo. In wider Brazil, 3DBS also distributes chips and pumps that circulate nutrients, which have been manufactured since 2019 by the German corporation Tissue-Use, whom 3DBS represents in the country. "We are invested in the growth of tissue and chip use in view of the need to standardize toxicity tests and other possible applications in the early stages of emergence," observes business administrator and Strategy and Innovation Director Pedro Massaguer. ■

OTHER APPLICATIONS

At the National Service for Industrial Training (SENAI) Manufacturing and Technology Integrated Campus (CIMATEC) in Salvador, Bahia State, materials engineer Josiane Barbosa uses a 3DBS bioprinter to test different formulations of meat produced from bovine or vegetable protein cells. "Bioprinting facilitates the reproduction of products with the required dimensions and geometry. This also helps with cell adhesion, given the layered arrangement, which is more difficult to achieve using manual techniques," she says.

At the beginning of October, the Brazilian Agricultural Research Corporation (EMBRAPA) Genetic Resources and Biotechnology wing in Brasília transformed vegetable-based ingredients such as soy flour, fava beans, and chickpeas into fish fillet substitutes. If successful, this research may result in the production of new foods, which would be primarily geared toward the vegetarian and vegan markets.

There have also been other advances in this area. In a study published in *Science Advances* in October, Brazilian and American researchers reported the development of skin tissue with structures similar to hair follicles by means of bioprinting. If this technique advances, it may provide cells that could help with wound treatment or with performing grafts, given that it is the follicle base cells that initiate healing.

The scientific projects and articles consulted for this report are listed in the online version.