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Artificial skin

Skin replacements could be used as grafts to treat burns and severe lesions

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Approximately one million burn injuries are recorded in Brazil each year. Of these individuals, 10% seek medical attention at hospitals and 2,500 die. Accidents involving fire are the second leading cause of infant death in Brazil and the United States. Therefore, the production of skin replacements in the laboratory for use as skin grafts has been a major focus of research in the last 30 years. Scientists in many countries are attempting to develop a type of artificial skin that can be successfully applied to individuals with severe injuries. Here, in Brazil, the work conducted by a team of researchers at the University of Campinas (Unicamp) is noteworthy. In laboratory tests they have proved the effectiveness of a three-dimensional skin replacement produced using a substance extracted from a tree native to Brazil, the copaiba (*Copaifera langsdorffii*). Its development began during the course of doctoral studies in biology by Ana Luiza Garcia Millás, who is with the Department of Materials and Bioprocess Engineering at Unicamp's School of Chemical Engineering. She has a FAPESP fellowship and in September her study was awarded first prize for innovation at the 8th National Innovation Meeting on Drugs and Medicines sponsored by the Institute of Research and Development in Drugs and Medicines in conjunction with the Brazilian Pro-Technological Innovation Society (PROTEC).

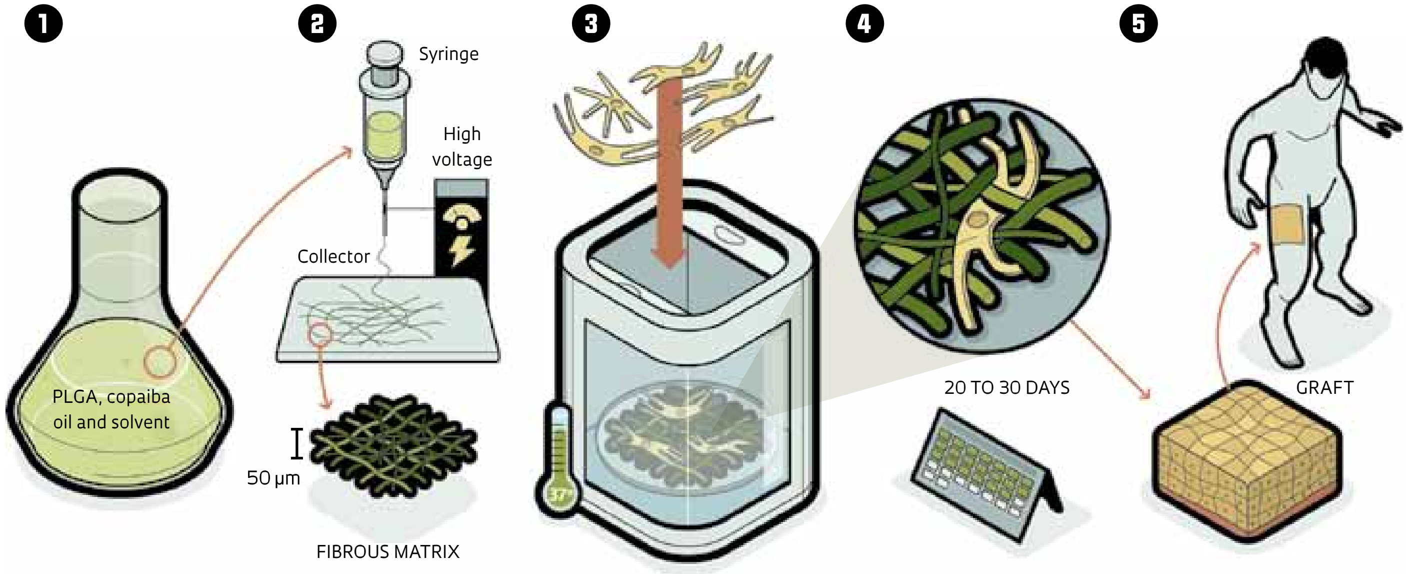
"The treatment of burns and extensive and severe skin lesions is a challenge for regenerative medicine. There are some skin replacement alternatives, but none meets 100% of the demand and the need for proper healing of scars. Our goal is to create an artificial skin that can be absorbed by the body and solve chronic problems such as ulcers, deep scars and third-degree burns," says Millás. "We want to develop a 3D skin replacement, which, in addition to its reparative role, also has a regenerative function, is aesthetically pleasing, and helps the healing process."

The new artificial skin is produced from a solution consisting of the absorbable polymer PLGA (poly-lactic-co-glycolic acid), copaiba oleoresin

Copaiba trunk: raw material for oleoresin, which facilitates skin regeneration in the treatment of burns

Synthetic graft

Principal stages of the development of the graft to be used in skin implants



POLYMER SOLUTION
The first step is the preparation of a solution consisting of bioreabsorbable polymer pellets of poly (lactic-co-glycolic acid) (PLGA), copaiba oleoresin and a solvent

ELECTROSPINNING
The polymer solution is placed in a syringe and converted into fiber via the electrospinning technique.
The result is a fibrous matrix (or scaffold) formed by filaments. The fibrous polymeric scaffold is sterilized with gamma or ultraviolet rays

CELL CULTURE
In an oven at 37 degrees Celsius that allows gas exchange, the patient's fibroblasts—the cells responsible for collagen synthesis—are placed on the scaffold. After attaching to the substrate, they grow, proliferate and differentiate

GROWTH
The pore size of the fibrous matrix allows fibroblasts to migrate and proliferate within it, through connection to each other and growing in layers to form a three-dimensional structure. This process takes 20 to 30 days

IMPLANT
Finally, the artificial skin (or replacement skin) formed by the combined polymeric scaffold and dermal cells is ready to be implanted in patients with severe skin lesions, such as third-degree burns, ulcers and bedsores

SOURCE ANA LUIZA GARCIA MILLÁS / UNICAMP

and a solvent. PLGA, which is widely used in the manufacture of implants, is gradually degraded and absorbed by the body. Once the polymer solution is ready, it is converted into a fiber through a technique known as electrospinning. The structure resulting from this process, referred to as a scaffold, will serve as a support or a three-dimensional cellular frame mimicking the architecture of the skin. Then, fibroblasts, a type of cell found in the dermis (the deepest part of the skin) are withdrawn via biopsy from the burned patient and grown on the fibrous structure, which a few days later is implanted in the patient.

According to Benedicto de Campos Vidal, emeritus professor at Unicamp's Institute of Biology and an expert on collagen, the in vitro results achieved to date are very promising and have led to important findings: the cells are adhering, proliferating, differentiating and apparently producing collagen, a key protein in the healing process. "Everything indicates that fibroblasts are resulting in a collagen matrix. This is key to the success of the research," says Vidal. The new cell structure functions as a support that allows the epidermis, the uppermost component of the skin, to proliferate. In addition to working with the patient's own cells, Millás also plans to use fibroblasts from third parties. "The advantage of using cells taken from others is the ability to produce artificial skin on a large scale for a skin bank. The downside is the increased risk of rejection."

A key aspect of this research is the electrospinning technique, which has attracted interest in the field of tissue engineering owing to its ability to produce ultrafine fibers with a high surface-

INFOGRAPHIC ANA PAULA CAMPOS ILLUSTRATION ALEXANDRE AFFONSO

to-volume ratio without the need for expensive and complex instrumentation. It is applicable to a wide variety of both natural and synthetic polymers and is also noteworthy for allowing control of the diameter, porosity and topography of the filaments. Furthermore, it improves the efficiency of the transport of nutrients between the fiber matrix and the external environment.

Another research innovation involves embedding a natural substance with proven, but insufficiently studied, therapeutic properties in the skin replacement. Copaiba oleoresin, which has been used for medicinal purposes since the 16th century, acts as a healing agent with analgesic, anti-inflammatory and antimicrobial properties. "This is an innovative aspect of the work, along with using a polymer to produce the matrix that will be applied to the lesion," says Dr. Beatriz Puzzi, a dermatologist and coordinator of the Skin Cell Culture Laboratory of Unicamp's School of Medicine and Millás's doctoral supervisor. Embedding the copaiba oil into the matrix has a functional aim: to facilitate skin regeneration in the burn area. According to Millás, the substance collected from the trunk of the tree is referred to as an oleoresin because its composition is approximately 45% volatile essential oils and 55% resin.

In vitro tests have shown that the material is biocompatible. The next step is clinical trials in humans

SKIN PRINTER

Preclinical testing in animals and clinical trials in humans have not yet been performed, but the group already sees an opportunity to produce the material on a larger scale, using 3D digital printers in combination with the electrospinning technique. The idea of using such printers arose from the need to scale up production of the material and to handle the requirements of the scaffold for the implant. "We have done some tests combining the two techniques, 3D printing and electrospinning. It may be an alternative because the matrices are extremely fragile and difficult to handle," says Millás. "In vitro tests have already shown that the material is biocompatible and has great potential. I believe that clinical trials can be started within two years, and, if successful, marketing can begin in five."

The Unicamp innovation is similar to two products from American companies: Apligraf®, produced by Organogenesis, and OrCel®, produced by Forticell Bioscience. Both use bovine collagen and human fibroblasts. Millás's research grew out of a study begun during work toward her master's degree in 2010, entitled "Using Electrospinning Technology for the Production and Characterization of Cellulose Nanofibers Embedded with Natural Oil." This work led to a patent that calls for the use of fibers produced via electrospinning technology and embedded with essential oils not only as artificial skin and dressings but also as filters, fabrics and packaging for food and cosmetics. The development of the skin replacement was aided by a team of chemical engineers, including Professor Edison Bittencourt of Unicamp's School of Chemical Engineering, who is Millás's doctoral adviser, and John Vinícios Silveira, in addition to professors Maria Beatriz Puzzi and Benedicto Vidal, also of Unicamp.

A portion of the development of the artificial skin was carried out abroad. In 2012, Millás received funding for her graduate studies from the international mobility scholarship program of Banco Santander; she completed a sandwich program, in which part of her studies were conducted in England. "Bob Stevens was my adviser. He's a scientist and professor at Nottingham Trent University and a research collaborator at The Electrospinning Company. This company uses an electrospinning platform to develop fibrous biomaterials for regenerative medicine. While I was at the company, I made the decisions about which polymer to use and established all the criteria for the solutions and electrospinning equipment for producing the scaffolds. I also performed preliminary in vitro tests using primary lung fibroblasts." In 2013, Millás completed another sandwich program, this time with the Science Without Borders program at Cornell University in the United States. ■ Yuri Vasconcelos

Project

Development of bioactive scaffolds embedded with vegetable oils for skin tissue regeneration using electrospinning technology (No. 2012 / 09110-0); **Grant mechanism** Scholarship in Brazil - Regular - Doctorate; **Principal investigator** Edison Bittencourt (Unicamp); **Fellowship** Ana Luiza Garcia Millás (Unicamp); **Investment** R$116,615.19 (FAPESP).

Scientific article

Yusuf, M. *et al.* Platinum blue staining of cells grown in electrospun scaffolds. **Biotechniques**. V. 57, Nº. 3, p. 137-41. September 2014.