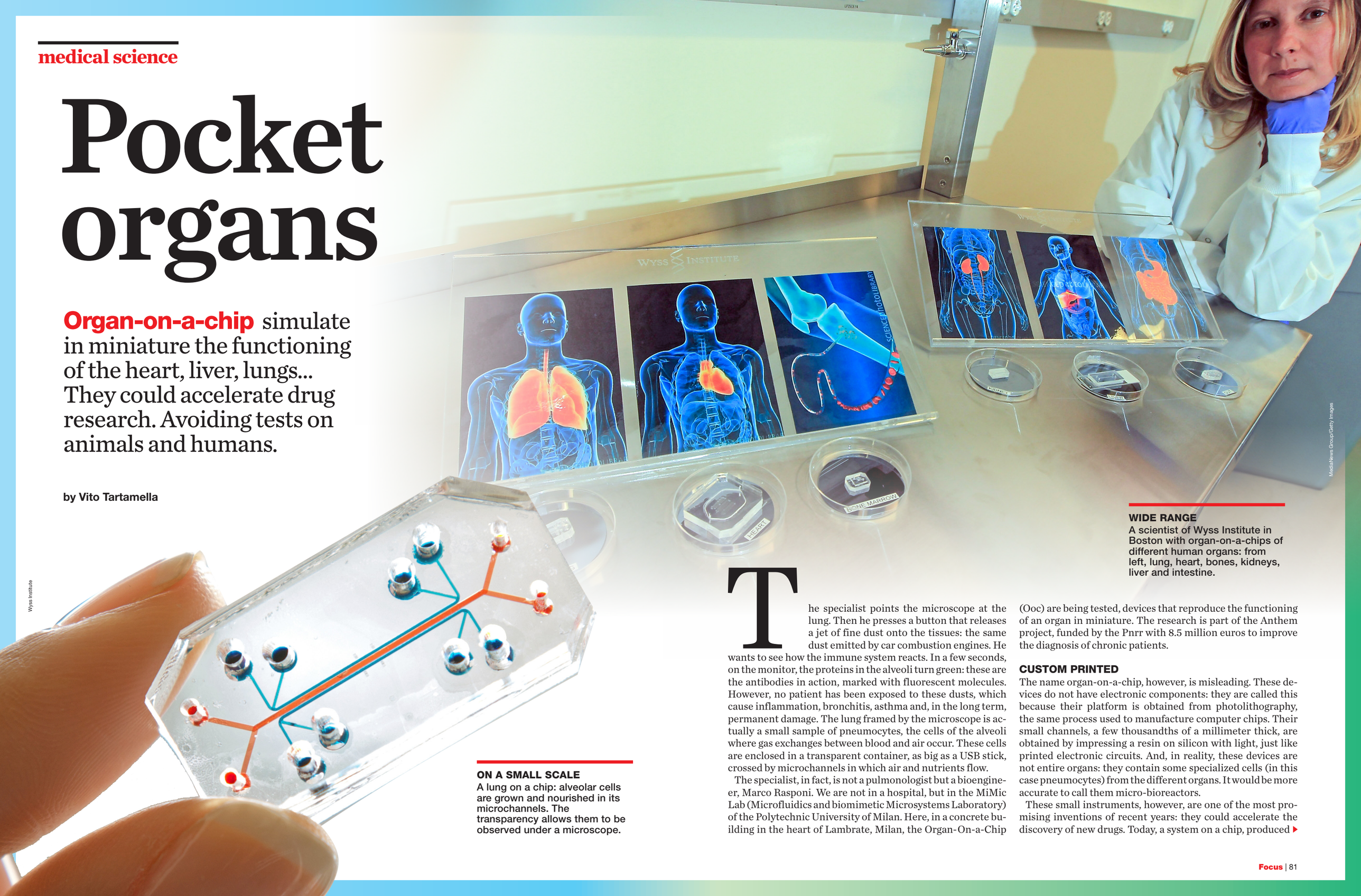
Pocket organs

Organ-on-a-chip simulate in miniature the functioning of the heart, liver, lungs... They could accelerate drug research. Avoiding tests on animals and humans.

by Vito Tartamella



Wyss Institute

ON A SMALL SCALE
A lung on a chip: alveolar cells are grown and nourished in its microchannels. The transparency allows them to be observed under a microscope.

WIDE RANGE
A scientist of Wyss Institute in Boston with organ-on-a-chips of different human organs: from left, lung, heart, bones, kidneys, liver and intestine.

MediaNews Group/Getty Images

The specialist points the microscope at the lung. Then he presses a button that releases a jet of fine dust onto the tissues: the same dust emitted by car combustion engines. He wants to see how the immune system reacts. In a few seconds, on the monitor, the proteins in the alveoli turn green: these are the antibodies in action, marked with fluorescent molecules. However, no patient has been exposed to these dusts, which cause inflammation, bronchitis, asthma and, in the long term, permanent damage. The lung framed by the microscope is actually a small sample of pneumocytes, the cells of the alveoli where gas exchanges between blood and air occur. These cells are enclosed in a transparent container, as big as a USB stick, crossed by microchannels in which air and nutrients flow.

The specialist, in fact, is not a pulmonologist but a bioengineer, Marco Rasponi. We are not in a hospital, but in the MiMic Lab (Microfluidics and biomimetic Microsystems Laboratory) of the Polytechnic University of Milan. Here, in a concrete building in the heart of Lambrate, Milan, the Organ-On-a-Chip (Ooc) are being tested, devices that reproduce the functioning of an organ in miniature. The research is part of the Anthem project, funded by the Pnrr with 8.5 million euros to improve the diagnosis of chronic patients.

CUSTOM PRINTED

The name organ-on-a-chip, however, is misleading. These devices do not have electronic components: they are called this because their platform is obtained from photolithography, the same process used to manufacture computer chips. Their small channels, a few thousandths of a millimeter thick, are obtained by impressing a resin on silicon with light, just like printed electronic circuits. And, in reality, these devices are not entire organs: they contain some specialized cells (in this case pneumocytes) from the different organs. It would be more accurate to call them micro-bioreactors.

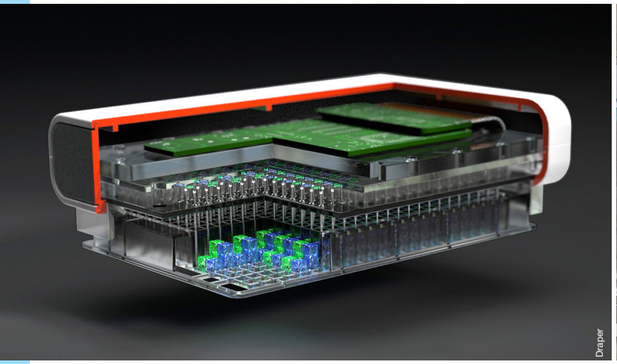
These small instruments, however, are one of the most promising inventions of recent years: they could accelerate the discovery of new drugs. Today, a system on a chip, produced ▸

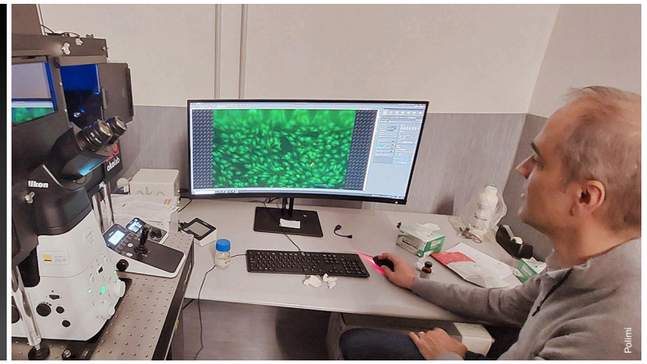
THE STEPS TO DEVELOP A NEW DRUG

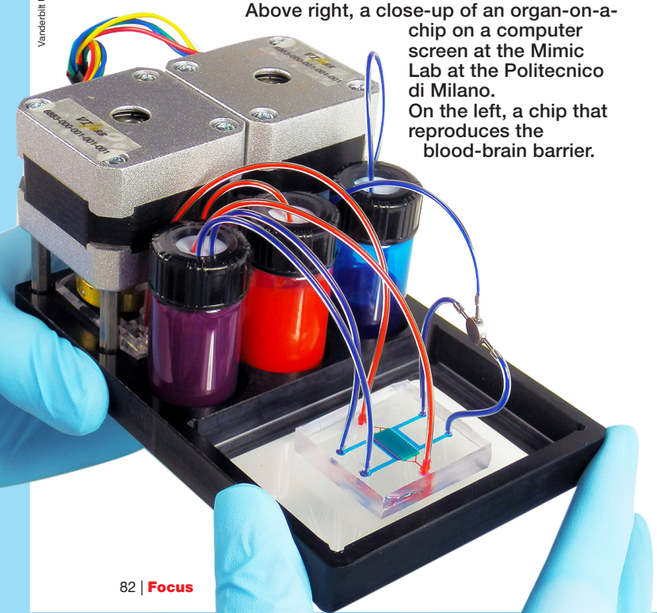
Once a promising molecule is found, these steps are followed:

1 SCREENING	2 IN VITRO TESTS		
Once a possible therapeutic target (protein, receptor, enzyme) has been identified, thousands of chemical compounds are tested in digital libraries to evaluate which ones can have an effect on the target.	**2D coltures:** a Petri dish is used, a glass or plastic dish, on which human cells are grown. They are made to react with the new molecule to understand more effective doses and formulas.	**Organoid**: cultivation of human cells on a 3D support (hydrogel) that mimics the structure of an organ, on which the effects of new molecules are observed.	**Organ-on-chip:** the behavior of human cells on a nanometric scale is observed, fed by fluids (blood) and gases (O_2, CO_2).
Disadvantages: Our knowledge of human physiology is limited to allow reliable a priori predictions.	Disadvantages: Only allows you to observe simple interactions between cells.	Disadvantages: It involves greater technical difficulties.	Disadvantages: Complicated and expensive to manage, lack of shared standards.

3 ANIMAL TESTING	4 CLINICAL TESTS	5 APPROVAL	6 MONITORING
The effect of a new drug is tested on a live animal (rats, domestic chickens, rabbits, monkeys) to evaluate its toxicity.	**Phase 1:** the safety of a drug is tested on 20-100 people (1 year). **Phase 2**: the effectiveness of the drug is verified on 100-500 patients (2 years). **Phase 3:** the effectiveness of the drug is compared with a standard treatment or a placebo in 1,000-3,000 patients (4 years).	The regulatory agency (EMA for Europe, FDA for the US) approves the drug's entry onto the market. Typically 12 years have passed since the molecule was identified. And an average of 1-2 billion euros have been spent. 90% of candidate drugs that enter clinical trials fail, due to lack of efficacy or unexpected toxicity.	**Phase 4:** the long-term effects of a drug that has already been approved and placed on the market are monitored.
Disadvantages: the method raises ethical issues (lives are sacrificed). And the results are not automatically applicable to humans.			


Draper


Polimi


Vanderbilt University

MODELS
Above, Predict96, an organ-on-a-chip capable of testing a new drug on 96 tissues simultaneously.
Above right, a close-up of an organ-on-a-chip on a computer screen at the Mimic Lab at the Politecnico di Milano.
On the left, a chip that reproduces the blood-brain barrier.

by the American Draper, can test hundreds of molecules simultaneously on a hundred different tissue samples. Without putting living beings at risk.

Oocs will be able to reveal how diseases develop, avoiding exposure to dangerous substances, such as the fine dust mentioned at the beginning, in which animals or human volunteers are not exposed. Today, in fact, 90% of new drugs that enter clinical trials (*see box above*) fail, due to lack of efficacy or unexpected toxicity. And tests on animals do not always predict lethal effects: in 2016 in France, an experimental drug to treat chronic pain, BIA 10-2474, after being tested on rats and monkeys, was administered to 128 healthy volunteers. One of them died, another 5 suffered permanent neurological damage. OOCs will be able to avoid these risks: during the pandemic, amodiaquine, an antimalarial drug, was tested on these devices, discovering that it inhibits Covid infection.

SMALL CHANNELS

How did we get here? The Polytechnic University of Milan is, with the Joint Research Center of Ispra and the Cnr, the organization that does the most research in the field in Italy. We are the fourth European country in terms of number of publications after Germany, the United Kingdom and the Netherlands. But OOCs come from Harvard University, in the United States. Here in 2004 two Asian researchers, Shuichi Takayama and Dongeun (Dan) Huh, wanted to imitate the functions of the lung on a microfluidic device. Microfluidics studies the behavior of fluids (blood, cellular tissues) on a microscopic scale: that is, it makes liquids flow in small channels that are micrometers thick (thousandths of a millimeter) to see how they behave under the microscope, in a 3D environment similar to capillaries. Rapid tests for Covid work with this technique, for example.

Takayama and Huh wanted to study what happens in the lungs when a virus causes inflammation and the tissues produce more mucus. So they grew lung cells in a microfluidic device on a porous membrane exposed to air. When a virus was inserted into the device, the tissue reacted by producing more mucus that blocked the channels: when air flowed through the channels, it broke these "plugs" producing a sound, a crackling, caused by the death of the cells.

MUSEUM PIECES

The device had attracted the attention of a bioengineer, Donald Ingber, head of the Wyss Institute, a spin-off of the university: «I was amazed», Ingber said. «The mucus plugs, moving through the channels, generated the same crackling that doctors hear with a stethoscope in patients. Any medical student would ask their teacher what makes that sound, but until now it was only known that it is related to the presence of mucus».

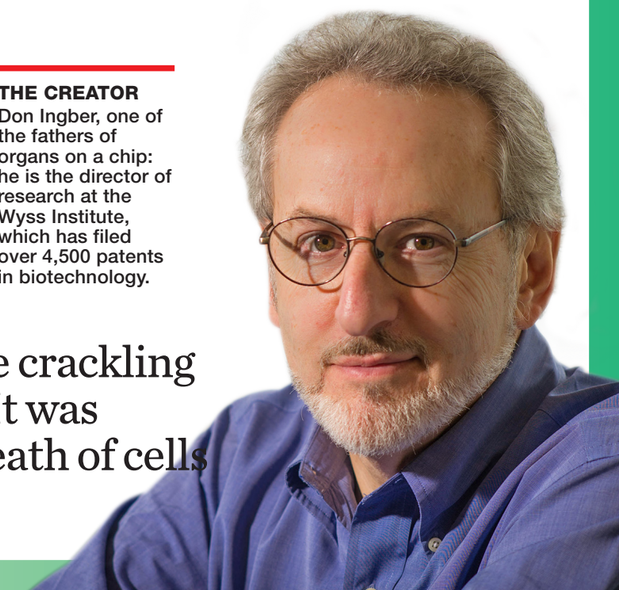
It was a revelation: it was possible to build a miniature model of the lungs, to realistically study how they work. Ingber and Huh focused on the alveoli: they managed to grow their specialized cells, the pneumocytes, exposing them to air and nutrients. And, to reproduce the dilation of the tissues that occurs during breathing, they changed the internal pressure (*see infographic on the next page*). When they introduced some bacteria into the device, the white blood cells phagocytized them, just as happens in the alveoli. The small device, in short, imitated the complex responses that occur in the lungs.

The study, published in the prestigious journal *Science* in 2010, went around the world. In in vitro experiments (the classic Petri dish) you can only grow the cells and make them react with other substances (drugs or pathogens), but the environment is two-dimensional and static. With organ-on-a-chips, on the other hand, microchannels allow the passage of nutrients, oxygen and drugs, simulating blood flow. It opened up the possibility, for the first time, of studying the functioning of an organ without invasive or dangerous procedures. And of preparing personalized drugs.

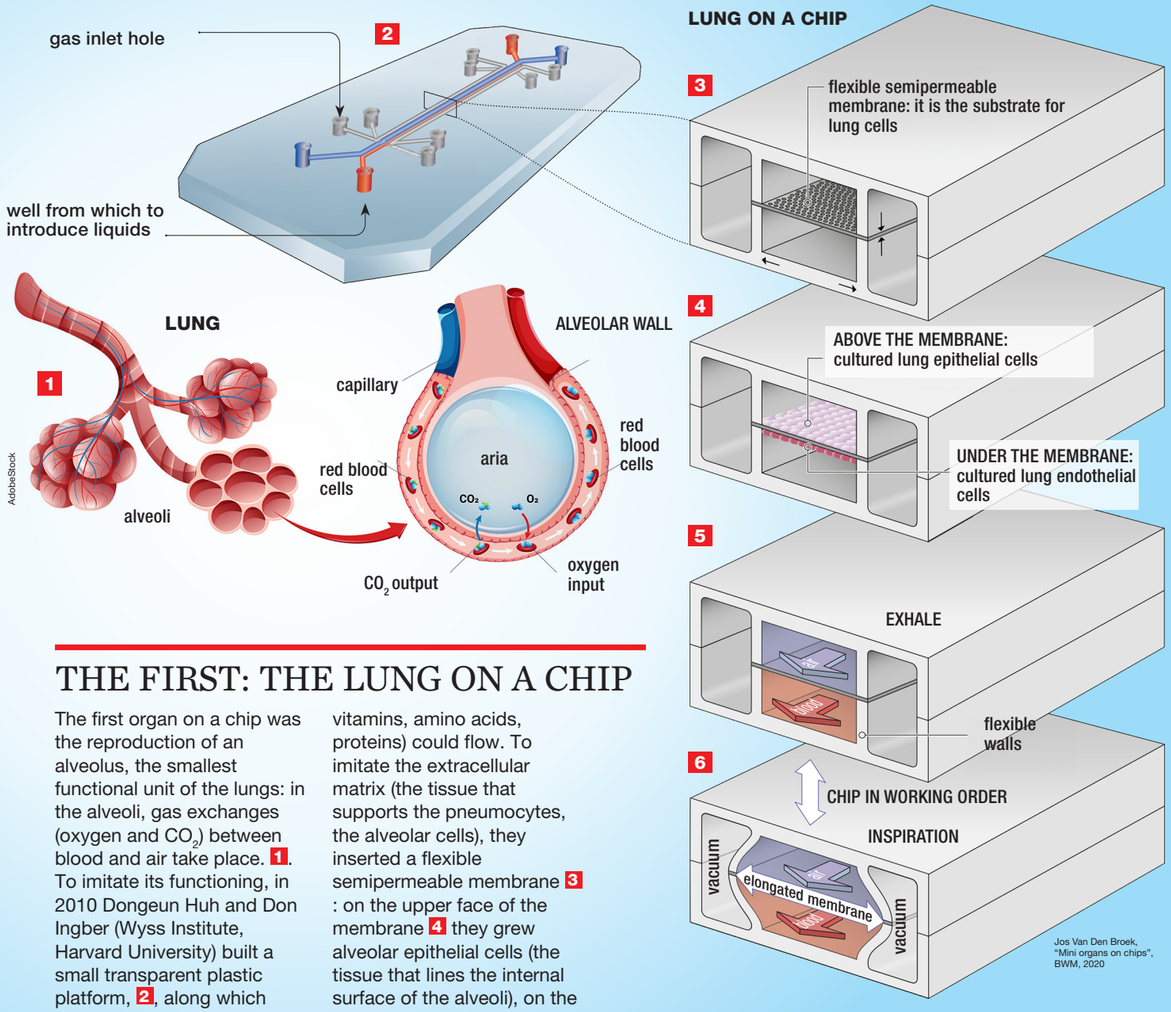
In fact, medicines do not work the same way for everyone: genetic heritage, sex, immune system, can reduce their effectiveness. In the future, however, to treat a heart patient, it will be possible to find the most effective drug by testing various molecules on an OOC with the cardiac cells of that patient. An epochal revolution. And also elegant: in 2015 the organ-on-a-chip prototypes were acquired by the Museum of Modern Art in New York and exhibited in a design show.

The system attracted the attention of two government agencies: NASA and Darpa, the Defense research agency. NASA wanted to find out why Apollo astronauts had lung infections once on Earth, and wanted to study the effects of microgravity on humans: so the OOCs were sent to the International Space Station. Darpa, on the other hand, put 37 million dollars on the table for studies on nuclear and biological accidents, impos- ▸

THE CREATOR
Don Ingber, one of the fathers of organs on a chip: he is the director of research at the Wyss Institute, which has filed over 4,500 patents in biotechnology.



The first model reproduces the crackling sound of mucus in the lungs. It was found to be produced by the death of cells



THE FIRST: THE LUNG ON A CHIP

The first organ on a chip was the reproduction of an alveolus, the smallest functional unit of the lungs: in the alveoli, gas exchanges (oxygen and CO_2) between blood and air take place. 1. To imitate its functioning, in 2010 Dongeun Huh and Don Ingber (Wyss Institute, Harvard University) built a small transparent plastic platform, 2, along which they printed small channels 200 micrometers (0.2 mm) wide, through which air and a blood simulator (saline solution with sugars, vitamins, amino acids, proteins) could flow. To imitate the extracellular matrix (the tissue that supports the pneumocytes, the alveolar cells), they inserted a flexible semipermeable membrane 3: on the upper face of the membrane 4 they grew alveolar epithelial cells (the tissue that lines the internal surface of the alveoli), on the lower face vascular endothelial cells (they line the inside of the pulmonary capillaries, which surround the alveoli). Then they made air and blood simulator flow along the microchannel 5: by applying vacuum to the side walls, they caused the membrane to lengthen 6, thus replicating the expansion of the lungs.

sible to simulate on human volunteers. It thus discovered that cells are not equally vulnerable to radiation: those of the bone marrow are the most sensitive, those of the intestine less so, while those of the lung resist more.

SCHOOL OF HEARTBEATS

So the race began to apply this technology to other organs, reproduced with a certain inventiveness: the intestine chip simulates peristalsis by compressing the internal walls. But for heart cells there is an even more ingenious procedure.

In the Polytechnic Laboratory, Professor Rasponi reports a regular ticking. It comes from a small metal cube, connected to a chip with a small tube: «It's a pneumatic solenoid valve», he explains. «It's used to train the heart cells, the cardiomyocytes, to beat regularly: it provides a flow of compressed air, once a second. The cells, joined by structures that transmit electrical impulses, thus learn to contract in time, synchronizing with each other: it takes a week to train them». The procedure was patented by Rasponi in 2015.

To study knee arthritis, instead, the walls of the device (which contains chondrocytes, cartilage cells) are compressed for two hours with a membrane activated by compressed air. «In this way we simulate the effects of a long walk», says Rasponi, who is vice president of the European Society of Oocs (EuroOocs). Today, almost all organs are reproduced: even the vagina (to study bacterial infections), lymph nodes, the eye, mammary glands, gums... According to Grand View Research, the global OOC market is now worth 157.3 million dollars, and is expected to grow by

TESTING IN PROGRESS
Right, bone marrow chip magnified under a microscope. Below, a scientist at work at Draper, one of the largest biotech companies in the United States. It developed the multi-tissue organ-on-a-chip.


Draper


Nasa

35% per year in the next 5 years, especially in toxicology studies. But where do the cells used in these devices come from? «All it takes is a blood or skin sample from a donor. Their cells are modified using genes that "turn off" the characteristics of the adult cell and "restart" them to a stem state. These stem cells are then grown to become specialized cells (cardiac, renal, hepatic and so on)», explains Rasponi.

What are the next steps? OOCs could help test new therapies for rare diseases, most of which cannot be replicated in animals. OOCs are ideal platforms to overcome this limitation: they require few cells and can replicate the donor's pathologies. «In the European Phoenix project we are developing platforms to study rare diseases of the heart and the neuromuscular system and thus develop targeted therapies», says Rasponi.

REPROGRAMMED CELLS

But in addition to the promises, in these 15 years the technology has shown several limitations: «Although inert substances are used for chips (a plastic, polydimethylsiloxane, ed.) in some cases drugs have reacted chemically with them. Much research is still needed to avoid these risks», observes Ana Lleo De Nalda, a hepatologist at Humanitas in Rozzano who is studying a very aggressive cancer of the bile ducts on chips. «Today chips depend a lot on the operator's ability to cultivate and keep the cells alive. However, they are useful for selecting new molecules to test without risks, especially on diseased tissue». Another limitation is the use of specialized cells obtained from "genetically reprogrammed" stem cells: «They are less differentiated than mature cells, they remain in a state similar to the fetal one. Therefore they can respond to stimuli differently than the original adult cells», warns Christodoulos Xinaris, research coordinator at the Mario Negri Institute in Milan. «Furthermore, it should be remembered that organs are only reproduced to a minimal extent: in a kidney there are at least 28 different types of cells and each one performs a different task, but in a chip only one or two types are tested at a time. And this limit also applies to "body-on-chips", those that connect different organs: they only replicate some aspects of each. In other words, an organism is too complicated to be reproduced on a device. Everyone, researchers and pharmaceutical companies, would like to avoid the use of animals because in addition to the ethical implications, it is expensive and subject to very strict rules. But unfortunately research cannot yet do without living organisms».

They allow studies on toxicity and diseases, but do not yet have validated standards

COMMERCIAL BATTLE

Last September, the FDA approved the first research to study, on a liver on a chip, the damage induced by drugs. But it is an isolated case: the FDA and the European Medicines Agency (EMA), while hoping for the use of OOCs for research, have not yet indicated scientifically validated standards for their use. Today, there are different chip models, but they are all built with different specifications (size, connections, ingredients, procedures): «To validate the use of these tools instead of animals», warns Ingber in *Nature review genetics*, «a large-scale evaluation on hundreds of devices with the same design that use the same protocols is needed. Each chip will have to be validated for a specific context of use. And the winner will be decided on the commercial battlefield».

To get to personalized medicine (and without guinea pigs) billions of investments and years of research are still needed. But it is worth it. F