B·DEBATE International Center for Scientific Debate BARCELONA

IBEC Institute for Bioengineering of Catalonia EXCELENCIA SEVERO OCHOA

Synopsis

FUTURE TOOLS FOR BIOMEDICAL RESEARCH

IN VITRO, IN SILICO AND IN VIVO DISEASE MODELING

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NEW TOOLS
FOR FUTURE RESEARCH

Biomedical research has advanced leaps and bounds in recent years, but there are still many roads ahead to explore. One thing that is normally considered a limiting factor is the technology used in research, which not only gives us access to more information but also makes it more reliable.

One controversial research tool is the use of animals, the ethics of which is much questioned, as is their ability to truly reflect the human body. Instead of or in addition to animal research, scientists are developing mini lab organs, organoids and "organs-on-a-chip" that allow for more direct, realistic experimentation; using iPS cells that are reprogrammed to return them to nearly the same state they started off at in an embryo; and working with mathematical models to create a "virtual human" that can be studied directly on a computer. At the same time, for example, mice modified gene by gene are studied to identify functions not yet known.

To debate these advances, some of the top international experts met for a session of B·Debate, an initiative of Biocat and the "la Caixa" Foundation to promote scientific debate.

CONCLUSIONS:

- ✓ Citizens' initiatives are working to prohibit the use of animals in research. However, the European Commission says this isn't currently possible and establishes a series of recommendations to rationalize use.
- ✓ Scientists are working to develop new tools for more reliable research, these include stem cells, organoids and even "organs-on-a-chip".
- ✓ Projects like Virtual Physiological Human aim to study the human body using computer-generated mathematical models.
- ✓ In the meantime, it is still necessary to use animals. In this regard, large-scale projects have been launched to improve how they are used: for example, identifying the function of each of a mouse's 20,000 genes, extrapolating them and comparing them to human genes.

TOWARDS RESEARCH WITHOUT ANIMALS?

In June 2015, the **European Commission** rejected a citizens' initiative that had collected nearly 1.2 million signatures to ban animal testing. Their reasons for calling for a ban on animal testing are based on ethics and questionable reliability. The Commission, however, believes that "a complete ban on animal research in the EU would be premature", and cited a directive from 2010 that establishes stricter regulation of the use of animals (the directive refers to mammals, as flies and fish aren't considered *in vivo* organisms). Spanish scientists have curbed the use of animals in research, down from 1.4 millions in 2009 to 920,000 in 2013, in part as a result of the new laws.

Elisabet Berggren, head of toxicology at the **Institute for Health and Consumer Protection**, believes "it may be possible to substitute *in vitro* testing for animals in pre-clinical trials. But to do so we have to think and plan experiments better." Berggren also mentioned two recommendations from the European Commission: the need to share more data and to improve development of alternative methods. One of the initiatives working towards the latter is the SEURAT-1 project, created as a result of the ban on using any type of research animal for cosmetics, which aims to develop methods that can also be applied in other areas.

Nevertheless, other experts aren't so optimistic, Fátima Bosch, director of the Autonomous University of Barcelona **Center for Animal Biotechnology and Gene Therapy**, says, "Some of these alternative methods could possibly work to assess drug toxicity, but not efficacy. That requires *in vivo* studies."

This opinion is shared by Miquel Borràs, professor of toxicology at the **University of Barcelona**. "Alternative methods make all the sense in the world in the early stages of drug development but not later on. And this isn't just a whim, animal studies are needed to test a therapy."

The industry itself is interested in moving beyond animal testing, because it tends to take longer and be more expensive. Therefore, to limit these inconveniences, they are beginning to use mice modified to develop tumors much more quickly, for example. But Borrás wonders, "What is the scientific relevance of these models? When we say *in vivo* research is imprecise, that is true (two thirds of all drugs approved in animals end up failing). But, careful, at least for now *ex vivo* is much more imprecise," he warns.

REGENERATIVE MEDICINE AS THE BASE OF RESEARCH IN THE FUTURE

When we talk about stem cells and laboratory organs, we normally think of transplants and regenerative medicine. But the same tools that can be used to create new tissue are also a great source of hope for research.

“Most treatments today have been designed with the *average patient* in mind,” says Josep Samitier, director of the **Institute for Bioengineering of Catalonia** (IBEC) and one of the scientific leaders of this B·Debate. However, “this is changing with precision medicine, which takes into account the differences between individuals in terms of genetics, environment and lifestyle.”

iPS (induced pluripotent cells) are the great hope for regenerative medicine and offer new possibilities for the much sought-after precision medicine. These cells, obtained for the first time in 2006 (and which Japanese scientist Yamanaka won the Nobel Prize in Medicine for in 2012), take us back to our origins. By adding just four genes, we turn differentiated cells (*adult* cells, in a manner of speaking) from various tissues into cells that are practically embryonic, which are much more flexible and able to become almost any type of cell. This is a huge advantage in the era of personalized medicine because it means you can use the patient’s own cells, or those of patients with similar characteristics. This has been done by the team led by Ángel Raya, director of the **Center of Regenerative Medicine in Barcelona**, with Parkinson, analyzing different forms of the disease using iPS cells from the skin that have been transformed into neurons. This isn't without its problems, however, but Raya says, “The great advantage of this technique is that we can get the patient’s cells from when they were born, long before they developed the disease, and that allows us to recreate how it developed.”

Another complementary option, also based on pluripotent cells, is to recreate organs in the lab, simulating cell types and the structures they make up. This is one of the latest goals of regenerative medicine, but also of research itself, because it would allow the characteristics and environment of our tissues to be more faithfully reproduced. Although we are still far from creating functional organs, there are already small *organoids* of the heart, kidneys and even brain, among others, that are generally built by filling a three-dimensional structure with cells. James Kirkpatrick, professor emeritus in pathology at the **Johannes Gutenberg University of Mainz**, admits there are still difficulties in “the

use of different cell types, like recreating vascularization and barriers," the areas of exchange between organs. There's also the question of size, "Without blood vessels, you have to *feed* them through diffusion and that means they have to be small," says Samitier.

Another alternative is "organs-on-chips", recreating the behavior of an organ in what is known as microfluid systems, small chambers filled with different types of cells that communicate amongst themselves and promises to overcome some of the difficulties of 3D organoids. For example, they can recreate physical forces like compression and reproduce cell-transport systems. As Roger Kamm, professor of Biological and Mechanical Engineering at **MIT in Massachusetts**, says, "They are true engineering systems, unlike organoids, in which nature is left to do its work." Samitier believes these organs-on-chips "will be much more useful because they offer greater freedom and trials with more drugs, while organoids will surely be more relevant in regenerative medicine." In either case, Kamm is both cautious and optimistic, "There are already models, but they need a lot of improvement. Pharmaceutical companies will end up adapting them in order to use them, but that's still a long way off."

ANIMAL MODELS (STILL) NECESSARY

Despite all the advances in new models, animals are still considered necessary and are a constant in research. Huge projects have even been created to delve deeper into the information they give us. For example, to untangle the function of all those genes we still don't understand, which is the majority. And, while we're at it, to check similarities between their functions in humans and mice, the sword of Damocles of animal testing.

This is the case, for example, of the International Mouse Phenotyping Consortium (IMPC), a huge consortium whose aim seems simple but is really quite complex: to check, one by one, the effects of eliminating or modifying each of the 20,000 genes in a mouse. So far, they've finished 2,500, and hope to hit 5,000 within the next few years. These studies have helped find model mice for various rare diseases that could hardly be studied, checking that at least two thirds of the genes don't have a unique function. The organizers of the project proposed it serve as a foundation for the ambitious Initiative on Precision Medicine launched by President Obama to analyze a mind-blowing amount of data from at least one million people over the coming years.

Animal testing is also used to do research into biological issues that seemed to be a closed case, like inheriting acquired traits. As Martin M. Hrabe de Angelis, director of the **Institute for Experimental Genetics in Munich**, explains, "9% of the population is diabetic. By 2030, this figure is expected to hit 64%." It is such a huge epidemic that there may be influencing factors beyond lifestyle. De Angelis, founder of the **German Mouse Clinic**, which is also a member of the IMPC, is studying whether there is a basis for "non-genetic transfer of the disease, depending on diet" (so, epigenetic). Some studies indicate that grandparents with a diet high in fat tend to have diabetic grandchildren, and studies with animals have shown several times that if fathers (only men, so there is no impact from gestation) eat that type of diet, their children are more likely to have diabetes. And this trend could be passed down through changes in sperm.

But none of this is clear yet. Nature tends to protect itself from these changes: what is known as the Weissmann barrier, for example, when gametes are formed (eggs and sperm) nearly all markers on an individual's DNA are erased. Perhaps some may be passed on, but this isn't easy to study, as other factors must be taken into account, such as uterine environment, difference in care received and the composition of breast milk. For now, as Josep Samitier confirms, "Some studies could be conducted without animals, thus rationalizing and decreasing their use. But they are still needed, at least before a drug can be tested in humans."

Towards a virtual human: cells or computers

The Virtual Physiological Human (VPH) aims to study the human body based on computer-generated mathematical models. This would make it possible to not only better understand it but also to design personalized treatment plans based on our most particular characteristics. In the words of Josep Samitier, it would be like "taking all the information available and designing a plane from its parts and systems, including a flight simulator that would let you practice flying." In practice, ambitions aren't as high and are limited to areas like diabetes and osteoporosis, but the goal is very ambitious and that makes it controversial. At B·Debate, scientists from the field of biology like Ángel Raya doubted such an optimistic application of mathematical models is possible, saying that mathematics isn't enough to study the causes of a disease, the cell and the real world are also needed. However Liesbet Geris, professor of biomechanics at the universities of Liège and Leuven and a member of the VPH project, believes that it isn't a competition, it's a collaboration. Mathematical models use information from experimental biology. Plus, as we see in nature, "Models can also have emerging properties. This happens, for example, in gene networks, networks that can't fit in just one head."