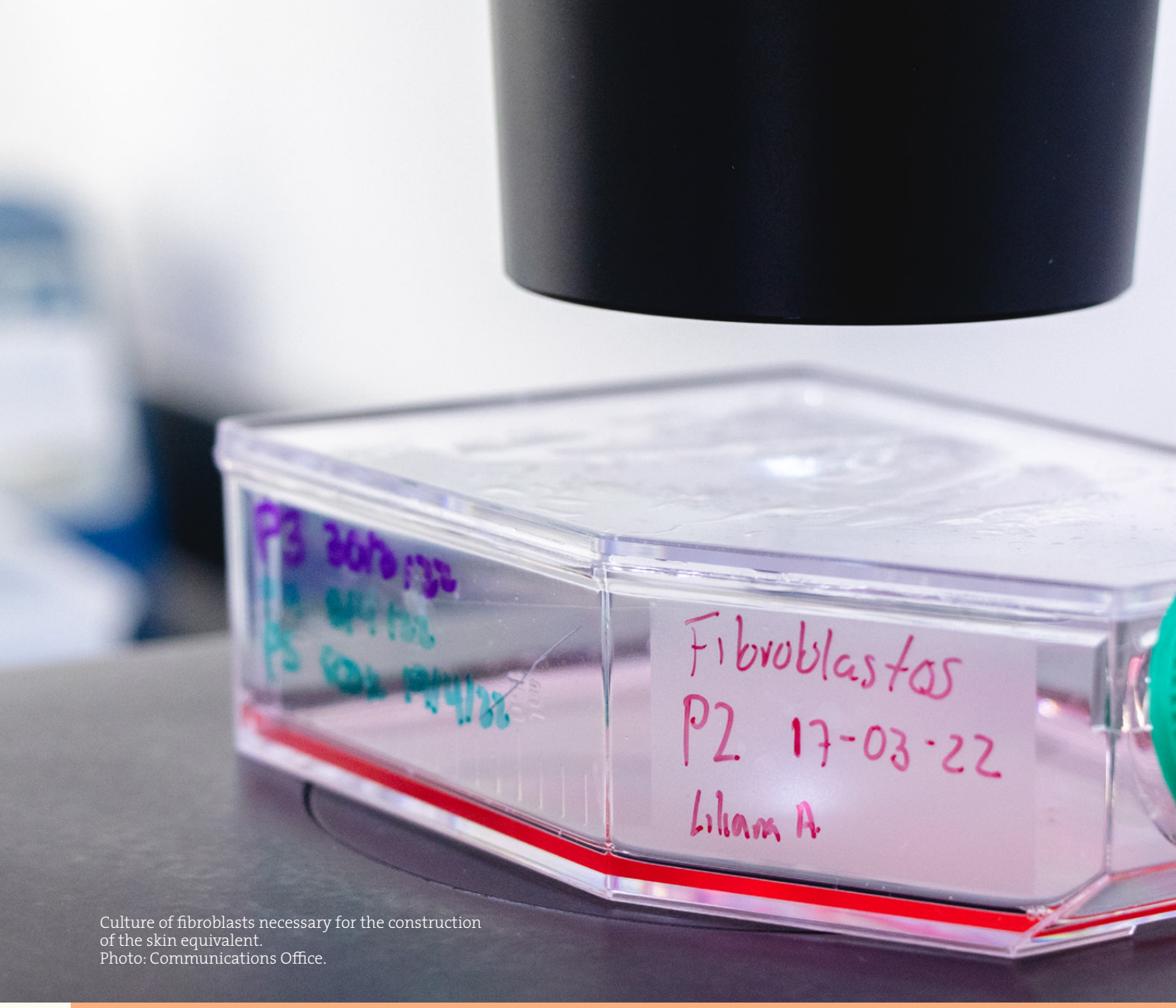

Culture of fibroblasts necessary for the construction of the skin equivalent.
Photo: Communications Office.

Creatin
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Skin substitutes are reconstructions made from human cells. They can help study human skin and test potentially harmful substances, which makes them a powerful alternative to the use of laboratory animals.

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Incredible as it may seem, every hour we lose more than one million dead cells from the surface of our skin. Therefore, new cells must constantly be born to replace those lost and form the outermost layer of the skin: the epidermis. This is the visible part of the skin and is mostly composed of cells called keratinocytes. These are constantly dividing and changing function and shape as they make their way to the surface of the skin. There they stay briefly and then begin to die and slough off, which causes the whole cycle to repeat itself.

We find the innermost layer of the skin known as the dermis beneath the epidermis. It is composed mainly of cells called fibroblasts, which are responsible for producing proteins and other components that form the network where the cells reside, i.e. the extracellular matrix (ECM).

These two layers work together to cover the entire body and protect us against harmful external agents. Interestingly, it was thought until recently that the skin had no immune role, and it was only an envelope that protected the body's organs. However, it is now known that the skin actively participates in the immune system thanks to the presence of so-called dendritic cells. These interact with foreign agents and can trigger an immune response.

Production of Skin Substitutes

At GITTC, we conducted a study in which we sought to create skin substitutes with an immune component to evaluate the sensitizing potential of chemical substances. The first step was to obtain the cells. We started with blood and skin fragments obtained from biopsies or reduction surgeries. Thanks to enzyme treatments, which break the bonds between cells, and between cells and the ECM, we were able to obtain keratinocytes and fibroblasts from the biopsies. On the other hand, since blood contains many different types of cells, we used the density gradient method to separate the different cell populations based on their density. Thus, we first obtained a mixture of lymphocytes and monocytes, which are special cells of the immune system. Then, we isolated the monocytes by magnetic separation. In this case, we use magnetic spheres that adhere specifically to the monocytes, which are then isolated thanks to a magnet.

We then proceeded with the construction of the skin substitute. For this, we had to recreate both the dermis and the epidermis. For the dermis, we made a fibrin gel, into which we incorporated the fibroblasts. Fibrin is a blood plasma protein and is involved in the formation of blood clots. In our study, we took blood plasma, incorporated the fibroblasts and mimicked the clotting process by adding calcium. We thus obtained a gel with embedded fibroblasts and clot-like characteristics. This became our dermal substitute.

Construction process of the skin equivalent. Photograph: Communications Office.

For the creation of the epidermis, we used fibrin gel as a support to seed keratinocytes in some cases, and keratinocytes and monocytes in others. These cultures were fed for 21 days since this is the time it takes for keratinocytes to grow and change their function, and thus form the epidermis.

The Potential of Monocytes in Skin Substitutes

After analyzing the skin substitutes, we found that the physical characteristics of the substitute were similar to those of human skin and the presence of keratinocytes and fibroblasts led the monocytes to become dendritic cells, i.e., cells of the immune system responsible for initiating the sensitization response. As expected, these were not found in cultures generated with keratinocytes alone. This result indicated that it is possible to use monocytes to obtain skin substitutes to study skin immunology.

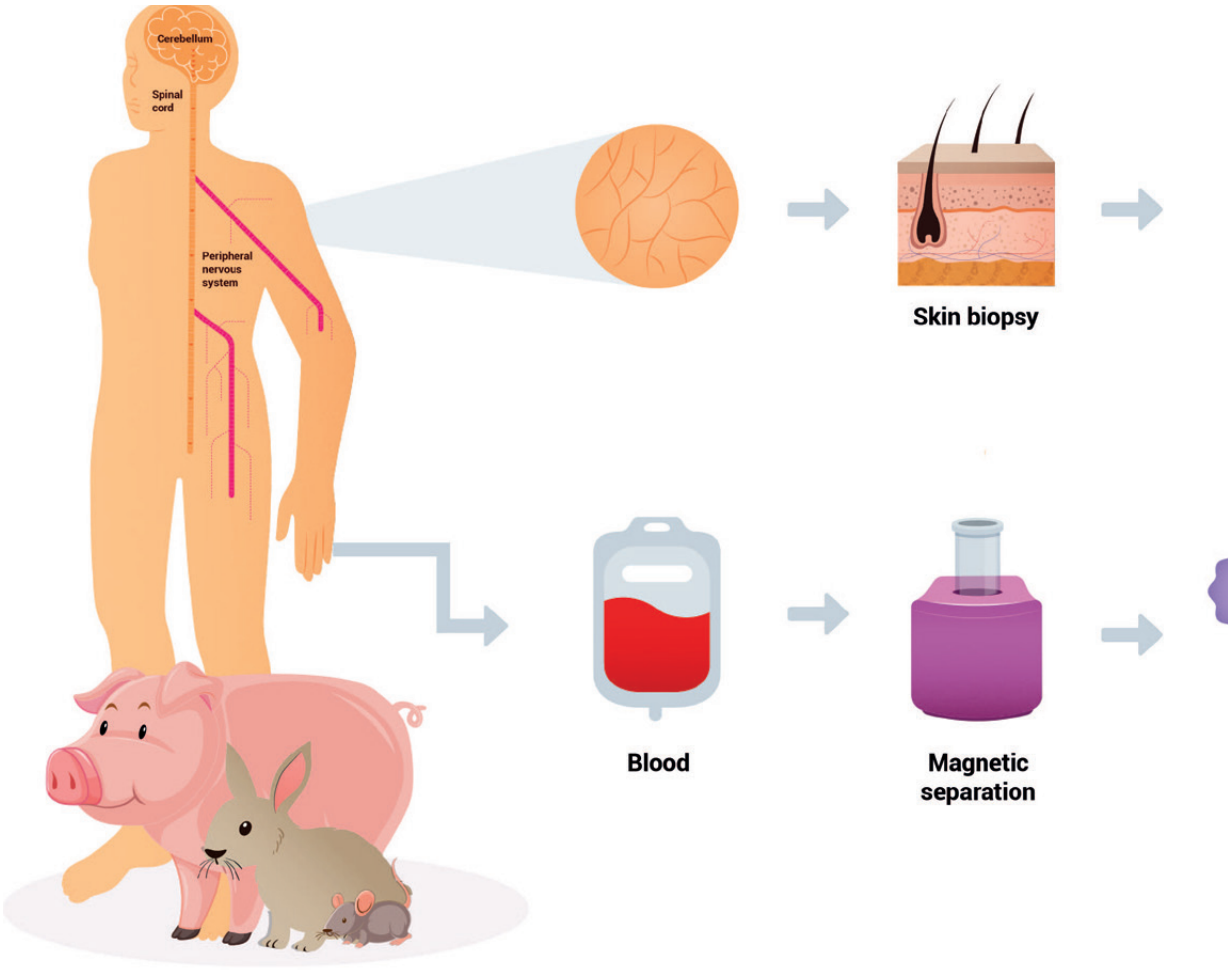
We then evaluated the ability of the skin substitutes to respond to sensitizing substances. For this, we applied formaldehyde and manganese chloride, two known sensitizers. At the same time, we applied saline as a control substance, which allowed us to check that the effects detected were not the result of other culture conditions.

When a sensitizer comes into contact with the skin, dendritic cells are activated and migrate to the lymph nodes. In doing this, they pass through the dermis. For this reason, we decided to analyze the location of these cells in our substitutes after exposure to the test substances. We observed that there was a migration of dendritic cells for the sensitizers. They exhibited a different location from that of the control.

The Promise of Skin Substitutes with Immune System Cells

The results of our study indicated that skin substitutes composed of keratinocytes and fibroblasts can promote the differentiation of monocytes in skin immune cells. This is probably because of molecules and factors released by keratinocytes and fibroblasts that "command" monocytes to become dendritic cells, most likely of different types. Additionally, these cells could have migrated through the fibrin gel in response to sensitization, which is close to what happens in human skin.

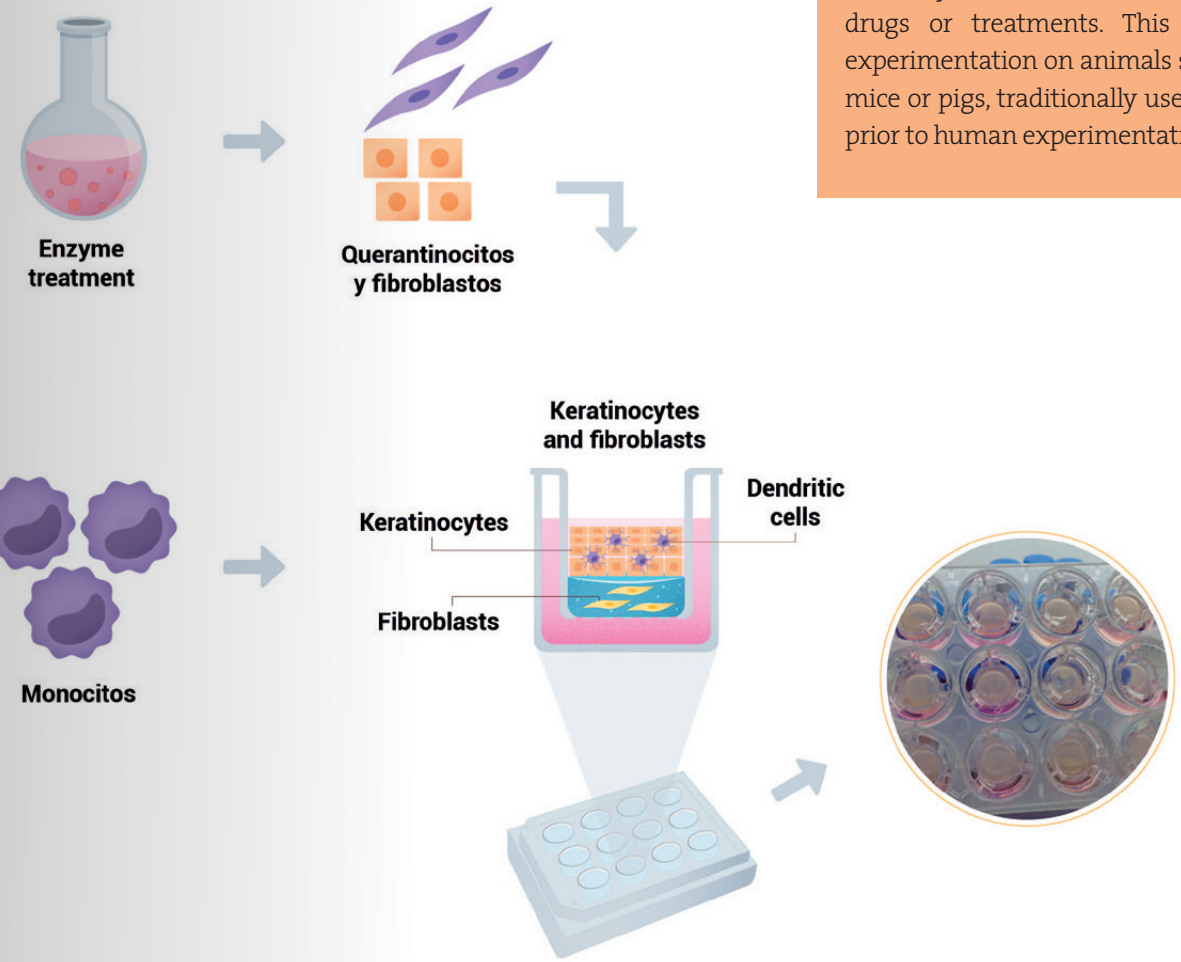
To our knowledge, this methodology has not been used before and represents a simpler method to obtain dendritic cells in substitutes or skin equivalents. Additionally, it highlights the possibility of obtaining different types of immune cells in a substitute and from a single precursor such as monocytes, which improves on current skin models.



In addition to the immune system, skin substitutes have numerous applications in different fields. For example, apart from sensitizers, they can be used to evaluate irritating or corrosive substances, model diseases such as cancer or psoriasis (and thus develop possible treatments), study skin pigmentation by adding another type of cells called melanocytes and even as skin grafts for people who have suffered burns.

At present, commercially available human skin models have high costs and involve long import and legalization procedures to be used in Colombia. Besides, their transportation may result in a possible loss of product quality. Additionally, most are composed only of keratinocytes, which excludes their use in the field of immunology. Thus, with this project, we seek to obtain a local model that enables the development and commercialization of products in Colombia. X

Skin substitutes are an innovative alternative to study how the skin would react to drugs or treatments. This could replace experimentation on animals such as rabbits, mice or pigs, traditionally used in the stages prior to human experimentation.



Glossary

Dendritic cells: special type of immune cells that process antigens (molecules that induce an immune response) and present them to other cells of the immune system.

Enzyme: protein that accelerates the speed of chemical reactions in living beings.

Density gradient: fluid whose density changes and which is used in the separation of different types of cells by centrifugation.

Blood plasma: liquid portion of blood.

Lymph nodes: structures that filter substances and contain immune system cells.