The reconstruction of psoriatic skin

Joint leaders of a groundbreaking study looking into the treatment of conditions like psoriasis, **Professors Roxane Pouliot** and **Michèle Auger** explain how their skin model stands to make a radical impact on the non-invasive approach offered by the transdermal drug industry

Could you begin by summarising the project's major aims and objectives? What led you to follow this line of investigation?

The objective of our work is to use techniques such as infrared and Raman spectroscopies to investigate the lipid phase transitions, the lipid chain order and orientation, the protein secondary structure as well as the morphology and hydration of healthy and psoriatic skin substitutes. These properties are compared for skin models derived from normal cells with those derived from biopsies taken from chronic psoriatic plaques as well as those from skin free of plaques. These systems are made with a variety of cell lines and the results obtained with the skin substitutes are compared with those obtained with healthy and psoriatic skin biopsies.

The main objective of the research programme overall is to develop skin substitutes that could provide a better understanding of psoriasis and be used for drug testing.

What are the benefits of using transdermal patches for medical administration over other methods such as oral, topical, intravenous, or intramuscular delivery?

Transdermal administration of drugs is a non-invasive technique which evaluates on a local or systemic level, topical drug activity or the safety of cosmetics and other agents that are likely to make contact with the epidermis. *In vitro* tests of transport through the skin are one of the initial steps in elaborating a transdermal drug. To that end, several instrumental models have been considered to avoid, among other things, tests on living animals. The Franz diffusion cell system is recognised as an excellent tool to perform *in vitro* studies on transdermal drug diffusion.

How have your recent developments in tissue engineering led to advancements in medical testing for patients with severe skin diseases, and psoriasis in particular?

The pathological cutaneous substitute model is an outstanding novelty for the percutaneous absorption field of research. Being a non-invasive method for tissues, the passage of substances through the skin allows a better understanding of the anti-psoriatic activity of tested molecules. For now, psoriasis is the targeted pathology, but it will be conceivable to widen this expertise to other skin diseases. This research is part of an international context particularly favourable to transdermal drug administration. Indeed, physicians and pharmacists are now looking for treatments which facilitate patient compliance. The popularity and ease of use of transdermal patches show the appeal of what research in this field offers.

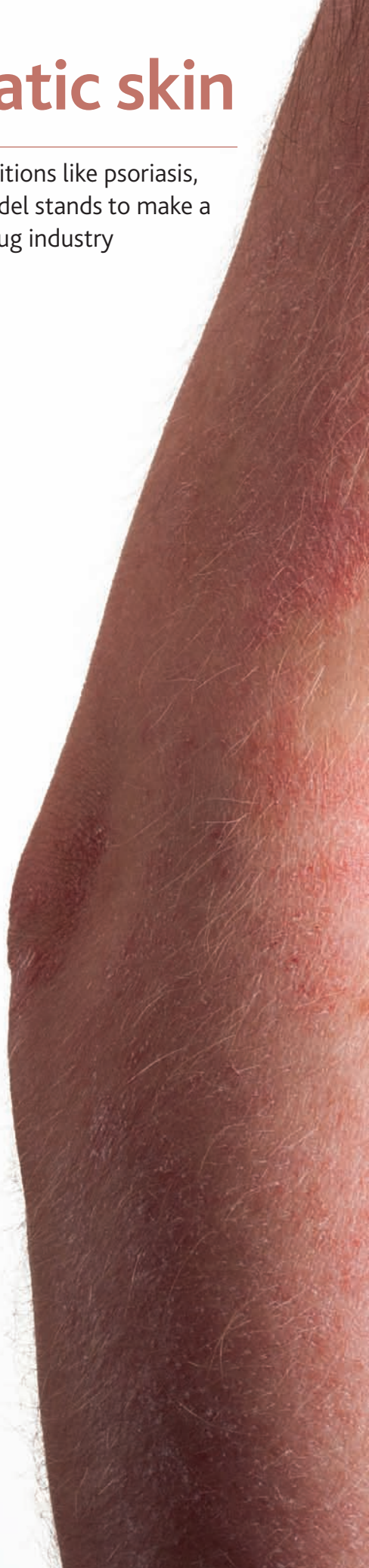
What results have you drawn from the project so far?

Our results indicate that the self-assembly method is a very powerful technique to prepare skin substitutes since it allows the study of the effects of various cell types (fibroblasts and keratinocytes) and of variation of culture medium on skin organisation. We have also demonstrated that both infrared and Raman microspectroscopies can be used to probe skin substitutes as a function of depth. This is the first time that information about lipid organisation and protein structure has been obtained as a function of depth for both healthy and psoriatic substitutes prepared by the self-assembly method.

On a personal note, what would you say have been your most successful moments during your research career?

Training graduate students and seeing them develop as independent researchers is certainly a very gratifying moment in our careers. As mentioned above, our projects are truly interdisciplinary and training students in such environments, although very demanding, is also very fulfilling.

One of the main achievements in Roxane Pouliot's research group was the development of a psoriatic skin model which allows dissecting step by step the mechanisms of this pathology. We have shown for the first time that pathological substitutes produced by the self-assembly method can be treated with an anti-psoriatic molecule and react positively to the treatment, such as observed in psoriatic skin *in vivo*. Our functional study suggested that this model could be used to better understand the mechanisms through which retinoic acid regulates cellular physiology in psoriatic skin. In the future, we would like to see our model of psoriatic skin becoming an effective and innovative dermopharmaceutical tool for the screening of new treatments.



PROFESSORS ROXANE POULIOT AND MICHÈLE AUGER

A second skin

Skin conditions like psoriasis are so complex and unpredictable that assessing treatment has so far been problematic. Now, a pioneering research project based at **Université Laval**, Canada, is developing replica diseased skin that could pave the way for safer and more effective testing

PSORIASIS IS A skin condition for which there is no cure that affects an estimated 80 million people worldwide. Its severity varies greatly, depending on the individual. Presenting itself through red and flaky patches, some people experience it as nothing more than a minor irritation, but for others it has a major impact on their quality of life.

THE STORY SO FAR

Up until now, testing the treatment for skin conditions like psoriasis has come across significant barriers. Traditionally, *in vitro* and *in vivo* experiments have been the primary methods adopted for investigating these conditions, but they may be limited either by prohibitive cost, or by the differentiation between the characteristics when studied in isolation, from actual diseased skin.

By using new techniques, a team led by Professors Roxane Pouliot and Michèle Auger from Université Laval in Canada have been working on the reconstruction and characterisation of psoriatic skin. They claim it will revolutionise the understanding of psoriasis, which is still very limited because of its complex and unpredictable nature: "To date, many studies demonstrate that psoriasis is undoubtedly an immune-mediated disease," Pouliot explains. "However, the knowledge of the exact role of the major cell types involved remains very fragmentary. Our *in vitro* model will be invaluable in understanding the respective role of soluble factors and direct cell-to-cell contact. The study will also help us to understand the disease and to discover new pharmacologic targets."

> The need for innovative and effective tools to evaluate new dermopharmaceutical formulations for psoriasis is essential

THE METHOD

Before transdermal products reach the market, they must be thoroughly analysed. One method, the 'Franz Diffusion Cell' system, is known to be particularly effective. First popularised by Dr Thomas Franz, it has been applied to a number of skin permeation studies, including topical and transdermal drug delivery formulations, as well as opthalmics, cosmetics, skin care products and pesticides. The vertical diffusion cell system is an ideal tool for quality control of topical preparations. A skin sample is placed on the glass cell, fixed with a clamp, and the test substance is set upon it. The substance then diffuses through the skin substitute and samples can be taken from the cell's receiving compartment at precise time intervals. Thus, it is possible to follow the drug *in vitro* going through the sample, assess the amount of product passing across the sample per unit time and area, and compare these values with those reported in the literature.

Despite this method being a widely recognised tool for performing *in vitro* studies on transdermal drugs, the use of artificial membranes, animal or healthy human skin poses a myriad of problems. Healthy human skin does not possess the same skin characteristics of people that will receive these treatments. Artificial membranes are even less likely to be close enough to human skin for testing to be effective. Furthermore, aside from potential ethical issues, no animal model accurately and suitably develops psoriasis and getting hold of psoriatic skin *in vivo* is both difficult and expensive. "The need for innovative and effective tools to evaluate new dermopharmaceutical formulations is therefore essential," Pouliot points out. "The objective of this project is the development and characterisation of reconstructed psoriatic skin."

THE NATURE OF SKIN

The cells that make up the skin have a life cycle. The body produces new cells in the deepest skin level and these skin cells gradually move up until they reach the outermost layer. Subsequently they die and flake off and the entire process normally takes around 21-28 days. When someone suffers from psoriasis,

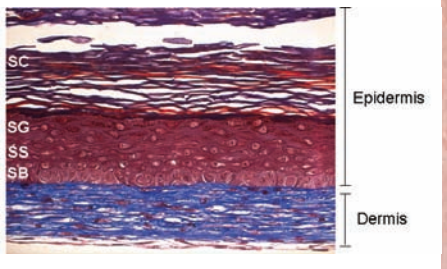


FIGURE 1. Histological features of self-assembled skin substitutes showing the different cutaneous layers. Magnification 40X. From Jean J, Garcia-Pérez ME, Pouliot R (2011) Bioengineered Skin: The Self-Assembly Approach. *J Tissue Sci Eng* S5:001.

DEVELOPMENT AND CHARACTERISATION OF RECONSTRUCTED PSORIATIC SKIN

OBJECTIVES

The objective of this work is to probe, using infrared and Raman spectroscopies, the lipid phase transitions and chain order, the protein secondary structure as well as the hydration of healthy and psoriatic skin substitutes. In addition, these techniques are used to investigate the skin substitutes at different depths and to investigate the spatial distribution of drugs in the substitutes.

KEY COLLABORATORS

Professor Gaétan Laroche, Department of Mining, Metallurgical and Materials Engineering, Université Laval

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MICHÈLE AUGER joined the Department of Chemistry at Université Laval in 1991 where she was promoted to Full Professor in 2000. She is internationally recognised for her work on the study of biomolecules using diverse spectroscopic techniques, mainly solid-state nuclear magnetic resonance and vibrational spectroscopy.

ROXANE POULIOT joined the Faculty of Pharmacy at Université Laval in 2002 where she was promoted to Full Professor in 2011. Professor Pouliot's research efforts are focused on the development of a new model of pathologic skin, which is made of cells isolated from patients biopsies affected with severe skin diseases such as psoriasis.

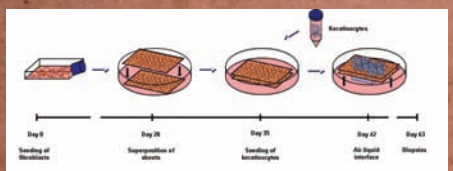

FIGURE 2. Scheme of the self-assembly approach. From Jean J, Garcia-Pérez ME, Pouliot R (2011) Bioengineered Skin: The Self-Assembly Approach. *J Tissue Sci Eng* S5:001.

the process speeds up and only takes two to six days. As a result, cells that are not fully mature build up rapidly on the surface of the skin, causing red, crusty patches covered with silvery scales. Psoriasis can occur on any part of the body, but is most common on the elbows, knees, lower back and scalp and can cause itching and burning. Psoriasis affects around 2 per cent of people in the US and it can start at any age, but most often develops between the ages of 11 and 45.

TISSUE ENGINEERING

Recent biotechnological progress in the tissue engineering field has allowed researchers to conceive, develop and produce biomaterials which can replace tissues or organs. So far, they have made successful attempts at reconstruction that can be applied to skin, blood vessels, cartilage and bones. Pouliot's team has found that their method could easily be modified to produce substitutes which are made with cells isolated from patients affected with severe skin diseases such as psoriasis. "It was the first time that infrared and Raman microspectroscopies were used in our laboratories to investigate skin substitutes," Auger recalls. "The results obtained in this project will lead to a better understanding of psoriasis and the development of improved pathological substitutes. Our results meet our expectations so far."

INFRARED AND RAMAN MICROSPECTROSCOPIES

Raman microspectroscopies, named after Sir C V Raman, are used to study vibrational and rotational modes in a system. It relies on a Raman scattering of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations, resulting in the energy of the laser photons being shifted up or down. This shift in energy gives information about the vibrational modes in the system. Infrared spectroscopy yields similar but complementary information.

Encouragingly, Auger also points out that for the first time, using such techniques, it will be possible to test transdermal drugs directly on pathological, reconstructed skin. Moreover, these pathological samples will be more efficient because of their reproducibility and their accessibility. The results from the proposed research will also lead to the development of new formulations conceived and appropriate for particular skin healing treatments.

OVERCOMING CHALLENGES

Despite its successes, the project has not come without its fair share of hurdles along the way: "One of the big challenges is the preparation of the skin substitutes as it is so time-consuming. The self-assembly approach takes about two months to perform," notes Pouliot; "Several substitutes can be prepared simultaneously; however, the fact that for most experiments, the substitutes have to be analysed within a few days after their preparation, also makes it difficult."

The interdisciplinary nature of Pouliot and Auger's collaboration was also the source of another barrier. The wider group of students and postdoctoral fellows involved had to be specially trained in cell culture, tissue engineering and advanced spectroscopic techniques that had never before been applied in their laboratories to study skin substitutes. That said, Auger reveals that such sharing of expertise soon became a key part of the success of their work, even to the point of making the most of local natural materials: "Roxane is also involved in the establishment of the possible utilisation of polyphenolic extracts from barks of Canadian wood species in psoriasis treatment, and the study of their antioxidant capacity and toxicological properties".

The future for this partnership looks promising. Certainly, amongst psoriasis sufferers, one in five of whom may have to endure frequent hospital admissions, there will no doubt be many who welcome the efforts of Pouliot and Auger in driving treatment advances forward.

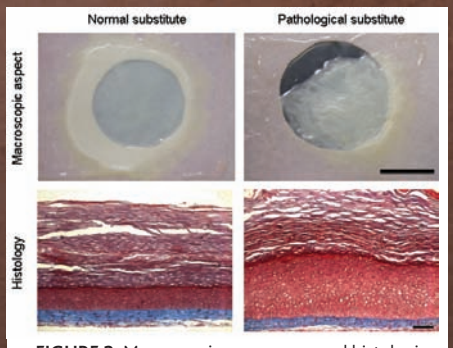

FIGURE 3. Macroscopic appearance and histological analysis of normal and pathological substitutes made by the self-assembly method (scale bars = 2.2 cm and 50 μm, respectively). Figure from Jean J, Garcia-Pérez ME, Pouliot R (2011) Bioengineered Skin: The Self-Assembly Approach. *J Tissue Sci Eng* S5:001.