Modelling psoriasis

Professor Roxane Pouliot's group is advancing the understanding of skin diseases. She talks about the importance of their unique psoriatic skin model and the emphasis she places on training students

Can you explain the overarching aims and objectives of the work carried out by the scientists in your research group?

My complete research programme consists of four parts: elaboration of new tools for better understanding the mechanisms of psoriasis; determination of cutaneous penetration and the biotransformation of antipsoriatic drugs; study of the physicochemical properties of the skin and its unique lipid composition; as well as the development of new antipsoriatic formulations. This programme is unique and brings a new dimension to my research centre and faculty – a specialised area of research in dermopharmacology. This field includes the study of skin tissue, its main diseases and their treatments. It also presents several benefits for population health such as the understanding of incurable conditions as well as developing effective medications to counter these disorders.

How has the development of your psoriatic skin model benefited the wider experimental research of the team?

Recent biotechnological progress in the tissue engineering field allows us to conceive, develop and produce biomaterials that can replace tissues or organs. It has been demonstrated that artificial reconstruction can be applied to several different tissues such as skin, cartilage, bone, cornea and blood vessels. Our research team has recently demonstrated that the auto-assembly method, which involves using tissue engineering to produce completely autologous skin substitutes with both dermis and epidermis, can be easily modified to produce pathological substitutes. Psoriatic skin equivalents are made with cells from biopsies of patients affected with severe skin diseases, such as psoriasis.

Are you able to summarise your findings that have come about from your characterisation of the psoriatic condition?

The data from our first study suggest that the macroscopic, histological and immunohistochemical characteristics of psoriasis are partially retained in the substitutes, thus providing a good model to investigate the mechanisms of abnormal keratinocyte growth and to study cell-to-cell interactions. Our results show that retinoic acid can modulate epidermal differentiation and proliferation with an antiproliferative potential in psoriatic substitutes, as observed in psoriatic skin *in vivo*. We have also demonstrated that uninvolved substitutes could highlight characteristics associated with both normal and involved psoriatic skin.

To what extent do you advance the research careers of the students and early-career scientists working in your lab?

Our team offers a high quality training environment for students and postdoctoral fellows. I am a member of the LOEX, a research centre internationally renowned in tissue engineering and artificial organs and also a member of Centre de Recherche sur les Matériaux Avancés (CERMA) and Centre Québécois sur les Matériaux Fonctionnels (CQMF). The involvement of my team with the LOEX, CERMA and CQMF allows students to work with leading research groups – in Canada and across the world – on tissue engineering and functional materials. The various seminar series organised by these centres, as well as their annual symposia, enable students to meet internationally renowned researchers in a variety of areas. In addition, the students regularly participate in joint activities such as group meetings and scientific discussions.

To motivate students in their research activities, my team always involves every student with our publications. We also strongly encourage them to participate in national and international meetings. Finally, my students have access to state-of-the-art scientific equipment shared by members of my group, the LOEX and CERMA.

How has a multidisciplinary approach proved important to the success of the project?

In collaboration with Dr François Berthod, we are using a new model to investigate the role of nerves in psoriasis, and it is the creation of this skin model that has enabled such research to take place. Our model is a unique tool to investigate this effect, since there is no ideal murine alternative to mimic the disease. We have successfully replicated, *in vitro*, most of the psoriatic phenotype using patients' cells. The reconstructed psoriatic skin can be used as a model to further investigate the interaction of sensory nerves, microvessels and lesional keratinocytes. In the future, we plan to incorporate human immune cells in order to analyse the impact of inflammation.

Skin deep

Recent biotechnological progress in tissue engineering has enabled scientists at **Université Laval** to expose some of the underlying causes of psoriasis, ultimately helping them to progress the development of novel and natural treatment options

PSORIASIS IS A COMPLEX chronic inflammatory skin disorder with unknown causes. It is a widespread disease that can have a damaging impact on patients, their families, and both the mental and physical healthcare systems. According to the Canadian Psoriasis Network, 1 million Canadians and 80 million people worldwide have the condition. Psoriasis is now understood by scientists to be an immunological condition and, as a result, much of the associated research has focused on the function of genes to enable the development of effective and targeted treatments. For a number of years, a research team led by Professor Roxane Pouliot at Université Laval's Faculty of Pharmacy has concentrated on deepening the understanding of psoriasis and the underlying disease mechanisms. Their aim is to ultimately support the creation of novel therapeutic options.

Pouliot's lab has had much success with its tissue engineering methodology, which the researchers have used to develop and characterise a new model of psoriatic substitutes. Making use of this, they have been progressing along a number of key pathways, including investigating the functionality and physicochemical properties of their novel psoriatic skin prototype.

Pouliot, whose multidisciplinary academic background includes postdoctoral studies in biomedical engineering at the Harvard–MIT Division of Health Sciences and Technology, and in biophysics at Université Laval, explains that psoriasis is characterised by an abnormal keratinocyte differentiation, which affects its pathogenesis. "This results in the overexpression of the enzyme transglutaminase type I and involucrin, which causes the development of the cornified tissue observed in psoriatic lesions." Keratin expression is also disrupted, leading to the dysregulation of several forms of this important skin constituent - K6 and K16 are upregulated in psoriatic epidermis, whereas K1 and K10 – markers of terminal differentiation – are downregulated. It is the role of the keratinocytes which drives her research forward toward the identification of the underlying causes of the disease.

MODELLING PSORIASIS MECHANISMS

Many of the instrumental models used to test transdermal drug administration are based on healthy human or animal skin, or artificial membranes; none of which have the same properties as diseased human skin. This presents significant challenges for researchers who try to avoid testing transdermal treatments on living animals but want to ensure that drug formulations are appropriate for a particular patient's situation, such as ethnicity. This conflict led Pouliot's group to develop an innovative psoriatic skin model that has proven essential for advancing our understanding of the mechanisms of the condition and for enabling the evaluation of new dermopharmaceutical treatments. Some of the research underway has involved looking at the physicochemical properties of the skin substitute, and a recent research article, published by the group this year in *Analytical and Bioanalytical Chemistry* confirmed that the substitutes used in their model reproduce essential features of real skin and, as such, provide appropriate biomimetics.

Ongoing investigations also consider the regulatory role of the interactions between T lymphocytes and keratinocytes on the production of immune factors. Various experimental tests were carried out to assess the cytokines and chemokines, including the use of an enzyme-linked immunosorbent assay. The results are complex and multifaceted, but show that direct cellular contact is needed for a functional interaction between keratinocytes and T lymphocytes. "Our work indicates that there is a reciprocal influence that depends on cytokine and chemokine type," Pouliot clarifies. This study was based on previous work with lymphocytes and was used to help set a robust framework for the development of the inflammation model.

UNLOCKING ANTIOXIDANT PROPERTIES

For the first time, in 2010, the Laval-based researchers attempted to establish opportunities for using polyphenolic extracts from the bark of Canadian tree species in psoriasis treatment. Their aim was to learn more about their antioxidant capacity, their toxicological properties and effect on proliferation of both normal and psoriatic keratinocytes by using a number of different analytical techniques, including MTT assay and trypan blue dye exclusion. Their work led to the discovery that the bark from the Canadian black spruce species (*Picea mariana*) had the most value when its extract was obtained using hot water extraction techniques.

Pouliot's group has developed an innovative psoriatic skin model that has proven essential for advancing our understanding of the condition

This line of inquiry has been advanced more recently by studying the anti-inflammatory and antioxidant properties of *P. mariana* bark extract. The researchers isolated and characterised major compounds of the ethyl acetate soluble fraction using a number of advanced analytical techniques, including high performance liquid chromatography, nuclear magnetic resonance spectroscopy and mass spectrometry. Results identified 28 compounds, some of which have never before been reported in the *Picea* genus. "This study provides novel information about the identity of major compounds present in the *P. mariana* bark extract, which is essential for understanding its anti-inflammatory and nutraceutical potential," highlights Pouliot. Publication of their findings in the international journal *Food Chemistry* has enabled them to widely disseminate this information and build support for their innovative work.

Importantly, a phenol-based therapy such as the one offered by *P. mariana* bark provides a number of novel applications because of the manner in which it impacts upon the psoriatic skin condition. "It has the potential to influence keratinocytes and to downregulate mediators of acute and chronic inflammation associated with the pathogenesis of psoriasis," notes Pouliot. Most of the therapeutic effects, demonstrated during testing of this potential psoriasis remedy, can be credited to a number of polyphenols or antioxidants. In fact, through *in vitro* studies, they have observed that the polyphenols' inhibitory impact appears to be more effective than some existing treatments. This work is helping the team make progress towards their vision of developing a new antipsoriatic skin formulation. The next steps towards this realisation require some in-depth studies on the immunological impact of the treatment. "It remains to be elucidated whether the immunopharmacological effects of these constituents could be attributed to individual, additive or synergistic mechanisms," Pouliot explains.

FUTURE PROOFING

The opportunity to share the knowledge gained with other experts within this field is very important to members of the lab and, to this end, they participate in the yearly conference of the Tissue Engineering and Regenerative Medicine International Society, where, this year, they presented their latest work on angiogenesis – a key characteristic of psoriasis. This is an important area because, as Pouliot elaborates: "The antiangiogenic effect is necessary for the healing of lesions, so this part of the research aims to develop capillarised psoriatic skin substitutes for antiangiogenic drug research". Once the outward symptoms of psoriasis have been successfully treated, the cutaneous microcirculation of the skin remains altered for a longer period. This deficit can be addressed using antiangiogenic therapies.

This research employs some of the latest advances in biomedical technology, particularly in the field of skin tissue engineering. The interdisciplinary nature of this research programme, which includes significant contributions from students and postdoctoral fellows, benefits from a unique and invaluable combination of expertise in the areas of tissue engineering, psoriasis, percutaneous absorption of drugs and several state-of-the-art spectroscopic techniques. This is exciting work for many students. It ensures that future generations of researchers are actively engaged in this important field and facilitates progress towards an effective, targeted treatment for psoriasis.

UNDERSTANDING THE MECHANISMS BEHIND THE DEVELOPMENT OF PSORIASIS AND THE FACILITATION OF NEW TREATMENTS

OBJECTIVES

To validate a new model of psoriatic substitutes and characterise this proliferative and pathological skin condition. Once the model has been validated it will be used to study the antioxidant properties of toxicological and antiproliferative polyphenolic extracts from the bark of Canadian tree species.

KEY COLLABORATORS

Canada: **Dr Jacques Soucy**, St-Sacrement Hospital, Quebec • **Professor Yves Desjardins**, Institut sur la Nutrition et les Aliments Fonctionnels, Université Laval • **Professor Michèle Auger**; **Professor Gaétan Laroche**, Faculty of Sciences and Engineering, Université Laval • **Professor René C Gaudreault**; **Professor François Berthod**, Faculty of Medicine, Université Laval

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ROXANE POULIOT is a professor in the Faculty of Pharmacy at Université Laval, where she also completed her BSc and MSc studies in Chemistry. Since studying part of her doctorate in Pharmacy at University Paris XI, she has also undertaken two postdoctoral research fellowships, one in collaboration with DiagnoCure Inc. and the other in Biochemical Engineering at Harvard-MIT-Division of Health Sciences and Technology. She was recently invited as an editorial board member of the *Journal of Pharmaceutics and Drug Development*. Pouliot has published a book on Dermatology-Laboratory and Clinical Research entitled: *Psoriasis: Causes, Treatments and Pathological Models.*