3D Modeling of Dermal Macrophages-Containing Dermis Equivalent

N. Bechetoille[1], P. Séroul[2], A. Boher[1], S. Charpy[2], M. Jomier[2], V. André-Frei[1]
[1] BASF Beauty Care Solutions France SAS, 32 rue Saint Jean de Dieu, F-69336 Lyon Cedex 07, FRANCE
[2] Newtone Technologies, 17 cours de la Liberté, F-69003 Lyon, France

nicolas.bechetoille@basf.com

INTRODUCTION

In vitro 3D reconstructed skin culture systems are useful tools for investigative purposes and for ingredient evaluation.

We previously succeeded in integrating monocyte-derived dermal type macrophages (MDdM) into 3D reconstructed dermis equivalent (Bechetoille et *al*., 2011).

However, classical visualization method such as histology and immunochemistry only provide limited information as for the functionality of MDdM. Medical imaging already used in neurosciences offer accurate lime and space resolutions and could thus inspire skin science.

Aim of the study

We used innovative real-lime laser scanning microscopy together with dedicated 3D image processing software to further assess the functionality (i.e., spreading and phagocytosis) of MDdM once integrated within 3D reconstructed dermis equivalent.

MATERIALS & METHODS

Production of monocyte-derived dermal macrophages (MDdM)

Isolation of monocytes from peripheral blood mononuclear cells of healthy volunteers by the standard Ficoll-Paque method.
Immediate separation by positive magnetic selection (CD14 Micro Beads; Miltenyi Biotec).
7-day culture of purified CD14+ monocytes in complete medium supplemented with GM-CSF, M-CSF and IL-10, as described by Kwan et al., 2008.

Dermis equivalent MIMEDERM™

Normal human fibroblasts seeded on collagen-GAGs- chitosan culture matrix (Mimedisc™, BASF Beauty Creations, France) and cultured for 14 days.
Seeding of MDdM on dermis equivalent Mimederm™ for additional 1-week culture without exogenous cytokines.
Stimulation of MDdM-containing dermis equivalents Mimederm ™ by TNF-□ the last two days of culture.

Phagocytosis

The last day of culture, fluorescent bead (3µm) were added onto the surface of dermis equivalent MIMEDERM™. Before performing observation by laser scanning microscopy, 1µM of calceine was added to the culture.

3D imaging processing software

Beads were automatically detected and segmented using DeepSkin® 3D image processing software (Newtone Technologies, Lyon, France).2D / 3D real-time volume rendering and navigation allow users to visualize in the same time the segmented beads and the structures of the equivalent dermis.
The number of beads, inside or outside MDdM was automatically computed.

RESULTS

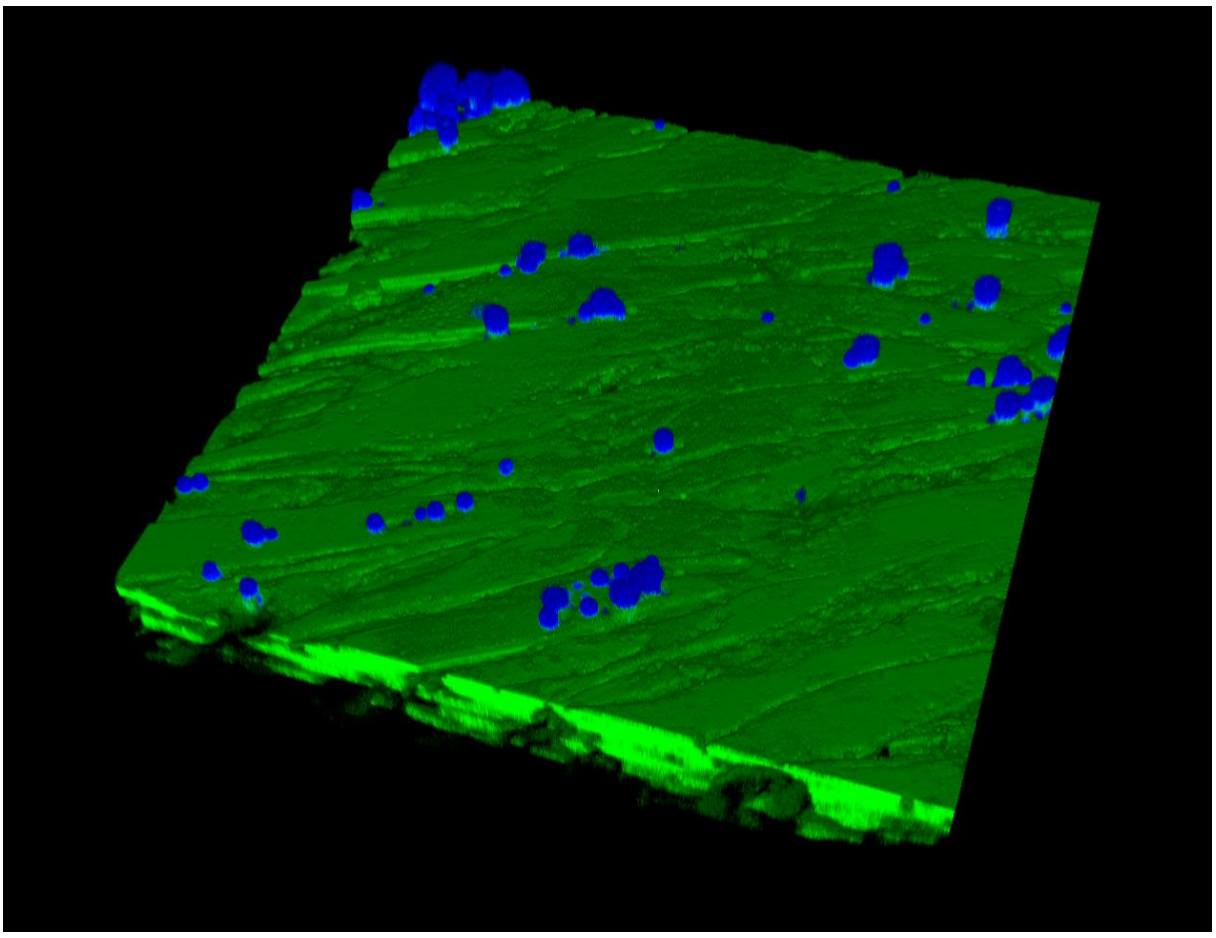

Figure 1: Location of MDdM incorporated in 3D dermis equivalent
Because displaying high phagocytosis properties, MDdM can be easily observable as calceine-positive (blue) cells. After 1 week of culture within dermis equivalents (green), MDdM were found to be mainly located at the surface and outermost area of dermis equivalents.

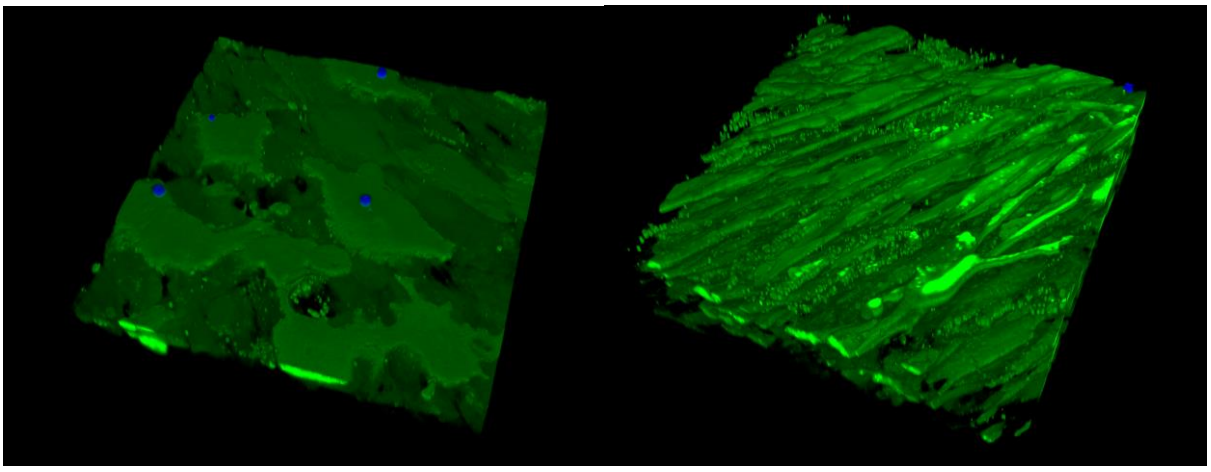

Figure 2: Modulation of MDdM phagocytosis properties within 3D dermis equivalent
As shown using specific image segmentation and dedicated 3D reconstruction, fluorescent beads (blue) were found in macrophages (4 beads/dermis equivalent). Only 1 fluorescent bead (blue) was detected in dermis equivalent without macrophages.

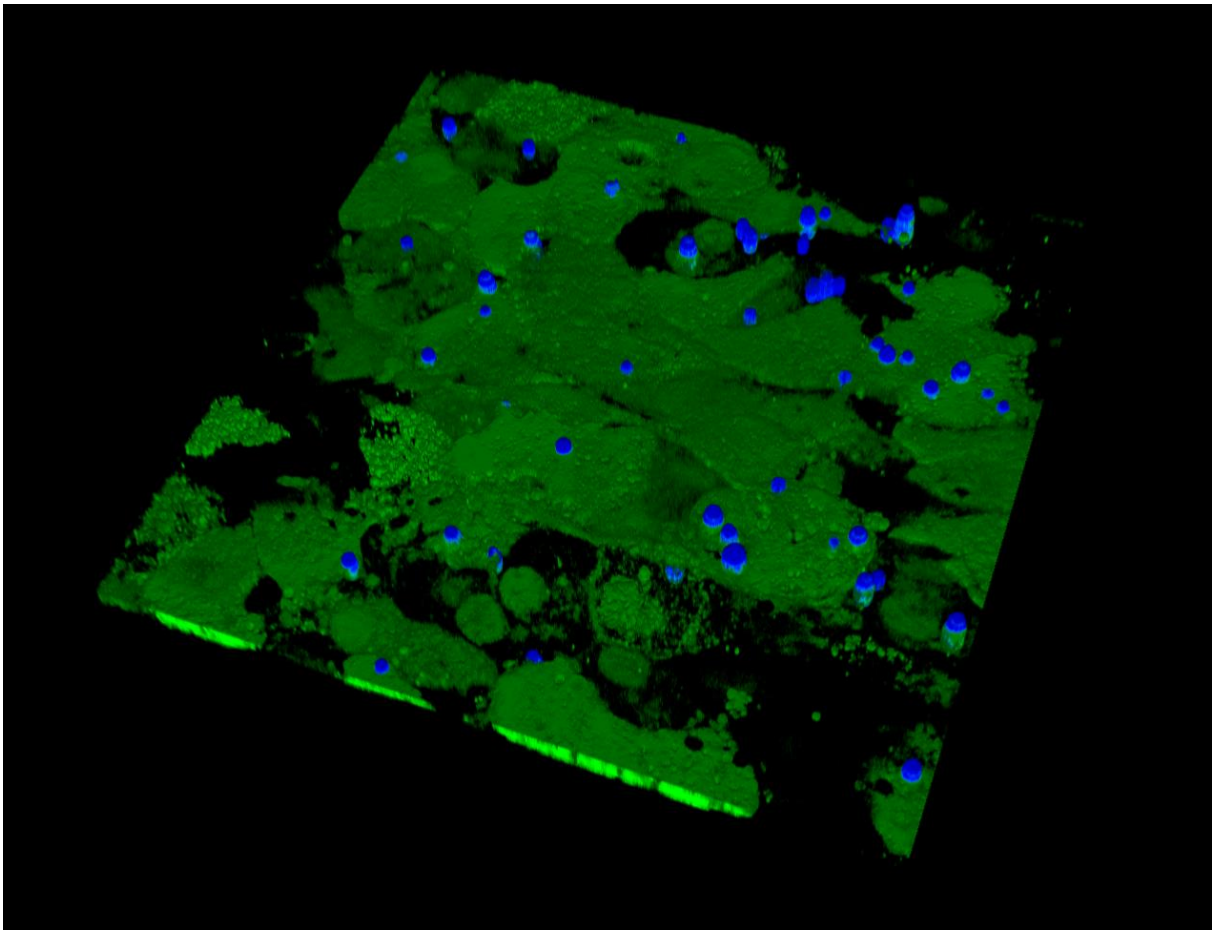

Figure 3: Modulation of MDdM phagocytosis properties within 3D dermis equivalent after TNF-α stimulation
The number of fluorescent beads phagocytosed by macrophages increased 17-fold (68 beads/dermis equivalent).

PERSPECTIVES

Whereas classical imaging methods provide limited functional data, these results show that advanced image processing algorithms with color specific segmentation can bring functional proofs (i.e., automatic quantification of fluorescent beads phagocytosed by MDdM).
Advanced image processing algorithms could be useful tools to find parameters which would allow to further investigate cell behavior.
The relationship and potential cross-talk between macrophages and their environment are currently deeply investigated in this way.