Maxime Lègues has been nominated for the Henry Maso Award for the work on this paper.

IFSCC INTERNATIONAL FEDERATION OF SOCIETIES OF COSMETIC CHEMISTS

The World's First 3D Bioprinted Immune Skin Model Suitable for Screening Drugs and Ingredients for Normal and Inflamed Skin

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INTRODUCTION

Human, and indeed all mammalian, evolution has led to a barrier to the outside environment with two main roles: firstly, water retention and secondly, a barrier to infections. It is normally an efficient barrier, whether at the epithelial or endothelial level.

Skin homeostasis relies heavily on the immunological interaction between cell types created in the bone marrow and those endogenous to the skin. This daily interaction is not just to fight infections from outside but also to monitor the daily clearance rate of cell turnover from aging and damaged epidermal and dermal cell types.

Abstract

Cosmetic regulations have accelerated making reliable safety and performance testing a priority. Allergy and sensitive skin research need better ingredient testing at all development stages. Therefore, we created 3D full-size bioprinted human skin models containing immune cells. Inflammatory M1 and M2 immune system roles prompted us to produce suitable models for safety and efficacy screening.

Same-donor keratinocyte and fibroblast populations were produced along with monocytes partially differentiated toward macrophage M0, M1, M2a, or M2c phenotypes. Final bioprinted models allowed maturation with LPS and/or inflammatory cocktails mimicking early or acute stages of atopic dermatitis. Response was evaluated by toxicity, cytokine release and histology.

Bioprinted models had good three-dimensional structure, with defined epidermis, dermis and differentiated macrophages. M2 were more motile than M1 and able to migrate more toward the epidermis. Cytokine production was measurable, and a defining distinction between M1 and M2 was differences between IL-1 and TNF production (higher in M1). Our proinflammatory M1 and anti-inflammatory M2 models were dose-responsive, reproducible and suitable for safety and efficacy screening of ingredients on inflamed skin conditions such as atopic dermatitis. With global pollution skin inflammation on the rise, our models are increasingly important in testing strategies for effective advanced formulations.

Key to the role of this homeostasis in controlling both innate and active immunity are macrophages. The fluidity of macrophage development between naïve monocytes, resident through M0 skin, and finally mature M1 and M2 active macrophages is a beautifully orchestrated symphony of communication between cells in the skin [1-3]. Cytokines, chemokines, and cell-to-cell interaction monitor skin health and respond to attack, trauma, and invasion at impressive speed [4-6].

However, for those who have allergies, or slow-developing long-term infections, the organization can be dysregulated by the same small molecules and cells that would normally try to help. It is not difficult in sensitive-skin patients for a circular loop of chronic irritation to form. Many cosmetic and dermatological strategies, not least in tackling conditions such as atopic dermatitis and psoriasis, are aimed at calming the epidermal environment, which can so easily be activated in sensitive skin. However, day-to-day inflammatory events are increasing generally, even in pathologically 'normal' skin. Many such strategies were previously aimed at strengthening the epidermal barrier to attack, but with limited success. Now, however, advanced cosmetics are investigating the immune-skin cell axis, aiming for a longer-term effect.

Historically, researchers used basic immunology cell interaction tests to try to

understand how to calm sensitive skin. However, that has limitations in the 'top-down' structure of skin response to external challenge, and indeed 'down-top' delivery of nutrients, oxygen, and immune system cells [7].

For this reason, we have created a human bioprinted immunized macrophage model of skin that researchers can use to help screen ingredients and finished products for the cosmetic and pharmaceutical industries. The model, which can be made even from one patient and any age-group or original phototype, allows the *in situ* development of naïve macrophages toward either an M1 or M2 phenotype, allowing an effective and rapid evaluation of a product prior to human donor testing. This screening system is human-orientated and was challenged with key molecules of irritation to evaluate the efficacy of the model for useful data acquisition.

The revolution of bioprinting gives hope for more rapid testing and better choices in skin barrier protection research. Further, combining our knowledge of skin with advanced immunology has provided the world's first efficient bioprinted skin model for this purpose.

EXPERIMENTAL

Sample collection

Skin samples and buffy coat were obtained from human donors following informed consent, and applicable French and European ethical guidelines and regulations from local hospitals in Lyon, France.

Cell isolation and amplification

a) Isolation

Fibroblasts and keratinocytes were isolated from human skin donors using enzymatic dissociation and grown in 6-well plates (Costar 3516; Corning, New York, USA) using RPMI 1640 medium (SH30096.01; HyClone supplemented with 15 % FBS (SH30109.03; HyClone,) and cyprofloxacin (4 µg/mL) and EpiLife medium (MEP1500CA; Gibco) supplemented with Human Keratinocyte Growth Supplement (HKGS) respectively (all *via* Thermofisher, Dardilly, France).

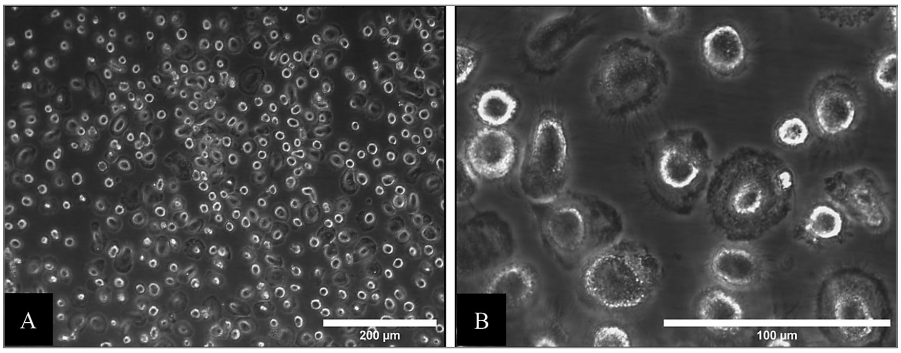

Figure 1 Differentiation of isolated monocytes into M1 macrophages
CD14 monocytes growing and differentiating into M1 macrophages observed with an optical microscope (Zeiss Primovert - Axiocam 105 color camera) at x20 (A) and x40 (B) after 11 days of differentiation.

Peripheral blood mononuclear cells (PBMC) were isolated first from buffy coat using Ficoll (17-1440-02; GE Healthcare, Buc, France) and counted on blood counter (MS9-5 MELET SCHLOESING, Osny, France). Then, CD14 monocytes were positively selected and isolated following CD14 MicroBeads (human) kit (130-050-201; Miltenyi Biotec, Paris, France). To verify the performance of the kit and ensure good isolation of CD14+ monocytes, flow cytometry was performed using a BD FACSVerse cytometer (Becton Dickinson, Grenoble, France). During the entire isolation process, the viability of all cells was tested using a LUNA-FL Dual Fluorescence Cell Counter (Logos Biosystems, Villeneuve d'Ascq, France).

b) Amplification and activation

Human fibroblasts were amplified using RPMI 1640 medium supplemented with 15 % FBS and cyprofloxacin (4 µg/mL) (All Thermofisher, Dardilly, France). Keratinocytes were grown in EpiLife medium supplemented with HKGS (Thermofisher). Monocytes were cultured using either M1-Macrophage Generation medium DXF, either M2- Macrophage Generation medium DXF kit (C-28055; C-28056; PromoCell, Fontenay-sous-Bois, France) to generate both M1 and M2 populations [1-3]. After 7 days, M1 and M2 macrophages were activated according to the differentiation kit recommendations using lipopolysaccharides (LPS) (L4130; Sigma Aldrich, Saint-Quentin-Fallavier, France) and IL-4 (130-095-373; Miltenyi), respectively ***(Figure 1)***. To ensure good differentiation of CD14+ monocytes in M0/M1/M2 macrophages, flow cytometry was performed using CD14 (V450 Mouse Anti-Human CD14; 560350; BD Horizon, CD80 (APC-H7 Mouse Anti-Human CD80; 561134; BD Pharmingen, Grenoble, France), CD163 (PE Mouse Anti-Human CD163; 560933; BD Pharmingen) and CD206 (APC Mouse Anti-Human CD206; 561763; BD Pharmingen) as screening markers.

3D bioprinting

3D bioprinted skin models were designed and 'software-sliced' beforehand on a computer using SketchUp (https://www.sketchup.com/) paid and Slic3r (https://slic3r.org/) opensource software respectively to generate a G-code file. Amplified cells were separately mixed with a bio-ink suitable for skin development and 3D printed (Bio X, CELLINK, Gothenburg, Sweden) in 12-well culture plates according to CTIBiotech optimization protocols. The bioink is a combination of nanofibrillar cellulose, sodium alginate, fibrinogen, mannitol and HEPES, adjusted to the skin microenvironment.

Maturation, differentiation, and viability of 3D bioprinted models

Printed models were grown over 21 days in Transwell culture inserts (Thermofisher, Dardilly, France), including dermal maturation, epidermis differentiation, air-liquid interface, and cornification steps of the bioprinted skin. The viability of cells was monitored with viability/cytotoxicity kit (L3224; Invitrogen, Thermofisher) and read on fluorescence microscope (Eclipse Ti, Nikon, Melville, USA) using FITC and TRITC channels.

Induction of inflammation and treatments

To assess the inflammatory response, models were systemically treated with different conditions every two days for six days: LPS 5 µg/ml; DMSO 0.1%; PBS; LPS 5 µg/mL and dexamethasone (dex) 100 µM (all Sigma). Each condition was performed in triplicates.

Cytokine levels

Following maturation and treatment steps, supernatants were collected and analyzed using Cytometric Bead Array (CBA) Human Inflammatory Cytokines kit from BD on BD FACSVerse cytometer. Human IL-6 and human IL-8 cytokines were also assessed with ELISA kits (Abcam, Paris, France).

Histology

At the end of the experiment, 3D bioprinted immune skin models were rinsed with 1X PBS (Corning) and fixed for 24 hours with buffered formaldehyde solution (Sigma Aldrich). After dehydration with ethanol, acetone and xylene, immune skin models were embedded in paraffin and sectioned into slices 5 µm thick (all Sigma Aldrich). Hematoxylin, eosin, saffron (HES) (Sigma Aldrich) and CD68 (Abcam) immunostaining were performed on the different conditions. Observations and acquisitions were carried out using a Leica DM2000 optical microscope and a Leica DMLB fluorescence microscope controlled by image acquisition software (LAS v4.2, Leica, Nanterre, France).

RESULTS

3D bioprinted full skin models reveal good dermis/epidermis development

a) Structure

3D bioprinted skin models displayed a good structure and viability after 14 days' growth ***(Figure 2)***. After 21 days of maturation (which varies between human skin donors), a multilayered, differentiated and cornified epidermis gradually developed on a rich dermis, as revealed by the histology analysis ***(Figure 2D)***. The dermal/epidermal junction (DEJ) was defined, separating organized differentiated keratinocytes from fibroblasts distributed in the dermal matrix with keratinocytes becoming denser at the junction.

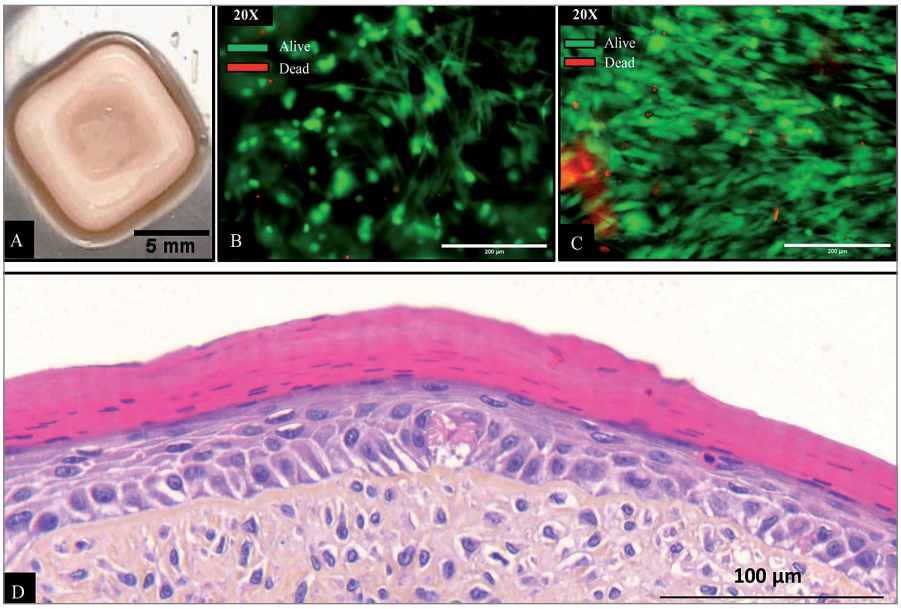

Figure 2 Structure and development of 3D bioprinted skin models
3D bioprinted skin models (A) matured over 15 to 21 days and viability of cells was checked using live/dead assays at day 6 (B) and day 14 on an inverted microscope focusing on the dermal region as an example (C). Hematoxylin, eosin, saffron stainings (HES) were performed after 21 days to assess the histology structure of models.

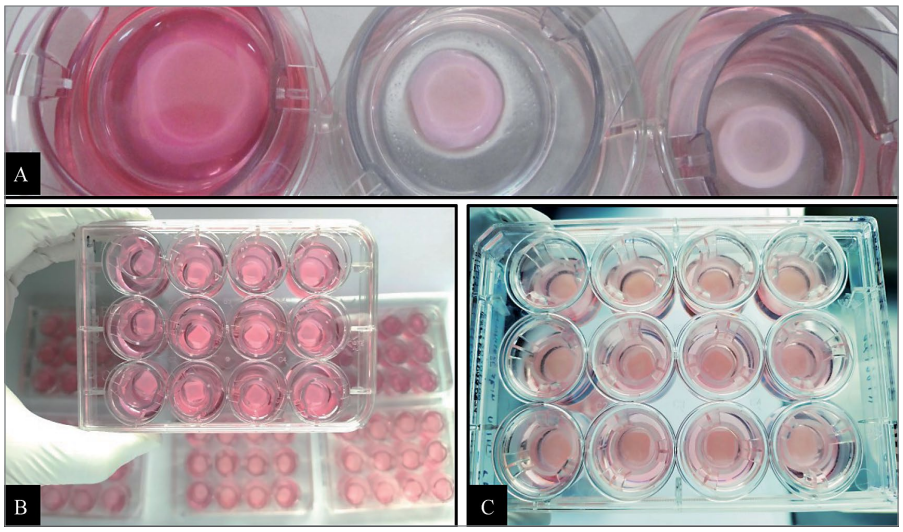

Figure 3 Reproducibility of 3D bioprinting
Manual skin models in our laboratory (A) *versus* 3D bioprinted skin models (B-C) maturing in 12-well plates. Pictures taken with a standard photo camera. Size reproducibility and quality are increased with bioprinting.

b) Reproducibility

One of the biggest advantages of 3D printing over manual skin models is reproducibility. Furthermore, it is possible to generate more models in less time than manually. Indeed, more than a hundred identical skin models had been 3D printed within two hours compared with just a few heterogeneous skin models produced manually ***(Figure 3)***. 3D printing provides gains in terms of time and reproducibility, enabling more efficient drug screening and testing of ingredients.

3D bioprinted immunized skin models show defined challenge response

a) Generation of M1 and M2 macrophages

To assess the level of monocyte differentiation, flow cytometry had been carried out

using CD14, CD80, CD163 and CD206 screening markers. M1 macrophages are known to be CD80+; CD163-; CD206+ phenotype, whereas M2 macrophages are known to be CD80-/low; CD163+; CD206+ [4-5-6]. Following the differentiation protocol, 97.12 % of the recorded cells were M1 phenotype, while only 26.99 % were gated in M2 phenotype ***(Figure 4)***, highlighting that M2 macrophages are more difficult to succeed in the differentiation step and are more difficult to generate in numbers. This is most likely due to the larger size and complexity of the M2 macrophage cell.

b) Macrophage integration into bioprinted skin

To demonstrate the viability of macrophages in the 3D bioprinted skin model, histology analysis was performed. HES stainings revealed good structure of the model and macrophages were well differentiated, with rounded kidney-shaped nuclei, highlighted by the DAPI staining on immunofluorescence ***(Figure 5)***. Although macrophages were highlighted in the dermis by CD68 immunofluorescence staining, they had a tendency to migrate toward the top of the model, in the direction of oxygen flow, which is normal for hematological cells of this type ***(Figure 5C, D)***. Compared with the negative control ***(Figure 5C)***, the model treated with LPS looked irritated and damaged, especially in relation to the epidermis. In addition, more macrophages were aggregated in the epidermis for the LPS-treated model, suggesting that macrophages migrated on account of the inflammatory response. Macrophages of the M2 type are harder to visualize than M1 type, owing to the larger diffuse type cytoplasm and cytoplasmic extensions. Migration after topical application and in response to oxygen tension is also observed with this type of hematological cell.

c) TNF, IL-10, IL-1β and IL-8 cytokines

Histology analysis showed that 3D bioprinted models treated with LPS (5 µg/mL) exhibited an irritated dermis and damaged epidermis ***(Figure 5D)***. Interrelated, secreted cytokines harvested in the culture medium indicated a significant inflammatory response to the LPS stimulation ***(Figure 6)***. Although the presence of macrophages in the skin model did not increase the level of secreted cytokines, TNF, IL-10, IL-1b and IL-8 concentrations were significantly higher after inflammation stimulation. Indeed, TNF concentration increased from 2.4 pg/mL for the non-stimulated condition "3D Skin + Mac" to 32.4 pg/mL for the LPS stimulated condition ***(Figure 6A)***. Identically, IL-10 and IL-1β cytokines concentrations were twice as high after stimulation ***(Figures 6B, C)***. Finally, the IL-8 cytokine level was more than tenfold, ranging from 10.06 µg/mL without stimulation to 113.36 µg/mL after induction of inflammation ***(Figure 6D)***. Additionally, treatment with dexamethasone seemed to decrease the level of human inflammatory secreted cytokines compared with the stimulated condition, especially in relation to TNF and IL-8 cytokines.

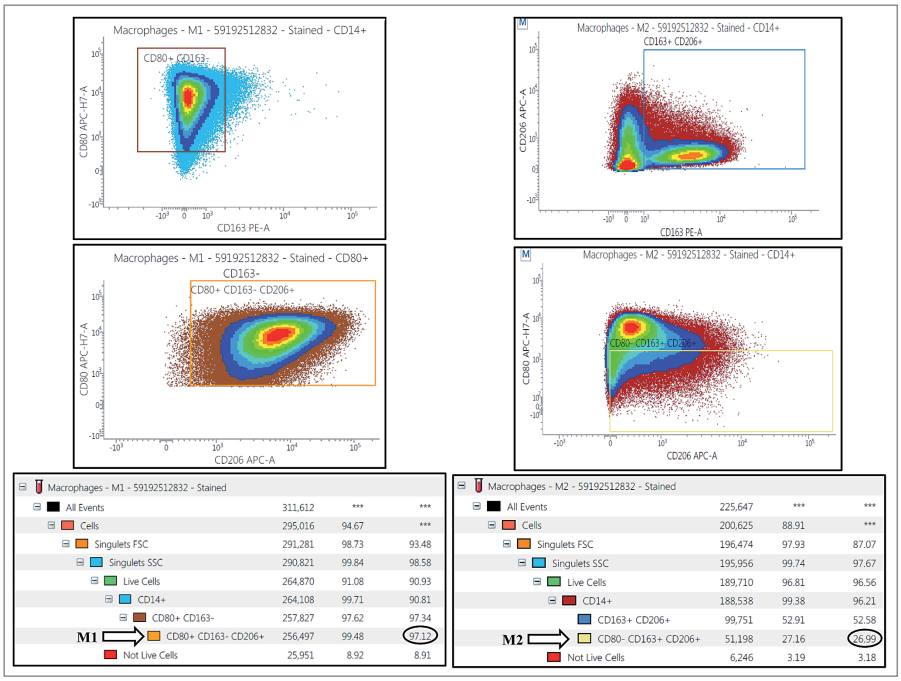

Figure 4 Flow cytometric analysis of M1/M2 macrophages differentiated from donor monocytes M1 (left) and M2 (right) macrophage differentiation using flow cytometry (BD FACSVerse cytometer and results analyzed on BD FACSuite software); populations were screened using CD14, CD80, CD163 and CD206 markers.

DISCUSSION

The global population is now close to 8 billion, with a growing number concentrated in cities. This is also accompanied by an increased burden of skin disease and sensitivity, possibly due to diet, lifestyle, and pollution [8]. The need for solutions to prevention and treatment of non-fatal skin disease rises each year, but the availability of suitable and affordable screening systems is not keeping up with need, in terms of innovation, speed and cost.

Bioprinting, while not new, has had limitations in the world of dermatology, with many applications limited to printing a model of either dermis (with fibroblasts) or epidermis (with keratinocytes). There is much left to be done in this area of skin research, but the growing need for more complex *ex vivo* skin models and *in vitro* testing rises each year owing to safety concerns and regulations. All areas of ingredient manufacturing are increasingly required to evaluate the safety of their products [9]

In our work, we aim to create innovation in skin testing and to bridge the gap between the development of ingredients and testing on human donors. Here we combine our knowledge in dermatology with immunology, hematology, and stem cell research to assist in creating complex screening models [10-12]. Particularly important in this is our aim, year on year, to increasingly remove animal products from

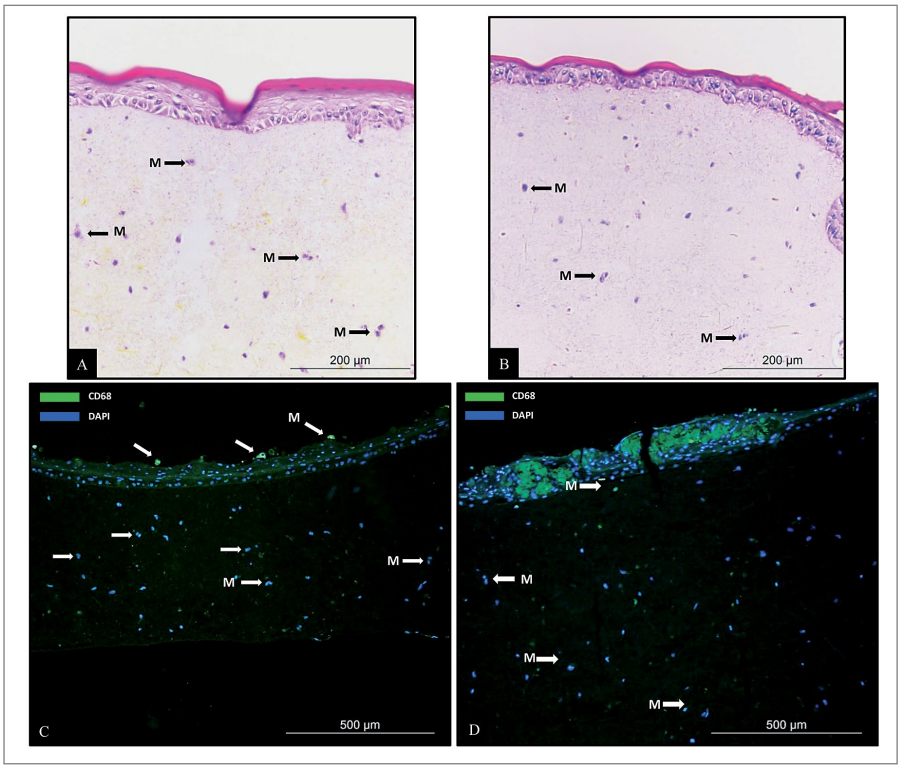

Figure 5 3D bioprinted immune skin models show macrophage activation and response to challenge
The structure of 3D bioprinted immune skin models was observed after HES staining (A-B).
A: 3D bioprinted skin model including M1 macrophages; B: 3D bioprinted model including M2 macrophages. Immunofluorescence (green = CD68; blue = DAPI) was performed to highlight macrophages in the models (C-D). C: 3D bioprinted immune skin model treated with PBS;
D: 3D bioprinted immune skin model treated with LPS 5 µg/mL. Arrows with "M" show examples of macrophages in the model.

our work and create human-compatible systems and models. Indeed, it has been an important aim in the skin research community for many years to have useful *in vitro* skin models for testing [13]. Advances in engineering technologies have led to bioprinting technologies, and these can now be applied to creating useful skin models [14-16].

Macrophages were chosen in this study because of their extreme importance in the immunological kinetics of the skin. The adaptability between the innate and adaptive immune processes can be interrupted in skin kinetics, leading to the chronic skin irritation that is seen in atopic dermatitis, and is of particular concern in the pediatric setting [7, 17].

Here, we achieved successful 3-dimensional (3D) skin printing, often described as 'full-skin models', but with the added advantage of having either the mature M1 or M2 macrophage system included ***(Figure 5)***. With our methodology, it is possible to print many such models – and certainly over 100 models – in a few hours, giving hope of more automated screening preparation systems. Reproducibility is a further advantage of bioprinting. Of particular note is the fact that our models achieve a sinusoidal epidermis/dermis structure, more typical of pathological of skin ***(Figures 5A, B)***, which many bioprinted models have historically failed to do.

Macrophages in our models reached a suitable maturity, which was demonstrated in their reaction during the challenge phases of our experiments ***(Figures 5, 6)***. Macrophages are oxygen-hungry cells, and there was a tendency to move toward the surface of the model, where oxygen is more abundant in an *in vitro* system. We intend to improve this with advanced cornification and drying steps in future work. We believe that a longer cornification period will help to alleviate this movement north and allow a more even distribution of the macrophages throughout the skin model. That being said, the migration of macrophages north in atopic dermatitis is also a normal process, and our model does well to demonstrate that during the challenge phases, ***(Figure 5D)***, which actually makes for a perfect screening model for cosmetic and/or pharmaceutical ingredients that are being developed to calm irritated skin. Further to that, the specificity of cytokine release (example in ***Figure 6***) demonstrates that the models are sensitive testing systems, with good response to LPS and dexamethasone challenges.

In our model development, growth of the M1 models was easier than the M2 models. M2 macrophages are delicate cells and not as robust as the M1 system, but we are now developing an *in situ* maturation phase specific to the M2 system to optimize this fragility. This work is part of our advancement of 3D bioprinted models with added microbiota, since we believe that this combination is important for a true model of diseased and burnt skin [18]. Further, our models have the advantage of being able to use the same donors. Historically, a disadvantage of 3D *in vitro* skin models was the use of pooled donor keratinocytes and fibroblasts, but we have overcome this hurdle effectively. Further, our models have no limitations in terms of ethic source and skin phototype, allowing for globalized testing parameters important for product testing [19].

The modern technology convergence of bioprinting with modern flow cytometric bead array has been a particular advantage in the reproducibility and speed of testing. We believe that this combination allows for a more rapid answer to both toxicology and safety testing, not least for the development of botanical alternatives to pharmaceutics in atopic dermatitis treatments. However, the move towards botanical natural treatment options requires *in vitro* testing which is quite strict and accurate to overcome regulatory hurdles [20, 21]. A more routine and optimized screening system also means less human donor testing and cheaper routes to market.

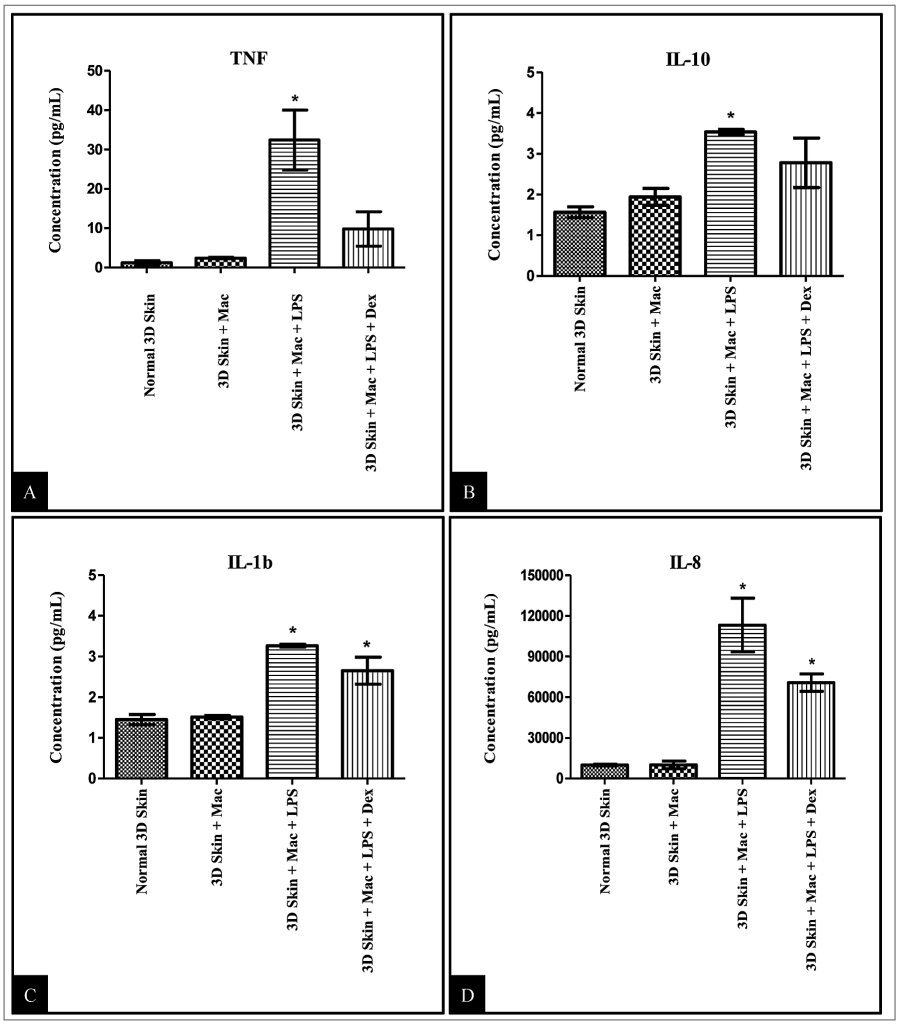

Figure 6 3D bioprinted inflammatory skin models display accurate cytokine profiling
Secreted cytokines were quantified with Cytometric Bead Array (CBA) kit from BD and ELISA assay. Four different conditions assessed:
"Normal 3D Skin" = Full skin model; "3D Skin + Mac" = Full skin model including M1 macrophages representing negative control; "3D Skin + Mac + LPS" = LPS stimulated (5 µg/mL) full skin model including M1 macrophages; "3D Skin + Mac + LPS + Dex" = LPS (5 µg/mL) stimulated full skin model including M1 macrophages treated with dexamethasone (100 µM). (A) TNF; (B) IL-10; (C) IL-1β; (D) IL-8. Error bars represent ±SE. Asterisks "*" highlight averages significantly different from negative control (p value < 0.05 – ANOVA Tukey-Kramer statistical test).

CONCLUSION

We have developed an innovative immunized mature macrophage-based full-skin *in vitro* testing system for the screening of cosmetic ingredients designed either to reduce inflammation or to prevent it. The applications to the cosmetic industry are significant and bring hope of optimized and sophisticated cosmetics of the future.

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References

[1] *Biedermann, T., Röcken, M. and Carballido, J.M.,* TH1 and TH2 lymphocyte development and regulation of TH cell-mediated immune responses of the skin, Journal of Investigative Dermatolology Symposium Proceedings, **9**(1) (2004), 5-14.

[2] *Mia, S., Warnecke, A., Zhang, X.M., Malmström, V., Harris, R.A,* An optimized protocol for human M2 macrophages using M-CSF and IL-4/IL-10/TGF-β yields a dominant immunosuppressive phenotype, Scandinavian Journal of Immunology, **79**(5) (2014), 305-314.

[3] *Saqib, U., Sarkar, S., Suk, K., Mohammad, O., Baig, M.S., Savai, R.,* Phytochemicals as modulators of M1-M2 macrophages in inflammation, Oncotarget, **9**(25) (2018), 17937-17950.

[4] *Rafiul, H.A.S.M., Moriyama, M., Kubota, K., Ishiguro, N., Sakamoto, M., Chinju, A., Mochizuki, K., Sakamoto, T., Kaneko, N., Munemura, R., Maehara, T., Tanaka, A., Hayashida, J.N., Kawano. S., Kiyoshima. T., Nakamura. S.,* CD206 + tumor-associated macrophages promote proliferation and invasion in oral squamous cell carcinoma *via* EGF production, Scientific Reports, **9**(1) (2019), 14611.

[5] *Kubota, K., Moriyama, M., Furukawa, S., Rafiul, H.A.S.M., Maruse, Y., Jinno, T., Tanaka, A., Ohta, M., Ishiguro, N., Yamauchi, M., Sakamoto, M., Maehara, T., Hayashida, J.N., Kawano, S., Kiyoshima, T., Nakamura, S.,* CD163 + CD204 + tumor-associated macrophages contribute to T cell regulation *via* interleukin-10 and PD-L1 production in oral squamous cell carcinoma, Scientific Reports, **7**(1) (2017), 1755.

[6] *Raggi, F., Pelassa, S., Pierobon, D., Penco, F., Gattorno, M., Novelli, F., Eva, A., Varesio, L., Giovarelli, M., Bosco, M.C.,* Regulation of Human Macrophage M1-M2 Polarization Balance by Hypoxia and

the Triggering Receptor Expressed on Myeloid Cells-1, Frontiers in Immunology, **8** (2017),1097.

[7] *Ribet, V., Gurdak, M., Ferret, P.J., Brinio, E., Labadie, F.G., Rossi, A.B.,* Stepwise approach of development of dermocosmetic products in healthy and atopic dermatitis paediatric population: safety evaluation, clinical development and postmarket surveillance, Journal of the European Academy of Dermatology and Venereology, **33**(12) (2019), 2319-2326.

[8] *Seth, D., Cheldize, K., Brown, D., Freeman, E.,* Global Burden of Skin Disease: Inequities and Innovations, Current Dermatology Reports, **6**(3) (2017), 204-210.

[9] *Henkler, F., Tralau, T., Tentschert, J., Kneuer, C., Haase, A., Platzek, T., Luch, A., Götz, M.,* Risk assessment of nanomaterials in cosmetics: a European union perspective, Archives in Toxicology, **86** (11) (2012),1641-1646.

[10] *Jurga, M., Forraz, N., McGuckin, C.P.,* Artificial tissues from cord and cord blood stem cells for multi-organ regenerative medicine: viable alternatives to animal *in vitro* toxicology, Alternatives to Laboratory Animals, **38**(2) (2010), May 183-92.

[11] *Mueller, A.A., Forraz, N., Gueven, S., Atzeni, G., Degoul, O., Pagnon-Minot, A., Hartmann, D., Martin, I., Scherberich, A., McGuckin, C.,* Osteoblastic differentiation of Wharton jelly biopsy specimens and their mesenchymal stromal cells after serum-free culture, Plastic Reconstructive Surgery, **134** (2014) 59-69.

[12] *Fabre, H., Ducret, M., Degoul, O., Rodriguez, J., Perrier-Groult, E., Aubert-Foucher, E., Pasdeloup, M., Auxenfans, C., McGuckin, C., Forraz, N., Mallein-Gerin, F.,* Characterization of different sources of human MSCs expanded in serum-free conditions with quantification of chondrogenic induction in 3D, Stem cells international (2019).
doi.org/10.1155/2019/2186728

[13] *Kandarova, H., Liebsch, M., Genschow, E., Gerner, I., Traue, D., Slawik, B., Spielmann, H.,* Optimisation of the EpiDerm test protocol for the upcoming ECVAM validation study on *in vitro* skin irritation tests. Alternatives to Animal experimentation, **21**(3) (2004), 107-114.

[14] *Zhou, F., Hong, Y., Liang, R., Zhang, X., Liao, Y., Jiang, D., Zhang, J., Sheng, Z., Xie, C., Peng, Z., Zhuang, X., Bunpetch, V., Zou, Y., Huang, W., Zhang, Q., Alakpa, E.V., Zhang, S., Ouyang, H.,* Rapid printing of bio-inspired 3D tissue constructs for skin regeneration, Biomaterials, (2020) Nov;258:120287.
doi:10.1016/j.biomaterials.2020.120287.
Epub ahead of print 2020 Aug 14.

[15] ***Perez-Valle, A., Del Amo, C., Andia, I.,*** Overview of Current Advances in Extrusion Bioprinting for Skin Applications. International Journal of Molecular Science, (2020) Sep12;21(18):6679. Epub ahead of print.
doi: 10.3390/ijms21186679

[16] *Tan, C.T., Liang, K., Ngo, Z.H., Dube, C.T., Lim, C.Y.,* Application of 3D Bioprinting Technologies to the Management and Treatment of Diabetic Foot Ulcers, Biomedicines, **8**(10) (2020), Oct 21, 441.
doi: 10.3390/biomedicines8100441

[17] *Lee, D.H., Park, J..K, Choi, J., Jang, H., Seol, J.W.,* Anti-inflammatory effects of natural flavonoid diosmetin in IL-4 and LPS-induced macrophage activation and atopic dermatitis model, International Immunopharmacology, 89(Pt A) (2020), Oct 9, 107046.
doi: 10.1016/j.intimp.2020.107046
Online ahead of print.

[18] *Huitema, L., Phillips, T., Alexeev, V., Tomic-Canic, M., Pastar, I., Igoucheva, O.,* Intracellular escape strategies of Staphylococcus aureus in persistent cutaneous infections, Experimental Dermatology, (2020) Nov 11.
doi: 10.1111/exd.14235
Online ahead of print.
PMID: 33179358 Review.

[19] *Diridollou, S., de Rigal, J., Querleux, B., Leroy, F., Holloway Barbosa, V.,* Comparative study of the hydration of the stratum corneum between four ethnic groups: influence of age, International Journal of Dermatology, **46** Suppl 1 (2007), 11-4.
doi: 10.1111/j.1365-4632.2007.03455.x

[20] *Draelos, Z.D., Traub, M., Gold, M.H., Green, L.J., Amster, M., Barak-Shinar, D., Kircik, L.H.,* Validation of Botanical Treatment Efficiency for Adults and Children Suffering from Mild to Moderate Atopic Dermatitis, Journal of Drugs in Dermatology, **18**(6) (2019), Jun 1, 557.

[21] *Janeczek, M., Moy, L, Lake, E.P., Swan, J.,* Review of the Efficacy and Safety of Topical Mahonia aquifolium for the Treatment of Psoriasis and Atopic Dermatitis, Journal of Clinical and Aesthetic Dermatology, **12** (2018), 42-47.

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