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**TITLE:** COMPUTATIONAL MODELING PREDICTS IMPAIRED BARRIER FUNCTION AND HIGHER SENSITIVITY TO SKIN INFLAMMATION FOLLOWING PH ELEVATION

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## Abstract

Skin surface pH has been identified as a key regulator of the epidermal homeostasis through its action on serine protease activity. These enzymes, like kallikreins (KLK), are responsible for the degradation of corneodesmosomes, the protein structures linking together corneocytes, and are regulated by Lympho-Epithelial Kazal-Type-related Inhibitor (LEKTI). KLK activity increases at pH levels higher than physiological. An increase in skin surface pH has been observed in patients suffering from skin diseases characterized by impaired barrier function, like atopic dermatitis. In this work, we introduce an agent-based model of the epidermis to study the impact of a change in skin surface pH on the structural and physiological properties of the epidermis, through the LEKTI-KLK mechanism. We demonstrate that a less acidic pH, compared to the slightly acidic pH observed in healthy skin, is sufficient to significantly affect the water loss at the surface and the amount of irritant permeating through the epidermis. This weakening of the skin barrier function eventually results in a more intense skin inflammation following exposure to an external irritant. This work provides additional evidence that skin surface pH and serine proteases can be therapeutic targets to improve skin barrier integrity.

## Introduction

The main function of skin is to act as a barrier, protecting us both from losing water (dehydration) and from the penetration of external aggressors, like irritants or pathogens. Skin barrier dysfunction has been identified as one of the key elements involved in the pathology of several cutaneous diseases including atopic dermatitis (AD) <sup>1</sup>. This disease can manifest at any age but affects primarily children. Its worldwide prevalence among children aged 6 months to less than 6 years is 12.1% <sup>2</sup>. Patients suffer from chronic skin lesions characterized by intense itch, erythema, very dry and cracked skin.

The acidic pH at the surface of healthy skin contributes to its protection against invading pathogens <sup>3</sup>. Above all, skin surface pH is a central regulator of *stratum corneum* (SC) homeostasis through its action on enzymatic activity <sup>3</sup>. Under physiologic conditions, the serine protease enzymes, like kallikreins

(KLK), ensure epidermal renewal via the degradation of corneodesmosomes that link together the corneocytes, eventually leading to desquamation<sup>4</sup>. The activity of the several types of KLK present in the SC (KLK5, KLK7, KLK14), is highly sensitive to pH, increasing in less acidic environments<sup>5</sup>.

Skin surface pH is thought to play an important role in the compromised skin barrier related to AD. Indeed, an elevation of skin surface pH has been observed in patients with AD<sup>6</sup>. In certain cases this increase can be partly explained by a mutation of the *FLG* gene coding for filaggrin, eventually leading to a faulty production of natural moisturizing factors (NMF), some of which act as pH buffers<sup>7</sup>. Although *FLG* mutation has been identified as a major risk factor for AD, it is not the only factor influencing skin surface pH, keeping in mind that the proportion of *FLG* mutation carriers among AD patients varies between 14.2 and 56% depending on the study<sup>8</sup>.

The role of elevated pH in the pathogenesis of AD has been investigated with a murine model<sup>9</sup>, and skin surface pH was identified as a key therapeutic target to improve the skin condition and control AD symptoms. Nevertheless, the mechanisms and effects of a change in pH remain incompletely understood. The role of pH and more broadly, the interaction between skin barrier and inflammation has been previously studied with several ordinary differential equations (ODE) models<sup>10–12</sup>. While providing interesting insights, these approaches overlook the dynamic nature of skin structure and heterogeneous distribution of the different reactions throughout the epidermis. In the present work we introduce a dynamic agent-based computational model of the epidermis, integrating the interaction of KLK with the Lympho-Epithelial Kazal-Type-related Inhibitor (LEKTI), to demonstrate that an increase in skin surface pH alone, through the LEKTI-KLK mechanism, is sufficient to compromise the skin barrier, facilitating external irritant penetration and triggering skin inflammation.

## Methods

For this work, we build on the agent-based model of adult epidermis described by Stamatias *et al.*<sup>13</sup>. This model is developed using the modeling and simulation platform EPISIM<sup>14</sup>. It recapitulates the epidermis structure and the permeation of a topically applied substance as measured *in vivo*. The

substance is exchanged between neighboring agents according to the concentration gradient, weighted by a coefficient, taking values between 0 and 1, representing both partition and diffusion. One agent represents the cell and its lipid surroundings. The different compositions of the SC and the viable epidermis (VE) are translated into two different inter agent exchange rates. Using depth profiles measured *in vivo* with Raman spectroscopy, the model has been calibrated to reproduce the observed behavior of specific compounds, by adjusting the values of the inter agent exchange rates. The model proposed by Stamatias *et al.*<sup>13</sup> also includes a barrier to water exchange between neighboring cells and to water loss at the surface depending on the water gradient and the amount of lipids and tight junctions<sup>15</sup>. Tight junctions are produced in the *stratum granulosum* (SG) and retained upon differentiation to SC cells. Lipids are released at the interface of SG and SC. In this model, the role of KLK is only considered indirectly through the decay of corneocyte-corneocyte adhesion forces. In order to study the impact of a change in pH, the KLK activity has to be added in the agent-based model more directly.

### **KLK-LEKTI module**

The ODE model described by Tanaka *et al.*<sup>10</sup> is adapted to focus only on the interactions of KLK with its inhibitor LEKTI (Supplementary 1). This model is then translated into an SBML (Systems Biology Markup Language) model<sup>16</sup> using the BIOCHAM software<sup>17,18</sup> to handle the differences in timescales (Supplementary 2). BIOCHAM also allows to check for the well-formedness and strictness conditions of the model, as defined by Fages *et al.*<sup>18</sup>. These conditions guarantee, among other things, the positivity of the system. The EPISIM platform then provides a method to integrate SBML models into the agent-based one, mapping the timescales<sup>14</sup>. In our case, the mapping is done so that the SBML model describing KLK activity has reached steady state over the course of one simulation step of the agent-based model. The pH influence on KLK activity is translated into two sets of parameters as described by Tanaka *et al.*<sup>10</sup>, representing healthy skin with an acidic pH (around 4.5) and compromised skin with a less acidic pH (around 6.5) (Supplementary 3). We selected one pH value in

the physiological range according to Proksch<sup>3</sup> and one above to represent a compromised skin barrier condition. To facilitate comparisons we followed the choice of pH values used by Tanaka *et al.*<sup>10</sup>. When integrating the KLK-LEKTI module into the agent-based model, the location of the reactions has to be taken into account. Biologically KLK and LEKTI are released at the interface of SG and SC, together with the lamellar bodies, a process that we call corneocytes maturation. Consequently, in the agent-based model, the LEKTI-KLK module is only active in the mature corneocytes, meaning those which have released all their lamellar bodies. The cleaving action of active KLK (KLK\*) on corneodesmosomes is modeled as a decay of adhesion forces, proportional to the amount of KLK\*. When the corneocyte-corneocyte adhesion goes below a certain threshold, the cell is desquamated and removed from the simulation. The action of KLK\* on adhesion forces is normalized to the quantity of KLK\* at steady state produced with the set of parameter values corresponding to physiological pH. This way, the previously validated epidermal structure is preserved.

### **Inflammation Module**

The inflammation module developed in this work represents the release of inflammatory molecules, such as cytokines, subsequently to the penetration of an irritant through the epidermis. The inflammatory reaction only begins when the irritant crosses the SC, as the corneocytes lost their nucleus when differentiating and cannot have any synthetic activity. Once the concentration of irritant around a cell of the VE rises above a certain threshold *irritation\_threshold* the cell starts producing inflammatory molecules (*[IM]*) according to:

$$\frac{d[IM]}{dt} = p_i + d_i [IM]$$

When the irritant concentration is lower than *irritation\_threshold*, the cell no longer produces inflammatory cytokines ( $p_i = 0$ ) but their degradation persists. The value for the *irritation\_threshold* is set to 0.1 so that a release of cytokines would be observed for both pH conditions, given the concentration of irritant applied at the surface equal to 1, selected for demonstration purpose. A

threshold value set too high, results in no cytokine release in both pH conditions. On the contrary, lowering the threshold leads to a higher response for both skin conditions, even though the comparison will remain qualitatively the same. The parameters  $p_i$  and  $d_i$  control the kinetics and magnitude of the inflammation. For inflammatory molecules to be produced, the value of  $p_i$  needs to be higher than the one of  $d_i$ . To allow for the ability to examine the dynamic changes in  $IM$  concentration within the time frame defined by the model, the values of  $p_i$  and  $d_i$  are set to 2 and 0.005 respectively. As they are the same values for both pH conditions, the qualitative comparison between them is not affected by changes of these values (Supplementary 4). Finally, the inflammatory cytokines are exchanged between neighboring cells according to the concentration gradient.

### **Simulations**

The simulations are done on a fully built and stable epidermis starting after 5000 simulation steps. Then the SBML model of KLK activity is switched on, and we let the model accommodate for another 500 steps to get stable thickness of the SC. The irritant is applied topically with a surface concentration of 1, inter-agent exchange rate equal to 0.58 in SC, and 0.29 in the VE. Depending on the scenario tested, the irritant is either removed after 50 simulation steps or continuously applied until the end of the simulation. In both cases during the application, the surface concentration of the irritant is constant, simulating the application of a patch on the skin. Simulations of the two models (physiological and less acidic pH) are compared in terms of model emerging properties, that is properties resulting from the agents' interactions, not directly encoded in the model. More specifically, we look at the thicknesses of different skin layers, the total amount of irritant permeated in the SC and VE, the resistance to water transport, water loss at skin surface and total amount of inflammatory cytokines produced.

### **Simulated water loss at the surface and resistance to water transport**

The simulated water loss at the skin surface is computed as the average of the *waterflow\_surface* parameter over all surface cells. This parameter value is proportional the intracellular water content,

the ratio of exposed cell surface area, the barrier properties of the cell depending on tight junctions and lipids, and evaporation rate. The resulting value for water loss at the skin surface and the simulated water depth profile are then used to compute the depth-dependent resistance to waterflow according to the method described by MDA van Logtestijn *et al.* <sup>19</sup>.

## Results

### Model overview

To study the impact of skin surface pH on skin barrier and inflammation, we introduce an agent-based model of the epidermis (Figure 1). This model builds on a previously presented model that considers explicitly keratinocyte proliferation, differentiation, and desquamation processes and can recapitulate skin structure, water barrier function, and penetration kinetics of an external substance introduced at the skin surface at a given time of the simulation <sup>13</sup>. This model is modified here by the introduction of KLK production, auto-activation (KLK\*), and inhibition by LEKTI, processes which affect the rate of corneocyte desquamation at the skin surface <sup>20,21</sup>. The mathematical model first described by Tanaka *et al.* <sup>10</sup> is adapted and integrated at the agent-level programming of the corneocytes, present only in the SC. This modification of the original agent-based model does not disturb the stability of the system and preserves the epidermal structure at skin surface pH of 4.5 (Supplementary 5).

The computational model highlights two coexisting mechanisms, both of which are able to lead to increased desquamation. Figure 2 shows that an increase in pH, affects the values of several parameters, resulting in higher KLK\* concentration at steady-state and eventually leading to increased corneodesmosomes degradation and faster desquamation. This process is inhibited by LEKTI. A threshold effect can be observed in the presence of LEKTI concentration sufficient to form a complex with all available KLK\*, completely preventing corneodesmosomes degradation. The model can be used to simulate the effect of irregular LEKTI production due to genetic mutations on *SPINK5* (the gene encoding for the LEKTI protein). Such mutations are sometimes present on AD patients <sup>22</sup>. *SPINK5* mutations result in loss of function of LEKTI and can be simulated in the model as a decrease of the *mL*

parameter, representing the basal production of LEKTI by keratinocytes. Figure 2 illustrates that at a given skin pH, the decrease of LEKTI production results in an increase of KLK\* concentration at steady-state, again leading to higher rate of corneocyte desquamation.

The agent-based model also allows for the simulation of permeation of a topically applied substance, as described by Stamatias *et al.*<sup>13</sup>. Here, we consider that the externally applied substance is an irritant, which can trigger skin inflammation depending on its concentration. The irritant is introduced in the model at the skin surface at a given time. It then diffuses through the epidermis following its concentration gradient. At the junction between the SC and the VE the inter-agent exchange rate is adjusted to reflect the compositional difference of the two layers (lipophilic SC compared to hydrophilic VE). Finally, the release of inflammatory molecules by keratinocytes in the VE is introduced in the model. This release is triggered when the irritant concentration inside the cell rises above a pre-defined threshold, simulating the release of inflammatory cytokines from cellular stores, which is the case for interleukin (IL)-1 $\alpha$  for example. This model was built as a qualitative tool to investigate the impact of skin pH, considering that all other influencing factors remain equal. The values of inflammation parameters were chosen only as example demonstrating the dynamics of the inflammatory reaction at two skin surface pH conditions (physiological and less acidic pH). A more precise simulation would require experimental data of irritant-induced inflammatory reaction. Unfortunately, there is a lack of available *in vivo* studies involving molecules that act as irritants without interfering with other skin functions. Overall, the agent-based model provided here integrates a refined desquamation process, permeation of an irritant substance and acute inflammation, in a dynamic and spatially discrete way. It enables to investigate the impact of a change in skin pH at tissue level through the study of the system's emerging properties. These are properties that were not directly coded but rather emerge from the interactions of agents. The emerging properties studied are robust to small variations of pH-dependent parameters (Supplementary 7).

### **Impact on epidermal structure**

Increased skin surface pH (and subsequent increase in KLK activity) has an immediate effect on the SC thickness (Figure 3). KLK\*, through its action of degrading corneodesmosomes, directly affects corneocyte adhesion and therefore the desquamation rate. The simulations demonstrate that increased KLK activity results in thinner SC by about 18% (Table 1). The model also predicts a thinning of the VE by about 6% (Table 1). This observation can be explained by the alteration of the calcium gradient controlling the differentiation process, consequently to SC thinning. This in turn results in weakening of the skin barrier function in both directions: outside-in (irritant permeation) and inside-out (water loss).

### **Weakening on the skin barrier function**

The skin's capacity to retain water is assessed in the model by two parameters: water loss at skin surface and resistance to water flow. At skin surface pH of 6.5, the water loss at the skin surface measured *in silico* is about 9% higher compared to a more physiologic condition of pH 4.5 (Table 1). Moreover, figure 3 shows a decrease in resistance to water flow, particularly in the lower part of the SC. These observations are in agreement with clinically reported results concerning changes in water transport through the SC following lipid removal, tape stripping or comparing AD to healthy skin<sup>23</sup>.

To evaluate the strength of the outside-in barrier, topical application of an external substance is simulated. Figure 3 shows the kinetics of the total substance amount that permeated in the VE. At a less acidic pH, the simulation predicts faster substance penetration and higher total permeated quantity at steady state compared to the physiological pH condition.

### **Resulting skin inflammation**

The impaired outside-in barrier function can have direct consequences on the release of inflammatory cytokines. Figure 4 shows the simulated production of inflammatory cytokines resulting from the permeation of an irritant into the epidermis, following continuous irritant exposure at the skin surface for 24 hours. At the peak of inflammation, the same amount of irritant applied at the surface induces an increase in the concentration of inflammatory cytokines by about 44% when skin pH is set at 6.5

compared to 4.5. Note that the production of inflammatory cytokines also starts earlier in the compromised skin model compared to healthy skin.

## Discussion

We developed a computational model of the epidermis taking into account the influence of pH on enzymatic activity as a tool to better understand and visualize the role played by skin surface pH on the skin barrier function. By simulating two scenarios differing only in terms of KLK activity level, our model illustrates the key role played by pH on the barrier. An increase of KLK activity alone, due to a less acidic pH, is sufficient to affect significantly both the skin capacity to retain water and its permeability to irritant. Moreover, this alteration of the skin barrier function can result in a more intense inflammatory reaction. The effects highlighted here can begin to explain the propensity to irritation and dryness observed in AD or irritant contact dermatitis related to an elevation of skin pH.

AD is a multifactorial disease involving the interplay between impaired skin barrier function, inflammation, and environmental factors<sup>1</sup>. Among those, the epidermal barrier dysfunction has been identified as “a primary event in the development of AD”<sup>24</sup>. Since skin pH is an important regulator of SC homeostasis and therefore skin barrier function, it has naturally been identified as a potential mechanism that can be used to improve the severity of symptoms<sup>25-27</sup>. It is thus important to consider the effect of a topical skincare product on skin surface pH, especially when the product is intended for atopic skin. While the model introduced here clearly demonstrates the effects of skin pH that are relevant to AD, it could be further developed to include additional aspects of AD pathology, including the involvement of microbiome dysbiosis<sup>28</sup>, the role of allergens<sup>29</sup>, or a more complex inflammation pathway involving specific cytokines (IL-4, IL-13, IL-33<sup>30,31</sup>) and Th2 cells. Moreover, the model can also be further developed to include aspects of AD lesional skin, like keratinocytes hyperproliferation, reduced secretion of lamellar granules (therefore less KLK), and SC hyperkeratosis<sup>32</sup>. The latter phenomenon however could be countered by an increased KLK expression stimulated by Th2

cytokines<sup>33</sup>. The integration of these additional modules in the model would necessarily require data, preferably measured *in vivo*, that currently do not exist.

In our qualitative model, the parameter values related to the inflammatory reaction were selected arbitrarily due to the lack of *in vivo* data involving irritants that trigger skin inflammation without disrupting skin barrier function. For example, surfactants like sodium lauryl sulphate (SLS) are disturbing skin lipid content and organization, effects that would need to be considered in the simulation separately. As presented in our previous work<sup>13</sup>, experimentally determined concentration profiles would be required for tuning the permeation parameters of an externally applied substance. If this substance is a skin irritant, this can be technically challenging. Furthermore, it is difficult to track quantitatively the production of inflammatory molecules *in vivo*. Nevertheless, parameter values related to the inflammatory reaction are shared by both pH conditions tested in this model, meaning that the qualitative comparison between them will remain the same. Due to the predicted alteration of the epidermal structure, the irritant substance will reach the VE sooner and in higher amounts, inducing a higher inflammatory response, regardless of the values given to these parameters.

Inflammation is a very complex physiological process involving different molecular cascades and activation sequences of several different cell types. In the present work we only considered the initial events of such an inflammatory cascade. In the future it is conceptually feasible to integrate in the model further downstream aspects of skin inflammation, for example to investigate the key role played by Th-2 cells in AD. Additionally, several studies have demonstrated that besides their activity on corneodesmosomes, certain kallikreins like KLK5 and KLK14, are able to activate proteinase-activated receptor-2 (PAR2)<sup>34,35</sup>. PAR2 is a transmembrane receptor present in keratinocytes that downregulates the lamellar bodies secretion<sup>36</sup> and triggers a proinflammatory and proallergic pathway upon activation<sup>35</sup>. Integrating this indirect influence of KLK on barrier formation and inflammation in the model would increase our understanding of the extent of KLK impact on epidermis homeostasis.

In this work we modeled the effects of a skin pH value as a constant within the SC. This approximation resulted in reasonable results, however a more detailed model would consider that pH actually follows a gradient in the SC, from neutral pH at the interface with SG to acidic at the skin surface<sup>37</sup>. As discussed earlier, the enzymatic reactions involving KLK and LEKTI are highly sensitive to pH<sup>20</sup>. In that case both the inhibition of KLK by LEKTI and the cleaving of corneodesmosomes by active KLK would be depth dependent. Additionally, the impact of pH on skin barrier, is not limited to its action on enzymatic activity. A recent study has shown that a more neutral pH prevents the right formation of the SC lipid structure, leading to even more water loss<sup>38</sup>. Such effects would go in the same direction and further support our conclusions.

The interplay between barrier integrity, inflammation and permeation of an external irritant has been previously studied through mathematical models. In that work the pH-dependent KLK activity was modeled using a system of ODEs to study its impact on barrier integrity and inflammation<sup>10,11</sup>. The adaptive immune response following infiltration of a pathogen in the epidermis was further developed to investigate AD pathogenesis<sup>39</sup>. Nakaoka *et al.* used a combination of ODEs with delay differential equations to represent the immune response to bacterial infection<sup>12</sup>. These approaches have in common the abstraction of the structure of the system and the location of the reactions. The assumption is that these reactions take place in a homogenous environment of fixed geometry. While making it easier to run extended model analysis, like parameter sensitivity, these types of models fail to account for the dynamic nature of the self-regenerating epidermal tissue, the structural alterations that can occur, and the heterogeneity of concentrations throughout the epidermis.

## **Conclusion**

We describe an agent-based model of the epidermis to investigate the critical role played by skin surface pH in the maintenance of skin barrier integrity. The model demonstrates that an increase in skin surface pH and the resulting overactivation of KLK are enough to weaken the skin capacity to retain water and prevent the permeation of an irritant. The model also predicts that the impaired barrier

function is followed by a higher sensitivity to skin inflammation. This computational tool could be used to better understand some of the pH-related mechanisms behind skin conditions such as atopic dermatitis or irritant contact dermatitis. It could also be used to simulate the impact on skin surface pH or barrier function of a skincare product aiming to prevent acute inflammation.

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## Table

**Table 1: Predicted properties of the system for the two conditions: physiologic pH (4.5) and less acidic pH (6.5).** VE: viable epidermis, SC: *stratum corneum*, AU: arbitrary unit

## Figures

**Figure 1: Graphical representation of the agent-based model and spatial distribution of the modules: barrier function, LEKTI-KLK interactions and desquamation process, irritant permeation, and acute inflammation.** (I) The water flows from the bottom of the epidermis to the surface according to its gradient. It is restrained towards the surface by the skin barrier, composed of tight junctions (in the SG and SC) and lipids (in the SC) <sup>15</sup>. (II) KLK and its inhibitor, LEKTI are released at the interface of SG and SC and interact in the SC. KLK self-activates into KLK\*, which can then either form a complex with LEKTI or contribute to the desquamation process by degrading corneocyte-corneocyte adhesion. (III) The irritant substance is introduced at the surface and diffuses through the epidermis according to its concentration gradient and the inter-agent exchange rates specific to the SC or the VE, reflecting the compositional difference of the two layers (lipophilic SC compared to hydrophilic VE) <sup>13</sup>. (IV) When the intra-cellular irritant concentration of VE cells rises above a pre-defined threshold, it triggers the release of inflammatory cytokines. The cells are colored according to their differentiation status on the left part (dark blue for stem cells, light blue for transient amplifying cells, light green for basal cells, light pink for spinous cells, dark pink for granular cells, and tan for corneocytes), the intra-cellular irritant concentration in the middle (the greener, the higher the irritant concentration), and the intra-cellular inflammatory cytokines concentration on the right (the redder, the higher the inflammatory cytokines concentration). VE: viable epidermis, SC: *stratum corneum*, SG: *stratum granulosum*, SS: *stratum spinosum*, SB: *stratum basale*, KLK: kallikreins, KLK\*: active kallikreins, LEKTI: Lympho-Epithelial Kazal-Type-related Inhibitor.

**Figure 2: Effect of pH and LEKTI inhibition on desquamation.** The amount of KLK\* at steady state increases with pH, represented by different colors according to the legend, for a given basal production

of LEKTI by keratinocytes,  $m_L$ . The model also predicts the complete inhibition of KLK\* by LEKTI when  $m_L$  gets above a certain value, that increases together with pH. The steady state amount of KLK\* directly affects the loss of corneocyte-corneocyte adhesion and thus desquamation. The dots correspond to the steady-state quantity of KLK\* predicted by the model for each value of  $m_L$  and with four sets of parameter values reflecting four values of pH (Supplementary 6). KLK\*: active kallikreins, LEKTI: Lympho-Epithelial Kazal-Type-related Inhibitor, AU: arbitrary units.

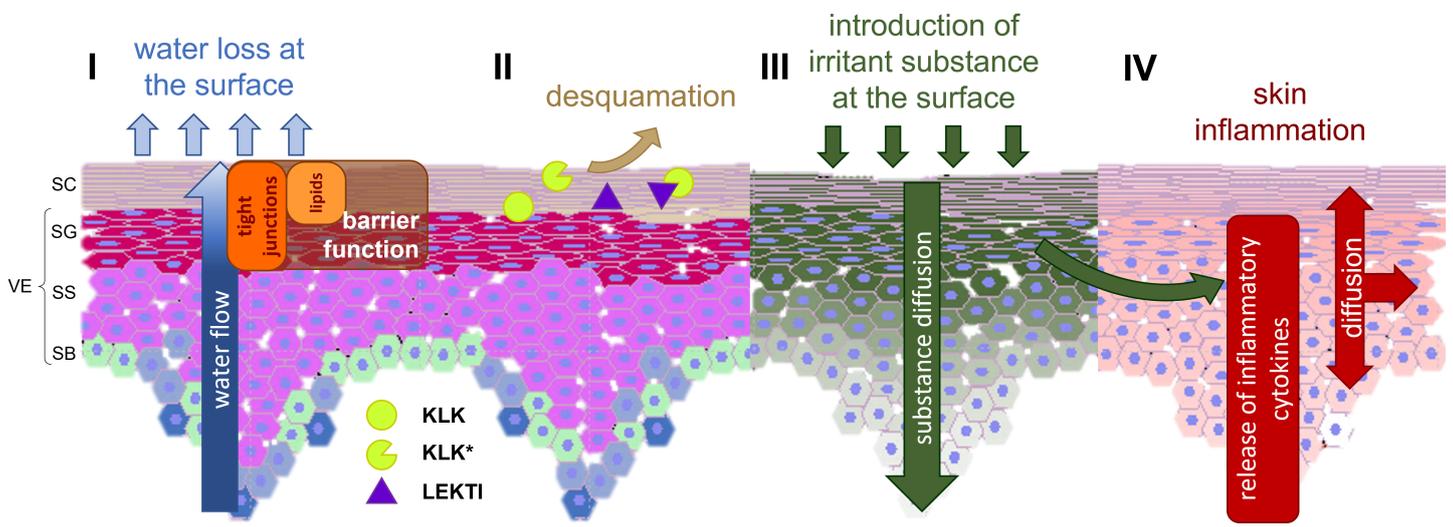
**Figure 3: The increase of skin surface pH results in the thinning of SC, with subsequent weakening of skin barrier function in both directions, inside-out and outside-in.** (A) The graphical output of the model for the two pH conditions (4.5 on the left and 6.5 on the right) show that the overall layered structure of the epidermis is preserved in both cases, while the SC is thinner for the less acidic pH condition. The cells are colored according to their differentiation status: dark blue for stem cells, light blue for transient amplifying cells, light green for basal cells, light pink for spinous cells, dark pink for granular cells, and tan for corneocytes. (B) The decrease in resistance to water flow (indicated with the black arrow) predicted for the less acidic pH condition (red) indicates a reduced capacity to retain water compared to the more physiologic pH condition (blue). The profiles are averaged over depth and time, and the x-axis is shown as the depth normalized to the SC thickness for easier comparison between the two pH conditions. The ribbon represents the SD of the resistance profile over time. (C) The model predicts both faster permeation (indicated by a steeper slope) and higher amount of irritant substance permeating in the VE with a less acidic pH (red), indicating a higher permeability of the epidermis to irritants, compared to the pH condition of 4.5 (blue). VE: viable epidermis, SC: *stratum corneum*.

**Figure 4: Impaired skin barrier function at higher pH causes a more intense inflammatory reaction.** (A) The model predicts that, the same amount of irritant applied at the surface at  $t=0$  and for 24 hours, permeates in higher quantity in the VE and therefore induces the production of more inflammatory cytokines with a skin surface pH of 6.5 compared to 4.5. The release of inflammatory cytokines also

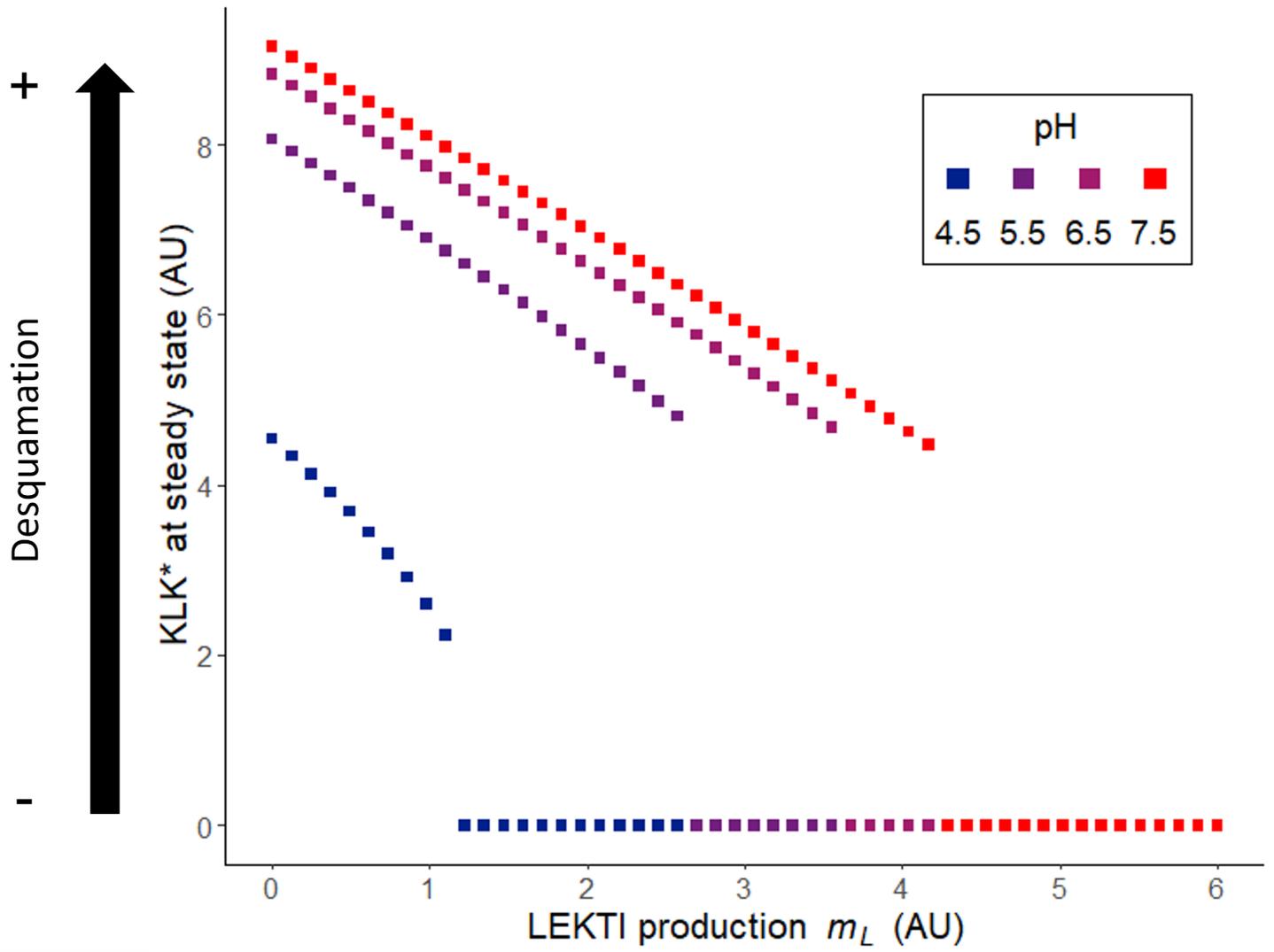
starts earlier with skin pH set to 6.5 ( $t=10$ ) compared to the more physiological pH condition of 4.5 ( $t=23$ ). The evolution of the total quantity of irritant and inflammatory cytokines in VE are shown with solid and dotted line respectively. They are colored according to the pH condition and normalized to the peaks of irritant and inflammatory cytokines with pH of 4.5. (B) Graphical output of the skin inflammation peak for both pH conditions: 4.5 on the left and 6.5 on the right. The cells are colored according to their concentration in inflammatory cytokines: the redder, the higher the concentration. VE: viable epidermis.

**Table 1:**

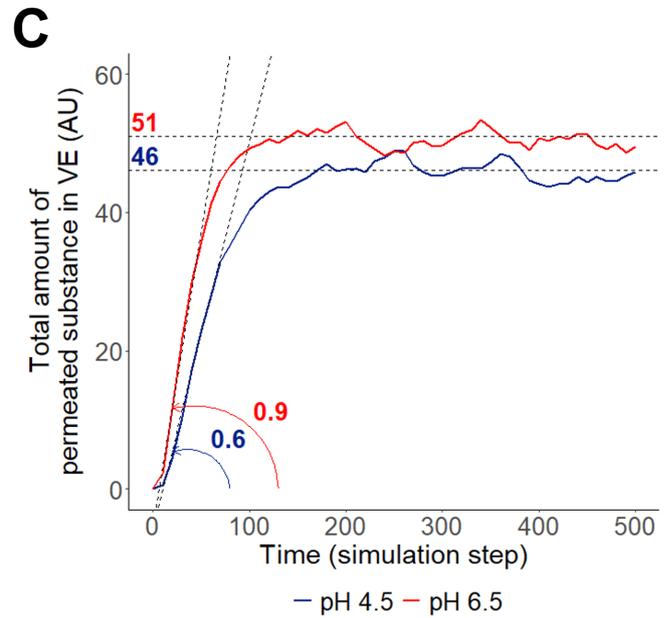
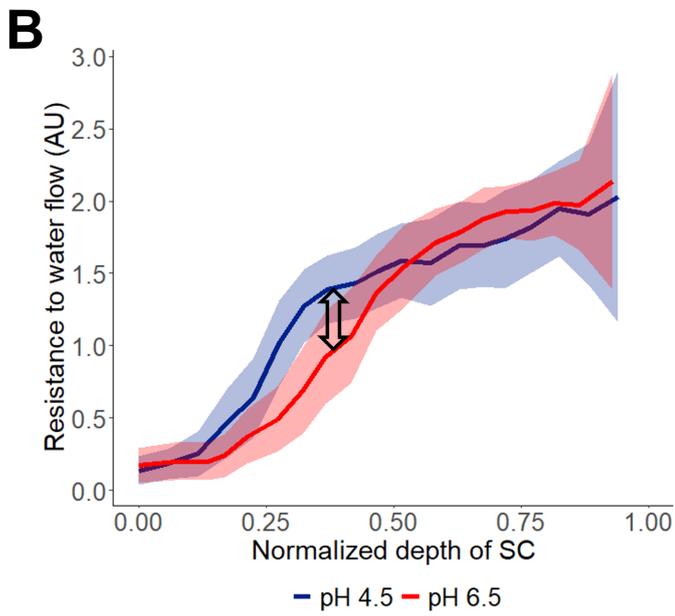
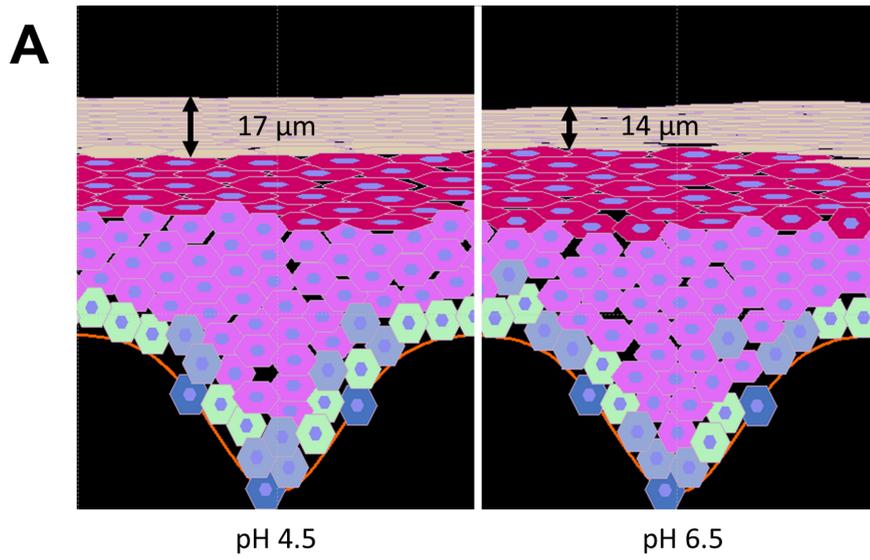
	SC thickness ( $\mu\text{m}$ )	VE thickness ( $\mu\text{m}$ )	water loss at skin surface (AU)	total substance in VE / thickness (AU)	total substance in SC / thickness (AU)	total cytokines at inflammation peak in VE (AU)
Physiologic pH (4.5)	17	85	1.1	0.54	9.1	5.5 E3
Less acidic pH (6.5)	14	80	1.2	0.64	7.9	7.9 E3
% difference	-18%	-6%	+9%	+19%	-13%	+44%



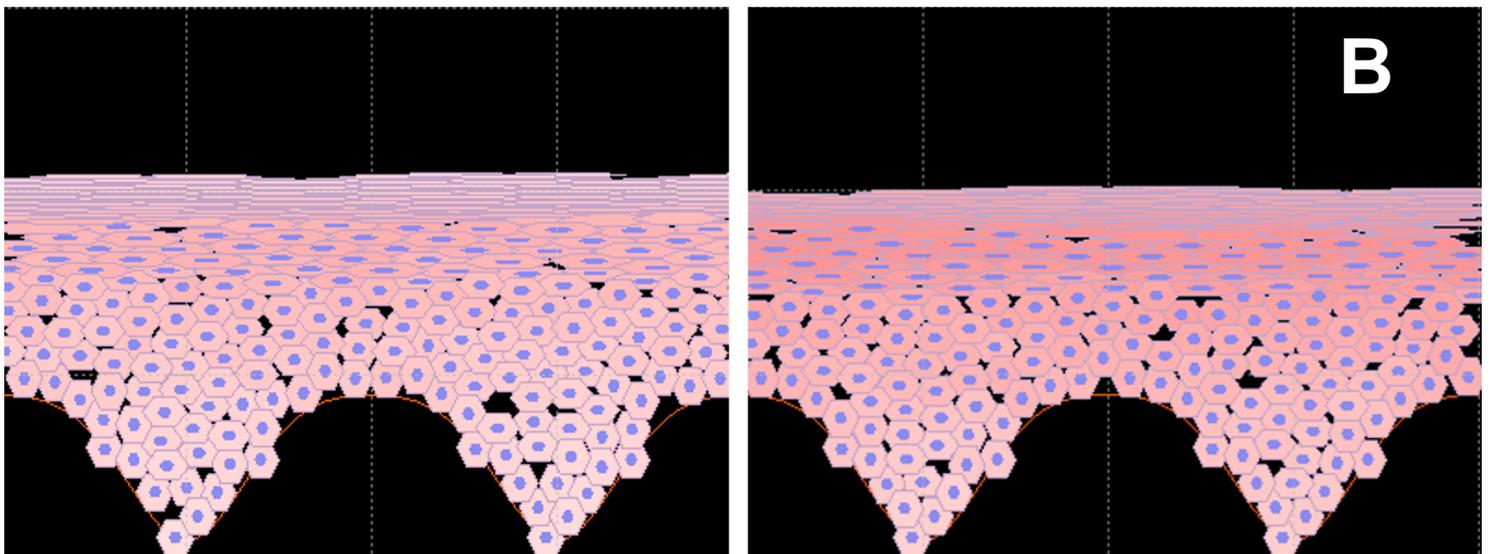
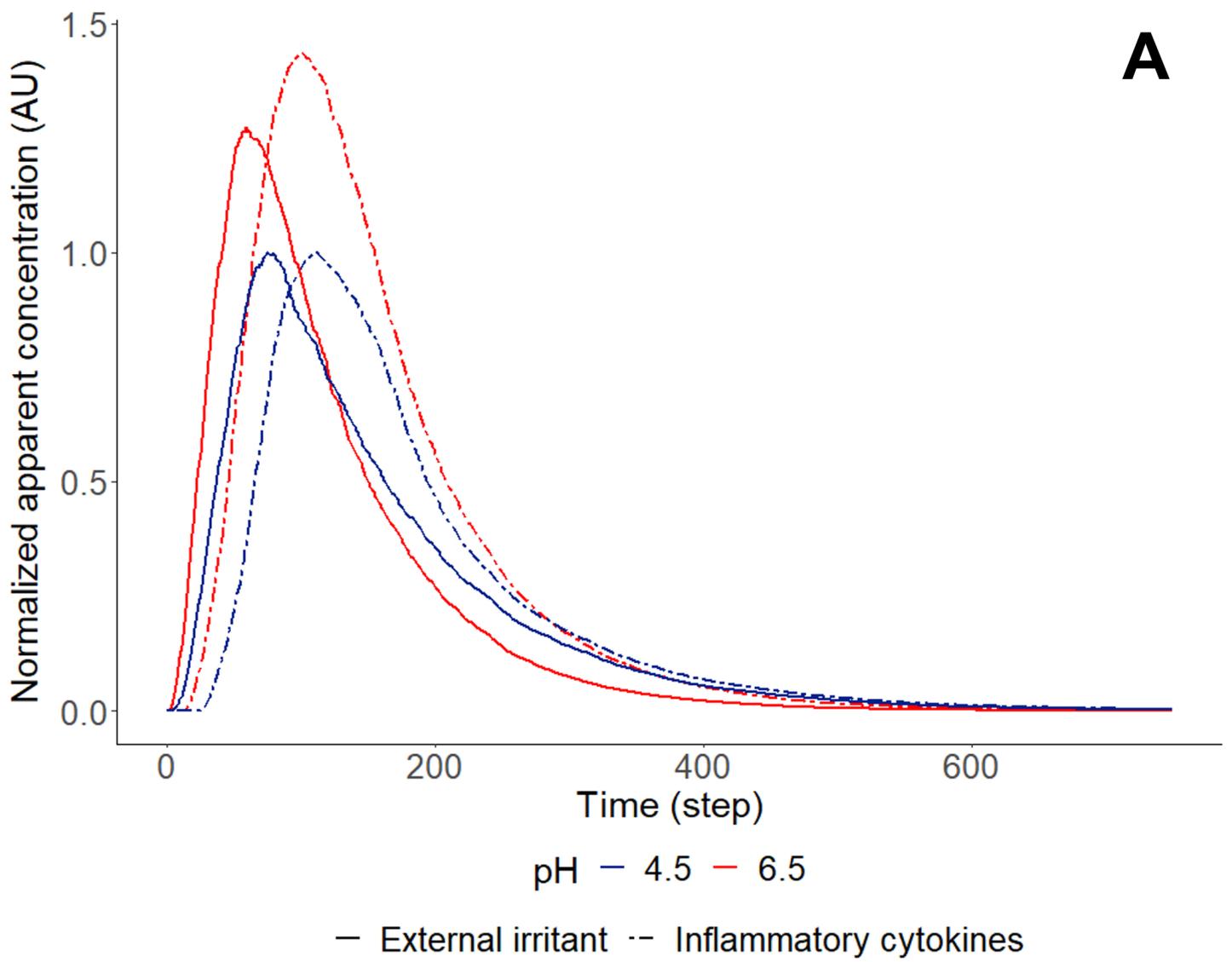
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