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Dendritic growth of protein gel in the course of sweat pore plugging by aluminium salts under physiological conditions

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Abstract

Are aluminium ions unavoidable in antiperspirants? In spite of strong consumer demand for alternatives to aluminium salts in cosmetics, these salts remain, to date, the only active substance in the market for antiperspirants. Such exclusivity is the direct result of the lack of understanding of how ions interact with sweat proteins. Here, we unveil the mechanism of antiperspirant action of aluminium salts, based on our observations through confocal microscopy and our numerical simulations of protein and ion motions in the sweat pore. We find that aluminium polycations move through Brownian diffusion in the vicinity of the walls of a sweat pore. They react with sweat proteins, and form a sparse dendritic protein gel in the pore through a diffusion-limited aggregation process. When the dendrites percolate, the gel suddenly densifies, thereby plugging the pore. Altogether, these results allow the understanding of why aluminium polycations are so efficient and why they are so difficult to replace.

Introduction

For thousands of years, antiperspirants have played an undeniable social role, for personal hygiene purposes but also in expressing voluntary control on one's body, and in not keeping others away with unpleasant odours: Pliny the Elder notably wrote in the 1st century of our era that "*Liquid alum has an astringent, hardening and corrosive property [...] It reduces odour from the armpits and perspiration*"¹. In this ancient statement on a cosmetic property, the active agent (alum) was a natural mixture of aluminium salts. In the last two thousand years, the formulation of these reactive substances has obviously changed a lot, but aluminium salts - sometimes associated with zirconium salts - are strangely still the only used antiperspirant principle known to us. This centuries-old consistency is a paradox in our modern society where science seeks to improve everything. It has been the direct consequence of our ignorance of the ionic interactions of aluminium salts in perspiring pores.

It was generally admitted that aluminium salts plug sweat pores when applied on human skin^{2,3}, but the mechanism was not established. Large soluble polycationic species⁴ such as $\text{Al}_{30}(\text{Al}_3\text{O}_8(\text{OH})_{56}(\text{H}_2\text{O})_{26})^{18+}$ (size ≈ 2 nm) were supposedly involved in pore plugging in a similar way as in the coagulation and flocculation of organic matter by aluminium salts in waste-water treatment⁵. In this context, new insights were uncovered recently when it was demonstrated definitively that sweat proteins precipitate due to the aluminium ions⁶, and the precipitate clogs the skin pore, preventing sweat from flowing out⁷. However, the underlying mechanism by which large numbers of aluminium

polycations could penetrate inside the pore against the flow of the sweat remained a mystery. Indeed, the average Péclet number for aluminium polycations is quite large, $Pe \approx 50$, for a flow rate⁸ of 0.5 nL/s in a pore diameter⁹ 50 μm . Consequently, all the ions present in the pore should immediately be swept away by sweat. The reality turned out to be much more imaginative than the few plausible mechanisms that had been suggested. Our main observation is described and discussed below. It finally paints a complete scenario of how the plug forms in the pore. Complementary results will be published in a separate article¹⁰.

Results and Discussion

We organised a multidisciplinary research project (microfluidics / confocal microscopy / X-ray scattering / numerical simulations) to investigate a model system replicating the effect of aluminium polycations in a pore where sweat flows. The first step of the project was to achieve a controllable and reproducible experimental model of the flow of human sweat in a skin pore. For this purpose, we used artificial sweat flowing at constant pressure in a 50 μm -wide microfluidic channel in PDMS (Fig. 1).

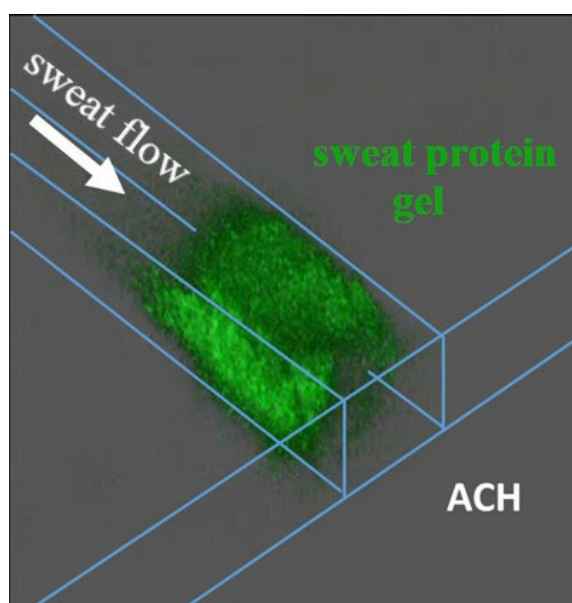


Fig. 1 Visual sketch of the microfluidic device modelling a perspiring sweat pore. Artificial sweat flows into the rectangular section channel and interacts with the ACH antiperspirant in a reservoir at the channel opening. The gel of proteins carried by sweat develops inside the pore. A real gel is seen here as the green cluster imaged by confocal microscopy (twisted view of 3D reconstruction of plug showing fluorescent aggregates located in a 50 μm PDMS sweat channel, 30 min after fluids contact).

The artificial sweat used here was a saline solution of dialyzed Fluorescein-labelled Bovin Serum Albumin (BSA-FITC) - a fluorescent conjugate of BSA - and the antiperspirant applied at the outlet of the channel was a solution of aluminium chlorohydrate (ACH). We had access to natural sweat to validate this model, and we found that artificial sweat with 15 μM BSA reproduces correctly the behaviours of natural sweat. The advantage of using artificial sweat is the easy real-time visualization of plug formation, because the brilliant green fluorescence of BSA-FITC is ideally suited for confocal microscopy analysis. Fig. 2 shows sections of the device obtained by confocal microscopy of a BSA protein gel at four successive flow and reaction times, with a spatial resolution of the order of 1 μm . The protein gel is seen to develop delicate dendritic and fluffy structures, which were not expected.

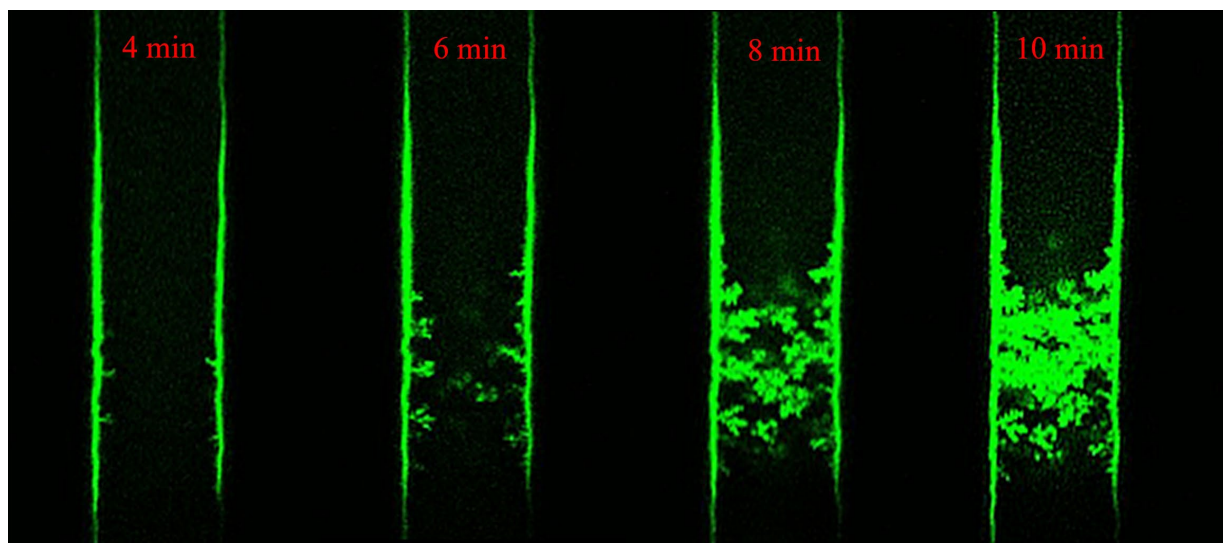


Fig. 2 Four successive photographs made through confocal microscopy. 5 μm deep sections in the middle of the PDMS pore with a rectangular section (pore width: 50 μm). Artificial sweat made of fluorescent 15 μM BSA-FITC flows at 0.6 nL/s. The ACH antiperspirant is initially located at the base of the pore (bottom of the pictures). The pictures were taken (from left to right) 4, 6, 8 and 10 minutes respectively after the time of first contact between the two fluids. In these photographs, we see the formation and growth of protein gel dendrites. In the photograph to the right, the dominant dendrites have started to coalesce to form the plug that will prevent the sweat from flowing out.

This kind of highly branched dendrites is known to occur when particles diffuse and stick to each other, either at the walls or on the already formed gel. This matches the "Diffusion-Limited Aggregation" (DLA)¹¹ algorithm in which fractal aggregates grow through capture of diffusing particles that are released from a static source and move through Brownian motion until they are caught (see Methods for details). We note the essential characteristics of this growth: Brownian diffusive motions leading to fractal structures. We also notice that the dendritic aggregates are very flexible, able to deform and twist without breaking when subjected to mechanical stress¹². This is in line with the observation that their growth is not greatly hampered by the flow of sweat. Besides, these fractal structures make it possible for these dendrites to spread out very quickly in the space between the walls of the pores.

Based on these observations, the following scenario then becomes evident: the flow of sweat is a Stokes flow (Reynolds number ≈ 0.025). It has a boundary condition of no flow at the walls. Accordingly, the Poiseuille formula then yields a low effective Péclet number near the pore walls (for example, the Péclet number is < 1 over a distance 0.1 μm from each wall) and diffusion can then become an efficient mean of transport for small particles such as aluminium ions (hydrodynamic radius ≈ 2 nm for Al_{30} polyoxocation). If the concentration of ions is large enough at the pore outlet, they can then move by counter-current diffusion along the walls to depths large enough to interact with the sweat proteins and generate dendrites made up of alternating proteins and aluminium polycations. Too close to the pore orifice, proteins are inactive because they are saturated with aluminium polycations attached to their surface; inside the pore, far enough from the orifice, the aluminium polycations are saturated with proteins¹⁰. This condition determines the actual depth of gel formation.

This two-component process is not standard in DLA, and we have validated it by numerical simulations where the reactive species (protein or aluminium polycation with the experimental relative concentrations) diffuse one at a time, and stick to each other according to the rule: protein on ion, or ion on protein. Fig. 3 gives an example of such numerical dendrites forming on a flat wall.

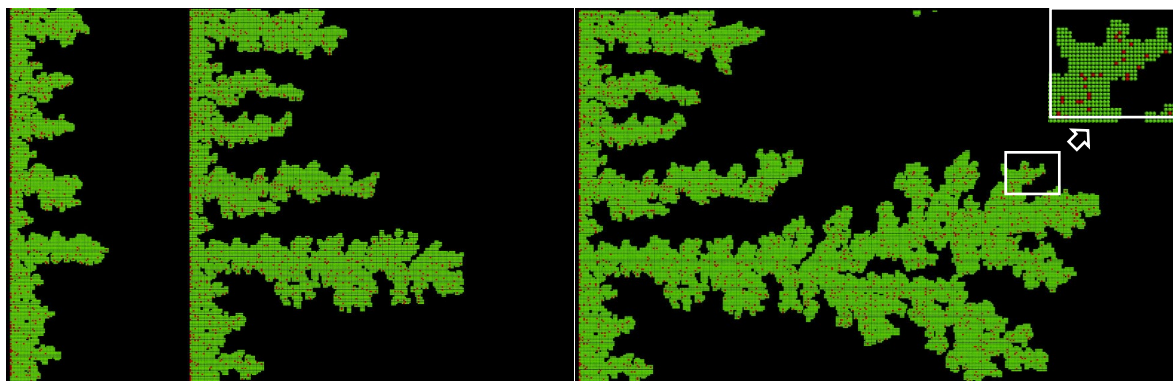


Fig. 3 Numerical simulation of dendrites growth on a plane through a 2-components 3D DLA process. Brownian motion is simulated as a random walk on a cubic lattice of 1 nm lattice step (see Methods for details). Three successive times are shown (from left to right: 30 000, 70 000 and 130 000 molecules deposited respectively). The width of each figure is 0.5 μm -wide and 0.025 μm -deep. ACH molecules (green dots, coordination number = 4, hydrodynamic radius = 1 nm) are 20 times more numerous here than sweat proteins (red dots, coordination number = 4, hydrodynamic radius = 3 nm). The diffusing molecules are launched one at a time from the right-hand side of each picture. Diffusing sweat proteins can only stick onto ACH molecules. Diffusing ACH molecules can only stick onto sweat proteins. Insert: enlargement of a tip of the main dendrite, in which one can make out the small red dots (sweat proteins), and the green dots (ACH molecules).

There are many consequences of this scenario. Let us take for example the dynamics of the process. The dendrites being generally mass fractals¹³ of fractal dimension, d_f , their mass, M , scales with their characteristic dimension, L , as the power law $M \propto L^{d_f}$, and their surface, S , scales similarly¹⁴ as: $S \propto L^{d_f}$. With the growth of dendrites following $dM/dt \propto S$, the mass of the gel must grow exponentially for these DLA mass-fractal dendrites, a result that we were able to verify experimentally by X-ray scattering during the growth phase of the dendrites in the pore (see Fig. 4).

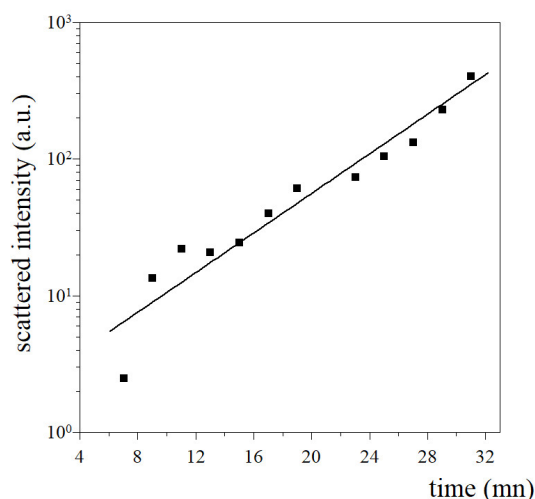


Fig. 4 Semi-logarithmic plot of the total X-ray intensity scattered by the protein gel inside the microfluidic. The T-junction was placed in front of the focused X-ray beam at the position of the plug using natural human sweat (similar result was found using artificial sweat¹⁰). For half an hour, the growth of the gel mass is exponential with time, corresponding to a two-decade increase in the intensity of the scattered signal.

Then, when the larger dendrites reach the centre of the pore, they percolate and intertwine, causing all of these dendrites to rapidly merge into a dense gel net. The plug forms and blocks the flow of sweat almost instantly.

This scenario brings together all the mechanisms that lead to clogging of a perspiring pore by aluminium salts, and it gives directions for where to search in order to reduce their amounts or to replace them entirely. Indeed, having proved that Brownian diffusion is both the origin of ion transport and that of their aggregation into gel structures, we understand now why it is natural that aluminium polycations are the most effective (because their size is quite small and their electric charges are very high), thus explaining their endured use since antiquity.

Through our work, we have now uncovered at least two possible avenues of research opening up with alternative strategies to reducing or replacing aluminium salts in antiperspirants. Incremental improvements could be obtained by using a two-stage process: 1) generate a gel at the entrance to the pore, thereby reducing the flow rate of the sweat; 2) then one would repeat the use of the same process with smaller quantities of aluminium ions for the same efficiency, or else use for the second stage other ions with larger hydrodynamic radii. More radical improvements would require replacing Brownian diffusion by another transport mechanism, such as the Marangoni effect which could increase counter-current flow of the active substance to reach deeper inside sweat duct.

Methods

Materials

Lactic acid, urea, sodium chloride and BSA-FITC conjugate were purchased from Sigma-Aldrich. Aluminium Chlorohydrate (ACH) was purchased from Elementis (reference Chlorhydrol 50). Artificial sweat used has the following composition: 0.5% (w/w) NaCl, 0.1% lactic acid, 0.1% urea and 0.1% BSA or BSA-FITC at pH 6.5 (adjusted with ammonia). BSA-FITC conjugate was subjected to extensive dialysis before use. Natural human sweat was collected from the armpit of volunteers after a sauna session and immediately kept frozen. All volunteers gave their written informed consent. The protocol complied with the Helsinki declaration and was approved by l'Oréal Research and Innovation Reading Committee.

Microfluidic set up

PDMS chips were manufactured according to classical published procedures. Fluigent pressure controllers MFCS-EX were connected using 500 μm inner diameter PEEK tubing. Sweat channel (55 μm high x 50 μm wide) were flowed at 0.6 $\text{nL}\cdot\text{s}^{-1}$ at the beginning of the experiments and then maintained at a constant pressure. ACH (15% w/w) was flowed at 60 $\text{nL}\cdot\text{s}^{-1}$ in the perpendicular channel (55 μm high x 400 μm wide) of the T-junction.

Confocal microscopy

Confocal microscopy images were acquired using confocal laser scanning microscope Leica DMI8 with excitation wavelength at 488 nm. Optical sections were obtained every 5 μm steps inside sweat channel to access to plug 3D structure. Image J software was used to process the captured images.

SAXS studies

SAXS measurements were performed at the coherent small angle X-ray scattering (cSAXS) beamline at the Swiss Light Source (Paul Scherrer Institute, Switzerland). The X-ray beam was focused to 20 x 30 μm^2 (vertical x horizontal) and the photon energy set on 11.7 keV ($\lambda = 1 \text{ \AA}$). Two-dimensional scattering patterns were collected by a Pilatus 2M detector. X-rays resistant chips were manufactured by the

combination of photolithography, soft lithography and replication processes¹⁰. The section of the channel was 50 x 50 μm . The T-junction was placed in front of the focused beam at the position of the plug.

3D off-lattice dendrite growth numerical model

In this model, space is discretized as a regular cubic lattice with lattice step 1 nm. The pore wall is a plane (W) of dimensions: 500 x 25 (in lattice step) with periodic boundary conditions. Each ACH molecule is a cube of edge 1 nm, and has 4 pending bonds. Sweat protein is a cube of edge length 3 nm, and has 4 pending bonds. At each growth step, a particle (either a sweat protein or an ACH molecule) is selected randomly according to their respective concentrations, and put in a random location of a plane parallel to the deposit plane (W) and far from it (1000 lattice steps). The selected molecule undergoes a random walk on the cubic lattice until one of the following events occur:

- if the diffusing molecule is an ACH molecule: the molecule can hit and stick onto the deposit plane (W), and the number of pending bonds of the ACH molecule is reduced by 1
- the diffusing molecule can hit and stick to an unsaturated molecule of the other species already part of the gel. The numbers of pending bonds of each of the two molecules are both reduced by 1
- if the diffusing particle reaches the distance 1500 from the base plan (W) before sticking to the wall or the deposit, the particle is discarded as too far from the wall.

The resulting gel is a complicated combination of sweat proteins and ACH molecules stuck together (the gel). Fig. 3 shows such numerical gel at three successive times: the resulting deposit is an ensemble of fractal, very irregular dendrites. One can note the screening effect: a big dendrite grows faster than the small ones around it because the diffusing particles feeding the deposit come from outside it.

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