What about a transmission of SARS-CoV-2 through a viral biofilm?

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Et si le SARS-CoV2 se transmettait sous forme de biofilm viral ?

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Since the onset of the Covid-19 pandemic caused by SARS-CoV-2 virus, the scientific community has endeavored to investigate the different routes for the virus transmission. Airborne transmission (aerosols, droplets) and direct contact with contaminated surfaces (fomites) or between individuals have been identified as the main modes of spread [1, 2]. In all studies on the subject, the only infectious entity taken into account is the isolated infectious viral particle. However, an intriguing hypothesis proposes that the virus could also be transmitted as a viral biofilm [3]. This hypothesis was put forward at the start of the pandemic, when patient tissues and then cell cultures were imaged [4, 5]. However, to date, no known work has addressed the question of the existence of SARS-CoV-2 viral biofilms and their involvement in virus transmission.

Biofilm formation is a process common to many microorganisms, including bacteria and fungi. While bacterial biofilms are well known and have been widely studied since their discovery in the 70s [6], viral borne biofilms is a relatively new concept [4, 5]. Biofilms are made up of microorganisms embedded in a composite extracellular matrix (ECM) composed of polysaccharides, proteins and in the case of bacterial biofilms, lipidic microvesicles and DNA molecules [6, 7]. These are often multi-species communities, which concentrate microorganisms locally, shield them against environmental constraints, and transmit them collectively by breaking up into microbial colonies in the original sense of the term [7]. Microorganisms' natural and dominant lifestyle is now considered to be in biofilm form, giving them selective benefits over their planktonic form (isolated microorganisms). It enables them to colonize various biotic and abiotic environments, even in the most extreme ecosystems [8, 9]. In the case of pathogenic microorganisms, biofilms are genuine reservoirs frequently associated with persistent infections, due to the protection they provide against immune responses and their reduced sensitivity to antimicrobial therapies [10].

Viral biofilms have been first described for the human retroviruses HTLV-1 (human T-cell leukaemia virus type 1) and HIV-1 (human immunodeficiency virus Type 1) [11, 12]. Comparable structures have also been reported for certain herpesviruses [13, 14]. These viral

arrangements promote collective and protected transmission, much like other microbial biofilms [11]. A significant difference is that the matrix constituents are secreted by the infected cell rather than the pathogen itself, with the composition of the biofilm being directly controlled by the virus and indirectly by the host response it triggers [11, 12]. Viral biofilms are highly adhesive, have higher infectivity and lower susceptibility to neutralizing antibodies compared to similar amount of individually released viral particles. They make an important contribution to direct and indirect transmission of viruses between T lymphocytes in cell culture [11, 12, 15]. They also provide protection against the action of antiretroviral drugs thanks to a mass effect, by facilitating the simultaneous entry of several viruses into a single cell. [12]. Moreover, studies on humanized mice indicate that HIV-1 may be disseminated in the host's lymphoid organs as biofilm, by cell contact during the migration of the infected cells into the host lymphoid tissues [16].

The hypothesis that SARS-CoV-2 may be transmitted in the form of a viral biofilm is built on numerous observations. Several studies have demonstrated the presence of viral aggregates on the surface of infected cells within patients' respiratory tract [17]. Additionally, viral aggregates have been observed in various types of cell cultures infected by SARS-CoV-2, including those that do not produce mucus [18]. In each case, a matrix that maintains viral particles in a three-dimensional structure was detected. This matrix may be the extracellular matrix (ECM) component of a viral biofilm, possibly combined or mixed with mucus and other secretions from respiratory tract cells. Taking a look at the various imaging studies reported in the literature (scanning electron microscopy among others), it is striking to note that the "collectives" of SARS-CoV-2 viral particles on the surface of infected cells are highly organized. [3]. Interestingly, video-microscopy of infected lung cells' surface enables to visualize their formation process. At first, individual viral particles cluster together to create spherical assemblies called 'nodes'. These nodes develop into 'branches', which consist of viral particles strings organized along threads of matrix structure. The highly organized interconnected 'networks' thus gradually created can detach '*en bloc*' from the cell. This live imaging study, together with observations that these viral structures are formed by different cell types including Vero cells, suggests that viral particles are released onto the cell surface along with ECM components to form a possible viral biofilm. Noteworthy, comparable structures (such as nodes, strings and networks) forming viral biofilms have been observed on the surface of HIV-1 and HTLV-1 infected lymphocytes. For both retroviruses, the composition of biofilms in extracellular matrix (ECM) turns out to be crucial for their architecture and infectivity [11, 12]. Specific components such as heparan sulfates and in particular collagens, whose expression is directly induced by the virus, have been identified as key factors in retroviral biofilm infectivity. These proteins play a crucial role in shaping the biofilm architecture produced by

HIV-1 and HTLV-1 infected cells [12, 19], in addition to contributing to TNF-β-mediated inflammatory processes.

Proteomic data also provide interesting information supporting the biofilm formation hypothesis by SARS-CoV-2 infected cells. Studies show that SARS-CoV-2 modulates the matrisome of the lung cells it infects [20]. Additionally, a study analyzing samples from patients with severe forms of the disease demonstrates a significant rise in collagen levels, which may be associated with the substantial inflammatory process observed in these patients, but also with the formation of a viral biofilm [21]. The fact that SARS-CoV-2 Spike protein interacts with ECM components, among which sulfated glycans on the cell surface [22] may also contribute to the SARS-CoV-2 viral particles organization as a viral biofilm on the cell surface. Although research on the matrix components of the observed biofilm-like structures and their role in viral infectivity is lacking, the available data strongly suggest viral biofilm formation by SARS-CoV-2.

If this were the case, SARS-CoV-2 biofilm present in human biological fluids such as saliva, sputum and bronchoalveolar fluids, could participate in optimal transmission of the virus between individuals. The presence of SARS-CoV-2 biofilm on contaminated surfaces could contribute to the reported resistance of the virus infectious capacity in the environment [1, 23, 24]. Along with mucosal secretions, the biofilm's matrix could help preserve virion integrity and infectivity. In addition, biofilm spread may improve SARS-CoV-2 infectivity by increasing the probability of a productive infection (local "quorum" effect), as for HIV-1 [12]. These traits, combined with a probably limited effectiveness of the immune system against this pool of viral particles (neutralizing antibodies among others), would allow SARS-CoV-2 to spread in an exceptionally efficient manner within its host. The fact that SARS-CoV-2 spreads via intercellular contacts, protected from neutralizing antibodies [25], further strengthens the SARS-CoV-2 biofilm hypothesis, as these properties are conferred by the viral biofilm in the case of HIV-1 and HTLV-1.

Furthermore, '*en bloc*' transmission in the form of a biofilm, which allows multiple viral genomes to be delivered simultaneously to the same cell, could have an impact on the viral genetic evolution over the course of the replicative cycles [5]. Considering the role of the biofilm in this process would make perfect sense, as recombination events between viral genomes have been shown to contribute to genetic variability in coronaviruses, including SARS-CoV-2 [26, 27]. It could also allow complementation between very often mutated or defective virions (viral quasi-species) and have important consequences on the viral adaptation to environmental constraints [13].

Reinforcing all these data with structural and functional studies to test these hypotheses would undoubtedly provide a better understanding of the transmission modalities of SARS-CoV-2, whose successive variants continue to circulate efficiently.

The viral biofilms formation could have noteworthy consequences on the development of innovative strategies for viral infections prevention and treatment. On the one hand, the viral biofilm is an antigenic structure composed of viral particles and matrix elements, with a very distinct composition and size as compared to individual viral particles. It should be taken into account when developing vaccine strategies, so that this pool of viruses, probably sheltered from the immune system, is also targeted by vaccines. On the other hand, the matrix elements of the SARS-CoV-2 biofilm essential for its structure and infectivity, could constitute interesting new antiviral targets for strategies seeking to limit both the transfer between individuals and the formation of viral reservoirs in the host. SARS-CoV-2 biofilm matrix components could in particular be targeted in addition to "classic" therapeutic approaches directly targeting the virus (additives to therapeutic formulations).

Even more speculatively, if the existence of a SARS-CoV-2 biofilm were validated, this would open up a new field of research into the role of the biofilm in certain poorly understood aspects of SARS-CoV-2-associated pathophysiology [28, 29]. In particular, it could be considered whether biofilm formation by SARS-CoV-2 contributes to the development of severe forms of the disease, most often correlated with a high viral load, or extra-pulmonary forms reported in the literature. The biofilm's replicative and/or distinct antigenic properties could contribute to the worsening of the disease. The inflammatory context induced by the virus, exacerbated in risk profile patient and in severe forms of the disease, could promote the formation of viral biofilm or reinforce its infectivity, thus generating an amplification loop of viral replication and pathology. In such scenario, the therapeutic approach using heparin and its derivatives, which favors recovery in patients with severe symptoms [30], could have a double beneficial effect: in addition to reducing infection-related thrombosis, it could also directly reduce the ECM-associated pool of SARS-CoV-2 since this molecules disrupt the structure and infectivity of retroviral biofilms [11, 12]. Finally, it could also be envisaged that transmission through viral biofilm promotes the persistence of SARS-CoV-2 in particular tissue compartments by protecting virions from the immune system. This could thus lead to the creation of tissue infectious reservoirs potentially involved in certain cases of long Covid [31].

In conclusion, the hypothesis that SARS-CoV-2 could be transmitted through a viral biofilm fully deserves further investigation. If confirmed, this discovery would provide a new and complementary perspective to current prevention and treatment strategies for Covid-19, which

could potentially be applied to other viral infections. Indeed, viruses from other families, notably respiratory viruses emitted in mucous secretions (such as Paramyxoviruses and Orthomyxoviruses), could also be transmitted in the form of biofilms, which would be interesting to explore. This hypothesis would also offer new perspectives for understanding the evolutionary processes of the virus and, in a more exploratory manner, certain aspects of the pathophysiology associated with SARS-CoV2. Finally, the integration of viruses into biofilms formed by other microorganisms from very different phyla, such as bacteria and fungi [32], raises the question of possible broad-spectrum interspecific cooperation within complex biofilms, and opens up a radically new field of clinical investigation.

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