Rédiger sa revue

Les points d'attention pour rédiger sa revue

La rédaction de votre revue doit tenir compte du travail déjà effectué en L1 en ce qui concerne chaque paragraphe rédigé mais doit s'enrichir des ajouts de deuxième année

- **Niveau L1** : un texte d'auto-évaluation est disponible sur la page moodle. La première question concerne les points centraux de rédaction corrigés en L1.
- **Introductions** : Une introduction complète est demandée pour introduire le travail de chaque équipe. Chaque membre de l'équipe réalise une micro-introduction de sa partie (3-4 phrases)
- **Conclusion** : Une conclusion complète est demandée pour conclure le travail de chaque équipe. Chaque membre de l'équipe réalise une micro-conclusion de sa partie (2-3 phrases)
- **Titres** : Vos titres doivent être explicites et attractifs (surtout le titre de votre travail d'équipe et de votre highlight)

Objectifs de la séance

- Travailler l'introduction et la conclusion d'équipe
- Travailler les titres

A. Avant la séance :

1) Bien retravailler vos problématiques avec une phrase de contextes/connaissances qui justifie votre approche. Cette phrase doit être référencée comme le reste de votre introduction.
2) Préparer les revues/livres avec les informations générales sur le thème
3) Venir avec l'ensemble de vos fiches et votre carnet de bord remplis

B. Pendant la séance :

1) Analyser une introduction (exemple à la suite)
   - Trouvez les trois parties vues en cours : Emmener le sujet, poser la problématique, annoncer le plan.
   - Trouvez les termes importants définis dans cette introduction
   - Soulignez la problématique
   - Surlignez les références dans le texte et dans la bibliographie
2) Rédigez introduction de groupe
   - Stabilisez votre problématique
   - Rédigez votre annonce de plan
   - Listez les mots à définir pour l'ensemble de vos highlights
   - Préparez des étapes de rédaction de la partie emmener le sujet. Cela doit constituer un entonnoir (du plus général au plus spécifique) qui emmène à votre problématique
3) Rédigez votre conclusion de groupe

- — Bilan réalisez une à deux phrases par highlight
- — Perspective : indiquez des travaux futurs/en cours ou dans un domaine proche

4) Rédigez vos titres

- — Allez sur le site de l'American Society of Microbiology et notez des titres de revue/partie/sous-parties qui vous semblent attractifs et explicites
- — Définissez votre titre d'équipe puis les titres de chaque highlight puis les titres de vos sous-parties

C. Après la séance la séance :

Terminez le travail de rédaction de votre revue et remplissez le carnet de bord. Utilisez le test d'auto-évaluation pour vous corriger.

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Surviving as a community: antibiotic tolerance and persistence in bacterial biofilms

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Abstract

Biofilms are surface-associated bacterial communities that play both beneficial and harmful roles in nature, in medicine, and in industry. Tolerant and persister cells are thought to underlie biofilm-related bacterial recurrence in medical and industrial contexts. Here, we review recent progress aimed at understanding the mechanical features that drive biofilm resilience and the biofilm formation process at single-cell resolution. We discuss findings regarding mechanisms underlying bacterial tolerance and persistence in biofilms and how these phenotypes are linked to antibiotic resistance. New strategies for combatting tolerance and persistence in biofilms and possible methods for biofilm eradication are highlighted to inspire future development.

Introduction

We live in societies made of individuals with interacting social connections and enduring architectural infrastructures. On a six order of magnitude smaller scale, bacterial cells also build microbial cities called biofilms in which individual cells and groups of cells interact and a global infrastructure is assembled. Biofilms are surface-attached communities of bacteria embedded in an extracellular matrix (Hall-Stoodley et al., 2004). Biofilms can be beneficial for health, for example, as normal components of plant, animal, and human microbiomes and they can be crucial for effective industrial processes such as wastewater treatment. However, often, biofilms cause major problems: in medicine, biofilms underlie chronic infections, and in industry, biofilms foul surfaces of pipes and clog filtration devices.

Biofilm eradication, whether in medicine or industry, is remarkably difficult. One feature thought to underlie biofilm tenacity is that biofilm communities can harbor tolerant and persister cells (Lewis, 2005): cells that can survive transient antibiotic treatment and that regrow when the antibiotic is withdrawn (Brauner et al., 2016). Indeed, both hyper-biofilm-

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forming mutants and mutants exhibiting enhanced persistence are isolated from patients with chronic infections (Hall-Stoodley et al., 2004; Lewis, 2010). In this Mini Review, we summarize recent progress in the understanding of biofilm formation, focusing on the mechanical attributes of biofilms that endow them with their remarkable resilience. We highlight progress aimed at defining mechanisms underlying the tolerant and persister phenotypes. Finally, we provide an overview of exciting new strategies for combatting harmful bacterial biofilms.

Biofilm matrices: mechanical shelters for bacterial cells

A defining feature of a biofilm is the presence of the extracellular matrix, made up of extracellular polymeric substances (EPS) secreted by the cells dwelling inside (Hall-Stoodley et al., 2004). The EPS is usually a mixture of polysaccharides, proteins, extracellular DNA (eDNA), and other minor components. The physical and chemical properties of the biofilm matrix constituents coupled with their particular interactions give rise to the global biofilm mechanical properties. These properties allow the matrix to shield the resident cells from desiccation, chemical perturbation, invasion by other bacteria, and killing by predators. The matrix also provides the mechanical properties necessary to protect the cells from external forces such as fluid shear and to ensure the biofilm community remains attached to a surface. In the context of infectious biofilms, neutrophils can only ingest pathogens smaller than 10 μm, thus, participating in the biofilm lifestyle protects individual bacteria and small bacterial clusters from neutrophil attack. Moreover, to access biofilm-dwelling bacteria, neutrophils need to first break biofilms (~100 μm) into smaller pieces. However, neutrophils can only exert stress up to ~1 kPa during phagocytosis (Kovach et al., 2017), so biofilm mechanics could potentially prevent neutrophils from making biofilm cells available for killing.

Tools and concepts from the rheology field have been adapted to quantitatively define biofilm mechanical properties. Rheology is the study of viscoelastic materials: materials that have both solid and liquid properties (Billings et al., 2015). For rheologic measurements, biofilms are sandwiched between parallel plates and subjected to shearing (Figure 1A, *Left*). These analyses define the elastic modulus, which is the stiffness of the biofilm at small deformation, and the yield strain, which is how much deformation a biofilm can sustain before it fails (Figure 1A, *Right*) (Kovach et al., 2017). The product of the elastic modulus and the yield strain defines the yield stress, which is the minimum force needed to cause a biofilm to fail. Below we summarize insight gained from recent rheological measurements of three model biofilm forming species: *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*.

V. cholerae is the causative agent of the pandemic disease cholerae. The major *V. cholerae* biofilm matrix component is the *Vibrio* polysaccharide (VPS) and there are three matrix proteins RbmA, Bap1, and RbmC (Teschler et al., 2015). Deletion of genes encoding matrix components, followed by rheological measurements, enabled the mechanical properties of *V. cholerae* biofilms to be defined (Yan et al., 2018). The *V. cholerae* biofilm can be described as a double-networked hydrogel with an elastic modulus of ~1kPa. One network is formed by the VPS polysaccharide reinforced by RbmC and Bap1, and the second network is

formed by the cells connected by RbmA (Figure 1B). Elimination of RbmA or RbmC/Bap1 weakens the dual network and reduces the elastic modulus. Elimination of all three matrix proteins causes the VPS to swell, resulting in an increased yield strain but at the expense of a highly reduced elastic modulus. Only when all the matrix components are present do *V. cholerae* biofilms possess a large enough yield stress (~100 Pa) to withstand the flow regimes they experience in their natural habitat, for example, on sinking marine snow.

P. aeruginosa is an opportunistic pathogen that forms chronic biofilm infections in patients with compromised immune systems, burns, in-dwelling devices, and cystic fibrosis (CF). Combinations of three polysaccharides can be present in the *P. aeruginosa* biofilm matrix: Psl, Pel, and alginate (Kovach et al., 2017). Rheological measurements using the model virulent strain PAO1 and isolates from CF lungs show that Psl, together with its cross-linking protein CdrA, are the main contributors to the biofilm elastic modulus (Kovach et al., 2017). Overproduction of Pel increases the biofilm yield strain but does not alter the elastic modulus. Pel is positively changed and it binds eDNA (Jennings et al., 2015). Pel-eDNA interactions could be instrumental in driving overall biofilm mechanics, but this aspect has not yet been studied. Mucoid *P. aeruginosa* biofilms that overproduce alginate are fluid-like, possessing reduced elastic modulus and reduced yield stress compared to PAO1 biofilms (Gloag et al., 2018). In CF patients, cells in *P. aeruginosa* biofilms tend to increase both alginate and Psl production. Alginate overproduction causes a decrease in yield stress that is compensated for via overproduction of Psl. Together, these alterations enable the biofilm to preserve its original yield stress (Kovach et al., 2017). It is possible that maintaining a minimum yield stress is required for *P. aeruginosa* biofilm cells to avoid immune clearance. The unique combinations of matrix components displayed by different *P. aeruginosa* strains suggest that such blends promote distinct biofilm mechanical properties, each presumably optimized for a particular environmental condition.

S. epidermidis is a member of the human skin microbiome but also occurs in medical device and hospital acquired infections. The main *S. epidermidis* matrix component is called polysaccharide intercellular adhesin (PIA), a positively charged polymer (Otto, 2009). At low pH, purified PIA in solution possesses concentration-dependent viscoelasticity that is well described by the classical model of associative polymers: polymers that can both physically entangle and chemically interact through hydrogen bonding (Ganesan et al., 2016). The main contribution to biofilm rheology, however, stems from chemical interactions, as the concentration of PIA in biofilms is too low for physical entanglement. At pH = 7 (or lower), PIA associates, becomes unstable in solution, and it phase separates together with the *S. epidermidis* cells to form biofilm-like structures with rheological properties similar to native *S. epidermidis* biofilms (Stewart et al., 2015). Indeed, simply increasing the pH above 7 was sufficient to stabilize PIA and make *S. epidermidis* biofilms more malleable. The strong pH-dependent phase behavior exhibited by PIA suggests *S. epidermidis* biofilms may possess distinct mechanical properties in particular local infection environments.

Biofilm architectures: from individual cells to macroscopic communities

Until recently, there was little understanding of how cells are arranged within biofilms and how 3D biofilm structures are built cell by cell. Custom high-resolution confocal microscopy technologies and companion imaging analysis algorithms were developed that enabled investigation of biofilms at single cell resolution. Initially, high-resolution confocal laser scanning microscopy was used to extract spatial information regarding fixed cells in *S. epidermidis* biofilms (Stewart et al., 2013). By tracking the centers of the spherical cells and analyzing the local cell density and cluster distribution, local biofilm compactness parameters were defined and were discovered to vary within an *S. epidermidis* biofilm. In regions with high and medium cell density, nearly all of the cells were present in a single cluster that exhibited characteristics of a dense disordered fluid. In regions of sparse cell density, cell clusters displayed open, fractal features similar to colloidal gels. Upon osmotic stresses (high salt concentration) or antibiotic challenge (vancomycin), however, the *S. epidermidis* biofilm structure only exhibited the low-density phenotype. The mechanism(s) driving these regional packing differences is unclear. We hypothesize that, as highlighted in the preceding section, local variations in pH or in PIA concentration that alter PIA solution behavior could lead to distinct biofilm packing density phenotypes.

Images of fixed *V. cholerae* cells obtained at different times during biofilm formation were acquired to learn how cell arrangement changes as biofilms mature (Drescher et al., 2016). The community transitions from a 2D branched morphology to a dense 3D cluster. In the mature *V. cholerae* biofilm cluster, vertical cells reside at the biofilm center and radially orientated cells are at the periphery. This entire sequence of structural transitions was subsequently visualized in living, growing *V. cholerae* biofilms (Figure 2A–B) (Yan et al., 2016). Mutagenesis coupled with matrix labeling showed that *V. cholerae* biofilms lacking cell-surface adhesion due to deletion of *rbmC* and *bap1* exhibit normal cell density but show no cell ordering. By contrast, biofilms lacking cell-cell connections due to deletion of *rbmA* display reduced cell packing density and enhanced vertical cell alignment.

To explore the forces driving structural transitions in *V. cholerae* biofilms, agent-based simulations were developed to investigate cell-surface interactions (Beroz et al., 2018). When a biofilm begins to form on a surface, it expands outward from the founder cell, as a one-cell-layer thick 2D film. During expansion, cells experience increasing mechanical pressure as they divide and push against their neighbors. These neighboring cells, in turn, resist the pushing force via surface adhesion. Ultimately, the pressure from pushing exceeds the cell-to-surface adhesion force and causes individual cells to reorient at the center of the biofilm where the pressure is the greatest. Cells transition from aligning parallel to aligning perpendicular to the substrate. When verticalized cells divide, they place their offspring further into the third dimension, thus the biofilm gradually transitions from a 2D surface layer to a mature 3D community (Figure 2C). To bolster these theoretical arguments, *V. cholerae* cell lengths were manipulated using chemicals. The timing of verticalization was altered: biofilms with shorter (longer) cells transitioned from 2D to 3D earlier (later) than cells of normal length, because shorter (longer) cells required lower (higher) critical forces to drive verticalization. A consequence of altering cell length was to change the overall width to height ratio of the resulting biofilm (Figure 2D).

Tolerance and persistence: how bacterial cells survive antibiotic challenge

Antibiotic resistance is caused by mutations that make a bacterial cell impervious to the toxic effect of the antibiotic, endowing that cell and its descendants with a selective growth advantage over non-resistant cells. Beyond classic resistance mechanisms, bacteria can display “tolerance”, the ability to survive transient exposure to high concentrations of an antibiotic (Brauner et al., 2016). Tolerant bacteria grow slower or have longer non-growing lag times when they exit stationary phase than their non-tolerant counterparts. Common targets of antibiotics, e.g., RNA polymerase, cell-wall biosynthetic enzymes, exhibit low activity in non-growing cells, and thus, slow-growing or non-growing cells can evade killing. In this respect, tolerance differs fundamentally from resistance, as resistance is usually specific to one antibiotic or one class of antibiotics.

Tolerant cells display a longer minimum duration of killing by an antibiotic than non-tolerant cells, enabling tolerant cells to enjoy a selective advantage during transient or periodic antibiotic treatment (Brauner et al., 2016). Indeed, tolerant *Escherichia coli* cells spontaneously arose after repeated cycles of ampicillin treatment (Fridman et al., 2014). The increased lag time the *E. coli* cells exhibited matched the duration of ampicillin exposure. Because antibiotic treatment usually occurs in timed doses, patients experience periodic fluctuations in antibiotic concentration, likely favoring the emergence of tolerant cells.

Mutations in genes encoding a methionyl-tRNA synthetase, ribose-phosphate diphosphokinase, and toxin-antitoxin (TA) modules all promote tolerance by extending lag phase (Fridman et al., 2014). It is hypothesized that a sequential relationship exists between bacterial tolerance and bacterial resistance. Indeed, in an experiment probing periodic ampicillin treatment that mimicked medical practice, resistant *E. coli* strains isolated at the end of the experiment all arose from ancestral, tolerant strains (Figure 3A) (Levin-Reisman et al., 2017). The logic is that tolerance mutations occur more frequently than resistance mutations due to a larger target size of the former: there are many genes that when mutated confer tolerance while mutations in only a few genes confer resistance to a particular antibiotic. Once a tolerant mutant becomes established in the population, its presence gives the rarer, resistance mutations more opportunities to occur (Brauner et al., 2016; Levin-Reisman et al., 2017).

Another form of tolerance, not obtained through heritable mutations but rather through phenotypic differentiation, is called persistence (Balaban et al., 2004; Lewis, 2005). Originally observed by Bigger (Bigger, 1944), bacterial persistence is receiving renewed interest due to its medical relevance, in particular, in the context of biofilms (Lewis, 2005). Time dependent antibiotic killing of a bacterial population shows that actively growing cells are killed first whereas persister cells are killed in a second phase at a much lower rate. Visualization of individual bacterial cells established that, prior to antibiotic treatment, an exponentially growing bacterial population contains a pre-existing fraction of non-growing cells (Balaban et al., 2004). It is this sub-population that survives antibiotic treatment and regrows after the antibiotic is withdrawn. Another source of persister cells are those that have become dormant during stationary phase. Such cells are simply carried over to the new culture upon sub-culturing.

The mechanisms driving subpopulations of cells to enter the persistent state are the subject of intense research and debate. One mechanism involves toxin-antitoxin (TA) modules (Lewis, 2005; Rotem et al., 2010). Indeed, the first identified high-persistence *E. coli* strain harbors a mutation in *hipAB* encoding a TA module (Moyed and Bertrand, 1983). The HipA toxin is a serine-protein kinase that phosphorylates GltX, a glutamyl-tRNA synthetase. HipA is inactivated by the companion antitoxin HipB (Schumacher et al., 2015). When HipA levels exceed a threshold in a cell due to stochastic fluctuations, protein synthesis is inhibited, and as a consequence, cell growth is arrested (Rotem et al., 2010). Growth-arrested cells can become persisters. The originally isolated high-persister strain possesses a mutation that impairs HipA-HipB binding (Schumacher et al., 2015), which increases the chances of cells of this strain entering the growth-arrested state. TA modules do not appear to underlie persistence in *Staphylococcus aureus* as elimination of all TAs had no effect on persister cell formation (Conlon et al., 2016). Rather, some *S. aureus* cells stochastically enter into stationary phase earlier than others to become persister cells. In this case, stationary phase entry is accompanied by a decrease in intracellular ATP levels, which, in turn, reduces the activity of ATP-dependent antibiotic targets (DNA gyrase, DNA topoisomerase, RNA polymerases, etc.). Therefore, stationary phase *S. aureus* cells are naturally prone to becoming persister cells. Lastly, genomic studies of *E. coli* and *P. aeruginosa* have identified many metabolic genes connected to persister cell formation (Amato et al., 2014).

Normally, persister cells make up from 10^{-2} to 10^{-5} of a population, so such cells might seemingly not be clinically relevant given that the goal of antibiotic treatment is to eliminate the majority of actively growing pathogens and to expect the immune system to clear the remainder (Lewis, 2010). However, persister cells may be dangerous to particular patient populations. In immunocompromised individuals, persister cells can likely regrow. In some diseases such as tuberculosis, antibiotic treatment must drive pathogens to very low numbers to achieve a clinical outcome. In such cases, persister cells could be problematic. In chronic infections, such as those in CF patients, high-persister mutants can be isolated after prolonged antibiotic treatment (Lewis, 2010). In these real-life cases, persister cells could be present and not eliminated by current drug regimes.

Tolerance and persistence in bacterial biofilms

The ability of biofilms to house tolerant and persister cells is proposed to underlie the difficulties encountered in eliminating biofilms during chronic infections (Lewis, 2005). Impeded antibiotic penetration into biofilms was initially proposed to be responsible, however it is now known that the matrix mesh size is much larger than antibiotic molecules (Ganesan et al., 2016; Yan et al., 2018), and most antibiotics do not interact strongly with biofilm matrix components (Spoering and Lewis, 2001). Rather, increased antibiotic tolerance and persistence in biofilms likely arises from altered physiology of biofilm cells. Cells buried inside thick biofilms could be in stationary phase, as penetration of nutrients and oxygen are known to be limited due to consumption by peripherally-located cells (Walters et al., 2003). Indeed, increasing evidence supports similarities between the physiological states of biofilm-dwelling cells and stationary phase planktonic cells. For example, the levels of persister cell formation by *P. aeruginosa* are comparable in the biofilm

state and in stationary phase (Spoering and Lewis, 2001). Likewise, antibiotic tolerance phenotypes of *S. aureus* biofilm cells, stationary phase planktonic cells, and persister cells are strikingly similar. (Waters et al., 2016). Nutrient starvation, a common environmental situation encountered during both biofilm formation and entrance into stationary phase could promote antibiotic tolerance and persister cell formation by triggering the stringent response (Nguyen et al., 2011). In *P. aeruginosa* biofilms, disabling the stringent response via deletion of both *relA* and *spoT* leads to a 1,000-fold reduction in cell survival upon antibiotic treatment. The Δ*relA* Δ*spoT* mutant cells possess impaired antioxidant defenses and increased oxidant production, which, together, sensitize the cells to antibiotic treatment and to nutrient limitation. Indeed, even in the absence of antibiotics, spontaneous death of the Δ*relA* Δ*spoT P. aeruginosa* cells occurs in the nutrient-limited interior regions of biofilm clusters (Figure 3B). Although there are some results suggesting specific genes drive persister cell formation exclusively in biofilms (Harrison et al., 2009), the current notion is that mechanisms underlying persister cell formation under planktonic conditions apply to persister cell formation in biofilms.

Even if the mechanisms giving rise to persister cells in biofilms and in planktonic environments are similar or identical, persister cells in *in vivo* biofilms could be particularly tenacious because the biofilm matrix provides a physical barrier that protects the persister cells from immune components (Lewis, 2005). As mentioned above, *in vivo* biofilm stiffness could exceed the maximum mechanical stress neutrophils are capable of exerting, in the present context, preventing neutrophils from accessing persister cells buried deep in the interior of a biofilm. Moreover, the rigidity of the biofilm matrix scaffold remains even if the majority of the biofilm cells have been killed by antibiotics (Zrelli et al., 2013). Such residual structures could harbor tolerant or persister cells that can regrow and cause recurrent infections.

New strategies to target tolerant and persister cells in biofilms

The notion that biofilms provide a “safe haven” for persister cells to arise and evade antibiotics and immune components suggests that entire biofilm structures must be removed from infection sites for successful elimination of pathogens. Mechanical debridement (scraping of biofilms from wounds) is the standard-of-care for chronic wounds (Gordon et al., 2017). However, it is difficult to completely remove all cells once a biofilm is sheared into pieces. Moreover, this arduous process can only be applied to infected regions that are exposed and thus amenable to mechanical manipulation. To address this challenge, a capillary peeling method has been developed in which liquid is slowly applied to a biofilm grown at an air-solid interface and capillary forces gently peel the biofilm off in its entirety (Yan et al., 2018). This method applies to a variety of surfaces including metals, hydrogels, and membranes and to biofilms formed by different bacterial species. This new method does not yet address situations in which biofilms are submerged or reside internally in patients.

In instances of internal biofilms, chemicals that induce biofilm dispersal have been pursued alone or in combination with antibiotic treatment. Examples include Dispersin B that degrades poly-N-acetylglucosamine, a common biofilm matrix component (McDougald et al., 2012). An unsaturated fatty acid produced by *P. aeruginosa*, *cis*-2-decenoic acid, can

trigger the dispersal of cells from biofilms formed by a range of bacteria including *P. aeruginosa* itself (Davies and Marques, 2009). Once the bacterial cells are dispersed into the solution, they become vulnerable to clearance by the immune system and/or killing by antibiotics. To date, Dispersin B is marketed as an ingredient in a wound care gel and as a medical device coating. In both cases, when Dispersin B is combined with antibiotics, the compound shows efficacy in prevention of bacterial infections (Kaplan, 2010).

Regarding targeting and eliminating tolerant or persistent cells, *E. coli* and *S. aureus* persister cells in biofilms can be re-sensitized to an aminoglycoside antibiotic by providing metabolites that generate a proton-motive force facilitating aminoglycoside uptake (Allison et al., 2011). Interestingly, the above dispersal promoting molecule *cis*-2-decenoic acid can also transform *P. aeruginosa* and *E. coli* persister cells from dormant to metabolically active (Marques et al., 2014). Again, this change renders the cells susceptible to antibiotics. Another good example is provided by the acyldepsipeptide antibiotic ADEP4, which kills persister cells in *S. aureus* biofilms via activation of ClpP (Conlon et al., 2013). ADEP4-activated ClpP exhibits promiscuous protease activity, cleaves essential proteins, and causes persister cells to die. Together with rifampicin, ADEP4 treatment successfully eradicated *S. aureus* biofilms both *in vitro* and in a mouse model.

Other clever strategies to eliminate chronic infections are being developed and have potential for use to combat persister cells in biofilms. Here, we provide one new example as a representative to highlight these emerging applications. The membranes of *S. aureus* and other Gram-positive bacteria contain functional membrane microdomains (FMM) similar to lipid rafts in eukaryotic membranes. The FMM of methicillin-resistant *S. aureus* (MRSA) contain a high level of staphyloxanthin, an essential membrane-bound antioxidant. Illuminating MRSA with blue light promotes degradation of staphyloxanthin and sensitizes MRSA to reactive oxygen attack, both in the planktonic and biofilm states. Treatment with light was effective in a mouse wound infection model (Dong et al., 2019). Whether such a mechanism is generalizable remains to be tested, and the delivery method (light in this case) might also restrict its use due to penetration issues for thick tissues.

Perspectives

Biofilm formation and persister cell formation can be viewed as two types of collective bacterial behaviors. In the case of biofilm formation, bacterial cells collectively produce extracellular matrices, a public good that profits the entire community. In the case of persister cell formation, the entire population benefits when, a subpopulation of cells survives a hostile environment in which the majority of cells perish. Combining these two collective lifestyles endows the bacteria with powerful mechanisms to survive harsh perturbations, including mechanical stress and antibiotic treatment. This good news for the bacteria presents humanity with a serious challenge regarding chronic infections.

To address this challenge, a deeper understanding of the biofilm formation process, biofilm mechanics, tolerance, and persistence is necessary. We expect new technologies to provide insight into how biofilm mechanics arise from particular steps in biofilm development and the features mechanics provide to these living structures. Simultaneous single-cell resolution

biofilm imaging and rheological measurements will allow interesting questions to be answered including: what happens at the single-cell level during biofilm failure or detachment? How does the biofilm internal structure evolve as the biofilm relaxes stress? How does local cellular configuration determine the local biofilm stiffness and do weak regions exist in biofilms that could be exploited to drive failure? Such measurements could provide a comprehensive understanding of biofilms as dynamic living materials.

Regarding tolerant and persister cells, additional evidence is required to confirm their presence in biofilms, especially *in vivo* during disease. Single-cell visualization of the process of tolerant/persister cell birth to non-growth to rejuvenation in a biofilm will yield valuable information. Questions that can be addressed include: Where and when do tolerant/persister cells arise in spatially-structured biofilms and what is their spatial distribution? Which cells enjoy the mechanical protection provided by the matrix, what selects them, and are their locations pre-defined or random? How do persistent/tolerant cells deal with debris left by cells that have been killed by antibiotics? Ultimately, a deep understanding of the behaviors of all cells in biofilms, both at the individual and collective levels, could lead to currently unimaginable strategies to combat harmful biofilms and to promote beneficial biofilms.

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Réaliser une figure : outil biorender

Les outils pour réaliser des figures

Sur ce TD, vous allez travailler sur un nouvel outil graphique **Biorender** qui a pour intérêt de proposer des dessins d'outils ou de structures biologiques à utiliser et combiner. Cet outil n'est pas un outil de dessin vectoriel et donc ne permet de garder la qualité du rendu si vous utilisez la figure pour l'agrandir. Il est donc pertinent d'utiliser chaque outil pour son utilisation :

- **Biorender**, spécialisé en biologie permet d'avoir des dessins esthétiques pré-réalisés
- **Inkscape/ adobe illustrator** : Dessin vectoriel. Vous avez intérêt à insérer les photos / images dans inkscape pour les agencer ensuite. Le texte ajouté sur Inkscape supportera mieux l'agrandissement. Cela est particulièrement utile pour les posters notamment.
- **GIMP/adoobe photoshop** : Travailler les contrastes de photo ou leur cadrage.
- **Tableurs** : permettent de réaliser des graphiques. Souvent les graphiques des articles sont à un format peu esthétique ou contiennent plus de données que ce que vous voulez exploiter. Vous pouvez utiliser le logiciel xyscan (TD2 - Modèles en SV) pour extraire les données et ensuite refaire le graphique.

La figure à réaliser

Le schéma graphique d'un article scientifique a pour objectif d'attirer l'attention sur le sujet et doit résumer les messages principaux du texte.

Objectifs de la séance

- Synthétiser les informations scientifiques des fiches bibliographiques dans une figure
- Créer un schéma professionnel pour présentation des travaux scientifiques

A. Avant la séance :

1) Avoir préparé, en TD Résolution de la SAE 31, la bibliographie relative à la thématique individuelle
2) Créer un compte BioRender.
3) Examiner attentivement deux documents de support pour la construction d'une représentation graphique.
   a) Support 1 : Un infographique crée par BioRender expliquant comment résumer un texte de manière graphique. Remarquez la disposition à utiliser, les couleurs, les flèches et les légendes.
   b) Support 2 : Les consignes pour créer un résumé graphique par l'éditeur scientifique « Cell », avec des exemples critiqués de quatre « graphical abstract ».

B. Pendant la séance :

1) Discussion d'un abstract et de sa représentation graphique. (20min)
   a) Commencer par examiner la représentation graphique.

b) Rédiger une phrase décrivant votre perception du schéma observé.

c) Lire ensuite le résumé et comparer votre description avec le résumé

d) Répondre aux questions : Recevez-vous le même message en lisant le résumée et en examinant la figure ? Quels éléments pourraient être améliorés dans la représentation graphique ?

2) Réalisation d'un brouillon de votre figure. (20min)

a) Commencer par rassembler les fiches bibliographiques réalisées sur la SAE 31 et décider si la figure à réaliser regroupera des méthodologies expérimentales (i.e. démontrer les instruments et les conditions de manipulation suite à une hypothèse) ou des conclusions scientifiques (i.e. présenter une figure de revue bibliographique qui regroupe des théories).

b) Définir 1 ou 2 phrases qui résument vos fiches bibliographiques ainsi que le texte construit lors de la SAE31.

c) Consulter les enseignantes pour vérifier les définitions qui doivent apparaitre sur le schéma.

d) Esquisser sur papier le contexte de ce que vous souhaitez représenter et les éléments clés à dessiner. Déterminer les formes, flèches et étiquettes pour clarifier les schémas. Sélectionner les couleurs et polices adaptés.

3) Seulement après avoir un brouillon sur papier, investiguer BioRender et les modèles à disposition pour dessiner votre schéma (50 mn).

a) Se rappeler des éléments indispensables pour son message.

b) Esquisser l'ordre de présentation et la numérotation des événements si c'est le cas.

C. Après la séance :

1) Terminer la figure

2) Remplir le carnet de Bord

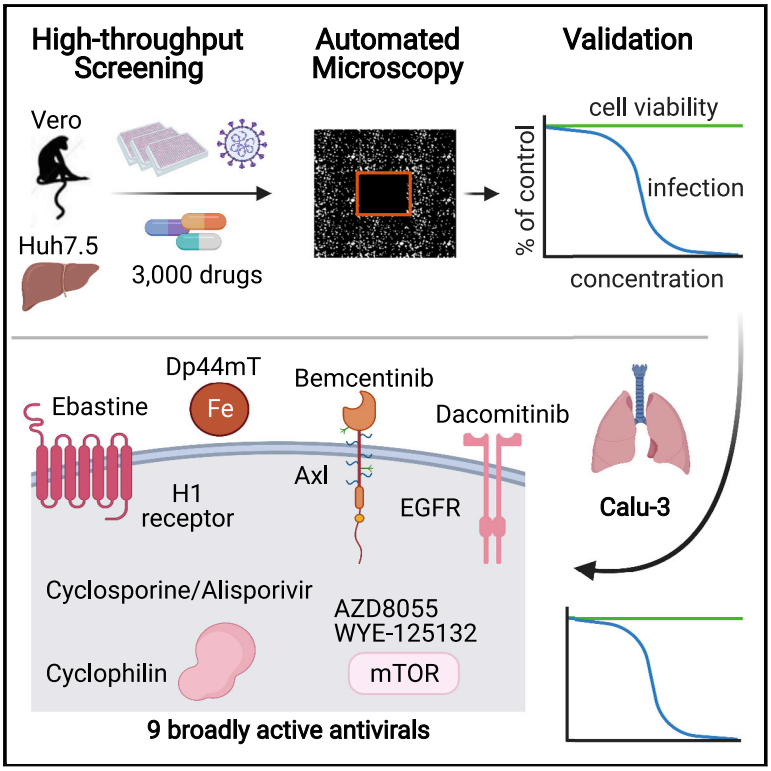
Exemple 1

Cell Reports

Resource

Drug repurposing screens reveal cell-type-specific entry pathways and FDA-approved drugs active against SARS-Cov-2

Graphical abstract



Highlights

- 3,000 compounds screened in two cell types against SARS-CoV-2
- Entry pathways are distinct in hepatocyte Huh7.5 and respiratory Calu-3 cells
- Only nine compounds that are active in Huh7.5 cells are active in Calu-3 cells
- Cyclosporin and cyclophilin inhibitors block SARS-CoV-2 infection in diverse cells

Authors

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In brief

There is an urgent need for antivirals to treat the newly emerged SARS-CoV-2. Dittmar et al. find nine host-directed drugs are antiviral in respiratory cells, seven of which have been given to humans, and three are FDA approved. We show host targets that have the potential for rapid clinical implementation.

Dittmar et al., 2021, Cell Reports *35*, 108959
April 6, 2021
https://doi.org/10.1016/j.celrep.2021.108959

CellPress

Cell Reports

CellPress
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Resource

Drug repurposing screens reveal cell-type-specific entry pathways and FDA-approved drugs active against SARS-Cov-2

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SUMMARY

There is an urgent need for antivirals to treat the newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To identify new candidates, we screen a repurposing library of ~3,000 drugs. Screening in Vero cells finds few antivirals, while screening in human Huh7.5 cells validates 23 diverse antiviral drugs. Extending our studies to lung epithelial cells, we find that there are major differences in drug sensitivity and entry pathways used by SARS-CoV-2 in these cells. Entry in lung epithelial Calu-3 cells is pH independent and requires TMPRSS2, while entry in Vero and Huh7.5 cells requires low pH and triggering by acid-dependent endosomal proteases. Moreover, we find nine drugs are antiviral in respiratory cells, seven of which have been used in humans, and three are US Food and Drug Administration (FDA) approved, including cyclosporine. We find that the antiviral activity of cyclosporine is targeting Cyclophilin rather than calcineurin, revealing essential host targets that have the potential for rapid clinical implementation.

INTRODUCTION

Coronaviruses represent a large group of medically relevant viruses that were historically associated with the common cold. However, in recent years, members of the coronavirus family have emerged from animal reservoirs into humans and have caused novel diseases (Cui et al., 2019). First, severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in China in 2003, followed by Middle East respiratory syndrome (MERS)-CoV in 2012 (de Wit et al., 2016; Weiss and Navas-Martin, 2005). Although SARS was in the end eradicated, MERS continues to cause infections in the Middle East. Beginning in December 2019 and continuing into January 2020, it became clear that a new respiratory virus was spreading in Wuhan, China. Rapid sequencing efforts revealed a coronavirus closely related to SARS, which was named SARS-CoV-2 (Wu et al., 2020). Unfortunately, this virus is highly infectious and has spread rapidly, creating a worldwide pandemic.

Identification of broadly acting SARS-CoV-2 antivirals is essential to clinically address SARS-CoV-2 infections. A potential route to candidate antivirals is through the deployment of drugs that show activity against related viruses. Previous studies found that the antiviral drug remdesivir, which was developed against the RNA-dependent RNA polymerase of Ebola virus, was also active against SARS-CoV-2 *in vitro*, with promising results in clinical trials (Beigel et al., 2020; Blanco-Melo et al., 2020; Warren et al., 2016). Chloroquine and its derivatives, including hydroxychloroquine, are approved for use in malaria, and many *in vitro* studies have found that these drugs are also active against coronaviruses, including SARS-CoV-2 (Liu et al., 2020; Wang et al., 2020). This led to early adoption of these agents to treat COVID-19 (the disease caused by SARS-CoV-2 infection); however, little efficacy of these agents has been demonstrated in subsequent clinical trials (Boulware et al., 2020). It remains unclear why these agents have not been more active in humans.

There are currently more than 3,000 US Food and Drug Administration (FDA)-approved drugs, as well as many others that have been tested in humans. We created an in-house library of 3,059 drugs, including ~1,000 FDA-approved drugs and ~2,100 drug-like molecules against defined molecular targets with validated pharmacological activity. In addition, we purchased drugs with reported anti-SARS-CoV-2 activity (e.g., remdesivir, lopinavir, azithromycin, etc.). Viruses encode unique proteins essential

Exemple 2

cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Drug Inhibition of SARS-CoV-2 Replication in Human Pluripotent Stem Cell–Derived Intestinal Organoids

Jana Krüger,[1,*] Rüdiger Groß,[2,*] Carina Conzelmann,[2,*] Janis A. Müller,[2,*] Lennart Koepke,[2] Konstantin M. J. Sparrer,[2] Tatjana Weil,[2] Desiree Schütz,[2] Thomas Seufferlein,[1] Thomas F. E. Barth,[3] Steffen Stenger,[4] Sandra Heller,[1,§] Jan Münch,[2,§] and Alexander Kleger[1,§]

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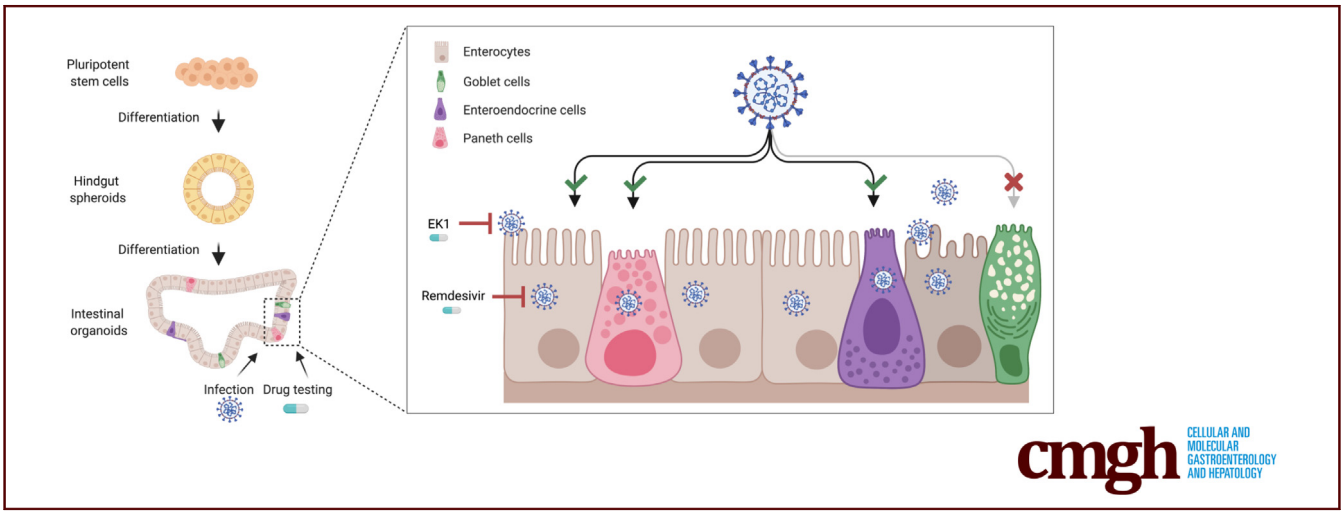

SUMMARY

Human pluripotent stem cell–derived intestinal organoids serve as an indefinite resource for organ-specific drug testing. SARS-CoV-2 infected and replicated within different cell types of the organoids, which was effectively inhibited by remdesivir and EK1 but not by famotidine.

BACKGROUND AND AIMS: The COVID-19 pandemic has spread worldwide and poses a severe health risk. While most patients present mild symptoms, descending pneumonia can lead to severe respiratory insufficiency. Up to 50% of patients show gastrointestinal symptoms like diarrhea or nausea, intriguingly associating with prolonged symptoms and increased severity. Thus, models to understand and validate drug efficiency in the gut of COVID-19 patients are of urgent need.

METHODS: Human intestinal organoids derived from pluripotent stem cells (PSC-HIOs) have led, due to their complexity in mimicking human intestinal architecture, to an unprecedented number of successful disease models including gastrointestinal infections. Here, we employed PSC-HIOs to dissect SARS-CoV-2 pathogenesis and its inhibition by remdesivir, one of the leading drugs investigated for treatment of COVID-19.

RESULTS: Immunostaining for viral entry receptor ACE2 and SARS-CoV-2 spike protein priming protease TMPRSS2 showed broad expression in the gastrointestinal tract with highest levels in the intestine, the latter faithfully recapitulated by PSC-HIOs. Organoids could be readily infected with SARS-CoV-2 followed by viral spread across entire PSC-HIOs, subsequently leading to organoid deterioration. However, SARS-CoV-2 spared goblet cells lacking ACE2 expression. Importantly, we challenged PSC-HIOs for drug testing capacity. Specifically, remdesivir effectively inhibited SARS-CoV-2 infection dose-dependently at low micromolar concentration and rescued PSC-HIO morphology.

CONCLUSIONS: Thus, PSC-HIOs are a valuable tool to study SARS-CoV-2 infection and to identify and validate drugs especially with potential action in the gut. *(Cell Mol Gastroenterol Hepatol 2021;11:935–948; https://doi.org/10.1016/j.jcmgh.2020.11.003)*

Keywords: SARS-CoV-2; COVID-19; Intestinal Organoids; Remdesivir; Famotidine.

Exemple 3

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Season-dependent effects of ZnO nanoparticles and elevated temperature on bioenergetics of the blue mussel *Mytilus edulis*

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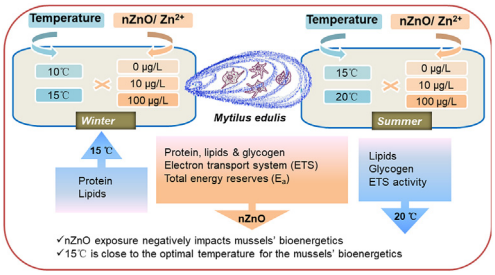
HIGHLIGHTS

- Combined effects of temperature and nZnO on mussels' bioenergetics were studied.
- In summer and winter, nZnO exposure depleted glycogen stores of the mussels.
- In summer, nZnO exposure suppressed mitochondrial activity and lipid levels.
- Warming (+5 °C) increased mussels' energy reserves in winter but not in summer.

GRAPHICAL ABSTRACT



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ABSTRACT

Input of ZnO nanoparticles (nZnO) from multiple sources have raised concerns about the potential toxic effects on estuarine and coastal organisms. The toxicity of nZnO and its interaction with common abiotic stressors (such as elevated temperature) are not well understood in these organisms. Here, we examined the bioenergetics responses of the blue mussel *Mytilus edulis* exposed for 21 days to different concentrations of nZnO or dissolved zinc (Zn^{2+}) **(0, 10, 100 μg l^{-1})** and two temperatures (ambient and 5 °C warmer) in winter and summer. Exposure to nZnO had little effect on the protein and lipid levels, but led to a significant depletion of carbohydrates and a decrease in the electron transport system (ETS) activity. Qualitatively similar but weaker effects were found for dissolved Zn. In winter mussels, elevated temperature (15 °C) led to elevated protein and lipid levels increasing the total energy content of the tissues. In contrast, elevated temperature (20 °C) resulted in a decrease in the lipid and carbohydrate levels and suppressed ETS in summer mussels. These data indicate that moderate warming in winter (but not in summer) might partially compensate for the bioenergetics stress caused by nZnO toxicity in *M. edulis* from temperate areas such as the Baltic Sea.

1. Introduction

With the global rise in nanotechnology, manufactured

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