Biofilm formation is intrinsic to the origin of life

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Abstract
Biofilm formation the built up of multicellular, often surface-associated, communities of autonomous cells, is the natural mode of growth of most microorganisms living on this planet today. Their tolerance against multiple environmental stresses makes biofilms refractory towards antimicrobial treatment strategies and the actions of the immune system. But how did biofilm formation arise? Here, I argue that the origin of the biofilm lifestyle has its foundation already in the fundamental energy preserving mechanisms that enabled the development of life on earth. Subsequently, prototypical biofilm formation has diversified concomitantly in composition and regulation with the expansion of prokaryotic organisms and their radiation by occupation of diverse ecological niches. This ancient origin of biofilm formation thus indicates that harnessing conditions have been the rule rather than the exception in microbial life, while upon the emergence of the association of microbes with higher organisms including the recent human pathogens, although being in a nutritional and stress-protecting heaven, some of these basic mechanisms of biofilm formation have been surprisingly conserved to promote sustained survival in new environments.

Keywords: serpentinization, fermentation, respiration, electron transfer, energy conservation, biofilm formation, extracellular matrix

Introduction

Global devastating acute and even chronic bacterial infections such as medieval plague caused by *Yersinia pestis*, pandemic cholera caused by *Vibrio cholerae*, whooping cough caused by *Bordetella pertussis* and tuberculosis caused by *Mycobacterium tuberculosis* have contributed to imprint our anthropocentric view of microbes as single-cell planktonic organisms acting in pure culture. In nature, rarely, if at all, single planktonic microbial cells are observed. Multicellular, surface or self-attached cell aggregates, biofilm forming microbes, consisting of autonomous cells of diverse phylogenetic origin constitute the majority of microbial life [1]. May it be in marine sediments, in the continental subsurface or in association with higher organisms such as plants, invertebrates and humans, biofilm forming organisms are predominant. Biofilm defined by Bill Costerton as 'microbes adhering to each other and/or to surfaces or interfaces with the aid of a self-produced (or environmentally derived) extracellular matrix' [2], these multicellular assemblies of self-autonomous cells undergo sophisticated genetically-programmed developments with tissue-like properties and a life cycle between multicellularity and the planktonic state directed by the integration of a multitude of environmental and intrinsic signals on various levels [3-7]. Rather the rule than the exception, more than one pathway leading to biofilm formation is encoded by microbial genomes with biofilm pathways and biosynthetic entities build up modular, although genetically-programmed, biofilm formation is highly flexible [8-13]. Considering this flexibility and plasticity, it might be challenging to develop strategies to tackle microbial biofilm infections, although commonly acting activators of biofilm formation such as the ubiquitous second messenger cyclic di-GMP have been identified [14, 15]. Refractoriness might thus be founded in the origin of biofilm formation which is proposed to be intimately coupled to the origin of life itsself. To understand the fundamentals of ancient metabolisms which are preserved until today in even evolved organisms might also aid tackling of chronic infections [16].

Evolution of life in close contact to surfaces

After the birth of our solar system and the proto-earth 4.6 and 4.5 billion years ago, respectively, life has been dated to originate approximately 3.8 Billion years ago. First indications for biofilm forming organisms have been traced back as to be as old as 3.5 to 3.8 Billion years [17, 18]. Here, I argue that biofilm formation is in fact intrinsic to the prerequisites and molecular mechanisms that enabled emergence of life. That is emergence of life at all of its stages including the fundamental principles of metabolism including energy conservation to the emergence of cellular organization to arise required solid catalytically effectual surfaces Thus already far before the emergence of the two structurally fundamental different, conceptually highly similar branches of early life, the eubacterial and archaebacterial protocells from the last universal common ancestor (LUCA), cells emerged from inorganic template predecessors in intimate surface-association [19, 20].

Irrespectively which environmental context has been most beneficial for the emergence of life, conditions such as found today in alkaline hydrothermal vents are considered to provide appropriate conditions for the cradle of life such as chemical disequilibria, natural gradients and transition metals at different redox stages for initial catalysis and thus are considered as one of the most likely origin [21, 22]. With its foundation in fundamental geochemical processes such as serpentinization, the transformation of minerals and water, the production and accumulation of inorganic molecules provided the basis for the transition to biochemical processes and the emergence of organic molecules [23]. Catalytic, electron-donating and/or accepting iron/nickel sulfur clusters subsequently enabled the acceleration of chemical reactions with inorganic gases CO_2 H_2 and NH_3 as substrates to provide the first organic molecules such as formate, methane, acetic acid, lactate and pyruvate. Indeed these molecules are even today central to carbon fixation and energy metabolism [24, 25].

With energy conservation as one of the prerequisites of life, initial chemical conservation of energy occurred by substrate-level phosphorylation which subsequently evolved into chemiosmotic energy conserving mechanisms at membrane that was coupled to the synthesis of ATP as a universal energy currency, driven by the built-up of ion, Na^+ and/or H^+ gradients, over an impermeable membrane. In the primeval Earth before the onset of life, these energy-conserving reactions have been initially facilitated through catalysis of the redox reactions on iron-sulfur mineral walls or mineral walls of similar transition metal composition, with conservation of energy in chemical form as a proton gradient over those semi-permeable inorganic walls [19, 21]. Nevertheless, these principles of bioenergetics are basically valid until today and organisms with rudimentary mechanisms of energy conservation are even found today [26]. As such obtain many organisms the energy for life in the form of the ubiquitous currency ATP by fermentation, substrate-level phosphorylation of energetically high molecules such as acetyl-phosphate and conserved energy over membranes without the sophisticated respiratory chains widespread today among bacteria.

Energy can be gained more effectively by respiration, which uses the energy of the electron flow to build up proton gradients by spatially separated energetically favored

redox reactions, diffusible membrane soluble redox compounds such as quinones and membrane-associated proteins containing iron-sulfur clusters, flavins and heme containing iron, with ferredoxin and cytochrome c, respectively, as the most ancient protostructures [27-30]. Respiration, which leads to a much higher energy gain than fermentation has been originally anaerobic and upon the availability of oxygen as an electron acceptor, became also aerobic significantly extended the range of available redox pairs, energy gain and ecological opportunities [31-33]. With the conservation of energy in a chemiosmotic H^+ or Na^+ membrane gradient, chemical energy in the form of ATP is ubiquitously gained by conceptually similar, structurally distinct ATP synthases [34, 35]. However, fermentation can be coupled to extracellular electron transfer which promotes more rapid proliferation and metabolism indicating that intrinsic redox reactions feedback on metabolism [36].

Similarly to the origin of simple central biomolecules and its energy conservation, emergence of other cellular components such as lipids are supposed to have required catalysis on autocatalytic surfaces [37, 38].
Extending this scenario, primitive forms of cellular life have intimately evolved as inherently sessile surface-associated life forms in direct contact with minerals. Indeed, also other fundamental metabolic processes such as anoxygenic photosynthesis thought to represent one of the earliest forms of metabolism on our planet and an alternative mode of energy conservation using light as the external energy sources is based on the oxidation of Fe^{2+} to Fe^{3+}, [39, 40]. Furthermore, Fe^{3+} has been recognized as the most ancient and a central electron acceptor [41] and Fe^{2+} is in a reverse reaction also used as an electron donor. Thus, surface association enabled various modes of energy conservation with subsequent development of a protocell and its growth connecting biofilm formation intimately to the origin of life. Considering the harsh conditions upon life emergence also puts forward the hypothesis that resource-restricted damaging-exposed environmental conditions, stress, has been the rule rather than the exception as experienced by some microbes today [42]. The early type of surface associated biofilm formation has been evolutionary maintained until today in microbes with microbes still respiring extensively using different iron redox stages in minerals [43-45]. Biofilm formation and the physiology of microbes has, however, evolved and diversified with the radiation of the two fundamentally different prokaryotic forms of life, the eubacteria and the archaebacteria which have access to all thermodynamically possible redox couples in minerals and in sdoluble form, the introduction of oxygen as an electron acceptors during the Great Oxygenation Event widened further the spectrum of potential redox pairs.

Although the most primitive metabolisms, as found in acetogens and methanogens, do neither possess heme, quinones or respiratory chains, build up of chemically stored energy requires ion pumps and membranes [46, 47]. Eventually, energy storage evoled towards quinone-based proton gradient generation using heme-flavin-FeS-containing cytochromes in respiratory chains including their regulatory components

[48-50]. In fact, the fundamental origin of the components of the respiratory chain is reflected by their combinatorial assembly using a redox protein construction kit [51].

Planktonic cells evolved secondary to surface-associated cells

What does this hypothesis has as consequence? Accordingly and most importantly, planktonic, not surface attached, cells evolved secondary to surface attached cells. Prerequisites that enabled the development of planktonic cells is the availability of electron acceptors in solution combined with high affinity terminal reductases.

With respect to biofilms, surface attached microbes have developed various modes of direct and indirect electron transfer to communicate with abiotic surfaces and with each other. Such deliver nanotubes, membrane extrusions with nanotubes and nanowires, proteinaceous type IV pili or archaella appendages, respectively, electrons to abiotic surfaces and, today, in biotechnological applications for current generation to electrons [52-55]. Furthermore, diffusible small redox active molecules emerge either synthesized endogenously and/or are provided exogenously from the environment to contribute efficiently to extracellular electron transfer. An example of the antibiotic pyocyanin and the humic model compound anthraquinone-2,6-disulfonate [56]. The biological significance of extracellular electron transfer might be equal as to small molecular metabolic waste products that can be catabolically used by other microorganisms and thus contribute to the optimal use of energetically higher molecules for energy conservation resulting in a macroorganism, for example, in cell dense sediments..

Thus the surface associated protocells might have been naked cells without an extracellular matrix surrounding them, different from the hallmark characteristic for todays' biofilms. So why and how did extracellular matrix production of surface attached biofilm cells evolve. While the 'how' cannot be answered directly, the production of an extracellular matrix might have provided an additional layer of protection when more diversified metabolic pathways that required protection emerged in surface-attached cells. Obviously, the advantages of extracellular matrix production were higher than the disadvantage; which are, for example, to be at a larger physical distance to the respective solid electron donors and acceptors. To overcome this hurdle, one can hypothesize that biofilm extracellular matrix components can associated with respective electron donors/acceptors for efficient energy gain [57]. As another aspect, upon division of protocells attached to a surface, not all cells in the multicellular structure have equal access to the redox pairs. Matrix production and other structures thereby enables physical closeness with efficient transfer of electrons and small molecules [58, 59]. Cellulose, a 1,4 beta-glucan with the most chemically insert sugar D-glucose might have been one of the first polysaccharide extracellular matrix components. Indeed, cellulose is considered to serve as a biosignature in rock records as an indication of microbial life. What is again surprising though is the conservation of such ancient components as cellulose production has been conserved in human pathogens such as *Salmonella typhimurium* where the microbes form cellulose-based biofilms even in immune cells [60-62].

Diversification of the biofilm response

The biofilm response in the presence of electron acceptors and other energy gain related signals has diversified. As proposed as the fundamental reaction and still observed basically for the need of direct energy gain from solid mineral electron acceptors [63, 64] is the promotion of sessility (biofilm establishment) over the opposite lifestyle motility (biofilm dispersal) as it is hypothesized to have occurred as the most fundamental behavior towards most ancient electron acceptors. However, this respiratory response has diversified and the response towards certain electron acceptors has reverted. For example does nitrate respiration lead to reducted biofilm formation, but trigger motility in order to promote invasion of the gut epithelial cell line and virulence [65-68]. Such a differentiated microbial behavior can extend to the strain level to ensure e.g. the differential occupation of ecological niches and the appropriate response to diverse environments [68, 69].

Discussion, Conclusions and Future directions

The ancient process of energy gain from solid reversibly redox active surfaces has many applications that reach beyond the understanding of the molecular mechanisms of biofilm formation to tackle chronic infections. One of the most prominent one is the gain of energy in microbial fuel cells [70, 71]. Another one is the gain of energy by electrosynthesis [72, 73]. As another example, biofilm may be developed that protect steel from corrosion [74]. However, also the colony morphotype biofilm model with its dense association of individual cells embedded into a honey-bee comb like extracellular matrix [75, 76, 77] can be seen in a different light, as a model not only for biofilm formation in microbial-dense environments such as the colon, but also for ancient biofilms on surfaces.

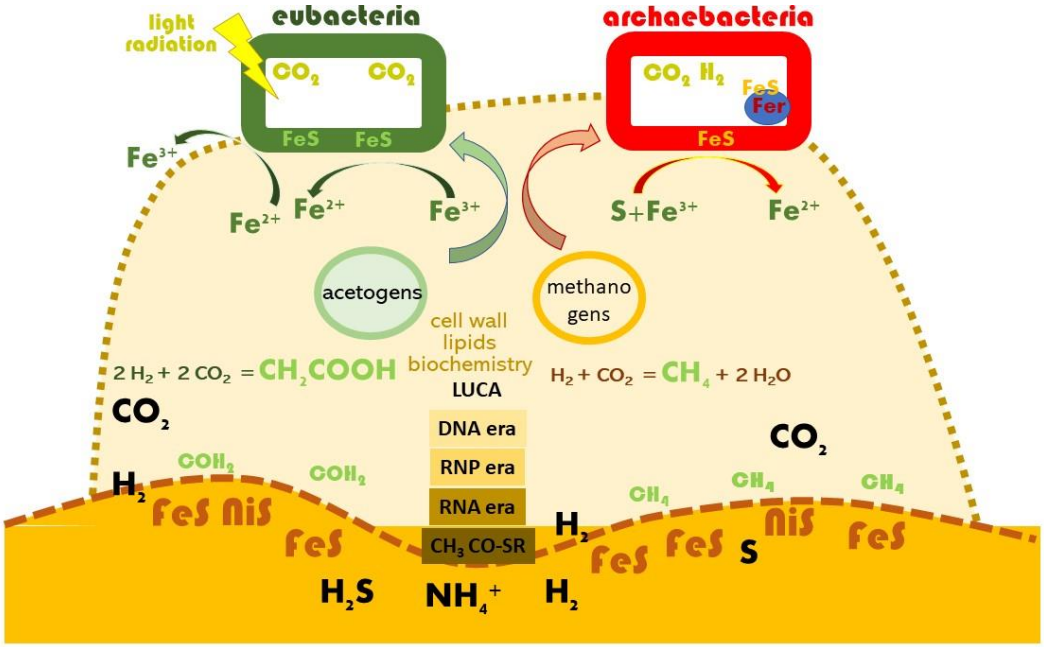

Figure 1. The biofilm mode of life has its origin in the development of life on catalytic surfaces (minerals) such as FeS and FeNiS clusters that are intimately associated with its emergence. On these and/or similar redox-active clusters in the presence of molecules created by geochemical reactions such as serpentinization such as hydrogen, H2; carbon dioxide, CO_2; NH_4^+ and H_2S the first organic molecules such as formamide, acetate and methan arised. Subsequently, upon the consecutive development of more complex organic molecules (the RNA, RNP and DNA era), a last universal common ancestor (LUCA) and the first biological membranes (probably organized around the semipermeable FeS walls that could already building up a ion gradient) and cell walls in protected compartments were similarly facilitated in contact with FeS and/or similar redox-active mineral clusters resulting in a primitive energy metabolism. Evolution would have eventually led to the incorporation of FeS clusters into respiratory proteins with the ferredoxin fold (Fer) central to the origin of ancient redox metabolism, as still universally found in all domains of life. Suggested primeval (prokaryotic) forms of life, a acetogenic and methanogenic life form on mineral surfaces would have been sessile, essentially constituting ancestral biofilms of the eubacterial and archaeabacterial line. Respiration with subsequent transformation of minerals using ferrous and ferric iron as the most ancient redox pairs, followed soon by sulfur and sulfur molecules in different redox stages. Over time, diversification of cellular life has led to populations of cells specialized on biofilm or planktonic lifestyles dependent on the availability of redox pairs and possibilities for electrohomeostasis. In the presence of respiratory electron acceptors, some bacterial species or strains take over the niche from competitors through (planktonic) population increase, thereby taking prevalent control of the metabolic resources available at the site. Freely adapted after [19].

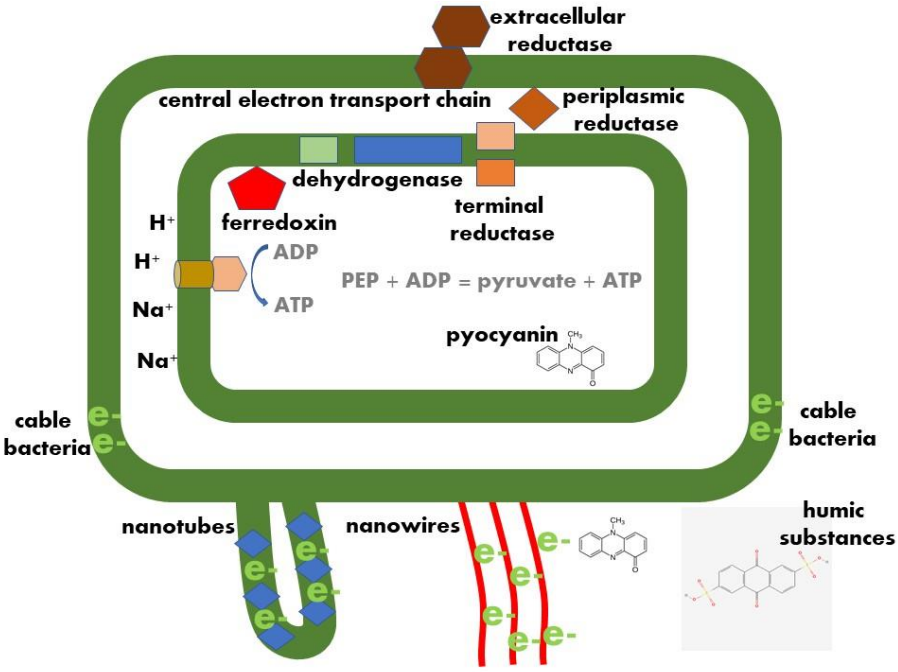

Figure 2. Different modes of electron transfer for energy gain. In respiration, an electron transport chain in the cytoplasmic membrane consisting of FeS cluster, flavin and heme containing integral membrane proteins and membrane diffusible quinone-based redox compounds transfer electrons along the redox gradient with the subsequent built up of an ion gradient, H^+ or Na^+, over the membrane for the production of the energy equivalent ATP. Substrate level phosphorylation is an alternative way to general ATP. The input into the central electron transport chain comes from different metabolic processes such as the catabolism of sugars and nucleotide biosynthesis performed by oxidoreductases, the most prominent the NAD(P)H/NAD^+ dehydrogenase complex, the succinate dehydrogenase complex, but also the dihydroorotate dehydrogenase in the nucleogenesis pathway. The presence and the concentration of the terminal electron acceptor determines the nature of the terminal reductase. For example, in *Escherichia coli*, there exist ten terminal reductases, integral membrane or periplasmic proteins, for substrates such as oxygen, dimethylsulfoxide, trimethylamine-N-oxide, fumarate, nitrate and nitrite. Reductases can however also be located in the outer membrane with the catalytic center facing the exterior. The periplasmic located CymA reductase of *Shewanella oneidensis* has been shown to respire ferric iron not only in its ingenious host, but also in *E. coli*. Other means to transport electrons along the redox gradient are intercellular nanotubes, nanowires (type IV pili in eubacteria or archaella in archaebacteria), intrinsic and extrinsic redox active substances such as pyocyanin and the humic model compound anthraquinone-2,6-disulfonate as well as an unknown mechanism in cable pili. PEP, phosphoenolpyruvate.

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