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Functional artificial free-standing yeast biofilms

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ABSTRACT

Here we report fabrication of artificial free-standing yeast biofilms built using sacrificial calcium carbonate-coated templates and layer-by-layer assembly of extracellular matrix-mimicking polyelectrolyte multilayers. The free-standing biofilms are freely floating multilayered films of oppositely charged polyelectrolytes and live cells incorporated in the polyelectrolyte layers. Such biofilms were initially formed on glass substrates of circular and ribbon-like shapes coated with thin layers of calcium carbonate microparticles. The templates were then coated with cationic and anionic polyelectrolytes to produce a supporting multilayered thin film. Then the yeast alone or mixed with various micro- and nanoparticle inclusions was deposited onto the multilayer composite films and further coated with outer polyelectrolyte multilayers. To detach the biofilms from the glass substrates the calcium carbonate layer was chemically dissolved yielding free-standing composite biofilms. These artificial biofilms to a certain degree mimic the primitive multicellular and colonial species. We have demonstrated the added functionality of the free-standing artificial biofilms containing magnetic, latex and silver micro- and nanoparticles. We have also developed "symbiotic" multicellular biofilms containing yeast and bacteria. This approach for fabrication of free-standing artificial biofilms can be potentially helpful in development of artificial colonial microorganisms composed of several different unicellular species and an important tool for growing cell cultures free of supporting substrates.

1. Introduction

In natural habitats microorganisms predominantly exist as biofilms which consist of living cells embedded in an extracellular matrix (ECM) based on glycoproteins or carbohydrates [1,2]. ECM allows microorganisms to (i) adhere to substrate surfaces and colonise the available surface space and (ii) integrate the same type of cells into colonies and protect their living environment from being populated by other species [1]. Biofilms colonise all accessible surfaces submerged into aqueous environments, including industrial and medical systems and devices, in addition, the severity of a number of human diseases strongly depends on biofilms formation in organs [3]. This makes the understanding of the mechanisms of biofilms formation crucial for the development of antimicrobial reagents and antibiotics. It is widely believed that in the biofilms the organization and differentiation processes of the microbial communities are regulated similarly to those in multicellular organisms [2]. The major challenge in studying the complex microbial communities is to reproduce *in vitro* the conditions which may occur in biofilms *in vivo* in order to predict the behaviour of constituent cells under experimental conditions.

Here we demonstrate for the first time that biofilms might be produced in a controllable way using polyelectrolytes and inorganic microcrystals as templates. Such artificial structures resembling natural biofilms may have several potential applications as cell sheets and can be further extended into the controllable assembly of mammal cells for tissue engineering. This work is concerned with the fabrication of artificial free-standing biofilms where the integrating matrix is composed of an appropriate composite of polyelectrolytes and solid inorganic or organic microparticles and nanoparticles. This allowed us (a) to prepare artificial biofilms free of the adjacent substrate and (b) to add functionality to the artificial biofilms integrating the cells by controlling the composition of the polyelectrolytes and the embedded solid particles. The prepared free-standing artificial biofilms can be regarded as a primitive version of colonial assemblies of cells and resemble to a certain

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