Collagen-immobilized polyimide membranes for retinal pigment epithelial cell adherence and proliferation

Degenerative retinal diseases are a leading cause of visual loss and irreversible blindness, particularly in the developed world. Retinal pigment cell (RPE) transplantation is nowadays considered the most promising therapeutic approach for certain retinal diseases, and the presence of a supportive scaffold has been considered essential to ensure the success of the implant. In this work, collagen IV was covalently immobilized to the surface of polyimide membranes, with the purpose of developing scaffold materials for RPE cell culture. The covalent modification method involved four steps: argon-plasma treatment, acrylic acid graft polymerization, surface activation, and finally immobilization of collagen type IV. Collagen-modified membranes did not become more rough but became significantly more hydrophilic than the unmodified and dip-coated controls. ARPE-19 cell morphology and attachment were studied by immunofluorescence staining and confocal microscopy. Covalently modified surfaces showed cell attachment and cell properties comparable to the uncoated and dip-coated controls. This work demonstrated the potential of collagen IV-immobilized polyimide membranes as substrates for the growth of ARPE-19 cells.

Transcription Initiation Controls Skewness of the Distribution of Intervals Between RNA Productions

Most regulation in transcription controls when and with which intensity genes are expressed. However, recent evidence suggests that control is also exerted on the noiseness of this process. Here, we use an empirically validated stochastic multi-step model of transcription to explore how its steps kinetics affect the skewness of the distribution of intervals between consecutive RNA productions in individual cells. From the simulations, we show that skewness is independent of the mean transcription rate, but differs widely with the fraction of time the RNA polymerase spends in the steps following open complex formation. Next, from qPCR and live, time-lapse, single-RNA microscopy measurements of multiple promoters, we validate our model predictions. Using the validated model, we then show that skewness affects, e.g., the fraction of time protein numbers are below a threshold. We conclude that skewness in transcription kinetics can be tuned by the rate-limiting steps in initiation and, thus, may be an evolvable decision-making parameter of genetic circuits.
Bioluminescent whole-cell reporter gene assays as screening tools in the identification of antimicrobial natural product extracts

We describe novel tools, bioluminescent whole-cell reporter gene assays, for facilitating the use of natural products in antimicrobial drug discovery. As proof-of-concept, a plant extract library was screened and follow-up experiments were carried out. Primary results can be obtained in 2-4 h with high sensitivity, leading to significant improvements of the process.

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Improved bioconversion of crude glycerol to hydrogen by statistical optimization of media components

Bioconversion of crude glycerol to hydrogen has gained importance as it addresses both sustainable energy production and waste disposal issues. Until recently, statistical optimizations of crude glycerol bioconversion to hydrogen have been greatly focused on pure strains. In this study, biohydrogen production from crude glycerol by an enriched microbial culture (predominated with Clostridium species) was improved by statistical optimization of media components. Plackett-Burman design identified MgCl2, H2O and KCl with negative effect on hydrogen production and selected NH4Cl, K2HPO4 and KH2PO4 as significant variables. Box-Behnken design indicated the optimal region beyond design area and studies were continued by ridge analysis. Central composite face centered design envisaged a maximal hydrogen yield of 1.41mol-H2
/mol-glycerol consumed at concentrations 4.40g/L and 2.27g/L for NH₄Cl and KH₂PO₄ respectively. Confirmation experiment with the optimized media (NH₄Cl, 4.40g/L; K₂HPO₄, 1.6g/L; KH₂PO₄, 2.27g/L; MgCl₂·6H₂O, 1.0g/L; KCl, 1.0g/L; Na-acetate·3H₂O, 1.0g/L and tryptone, 2.0g/L) revealed an excellent correlation between predicted and experimental hydrogen yield. Optimization of media components by design of experiments enhanced hydrogen yield by 29%.

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Integrated in vitro-in silico screening strategy for the discovery of antibacterial compounds
Multidrug-resistant bacterial infections are an increasing source of healthcare problems, and the research for new antibiotics is currently unable to respond to this challenge. In this work, we present a screening strategy that integrates cell-based high-throughput screening (HTS) with in silico analogue search for antimicrobial small-molecule drug discovery. We performed an HTS on a diverse chemical library by using an assay based on a bioluminescent Escherichia coli K-12
(pTetLux1) strain. The HTS yielded eight hit compounds with >50% inhibition. These hits were then used for structural similarity-based virtual screening, and of the 29 analogues selected for in vitro testing, four compounds displayed potential activity in the pTetLux1 assay. The 11 most active compounds from combined HTS and analogue search were further assessed for antimicrobial activity against clinically important strains of E. coli and Staphylococcus aureus and for in vitro cytotoxicity against human cells. Three of the compounds displayed antibacterial activity and low human cell cytotoxicity. Additionally, two compounds of the set fully inhibited S. aureus growth after 24 h, but also exhibited human cell cytotoxicity in vitro.

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