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Cellular communication via directed protrusion growth: Critical length-scales and membrane morphology

Haijiang Zhang [a,1], Anna Kim [a,b,1], Shijun Xu [a], Gavin D.M. Jeffries [a], Aldo Jesorka [a,*]

[a] Department of Chemical and Biological Engineering, Chalmers University of Technology, S-412-96 Göteborg, Sweden
[b] Department of Physiology and Pharmacology, Karolinska Institutet, SE-17177 Stockholm, Sweden

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ABSTRACT

We investigated the growth of cell protrusions from adherent HEK 293 cells and their capability to bridge cytophobic Teflon® AF microgaps, establishing a critical length scale, beyond which cells cannot probe free space. For this purpose, we employed a photolithography-based surface fabrication strategy for producing micropatterned substrates composed of glass and the amorphous polymer Teflon® AF. Cell protrusions growing from HEK 293 cells on these substrates were confined to extend on 2 μm wide glass lanes, intersected by Teflon® AF microgaps of various lengths between 2 and 16 μm. After 24 hours of incubation, the frequency of cell protrusions crossing the gap was found to be strongly dependent on the gap size. Gaps which are greater than 4 μm were found to be increasingly difficult to cross. Cell extensions crossing the microgaps either appeared as nanosized connections, in approximately 30% of all observed cases, or as microsized connections. Molecular transport in the established cell-to-cell connection across the microgap was investigated by activation of TRPM8 ion channels followed by supply of Ca^{2+} to one of the connected cells. The diffusion of the Ca^{2+} ions was visualized by means of a cell-permeant pre-fluorescent dye. We observed both open- and closed-ended intercellular connections in both nano- and microsized cell protrusions.

Introduction

Biological cells commonly migrate in response to external chemical or mechanical signals; wound healing, immune response, and tissue formation, all require the migration of cells towards specific locations. Many types of migrating cells feature filopodia, which are small, actin filament filled, plasma-membrane protrusions, typically microns in diameter [1]. One of their primary functions is to reach out from the cell, to sense and probe their environment [1,2]. They originate from cells during migration, appearing as protrusive structures at the leading edge of a motile cell. Despite extensive studies, the biological functions of filopodia and the mechanisms of their assembly are still not fully understood. Some recent studies on filopodia function include: cellular migration and connection [1], intercellular transport and interaction [3], virus transport [4], and gene delivery [5]. Filopodia are also implicated in cancer cell motility [6]. The primary biological role of filopodia appears to be the sensing of external mechanical and chemical cues, and have been found to sense signaling molecules at large distance away from the cell body [7]. Moreover, filopodia-like structures are also implicated in cell–cell communication in invertebrates [7,8].

* Corresponding author.
E-mail address: aldo@chalmers.se (A. Jesorka).
[1] These authors contributed equally to this work.

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Recently, we have demonstrated the ability to interconnect cells into a network by artificially generated nanotubes, using a micropipette assisted fabrication protocol [9]. The on-demand creation of these interconnections, facilitates the investigation of chemical species and organelle transfer between selected cells. However, our earlier investigation showed a high risk of cell damage during extrusion of cell tubes, stemming from the use of electrical pulses for membrane penetration by the glass pipette. The cells were found to reliably survive only two penetrations, which limited the possibility of generating larger cellular networks [9].

Tubular membrane interconnections have generated considerable interest, as open cell-to-cell bridges, and have been implicated in the transport of molecular cargo. The discovery of tunneling membrane nanotubes (TNTs) in 2004 as a new mode of exchange between mammalian cells [10] shed new light onto intercellular communication pathways. The formation process of TNTs between cells has previously been related to the formation of filopodia in a native cell growth environment [11,12]. Filopodia and TNTs have been found to have some structurally similar properties [13]. In this context, the possibility to generate networks of living cells, exploiting the growth and connectivity of cell protrusions would be a promising route to establish a controlled experimental environment for the study of intercellular communication.

In order to provide more insight into intercellular communication within artificial and natural cell networks, we present herein the growth of filopodia-like cell protrusions from HEK 293 adherent cells, guided by surface-fabricated microlanes. Particular focus was placed on investigating the ability of cell membrane protrusions to bridge cytophobic obstacles. Surface fabrication and cell culture protocols were established; and chemical transport through the established tubular connections was evaluated using interconnected pairs of cells.

In our experimental study, we directed the growth of cell protrusions in cytophobic Teflon® AF-framed glass microstructures, featuring 2 μm wide glass lanes intersected by a Teflon® AF microgap of varying lengths, from 2 μm to 16 μm (Supplementary Information S1). Teflon® AF (amorphous copolymer of polytetrafluoroethylene (PTFE) with 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole) is typically chosen as the surface coating material, as it features strong hydrophobicity, high gas permeability [14] and has very low autofluorescence, which is of key importance for fluorescence microscopy imaging. One major benefit of Teflon AF, as opposed to other surface mediated growth strategies [15], rendering it particularly suitable for our study, is its ability to guide cell growth, due to its cytophobic, but not cytotoxic, properties [16]. An optimized variant of a previously reported micropatterning technique was employed to produce Teflon® AF-framed glass microstructures [17], which were appropriately sized to accommodate up to three HEK 293 cells per circular region. Other examples of micropatterned surfaces for the study of filipodia-like cell protrusions exist [18–20], but none of them exploit the specific properties of Teflon® AF to guide growth while minimizing cell adhesion to undesired binding events.

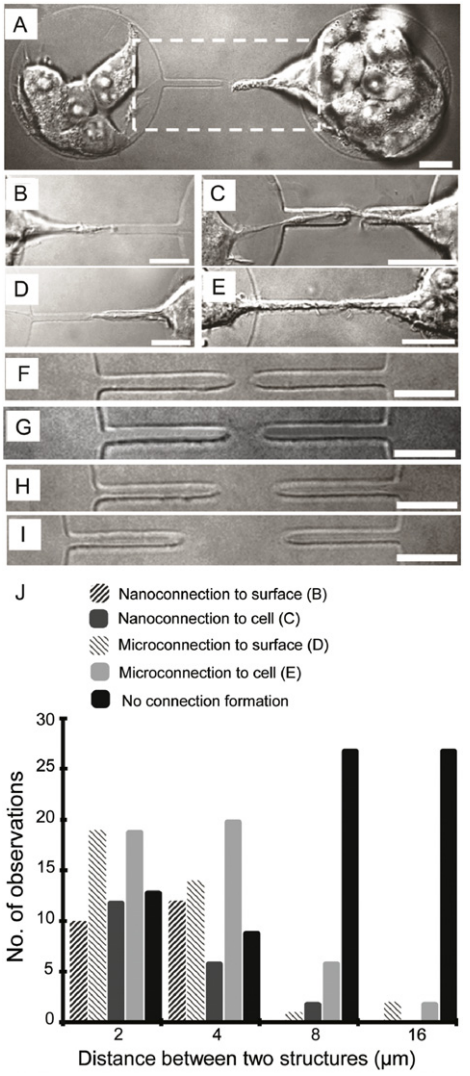

Fig. 1. Teflon® AF patterned glass cover slip and intercellular tubular growth. (A) Cell adhesion to the circular glass surface and filopodia-like protrusion growth along the Teflon-framed lane. (B–E) Bright-field images of the identified types of intercellular connections for microgaps of different length. (B) Image of a cell forming a nanosized bridge head to the glass surface across a microgap. (C) Image of a cell forming a nanoconnection with another cell across a microgap. (D) Image of a cell forming a microsized bridgehead to the glass surface across a microgap. (E) Image of a cell forming a microconnection with another cell across a microgap. (F–I) Images of various microgap separations between two structures (2, 4, 8 and 16 μm long, respectively). (J) A graph summarizing the total number of the different types of intercellular connections observed for different microgap lengths. The scale bars in panels A–I represent 10 μm.

On the micropatterned surfaces, cells directly adhere and grow on the exposed glass regions, where the development of cell protrusions along the Teflon® AF-framed lanes could be observed (Fig. 1(A)–(E)). We incorporated various Teflon® AF gap junction distances in the glass lanes (Fig. 1(F)–(I)), and fabricated them in large areas on each surface to enable many patterning variants to be present on each micropatterned surface (Supplementary Information Figure S2). Since this cytophobic gap does not support cell adhesion, the ability of the approaching mem-