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Leading opinion

Theoretical considerations on mechanisms of harvesting cells cultured on thermoresponsive polymer brushes☆

Avraham Halperin [a,]*, Martin Kröger [b,]*

[a] University of Grenoble 1/CNRS, LIPhy UMR 5588, BP 87, 38041 Grenoble, France
[b] Polymer Physics, Department of Materials, ETH Zurich, CH–8093 Zurich, Switzerland

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ABSTRACT

Poly (N-isopropylacrylamide) (PNIPAM) brushes and hydrogels serve as temperature-responsive cell culture substrates. The cells adhere at 37 °C and are detached by cooling to below the lower critical solution temperature $T_{LCST} \approx 32$ °C, an effect hitherto attributed to change in PNIPAM hydration. The article proposes a mechanism coupling the change of hydration to integrin mediated environmental sensing for cell culture on brushes and hydrogels in serum containing medium. Hydration is associated with swelling and higher osmotic pressure leading to two effects: (i) The lower osmotic pressure in the collapsed brush/hydrogel favors the adsorption of serum borne extracellular matrix (ECM) proteins enabling cell adhesion; (ii) Brush/hydrogel swelling at $T < T_{LCST}$ gives rise to a disjoining force f_{cell} due to confinement by the ventral membrane of a cell adhering via integrin-ECM bonds. f_{cell} places the integrin–ECM bonds under tension thus accelerating their dissociation and promoting desorption of ECM proteins. Self consistent field theory of PNIPAM brushes quantifies the effect of the polymerization degree N, the area per chain Σ, and the temperature, T on ECM adsorption, f_{cell} and the dissociation rate of integrin–ECM bonds. It suggests guidelines for tuning Σ and N to optimize adhesion at 37 °C and detachment at $T < T_{LCST}$. The mechanism rationalizes existing experimental results on the influence of the dry thickness and the RGD fraction on adhesion and detachment.

1. Introduction

Cultured anchorage dependent cells are often harvested using proteolitic enzymes [1] thus damaging the extracellular matrix (ECM), cell–cell junctions and cell surface receptors. Temperature responsive cell culture substrates enable "non invasive" detachment sparing these components and thus conducive to tissue engineering applications [2,3]. Among these substrates, surface layers of poly (N-isopropylacrylamide) (PNIPAM) received most attention leading to corresponding technological developments [4–7]. These layers allow cell adhesion and proliferation at 37 °C, above the lower critical transition temperature (LCST) of PNIPAM at $T_{LCST} \approx 32$ °C. The cells spontaneously detach upon lowering the temperature T to below the LCST and efficient harvesting is typically achieved at $T = 20$ °C. The current understanding of these effects involves two postulates [4,7–9]: (i) Both cell spreading at 37 °C and cell retraction at $T < T_{LCST}$ are metabolically active processes involving integrin mediated environmental sensing. (ii) The thermal switch between cell adhesive and cell detaching states is related to change in PNIPAM hydration. It is often discussed in terms of hydrophobicity/hydrophilicity as characterized by contact angle measurement. The mechanism allowing the cells to detect the hydration state of PNIPAM and react to it is currently unspecified. In the following we present theoretical considerations suggesting such mechanism focusing on the swelling degree as a measure for hydration. We further explore the mechanism's dependence on the structural parameters of the PNIPAM layers and suggest guidelines for optimizing brush performance allowing for cell adhesion/proliferation as well as efficient detachment.

Studies of PNIPAM based temperature-responsive cell culture substrates utilized diverse structures including PNIPAM co-adsorbed with collagen [10], surface hydrogel layers cross linked by electron beam (e-b) irradiation [6,11], plasma polymerized layers [12–15], brushes of terminally anchored chains [16–20] and

☆ *Editor's Note:* This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

* Corresponding authors.
E-mail addresses: avraham.halperin@ujf-grenoble.fr (A. Halperin), mk@mat.ethz.ch (M. Kröger).

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