Design of Polysaccharide Gel-Based Sensor to Quantify the Number of Receptor-Adhesion Ligand Bonds

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Introduction

Binding between cellular receptors and adhesion ligands in extracelllular matrices(ECM) plays a critical role in regulating a wide array of cellular activities. It has been believed that the number of receptor-ligand bonds mediates the response of cells to ECM, but this assumption has never been tested due to the lack of analytical tools, specifically for cells within a 3D microenvironment. This study demonstrates a bioactive polysaccharide-based gel system which allows one to measure the bond number between receptors of MC3T3 preosteoblasts and synthetic adhesion oligopeptides (denoted as RGD peptides) chemically bound to a gel matrix using a fluorescent resonance energy transfer (FRET) technique. This technique was used to examine how the number of receptor-ligand bonds varied with the presence of soluble factors (e.g., 2,3-butanedione monoxime to inhibit myosin and serum protein to compete with RGD peptides) and insoluble factors (e.g., total density of RGD peptides).

Materials and methods

Alginate molecules (LF 20/40, FMC Biopolymers) were modified with RGD peptides using aqueous carbodiimide chemistry, following a previously reported procedure[1]. Degree of substitution was varied from 1 to 20 peptides per alginate chain. Then, lysine residues in the number of RGD peptides were labeled with rhodamine succinimidylester (Invitrogen)[2]. Following the purification with dialysis, sterilization and lyophilization, 1 %(w/w) alginate solution reconstituted with α MEM (Invitrogen) was mixed with MC3T3 presoteoblasts. Cell membranes were stained with hexaaminofluorescein (Invitrogne). The gel matrix was prepared by mixing rhodamine-RGD-alginate and cells with $CaSO_4$ slurry. Cell-gel constructs were incubated in the fetal bovine serum (FBS)-free medium. For certain experiments, cell-gel constructs were incubated in medium containing FBS or 2,3-butanedionemonoimie (BDM).

The degree of energy transfer (D_{FRET}) was measured using a fluorometer (Jobin Yvon). Fluorescently labeled cell-gel disks were excited at a wavelength (λ) of 488 nm and the corresponding emission spectrum ranging from 500 to 650 nm was collected. D_{FRET} was calculated from the emission intensity of fluorescein in the absence ($\Gamma_{\text{fluorescein},0}$) and presence ($\Gamma_{\text{fluorescein}}$) of rhodamine-RGD-polymer following Equation (1)

$$D_{FRET} = \left[1 - \frac{\Gamma_{fluorescein}}{\Gamma_{fluorescein,0}}\right] \quad (1)$$

Results and Discussion

Encapsulating cells labeled with fluorescein in the rhodamine-RGD-alginate gel matrix greatly decreased the emission intensity of fluorescein, maximized at λ of 520 nm and increased the emission intensity of rhodamine, maximized at λ of 580 nm (Fig. 1a). A control experiment that exposed cells to free soluble RGD peptides followed by encapsulation in the gel matrix led to minimal changes in the emission intensity of fluorescein. This result confirms that fluorescein neighboring to cellular receptors is exclusively involved in the energy transfer to rhodamine coupled to RGD peptides. D_{FRET} and the bond number computed from D_{FRET}, using a calibration curve[2], were increased with the density of RGD peptides. Incubating cell-gel constructs in medium supplemented with FBS and BDM decreased D_{FRET} and the bond number, in consequence (Fig. 1b).

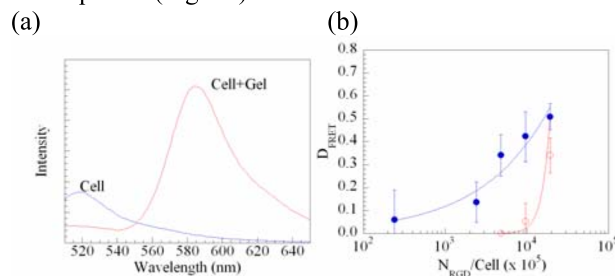

Figure 1 Fluorescent emission spectrum from fluorescently labeled cells encapsulated in the gel matrix (a). D_{FRET} was decreased in the presence of FBS in the cell culture medium (○) as compared with a condition without FBS (●).

Altogether, for the first time, this study demonstrates a gel system which allows one to monitor how the receptor-ligand bonds are varied with the cellular microenvironment. This method will be broadly useful in understating and predicting cellular behavior in given conditions, and in the rationale design of biomaterials to induce a specific cell response.

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