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Response to Yeo and Mrksich

Although some of their results were compelling, Yeo and Mrksich failed to make adequate use of controls, to provide sufficient explanation for their data, and to supply quantitative means for evaluating their conclusions. In analyzing the use of the Cys-Gly-Arg-Gly-Asp-Ser peptide (CGRGDS) containing the RGD sequence as a ligand for receptor-mediated adhesion of cells, Yeo and Mrksich would have benefited from a control ensuring that CGRGDS was indeed the mechanism causing cells to adhere to the self-assembled monolayers (SAMs).[1] One such control might include a single amino acid substitution in the RGD sequence knocking out CGRGDS function, where no observed cell adhesion would substantiate the necessity of the RGD ligand in cell adhesion.[2, 3] Yeo and Mrksich next synthesized electroactive O-silyl hydroquinone (SHQ) and disulfide quinone ester (QE) for the purpose of creating two electrochemically active cell-adhering tethers that respond to positive and negative potentials, respectively.[4] However, the authors use of triethylene glycol to inhibit nonspecific cell adhesion not mediated by SHQ or QE lacked controls substantiating triethylene glycol presence or efficacy, despite its subjection to the same electrochemical force that released the disulfide QE and SHQ.[5] In determining the presence and successful creation of disulfide QE and SHQ, controls were warranted in analyzing the mass spectrometry data for the purpose of accurately identifying the peaks on the mass spectrum.[6] When looking for the electrochemical release of RGD ligands with MALDI-TOF MS, data obtained after treatment with a CGRGDS solution show five substantial peaks and a multitude of smaller peaks.[4] However, Yeo and Mrksich, without a control MALDI assay, labeled two peaks as the released RGD ligands and left the remaining peaks and noise unexplained. As with the possibility of the electrochemical potentials interfering with triethylene glycol, another important problem lies in the unreliable nature of SAMs, especially with regard to electrochemical reactions. While SAMs were used as the platform to measure the electrochemical release of RGD, SAMs are known to be difficult to work with, as they oxidize quickly and frequently break down when voltage is applied, making their use for electrochemical applications tenuous.[7]

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Ismagilov et al.'s Chemical Model

The chemical model designed by Ismagilov et al. simplifies the *in vivo* network leading up to clotting with three chemical reactions intended to represent themes in hemostasis.[8] The series of initiation, inhibition, and precipitation reactions, substituting hydronium (H_3O) ions for clot-inducing factors, is composed of the second-order autocatalytic production of H_3O^+ ($S_2O_3^{2-} + 2ClO_2^- + H_2O = 2SO_4^{2-} + 2H_3O^+ + 2Cl^-$), the linear consumption of H_3O^+ ($4S_2O_3^{2-} + C1O_2^- + 4H_3O^+ = 2S_4O_6^{2-} + 2H_2O + C1^-$), and the gelling of sodium alginate (which produces a gel of alginic acid) in the excess of H_3O^+ ions, as initiated by a reaction between $Na_2S_2O_3$ and $NaClO_2$.[8, 9, 10, 11] An excess of chlorite (ClO_2^-) in a high pH solution causes "supercatalytic" behavior in chlorite-thiosulfate ($S_2O_3^{2-}$) reactions, as is seen in the autocatalytic production of H_3O^+ ions in the presence of UV-activated alginic acid.[12, 13] At a high enough pH, this catalytic behavior rapidly surpasses the linear consumption of ions, creating a threshold phenomenon that results in the gelling of sodium alginate at a particular acidity.[8] However, the authors' chemical model is based upon the assumption that mimicking the themes and kinetics of hemostasis is sufficient for modeling it. Furthermore, current research indicates that platelets, not chemical inhibitors, block tissue factor production, suggesting that hemostasis dynamics are characterized by more than a series of initiation, inhibition, and precipitation reactions.[14, 15, 16]

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Introduction to Circular Dichroism

Circular dichroism (CD) is a method of obtaining molecular structure from the differential absorption of right- and left-handed circularly polarized light.[17,18] CD can resolve the secondary and tertiary structures of protein as well as of RNA, and is particularly useful in determining conformational change of molecules, as CD signal changes as the average molecular population fluctuates between alpha-helix, beta-sheet, and random coil conformations.[19, 20] The absorption of light is translated into molecular structure visualization through the computerized algorithms CONTIN, SELCON, or CDSSTR.[20]

This ability to see the composition of the average molecular population (alpha-helix, beta-sheet, and coil conformations) also leads to circular dichroism's limitations, as a global view cannot give specific information about individual molecules. Also, CD relies on reference protein sets for interpretations of spectrometric data, and so accurate interpretations of structural conformation are limited by the diversity of the reference sets' structures.[20] As these reference sets are often verified through X-ray crystallography, CD in turn depends on previous crystallographic resolution of specific structures. One other limitation of using CD to characterize molecular structure is the light wavelength specificity necessary for visualizing a conformation, where macromolecules can be visualized in the near-UV wavelength range while smaller molecules such as RNA, can only be visualized at the far- to vacuum-UV wavelength range (~160nm).[21, 22] Accordingly, while CD can determine slight changes in secondary and tertiary structure of protein at the near-UV CD spectrum range, changes in RNA conformation would be better determined using small-angle X-ray scattering (SAXS) or cryo-electron microscopy (cryo-EM).[23, 24]

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