

## The cytoplasm's gel-like behavior contributes to human cell osmosensitivity

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Current models of cell volume regulation are almost exclusively based on the concept of free diffusion in the aqueous cytoplasm surrounded by a semi-permeable membrane, which controls solute fluxes with an array of channels and pumps. However, the cytoplasm is a matrix of large, crosslinked, charged polymers, making it a viscous, gel-like structure that restricts diffusion, retains water and, like non-living hydrogels, may have distinct swelling properties. The gel-like nature of the cytoplasm has not been fully documented for mammalian cells, and its impact on cellular processes, such as volume-sensing, remains unclear. Here, we demonstrate that gentle perforation of the surface membrane with digitonin preserves the gel-like character of the cytoplasm, enabling us to examine its biophysical properties at the single-cell level. Volume changes of intact and permeabilized, substrate-attached human epithelial cells were investigated by a light microscopy, 3D imaging technique developed in our laboratory. Upon membrane permeabilization, the cells swelled 2.2-fold due to the Donnan effect. In response to subsequent external osmolarity changes, permeabilized cells behaved like a highly-sensitive osmometer showing reversible volume alterations that were several-fold larger than those in intact cells, indicating the cytoplasm's high water-binding capacity. Binding or release of large amounts of water may allow rapid modulation of local fluidity, macromolecular crowding and activity of the intracellular environment, consistent with the cytoplasm's function as osmosensor. Permeabilized cell volume also displays bell-shaped pH-dependency, with a maximum at pH 7.4, similar to that observed in intact cells. Isoosmotic replacement of  $K^+$  by  $Mg^{2+}$  resulted in significant shrinkage of permeabilized cells, likely due to increased charge screening and ionic crosslinking effects. Our study demonstrates that the cytoplasm of single human cells behaves like a hydrogel, and its swelling properties contribute to intact cell volume responses. These findings are of fundamental importance in our understanding of cell osmosensitivity and volume regulatory processes.