Study suggests physical cause for cell death in dry preservation

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A new finding in experiments studying the dry preservation of living cells—a potentially revolutionary alternative to cryopreservation - has defined a clear limit where continuing dehydration kills cells. The data, combined with molecular dynamics simulations, provides insight into an important processing factor that has limited recent attempts at dry preservation.

"What we have done is identified what appears to be a materials constraint in our method of dry preservation. I think this new understanding suggests some interesting avenues to pursue in developing a successful process," said Gloria Elliott, Professor of Mechanical Engineering at the University of North Carolina at Charlotte, one of the study's authors.

The findings, reported in the July 8 issue of *Scientific Reports*, analyzes changes in the molecular arrangements of trehalose (a sugar) and water molecules during a typical dehydration process that they use to immobilize cells in a stable trehalose glass for long-term storage.

The dry preservation of cells is a potentially revolutionary biotechnology that would offer some key advantages over current cryopreservation methods that require maintenance at extremely low temperatures. Because dry preservation might allow the maintenance of material at normal ambient temperatures, dry preserved cells would be more cost effective to store and easier to transport and thus might allow far more material to be banked and available for use.

The preservation method that Elliot's research team is investigating involves suspending cells in a dilute trehalose solution, and then concentrating it by removing the water with a gentle microwave-assisted heating process so that a trehalose glass forms, immobilizing biological molecules, similar to freezing. The technique is suggested by various organisms in nature, such as brine shrimp and tardigrades ("water bears"), that synthesize sugars in response to environmental stress and have been known to survive stasis for many years in a desiccated state.

"People have studied these examples from nature and have discovered that they produce large amounts of sugars, specifically trehalose," Elliott noted. "Over the last couple of decades we've been trying to learn how they do that and mimic it to preserve mammalian cells."

The current study involves an attempt to dry preserve T-cells. The method involves using microwave energy to speed the removal of water molecules from cellular cytoplasm, avoiding crystallization of trehalose, which would cause damage to membranes and other cellular structures.

"We are increasing the viscosity of the solution and decreasing molecular mobility without inducing trehalose crystallization," Elliott said. "But that doesn't mean that there isn't anything going on molecularly. There is a hydrogen-bonded trehalose network developing which leads to the solid-like character, which contributes to the high glass-transition temperature of the matrix."

But, researchers have found, dehydration can continue only to a certain point - when the ratio of water mass to the total mass of other molecules approached 0.1, a cascade of other effects appear and all cells start dying.

"What we saw with this paper is that there is a fairly well defined limit where things change substantially during dehydration," Elliott noted.

"When we try to dehydrate nucleated cells with membranes and preserve whole cell functionality - we seem to hit a wall. We can dry, dry, dry to increasingly lower moisture contents with good viability and then we hit a particular moisture content at which everything dies. There is a very rapid fall-off."

The exact physical process that kills the cells was not possible to detect, but the team noted a correlation with a process that was observed to occur at the same point in molecular simulations.

"In molecular simulations, you see trehalose clusters form during dehydration. What was insightful here was that we saw the moisture content level at which we lose cell viability coincide with when all these small clusters of trehalose hook together to become one big network of trehalose molecules," she said.

"That seems to suggest a mechanical force on the cells. If all the molecules form one network, one big cluster, then the cells are going to be trapped in that rigidified network."

Given the fragility of cell structures, especially thin cellular membranes, the sudden transition to rigidity of the unified trehalose network may be fatal to the cells, the researchers hypothesize. "We may be squeezing the cells or mechanically shearing them when that final clustering event happens," Elliott said.

"This gives us a new working hypothesis for the nature of cell injury during dry preservation - that during drying processing of cells, there is a critical moisture content at which the network of sugar molecules rigidifies and can induce physical injury to cells," she said.

"It's a hypothesis we can test, and we have some interesting ideas regarding things we can do to solve the problem if the network's rigidity

is the problem."

More information: Lindong Weng et al, Effects of Water on Structure and Dynamics of Trehalose Glasses at Low Water Contents and its Relationship to Preservation Outcomes, *Scientific Reports* (2016). DOI: 10.1038/srep28795

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