**Theoretical Framework: Cryoprotection, Tissue-Tek Emmbedding and Sectioning**

**Cryoprotection**

The objective of cryopreservation is to uphold cellular viability and functionality at low temperatures. Cold temperatures elongate biological processes by decelerating biochemical reactions. However, these low temperatures can induce changes in the cellular properties of the preserved tissue.

The majority of detrimental chemical events associated with freezing stem from the osmotic characteristics of cells. Cells comprise water, organelles, dissolved salts, sugars, proteins, and lipids, enclosed by a semi-permeable membrane. Water tends to migrate through the cell membrane to maintain an equilibrium concentration of solutes both inside and outside the cell, thus establishing its osmolarity. Therefore, osmolarity stands as a critical parameter to consider for cryopreservation.

Cell membranes undergo the most significant damage during freezing processes due to the loss of fluidity in their lipid components. The transition from fluid to solid lipids occurs within a temperature range of 10°C to 16°C, leading to altered membrane functions and increased susceptibility at these temperatures.

To mitigate such damages, cryoprotectants come into play. These are water-soluble substances with low toxicity that lower the eutectic point (the temperature at which a solution can crystallize) of a given solution. This reduction implies that a specific concentration of solutes will be attained at a lower temperature, inducing cellular dehydration and minimizing the osmotic gradient. Biochemically, cryoprotectants can be classified into three types: alcohols, sugars (e.g., glucose, lactose, sucrose, saccharose), and dimethyl sulfoxide. They can also be categorized as either penetrating or non-penetrating agents based on cellular permeability. In our context, non-penetrating cryoprotectants like sucrose are of interest. This macromolecule acts as an osmotic force, driving cellular dehydration by elevating the osmolarity of the medium, prompting initial dehydration followed by subsequent rehydration.

In essence, to freeze cells for preservation, three steps are imperative: 1) subject the cells to a solution containing a cryoprotective agent (e.g., sucrose) to alter the medium's osmolarity; 2) regulate the cell's osmolarity to achieve iso-osmolarity with the external medium, thereby reducing the cells' eutectic point; 3) lower the temperature without surpassing the eutectic point (averting crystallization and consequent cellular damage) to maintain cellular integrity.

To attain cryoprotection, a 30% sucrose solution is utilized, into which rat brains are immersed.

Initially, due to the solution's density, the brain will float. Over time, the tissue will regulate its osmolarity, causing it to sink. The sinking of the brain indicates the completion of cryoprotection, rendering it ready for inclusion in Tissue-Tek. \*The estimated duration for this process is 48 hours.

**Embedding in Tissue-Tek**

After cryoprotection, the brain can be embedded in a Tissue-Tek solution for future cryopreservation. This allows the brain to be stored at -80°C for around 1 year.

Creating aluminum molds that match the brain's shape is advisable. This enhances visualization of the brain structure after freezing, making it easier to section in the cryostat.

**Sectioning**

When we're set to start sectioning the brain, we place it directly into the cryostat and secure it onto the chuck using Tissue-Tek. After mounting, we wait a few minutes for the tissue's temperature to adjust to the cryostat (BR\_IHCGuide).

Next, we proceed to cut the tissue into 30 μm thick sections. These slices are then individually placed into a box with wells.

Following this procedure, we utilize either an antifreeze solution or PBS, depending on how long they will be worked on.