Cryoprinting Hydrogels Outreach Activity

Nivedita Chandra Bose

Introduction

Three-dimensional bioprinting is a key technology for creating constructs using biocompatible materials like hydrogels and cells. Hydrogels, 3D networks of soft, water-absorbent polymers, mimic the natural extracellular matrix, making them ideal for tissue engineering. A notable innovation in this field is cryoprinting, which deposits materials layer by layer onto a cryoplate at temperatures below 0°C. This technique is effective for printing low-viscosity materials, cross-linking hydrogels to increase their stiffness, and has potential applications in tissue engineering, bioelectronics, and regenerative medicine.

In this educational outreach event, students will learn about cryoprinting and cross-linking hydrogels through an introductory presentation and an interactive activity. They will experience the cryoprinting process by manually extruding the hydrogel onto the cryoplate in the cryoprinting platform setup, observe the cross-linking of hydrogels, and compare the structural integrity and material properties of cross-linked versus non-cross-linked structures.

Materials

Material	Quantity
Cryoprinting platform setup (Peltier element cryoplate, aluminium cooling block, tubes, bucket, ice, water, water pump, voltage supply)	1/class
4% alginate with red food colouring	2ml /student
1ml plastic syringe	1/student
140mM Calcium Chloride solution	1ml/student
Tissue Paper	2 sheets/student
Nitrile gloves	2/student
18 GA Needle	2/class

Activity Overview

An initial presentation will provide background information on 3D bioprinting and cryoprinting, explaining the importance of hydrogels. It will highlight the properties of alginate- hydrogels (derived from brown seaweed) and the changes that occur when cross-linking them with calcium chloride. Students will be introduced to the cryoprinting platform and its components and shown examples of cryoprinted structures created in the lab using a 3D extrusion-based printer.

Students will be given syringes filled with alginate-hydrogel dyed with red food colouring. They will be asked to manually extrude shapes onto the tissue to try to create stable structures. They should observe that the structures do not remain intact like in Figure 1 below.

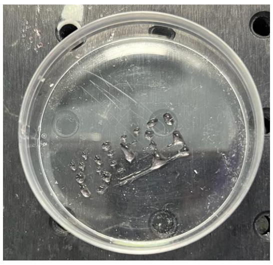

Figure 1: Alginate-hydrogel grid not holding its shape.

In groups of 2-3, students will manually extrude alginate hydrogel onto a cryoplate using the cryoprinting platform setup shown in Figure 2, and cross-link it with calcium chloride as depicted in Figure 3. They will observe how cold the cryoplate gets and how the extruded structure is preserved as the material freezes upon contact with the plate. They will also see how cross-linking the alginate hydrogel with calcium chloride when frozen, and then letting it thaw, makes it stiffer and gives it a jelly-like consistency. The students will discuss how the stiffness can be adjusted to match the stiffness of tissues in different organs and structures. The process of fabricating constructs and cross-linking them through cryoprinting will be explained. Discussions will cover how robotic arms and 3D printers can assist in the rapid and precise manufacturing of intricate structures using various materials for diverse applications.

Figure 2: Cryoprinting platform.

Figure 3: Cryoprinted alginate-hydrogel grid, crosslinked with calcium chloride and thawed.

Risk Assessment

This activity is generally safe. The part where students manually extrude alginate hydrogel from the syringe can be done without supervision since there is no needle involved. Alginate hydrogel is non-toxic and biocompatible, making it safe to touch. However, gloves are recommended to minimise mess and avoid ingestion.

When manually extruding alginate hydrogel and cross-linking on the cryoplate using the cryoprinting platform, supervision is required due to the cryoplate's very low temperature, which can reach -20°C. To prevent cold burns, students should wear gloves and be instructed not to touch the cryoplate for too long. Safety measures should be clearly communicated to ensure a secure, enjoyable, and educational experience for all students.