Preparation of samples for scanning electron microscopy

Product: BIOMIMESYS® 3D Cell Culture Hydroscaffold™

Format: 96 well-plate black/clear & 96 well-plate clear

Context: BIOMIMESYS® is a unique groundbreaking 3D cell culture technology which associates the behavior of a solid scaffold and of a hydrogel. It provides a cell culture microenvironment reproducing all aspects of human tissues, including matrix architecture, cellular organization, cell-cell and cell-matrix interactions. Depending on the organ, extracellular matrix components and their proportions may vary, allowing a more or less dense and compact cellular environment (different Elastic Moduli, porosities). BIOMIMESYS® matrices are made of Hyaluronic Acid (HA), the main glycosaminoglycan (GAG) of the ECM, collagens and adhesion proteins. Our patented manufacturing process allows to preserve the natural properties of HA and therefore synthesizing proprietary Hydroscaffold™.

Scanning electron microscopy allows you to see precise structures at a very low scale. You can prepare hydroscaffolds™ in order to see the chains or in order to see where the spheroids are located within the hydroscaffold and how they interact with the chains.

1. Protocol for optimal conservation of the *hydroscaffold* structure (may alter the spheroids and cell structure)

- Place the *hydroscaffolds™* using curved end-tweezers into a microtube containing a cold solution of glutaraldehyde 2.5%.
- Fixing 2 hours at 4°C
- Wash 3 times with distilled or ultraclean water
- Close the microtube containing the hydroscaffold and the distilled/ultraclean water and pierce the cap with a needle (to avoid overpressure into the tube when immersed in liquid nitrogen)
- Immerse the microtube into liquid nitrogen
- Place quickly the microtube in a lyophilizer and lyophilized (from overnight to 24 hours)
- Place the sample on a special rack for observation by scanning electron microscopy (carbon tape) and metallize prior to observation

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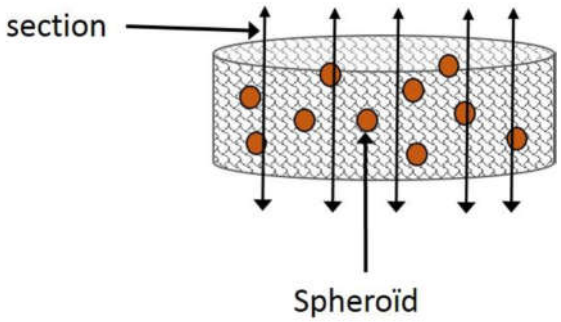
2. Protocol for optimal conservation of the cell aggregates and cells structure (sample preparation alters the structure of the hydroscaffold)

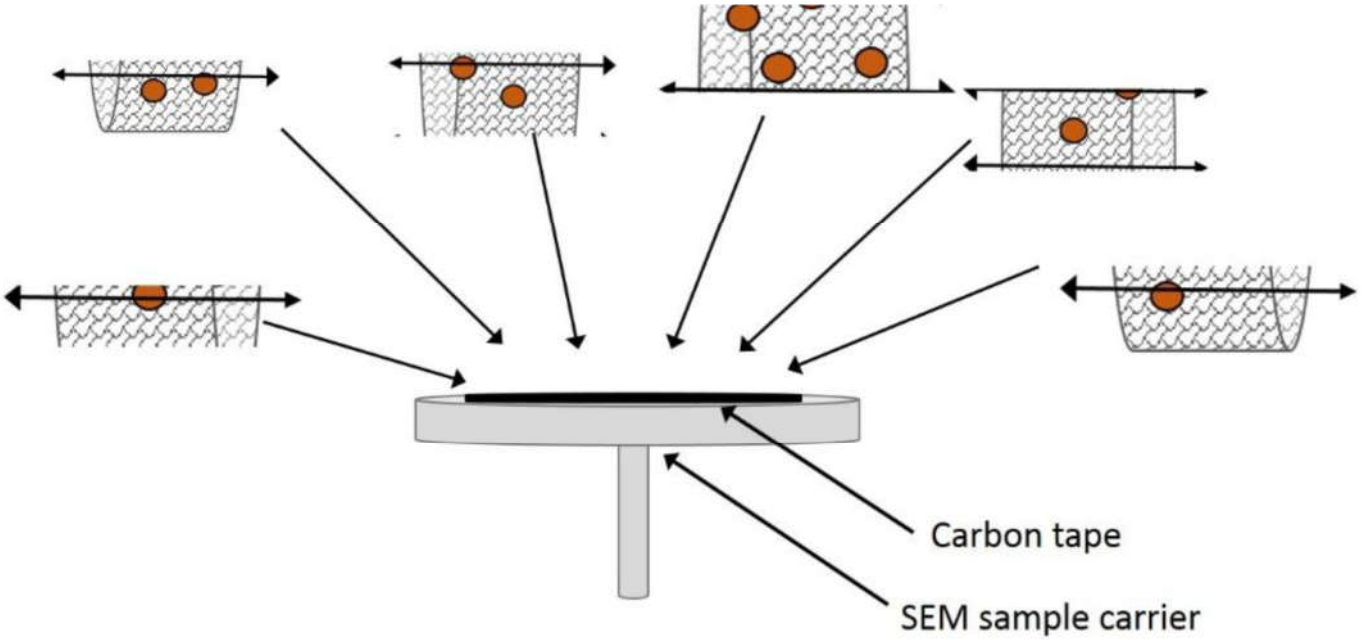
- Place the *hydroscaffolds*™ using curved end-tweezers into a microtube containing cold 2.5% glutaraldehyde (stored at 4°C)
- Fixing 2 hours at 4°C
- Wash twice with distilled or ultraclean water
- Dehydrate gradually the samples by successive baths in increasing concentrations of ethanol, at room temperature:

1. 30% ethanol, 30 minutes
2. 50% ethanol, 30 minutes
3. 70% ethanol, 30 minutes
4. 80% ethanol, 30 minutes
5. 90% ethanol, 30 minutes
6. 95% ethanol, 30 minutes
7. Absolute ethanol, 3x30 minutes

- Place the samples under vertical flow hood overnight to achieve ethanol evaporation
- As a result of the dehydration process, the *hydroscaffold* is shrunk, as well as most cellular aggregates who had grown inside the *hydroscaffolds*™. The SEM observation will likely require to cut the *hydroscaffold*, as shown on the scheme below:



- Put down the sample pieces on the carbon tape and on the SEM sample carrier, as described below:



- Proceed to the metallization

- Make your sample observations

Contact Information

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