Preparation of samples for scanning electron microscopy (SEM)

BIOMIMESYS® Hydroscaffold is compatible with scanning electron microscopy (SEM) analyses to investigate fine structural features of the hydroscaffold and cells grown inside. Two protocols can be carry out to have an optimal conservation of the hydroscaffold (part 1) or of the cells (part 2).

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

1. Place the hydroscaffold using fine forceps in a microtube containing a cold solution of glutaraldehyde 2.5%.
2. Fixing 2 hours at 4°C
3. 3 washes with distilled or ultraclean water
4. 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)
5. Then immerse the sample in the liquid nitrogen
6. Place quickly the sample in a lyophilizer and lyophilized (from overnight to 24 hours)
7. Then place the sample on a special rack for observation by scanning electron microscopy (carbon tape) and metallize prior to observation

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

1. Handle the hydroscaffold with fine forceps and place it in a microtube containing cold 2.5% glutaraldehyde (stored at 4°C)
2. Fixing 2 hours at 4°C
3. 2 washes with distilled or ultraclean water
4. Then dehydrate gradually the sample by successive baths in increasing concentrations of ethanol, at room temperature:

a. 30% ethanol, 30 minutes

b. 50% ethanol, 30 minutes 2/2

c. 70% ethanol, 30 minutes

d. 80% Ethanol 30 minutes

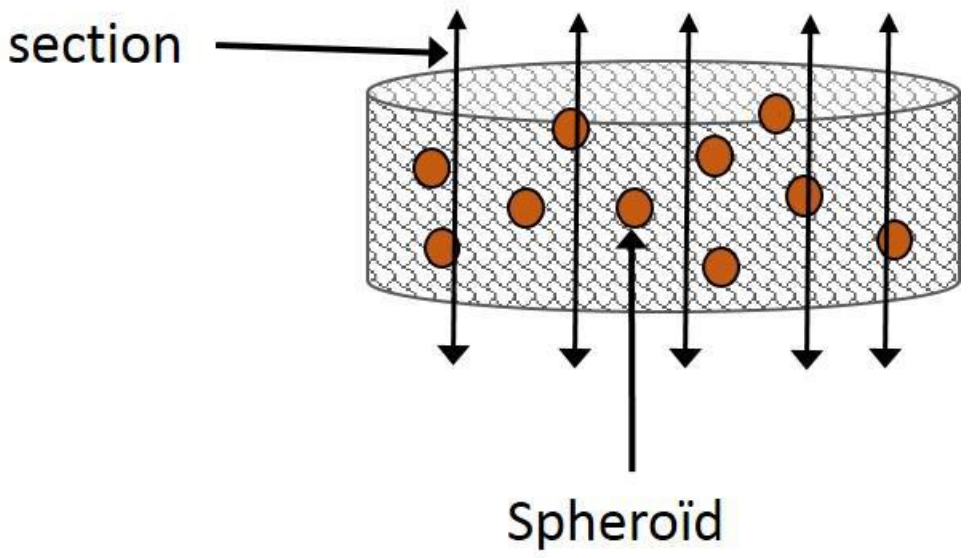
e. 90% Ethanol 30 minutes

f. 95% Ethanol, 30 minutes

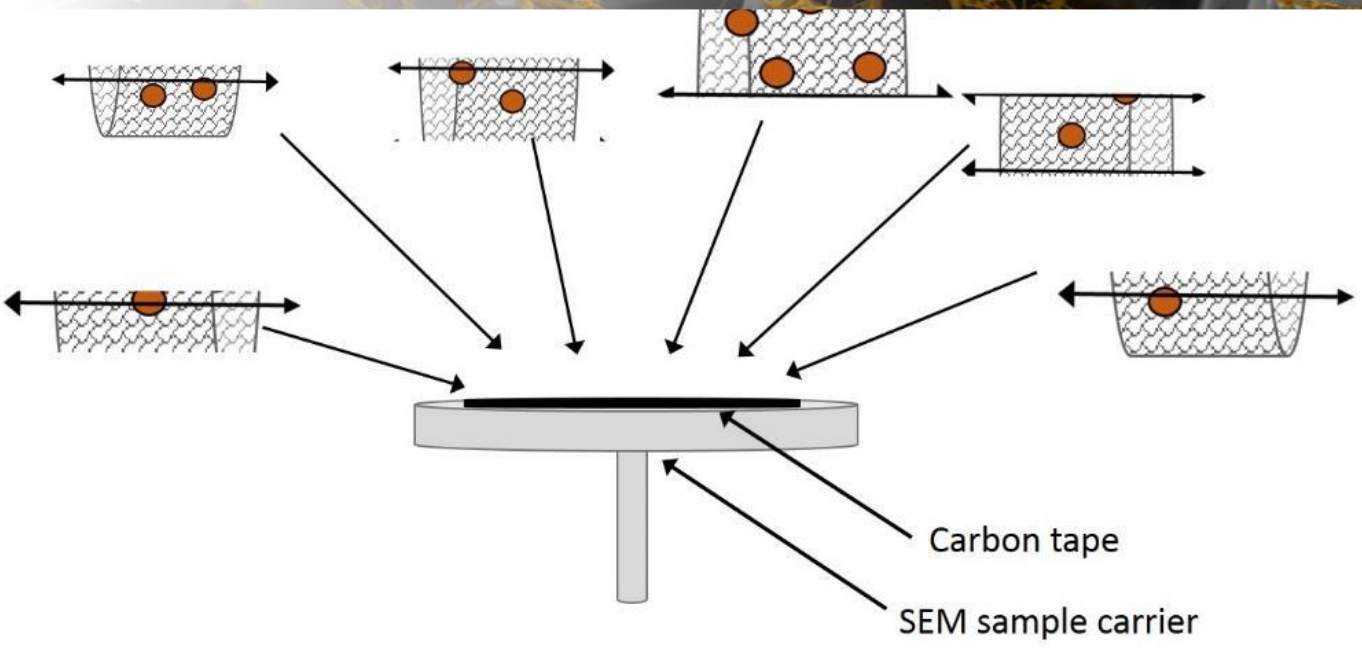
g. Absolute ethanol, 3x30 minutes

5. Place the sample under vertical flow hood overnight to achieve ethanol evaporation

6. As a result of the dehydration process, the hydroscaffold is shrunk, as well as most cellular aggregates who had grown inside the hydroscaffold. The SEM observation will likely require to cut the hydroscaffold, as shown on the scheme below: Protocol for optimal conservation of the cell aggregates and cells structure (sample preparation alters the structure of the hydroscaffold)



7. Then put down the sample pieces on the carbon tape and on the SEM sample carrier, as described below:



8. Then proceed to the metallization

9. Make your sample observations

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