No. 8: The origin of cells: enzymatic disintegration of tissue

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Our own cells Susi Pensa and Adi Herence have been living for quite some time happily alongside each other in the cell culture incubator.

Up to now they have thought little about their own origins.

Susi's figure is somewhat rotund and she lives floating in cell culture medium as a so-called suspension cell. Before she was promoted to a cell line, she had lived in the blood of an animal organism and simply allowed herself to be taken wherever the blood stream took her.

Looking at her own reflection in the silver incubator wall, Susi started wondering about the way Adi looked. He had a much broader but flatter constitution. And he stood quite firmly on the floor of the cell culture bottle with his lamellipodian feet. He doesn't even fall over if the bottle is moved abruptly.

"Adi, you look so different from the way I do, where do you come from?", asks Susi. "Well", answers Adi, "my origin is quite different from yours. Where I come from, cells live in a firm type of tissue. Each cell has its own spot between neighbouring cells. On our surface we have so-called cell adhesion proteins. These proteins help us to cling together. Ions of valency two such as Ca^{2+} provide additional stability to the molecules. The interstitial spaces are upholstered with matrix molecules and this makes it quite cosy for us".

Susi is impressed. "But how did you manage to leave your cell community in order to live in a cell culture?", asks Susi and continues: "I can be isolated quite simply through blood withdrawal".

"Yes, in our case cell biologist have quite a job", answers Adi. "When they tried to separate us mechanically, most of us were fatally injured". Adi then continued: "But once the researchers realised that it was the protein molecules that held us together, the method became clear: Now, these proteins are gently removed from our surfaces using proteases such as trypsin (these are enzymes that cleave proteins). This process causes us to lose firm contact with our neighbouring cells. Substances are then added that bind the ions; we are then subsequently suspended and this causes the tissue to disrupt into individual cells. This process is called enzymatic tissue disintegration.

If this process is carried out gently and carefully, we can survive. And as a reward, we can expect to continue our lives in a cell culture which is nice and warm and which contains medium and an excess of serum".

Protocol for the isolation of cells from the brain tissue of new-born rats using enzymatic disintegration

Preparation:

- PBS - phosphate buffer, Ca^{2+} and Mg^{2+}-free, 37 °C
- Trypsin - 2.5%, 4 °C
- DNAse (deoxyribonuclease) - 500 mg/ml, 4 °C
- Small sterile scissors
- Small glass funnel fitted with nylon gauze and sterilised in an autoclave (the gauze is fixed to the funnel with autoclave band, the

whole packed in aluminium foil); pore size of the gauze: 30 µm
- Bacteriological Petri dishes
- Tubes

Procedure:

The tissue should be removed under sterile conditions, freed from adhering blood by immersing twice in buffer and transferred to a Petri dish along with 5 ml of phosphate buffer (without Ca^{2+}, Mg^{2+}).

The tissue should be cut into small pieces using scissors. The smaller the pieces, the larger the surface available for the enzyme and hence the more efficient the enzymes can digest the cell surface molecules.

Enzymatic tissue disintegration is started by adding the enzyme: 500 µl of a 2.5% trypsin solution should be pipetted into the Petri dish and rapidly mixed with buffer by shaking the dish (final concentration of enzyme is thus 0.25%).

The enzymatic treatment of the tissue should be carried out on a heating plate pre-heated to 30 °C or in an incubator over a period of 5 minutes.

500 µl DNAse should be added in order to digest any DNA released from damaged cells. Free DNA tends to cause the cells to adhere and become a slimy mass.

The cells that are now positioned freely in the tissue community should be transferred to the buffer by pipetting and re-pipetting five times using a 10 ml pipette (mechanical treatment using sheer force). Formation of foam is frequently unavoidable; this should be reduced to a minimum in order to protect the cells.

The disintegration process can be checked by inversion microscoping.

Both enzymatic and mechanical treatment should then be repeated. There is however no need to repeat the DNAse treatment in most cases.
If the Petri dishes are placed on an inclined surface, the remaining pieces of tissue will sediment. The released cells are then located in the supernatant buffer and can be carefully removed and transferred to a tube.

The cells should then be mixed with the same volume of the cell culture medium. The serum trypsin inhibitor in the serum stops enzymatic digestion completely.

The cell suspension should then be filtered through a nylon gauze with a defined pore diameter of 30 µm in order to separate any residual tissue aggregates.

An aliquot of the cell suspension should be mixed with trypane blue solution. The number of cells and the proportion of dead cells should be determined in a counting chamber.

N.B.: The red blood cells (erythrocytes), recognisable in the case of mammals by their small size and typical dimples, represent a large number of the isolated cells depending on the type of tissue being processed. These must not be counted as otherwise this can falsify the yield. Erythrocytes disintegrate within a few days by themselves in the cell culture.

The cells should be centrifuged and inoculated in a culture vessel in the desired cell density. The medium should be changed after 24 hours.

Modifications:

The proportion of dead cells should be under 15%. In cases of poor yield or low vitality of the cells, the standard protocol should be modified as follows:

The trypsin concentration can either be increased by a factor of 10 or reduced: In the case of embryonic tissue which is extremely loose and sensitive, 0.025% trypsin is adequate and also increases the vitality of the cells. Epithelial cells on the other hand require 2.5% trypsin (final concentration).

If the yield is still too low when using this procedure, 3 to 4 harvests can be combined. Once the released cells have been removed, the remaining pieces of tissue can then be treated enzymatically according to the protocol. Cell yield and vitality should be separately determined for each harvest.

Some cell adhesion and cell substrate molecules require divalent ions in order to aggregate. Complex enhancers such as EDTA in the disintegration solution can improve the separation of cells. Watersoluble di-sodium salt of EDTA is better known under the trade name Versen.

Trypsin has a gentle effect and is usually extremely effective. However, in the case of some tissues, the enzyme pronase produces better results. Pronase should also be used in a concentration of 0.25%. However, as it is not deactivated by serum, it must be removed by carefully washing (twice).

The tissue of adults contains a high proportion of collagen. In such cases the use of the enzyme collagenase is recommended. Collagenase should also be used as a 0.25% solution and can be incubated simultaneously with trypsin. N.B.: The cheaper collagenase preparations contain other enzymes as impurities; these do not interfere in most cases but they can affect cell vitality in certain cases. For this reason, it is worthwhile investing a little more money to purchase a purer product.

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