Prospective

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Organ Engineering Using Stem Cells

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Introduction

Convalescent specific substitution organs can be developed in a research facility. No downtime of years to unearth the suitable organ donor. Tens of thousands of patients pass away every year around the globe waiting for the right organ transplant which never becomes a reality. Organ donors are low in numbers and the perfect fit is even more difficult to find. Organ rejection rates are also high.

Take an organ for specimen in this case a heart from a cadaver. Preferably, harvested within 6-8 hours after demise to circumvent any putrefaction. Heart is only considered for illustrative purposes in this write-up. Similar technique can be replicated to construct various other complex or ganoids & organs such as kidneys, liver, pancreas, stomach, intestine etc. in a lab.

Steps

Step

Decellularize the retrieved heart using a detergent. Cells are fundamentally a bubble of oil. So this detergent/soap destroys the cells and washes away all cells, DNA, lipids, soluble proteins, sugar & debris. The heart is now stripped of all its indigenous cells yet holds on to its basic structure. Detergents such as DLS, SLS, and SELS etc. can be utilized. In this demonstration we will use SLES solution with 5% concentration in distilled water. That is 2.5 gms SLES into 500 ml of purified water.

Step

Then chemically sterilize the ECM with 70% ethanol solution CH3-CH2-OH or C2H5OH. Keep the ECM in it for a day. Ethanol kills the pathogens by disintegrating their membrane, lipid bilayer and denaturing their proteins, and is effective against most bacteria, fungi and viruses. For foiling remaining unwanted infections if any, the culture media of new cells will also contain antibiotics.

Step

Work in a thoroughly sterile laminar flow hood/cabinet. Spray the work surface area with alcohol. After Decellularization we obtain an ECM scaffold which is a spongy matrix of collagen, fibronectin, cellulose & ligament. All living cells of the organ have been washed away and their DNA eradicated. Cellulose sponge is left behind. The ECM scaffolding has a white ghostly appearance. Cell seeding now we can put any type of cells into this ECM Scaffold & cultivate them.

Step

Then this scaffold is submerged in culture media. Culture media nourishes the cells with all the nutrients mandatory for their growth. The culture media to be utilized is Dulbecco's Modified Eagle Medium-DMEM. Dulbecco's Modified Eagle Medium (DMEM) is a universally used basal medium for supporting the growth of numerous different mammalian cells.

Step

Then add a mixture of penicillin and streptomycin antibiotics which will eliminate

any infecting harmful bacteria. The antibiotics penicillin and streptomycin are used to fend off bacterial contamination of cell cultures due to their successful united action against gram-positive and gram-negative bacteria. The growing cells remain in the pink of health. Allow the scaffold to sit in DEMEM solution mixed with everything else for about 5 days.

Step

We were cultivating cardiac stem cells simultaneously. Induced Pluripotent Stem Cells (IPSCS) can differentiate to cardio myocytes in vitro. Endogenous Cardiac Stem Cells (ECSCS) are tissue-specific stem progenitor cells harboured within the adult mammalian heart can also differentiate into a cardio myocytes. Mesenchymal stem cells from adult human bone marrow can also differentiate into a cardio myocytes phenotype in vitro. Any of these 3 types of stem cells can be manipulated to differentiate into cardio myocytes.

Step

Stem cells are then compelled to differentiate into the original cardiac cells of the patient. To generate cultures of specific types of differentiated cells like cardio myocytes we may change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by forcing the expression of specific genes. We can also add growth factors and signalling molecules to the stem cell culture.

Step

Place the organ on a sterile plate. Using P1000 pipettes inject cell suspension solution into the scaffolds. Also gently pump the cell suspension solution through the vascular tube attached to the organ. Cells will settle into their tissue. Zero Contamination should be ensured. Try and distribute the cells evenly into the internal structures of the organs and not just the outside surface.

Testing and Observation

Immuno fluorescence staining is done on a sample to only stain the living cells and not the cellulose, dead cells and other debris. Cell staining is a very versatile technique and, if the antigen is highly localized, can detect as few as a thousand antigen molecules in a cell. All cell locations within the organ can be traced.

Objective and Conclusion

This operation is known as recellularization. We can extract a small tissue sample from the patient's heart tissue through a biopsy and isolate the adult cardiac stem cells Endogenous Cardiac Stem Cells (ECSCS). Grow these stem cells in a petri dish. These stem cells can be then grown and allowed to multiply into millions of stem cells in a bioreactor. These stem cells are then allowed to develop and differentiate into cardio myocytes or heart tissue cells.

Contradictions

We can also use embryonic cells found in the blastocysts or zygote as stem cells by fully replacing/editing the DNA of the existing cells with the patient DNA. The nucleus which contains the genetic material is transferred with the patient's cell nucleus. These cells are pluripotent. This will give birth to a patient specific stem cell line genetically identical to the donor's cells. This is known as somatic cell nuclear transfer similar principle as to therapeutic cloning. However, this procedure remains controversial as it results in the loss of the human embryo.