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Advanced In-Vitro Systems Mimicking the In-Vivo conditions

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Abstract: Our problem is that invitro and invivo systems are hard to compare due to the differences in their complexity, as invitro systems are very simple compared to invivo conditions. Where, there is only one cell type which is cultivated under two-dimensional conditions with an optimized culture medium. In contrast to invivo test where, there is always more than one cell type involved, and cells act in a three-dimensional environment in a dynamic fashion surrounded by different fluids. In case of Invitro tests this condition is considered pathological environment to which cells adapt and respond giving different response than that given to normal environment. Further research is conducted aiming to cross the gap between in vitro and in vivo tests.

Keywords: multiple cell types, cell aggregate, cell organoid and body fluid mimicry.

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1. From single to multiple cell types.

Different cell types are cultivated in the same culture, either completely mixed or separated via membranes. It is known that an in vitro mixture of progenitor cells in different states of differentiation leads to more significant results than single cell types. If a material is placed in a multi-cell-type environment, each cell interacts with another in a synergistic way which is crucial for tissue formation and mainly for tissue repair. Different cell types affect cell proliferation, state of differentiation, and functionality of other cells.

The selected cell-type composition should depend on the application of the implant material. For example, if the tested material will be used in a bony environment. Then, the main cell types should include mesenchymal progenitor cells, osteoblasts, osteoclasts, fibroblasts and endothelial cells. **[1]**

Mohammadi et al., 2019 conducted a study to investigate if natural / synthetic polymeric nanofibers (Gel/PLCL nanofiber) can probably promote the proliferation of coculture (adipose derived stem cell ADS/ human fibroblast cells HFC). Where ADSs and HFCs were mixed and seeded on well plates coated with Gel/PLCL nanofiber and non-coated plates. Cell morphology and the interaction between cells and Gel/PLCL nanofiber were evaluated by field emission SEM & fluorescent microscopy. Fluorescence viability staining for detecting live cells was done after incubation of seeded samples with cells for seven days. Then, the samples were incubated by Calcein AM that stains alive cells in green for 30 min at 37°C and washed with PBS. The green- stained samples were subjected to fluorescent microscopy with a 490 nm excitation filter and a 520 nm emission filter. **[2]**

It was found that, nanofibers exhibited proper structural properties in terms of stability in cell proliferation and toxicity analysis processes. Gel/PLCL nanofiber promoted the growth and the adhesion of HFCs. Moreover, co-culture of ADSs/HFCs on the Gel/PLCL nanofiber increased cellular adhesion and proliferation synergistically compared to non-coated plate.[2]

2. **From 2D to 3D**

In invitro tests, single layer of cells is seeded on top of the tested material is done to evaluate its biocompatibility. While in in vivo conditions, an implant of the tested material is placed within a tissue. Thus, tissues will contact the implant. It is well known that cells in a 3D environment behave differently from those in a 2D environment.

Cell reaggregates are prepared of a defined cell number of one of the key cell types of the tissue that after implantation will contact the implant. it can be seen as a kind of organoid, that is placed on the test material and assessed. Growing cells in this situation have the choice to grow out vertically or laterally on the material. So, after placing the material in contact with the organoid, the cell expansion on the surface of the implant is measured. Moreover, cell morphology of the grown cells is assessed.

The point that might affect the outcome is the difference in the oxygen tension gradient. in vivo, oxygen tension probably has the lowest tension at the implant surface, while in this in vitro system, the lowest oxygen tension is in the centre of the reaggregate. Oxygen tension gradients may greatly affect cell migration activity and cell proliferation rate. **[1]**

3. **Oxygen tension**

Control and adaptation of oxygen tension in cell culture is hardly ever considered even though it is a very important factor in how cells react. For example, hypoxia is a pathological condition for most stem cell types, it is a key factor for chondrocyte development and behaviour. In native tissue, chondrocytes are exposed to oxygen concentrations ranging from 1–5%, since the distance to the vessels supplying the synovial membrane is exceptionally large compared to other tissues .

An elegant approach to mimic the in vivo oxygen tension gradient as in extracellular matrix environment is to place a **collagen gel layer** with embedded cells on top of the test material. With this set-up, another important

in vivo aspect would be taken into consideration the dimensionality (3D instead of 2D). Collagen gels with embedded cells are currently used for various investigations including effects of matrix stiffness, mechanical load and tissue engineering. **[1]**

4. **From culture medium to body fluid mimicry**.

Culture medium contains a mixture of salts, amino acids, dilute quantities of serum proteins and specific growth factors. While, in in vivo conditions, the cell environment is crowded with proteins resulting in a reduction of diffusion and local high concentrations of released cell products. In this environment, nonspecific reactions play an important role. For example, it was found that collagen was formed by cells in in vivo conditions while absent in invitro conditions, this was solved by adding charged macromolecules to the cultured medium. Moreover, it was found that the addition of macromolecules promotes the differentiation of various cell types. So, by adding macromolecules the original microenvironment can be better mimicked. **[1]**

5. **From static to dynamic**.

The in vivo situation is characterized by the presence of microcirculation in the area of the implanted material. Dolder et al. reported that proliferation and differentiation towards osteoblasts of rat bone marrow stromal cells was increased in the presence of a fluid flow. [3]

All these are single attempts aiming to improve the invitro test system by applying at least one key issue of the in vivo conditions. However, combining different issues in one test system would certainly help better mimicing in vivo conditions and improves the test system.

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