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ABSTRACTS

Experimental Surgery

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1

THE FUTURE IN HUMAN CELL CULTURE MEDIA

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Human cells culture has become an essential tool for scientific researches. The most widely used medias for human cell cultures are supplemented with fetal bovine serum (FBS) which contains most of the factors required for in vitro cell proliferation and development. Few types of cells grow in the absence of FBS and when that happens it is just for a short time. Even when growth occurs, its rate is always better when serum is added to cell culture media. This supplement is obtained from blood extracted of fetuses removed, under aseptic conditions, from cows found to be pregnant at the slaughter. FBS quality varies between batches and contains lots of undefined compounds witch carries the risk of undesired protein or pathogens presence in cell culture. Because of these risks, there is a worldwide concern in developing alternatives using autochthonous proteins or chemically defined compounds. One of the advantages in using FBS is its almost universal growth supplement effect in most types of human and animal cells. Therefore, the use of FBS reduces time and effort in developing specific media formulation for each cell type. Some of the disadvantages are: High cost; variable composition; presence of harmful toxins; contamination of human cell cultures by bovine contaminants; immune-reaction to foreign proteins; difficulty of the downstream purification of proteins products; ethical issue about animal suffering during blood extraction from the heart of the fetus still alive and without anaesthesia. Although there is no breakthrough discovery of a new alternative of cell culture media, with all development and the range of serum free products already on the market, it seems to be matter of time for the elimination of serum use in cell culture.

2

STUDY OF FIBROBLASTS PROLIFERATION IN HUMAN SERUM

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Cell culture is becoming an important tool in biotechnological researches. Despite of this, still today, those cells are universally supplemented by Fetal Calf Serum (FCS), a complex mixture that contains necessary growth factor's requirement to maintain the cell function and proliferation. This form of supplementation is far from ideal, once their qualities varies from batch to batch, its composition is not completely known, it may contain virus and prion contamination and may also cause immunologic complications. Ethical question concerning the obtainment and economics factors are also of great relevance. Due to those details, there is a world wide effort to find alternatives to the use of xenobiotic elements in cell cultures. In this study we assayed human serum as replacement for FCS in human fibroblasts culture. Human serum was obtained from blood of healthy 10 volunteers, submitted to serological evaluation. Fibroblasts were obtained from samples of healthy skin obtained from Tissue Bank of Hospital das Clinicas donated for research purpose. Fibroblasts were cultivated in multiwell plates with 10% FCS or 10% human serum DMEM. After 24, 48, 72 and 96 hours cells were counted in hemocytometric chamber. Results were expressed in mean ± standard deviation to obtain the proliferation cell curve. There was no statistical difference between both proliferation curves. Human serum fully supported growth and proliferation of human fibroblasts, showing its high potential as substitute for FCS in cell culture.

3

NEOSKIN DEVELOPMENT IN THE FETUS WITH THE USE OF A THREE LAYER GRAFT: NA ANIMAL MODEL FOR IN UTERO CLOSURE OF LARGE SKIN DEFECTS

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We have shown that a cellulose patch induces the formation of a neo-dura mater when placed above the defect spina bifida, in uterus, in the ovine model. "Large" meningomyeloceles are defects in which a tension-free skin closure is not possible by simple undermining of the skin edges. Thus, the treatment using only our previous cellulose graft is ineffective. Our purpose was to assess an alternative grafting technique for correction of a meningomyelocele-like defect in the ovine fetus. Four fetuses underwent a large (4 x 3 cm) full-thickness skin defect over the lumbar region at 105 days' gestation (term = 140 days). The fetus was returned to uterus and the membranes and myometrium were closed. The skin defect was repaired endoscopically under gasless fetoscopy. The biosynthetic cellulose was placed over the defect and the bilaminar artificial skin was placed over the cellulose. The skin was partially reapproximated with a continuous nylon suture. The uterus and abdomen were closed. At 120, 130 (n=2) and 135 days of gestation ewes were euthanized, fetuses were harvested and the lesion zones obtained. The structural organization of collagenous fibers was studied with the aid of the Picrosirius-polarization method. Cellular migration, neovascularisation, and remodelling/maturation of extracellular matrix were observed in the repair zone. New dermal components grew towards the top of the artificial skin and a newly formed epidermis grew beneath the new dermis layer, starting from both edges of the former epidermis. The entire gap of the defect was closed by new skin in specimens remaining until term. None of the specimens showed neoepidermis growing directly over the artificial skin dermal matrix; instead, the artificial matrix served as a scaffold over which the new dermal component could grow. These findings suggest that a neoskin can develop in the fetus with the aid of the 3-layer graft and that the fetus is able to reepithelialise even large skin defects.

4

EFFECT OF METILPREDNISOLONE IN PULMONARY PERIVASCULAR EDEMA, INFLAMMATORY INFILTRATE AND IMUNOHISTOCHIMIC EXPRESSION OF TGF-BETA AND VEGF IN THE REMAINING LUNG OF RATS SUBMITTED TO LEFT PNEUMONECTOMY.

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Objectives Pneumonectomy is associated to high rates of morbimortality and postpneumonectomy pulmonary edema is one of the leading