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CONTENTS

Cold atmospheric plasma (CAP) is has been considered as a safe treatment option for cancer therapy. Cancer cell viability is known to be inhibited by generation of reactive oxygen species (ROS) by CAP treatment and then induces apoptosis of cancer cells.
Photodynamic therapy (PDT) is also regarded as an safe and effective treatment option for cancer patient.
Photosensitizer such as chlorin e6 (Ce6) produces excessive ROS in cancer cells and this induces apoptotic death of cancer cells. The aim of this study is to study synergistic effect of PDT and CAP treatment against gastrointestinal cancer cells. HuCC-T1 human cholangiocarcinoma cells and CT26 colon cancer cells were maintained in RPMI1640 medium. cancer cells (1×10^4 cells/well) were exposed to various concentration of Ce6 and irradiated at 664nm light (2J/cm2) with or without CAP (60s). This was incubated for 1 day and viability was measured with MTT assay. ROS generation was investigated by DCFH-DA assay.
PDT treatment using Ce6 inhibited viability of CT26 cells and HuCC-T1 cells dose-dependently. Viability of cancer cells was properly decreased at higher than 1microgram/ml Ce6 concentration. Viability of cells was significantly inhibited when CAP was simultaneously treated to this cells. Futhermore, combination of Ce6 and CAP treatment also significantly increase ROS generation in cells.

Contribution ID: 730
15. Biomaterials, Cellular and Tissue Engineering, Artificial Organs
15.07. Current advances in stem cell biology

Cryopreservation of stem cells within intact alginate microspheres

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The possibility of long-term storage of clinically relevant cells combined with daily availability of genetically stable materials gives cryopreservation a high potential in modern cell therapeutics. Although cryopreservation of alginate-encapsulated cells is very promising for further clinical application, there is still no optimal cryopreservation protocol for freezing and thawing of cells within intact and mechanically stable alginate microspheres. In this work, a range of parameters such as alginate crosslinking time, concentration of dimethyl sulfoxide, its loading time and cooling rate were analysed. The structural integrity was evaluated upon freeze-thaw cycles using an Axio Imager M1m microscope with Linkam cryostage to identify an optimal combination. Verification of optimal cryopreservation protocol yielding intact capsules has been performed using multipotent stromal cells (MSCs) derived from the common marmoset (Callithrix jacchus). Freezing of alginate-encapsulated cells has been conducted using a controlled-rate freezer Planer Kryo 560-16. Analysis of cell viability after thawing and subsequent culture for 24 h (recultivation) has been performed using live-dead Calcein AM / Ethidium homodimer assay following image analysis using ImageJ and MicroVision software. Among the optimal conditions, the following ones yielded the highest cell viability of 60% after thawing: crosslinking time 15 min, 10% (v/v) of dimethyl sulfoxide, incubation time at 4°C of 45 min and a cooling rate of 2.5 K/min. Moreover, considering 75% of viable cells before cryopreservation, the viability analysis yielded 83% of encapsulated cells, which survived the cryopreservation process. The results of this study are the first report of cryopreservation of alginate-encapsulated cells within intact structure after thawing thus having a high potential for clinical application of mechanically stable alginate capsules for efficient treatment of rare diseases.
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15. Biomaterials, Cellular and Tissue Engineering, Artificial Organs
15.07. Current advances in stem cell biology

Modern semiautomatic setup for testing cell migration with impact to therapy of myocardial infarction

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Ischemic heart disease followed with myocardial infarction (MI) is one of the main cause of morbidity and mortality. Cardioprotection after MI is based on traditional surgery and pharmaceutical applications, and also on modern strategy to affect the leucocytes and progenitor/stem cell migration.
Modern therapeutical strategy based on activation of progenitor/stem cell migration or reduction of inflammatory cell migration in MI regions was proposed in last 15 years as a results of physiological observation on experimental animals. Published data from direct measurements of cells migration and evaluation of migration speed in different conditions are limited. We present a universal setup, which can be used for testing of the cell migration in cardiac or cardiac-like tissue. The setup brings a possibility of microenvironment setting and time-lapse experiments on cell migration.
The experimental setup was built on the platform of Leica TCS confocal microscope. The micro-chamber on microscope can adopt 2D or 3D substrate, which mimic various properties of the native cardiac tissue and distribution of chemoattractant. The micro-chamber was aseptic and connected to a temperature regulator, gas detectors and gas reservoirs (O2 and CO2). The cells tested for their migration potential were injected to some starting point on the substrate. The microscope allowed imaging of cells in micrometer-resolution every 2 minutes. Our software tools provided precise 2D and 3D tracking of moving cells and data export for statistical analysis. Mesenchymal stromal cells (MSC) were used for our experiments. Results show that geometry of tissue and gradient of chemoattractant had significant influence on direction of cell migration. Setting of hypoxic environment modified speed of cell migration.
In future, the setup will be rearranged to fully-automatic preclinical screening tool, which could be used for examination patient´s MSC and leucocytes.

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15. Biomaterials, Cellular and Tissue Engineering, Artificial Organs
15.07. Current advances in stem cell biology

Investigation of in vitro neural differentiation of olfactory mucosal mesenchymal stem cells in 2D and 3D

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Effective treatment methods for nerodegenerative diseases are not available yet. The lack of effective treatment methods has emerged as a serious and immediate problem that could potentially be solved by bioengineering applications such as neural tissue engineering. Neural regeneration can be achieved to a larger extent by tissue engineering approaches, such as supporting isolated cells with neurotrophic factors and extracellular matrices. Stem cells, because

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