UNIVERSITY OF BRESCIA

PhD Program
in
TECHNOLOGY FOR HEALTH

DEVELOPMENT OF A 3D-MODEL BASED ON HYDROGEL POLYMERIC SCAFFOLD (HYPS), BIOENGINEERED WITH HUMAN MESENCHYMAL STROMAL CELLS (HMSCS) FOR REGENERATIVE MEDICINE EMPLOYMENT

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Background

Regenerative medicine aims to restore normal tissue function by repairing or replacing damaged cells and tissues in patients. Scaffolds acts as a temporary matrix for cell proliferation and extracellular matrix deposition until the tissues are totally restored.
Mesenchymal Stromal Cells (MSCs) promotes tissue repair because of their proliferative potential, ability to migrate to injured tissues, as well as immunomodulatory and trophic effect.

Objectives

This project aims to develop a 3D model based on hydrogel-forming polymeric scaffolds (HyPSs) bioengineered with Human Mesenchymal Stromal Cells (hMSCs).

Methodologies

Different natural hydrogel scaffolds were characterized; A first series obtained from gelatin, hydroxyethylcellulose (HEC) and PEG (Polyethylene Glycol) as a cross-linker and a second series based on gelatin, different concentrations of chitosan (Ch) and PEG.
Scaffolds were characterized for their physico-chemical, thermo-mechanical and morphological properties. MSCs were seeded into the scaffolds, in both dry and wet (hydrated) states, at a cellular density of 1×10^6 cells/scaffolds and 4×10^6 cells/scaffolds. In this context, the MSCs proliferation in presence of Fetal Bovine Serum (FBS) versus HPL (Human Platelet Lysate) were tested. The expansion of MSC from bone marrow (BM) and adipose tissue (AT) was evaluated aiming to find the best source of MSCs. The viability of MSCs was determined using MTT cell proliferation assay after 2, 7 and 14 days of culture.
For inducing osteogenic and chondrogenic differentiation, cells/scaffolds constructs were cultured in 24-well plates for 3 weeks with osteogenic differentiation media consisting of a high-glucose DMEM supplemented with 10% FBS or 5% HPL, 10^{-7}M Dex, 25 mg/ml lascorbic acid, and 3mM NaH_2PO_4 or chondrogenic media composed of high-glucose DMEM supplemented with Dex 10^{-7} M, sodium pyruvate 1 mM, 50 µg/ml l-ascorbic acid-2- phosphate, 40 µg/ml proline, 1% ITS and 10 ng/ml of human transforming growth factor β_3.
These media were renewed every 3 days. Scaffolds without cells were used as controls.

Expected Results and Impact

Hydrogel scaffolds with Ch were selected to design a potential s ubstrate to support MSCs from different sources. Moreover, the use of HPL for cell expansion offers the possibility to obtain a safer cellular products without xenogenic contaminants and able to be transferred to clinical applications in humans.