Osteochondral Differentiation of MSCS In 3D Decellularized Wharton Jelly Hydrogel Scaffold in Tissue Engineering

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ABSTRACT

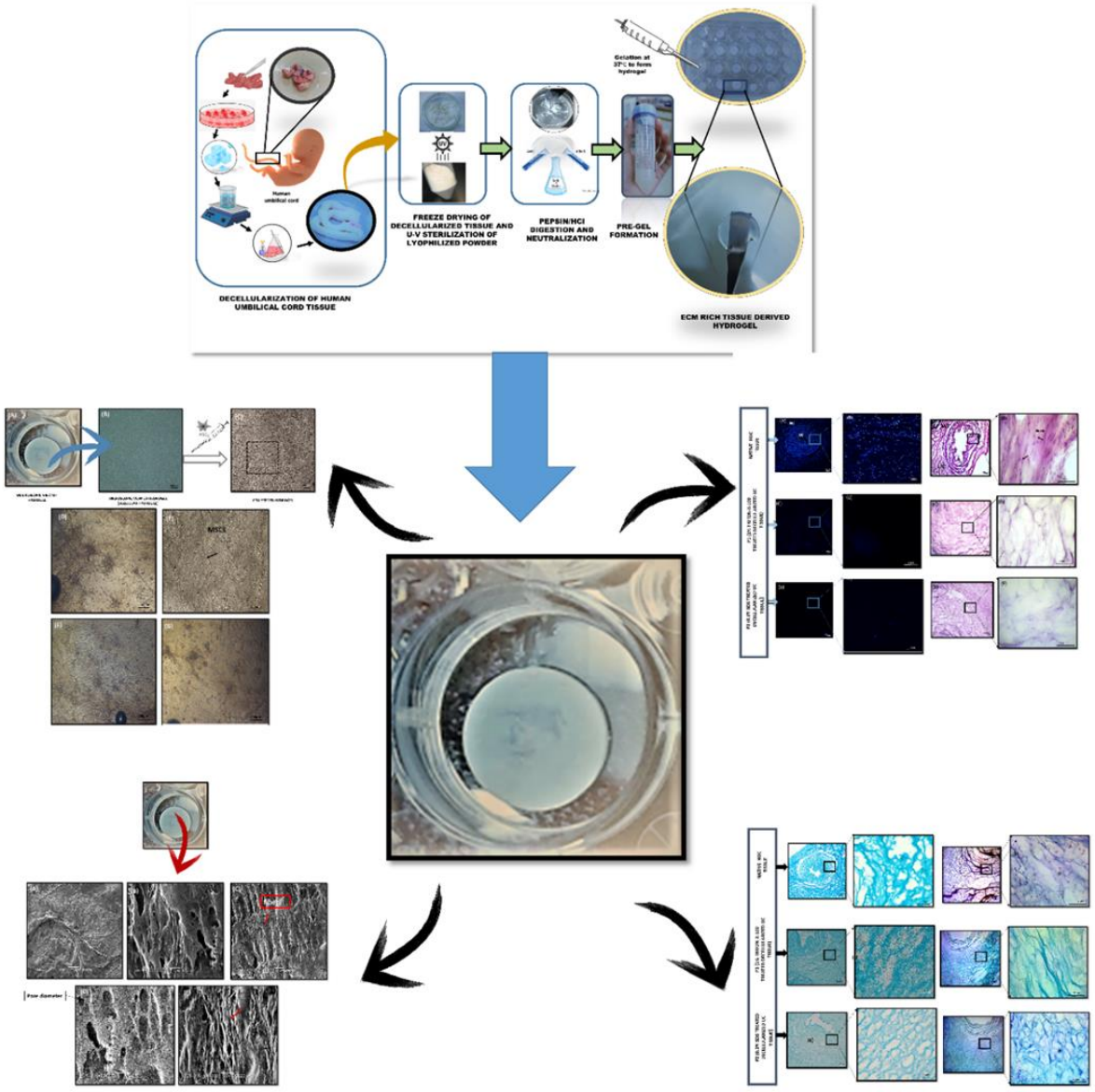
Background: Osteochondral (OC) lesions are worldwide medical issue for orthopedic surgeons which are linked with several clinical problems includes, acute trauma ,arthritis, subchondral insufficiency fractures and osteonecrosis(Takao et al. 2003),(Gorbachova et al. 2018). Current techniques used for the treatment of OC defects include autologous chondrocyte implantation (ACI), OC grafting, and a combination of both, based on the defects. However, there have been recent treatment approaches which provide better therapy for the regeneration and repair of OC lesion include stems cells seeded scaffold therapies in tissue engineering area using variety of synthetic and naturally derived scaffolds(Jacob, Shimomura, and Nakamura 2020).Extracellular matrix-derived hydrogels work as 3D scaffolding material and considered as close replica of native tissue microenvironment for invitro cells. its chemical and structural composition play an important role for attachment and proliferation of seeded cells (Solorio et al. 2015).

Objective: To analyze the culturing of MSCs on a 3D scaffold and their differentiation into Osteochondral lineage.

Methods: Decellularization of wharton jelly was evaluated by Hematoxylin and Eosin, alcian blue, and Masson's trichrome staining. Scaffold was characterized by scanning electron microscopy and FT-IR spectroscopy. MSCs were isolated from human umbilical cord tissue, and were characterized by immunostaining, flow cytometry, and trilineage differentiation. The MSCs capability to differentiate into an osteochondral lineage was determined by microscopy, alizarin red, alcian, blue staining, and osteochondral gene expression by quantitative PCR and by immunostaining.

Results: Decellularization was confirmed by determining DNA content and Extracellular matrix analysis. The scaffold was characterized through imaging and scanning electron microscopy for fibers formation and FT-IR analysis for cross linking. MSCs were characterized by cellular morphology, immunostaining for presence of MSCs markers Stro1, CD90, CD44, CD105, Vimentin, CD117, HLADR, and CD45. Immuno-phenotypical analysis showed positive expression for vimentin, CD73, CD90, and CD 117. Trilineage analysis showed osteogenic, adipogenic and chondrogenic differentiation. MSCs were seeded in Scaffold, and were evaluated using phase contrast microscopy, cell attachment and infiltration was observed. The cells proliferation and viability experiments displayed that hMSCs were viable and able to proliferate over time on seeded scaffold. hMSCs seeded on scaffold and cultured in stromal, osteogenic, and chondrogenic media for 28 days showed the upregulation of osteogenic and chondrogenic marker and calcium mineral deposition in scaffold. However, the Scaffold was more efficient in inducing hMSCs osteogenic differentiation than the Chondrogenic differentiation. Immunostaining showed the expression of osteogenic markers runx2, osteopontin, and osteocalcin in MSCs seeded on 3D hydrogel.

Conclusions: In conclusion, umbilical cord derived scaffold can be used as a 3D porous, bioactive, and biocompatible material that can effectively promote bone and cartilage tissue generation in vitro.



Keywords: Tissue derived hydrogel, Human Umbilical Cord, Scaffold, Mesenchymal Stem Cells, Gene Expression, Regeneration, Scaffold.

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