Adipose tissue-derived mesenchymal stem cells and chitosan/poly (vinyl alcohol) nanofibrous scaffolds for cartilage tissue engineering

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Abstract

Osteoarthritis (OA) has been defined as a chronic inflammatory joint disease characterized by progressive articular cartilage degeneration. Recently growing interest in regenerative medicine, using cell therapy and tissue engineering, where cellular components in combination with engineered scaffolds and bioactive materials were used to induce functional tissue regeneration. In the present study, nanofibrous scaffold based on chitosan (CS)/ poly (vinyl alcohol) (PVA) were used to develop biologically functionalized biomaterial to mimic the extracellular matrix, allowing the human adipose tissue derived mesenchymal stem cells (ADSCs) to proliferate and differentiate to chondrogenic cells. The morphology of the nanofibrous mat was examined using FESEM. The characteristic functional groups and the nature of the chemical bonds between atoms were evaluated using FTIR spectrum. Characterization of the seeded cells was morphologically evaluated by SEM and by flow cytometry for the expression of the stem cell surface markers. The differentiation potential was verified after chondrogenic induction by analyzing the expression of chondrogenic marker genes using real-time (RT PCR). Current study suggests a significant potential for the use of ADSCs with the nanofibrous scaffolds in improving the osteoarthritis pathology.

Background

Osteoarthritis is known to be chronic, debilitating joint disease. Mesenchymal stem cells (MSCs) have a new line for management of osteoarthritis in accordance with their capability of differentiation into chondrocytes, and the paracrine effects of secreted bioactive substances that might be more important than differentiated cells in enhancing repair responses.

The designing of scaffold to have composition, biological, mechanical and physiochemical properties that imitate extra cellular matrix (ECM) of the damaged tissue is considered one of the significant tools for tissue engineering.

(CS) is a naturally derived biodegradable polysaccharide commonly used in tissue engineering because of its biodegradable, biocompatible, and non-toxic properties.

(PVA) has good mechanical and chemical characteristics. PVA was used in controlled release systems and due to its biocompatible nature; it has a variety of biomedical uses.

The electrospinning was used for the manufacture of nanofibrous scaffolds composed of Cs/PVA that are suitable for the 3D cell cultures for tissue regeneration.

Objectives

- Fabrication of chitosan/polyvinyl alcohol (CS/PVA) nanofibers mats.
- The aim of the current study was to establish suitable physiologically and biochemically relevant microenvironment allowing ADSCs proliferation and differentiation into chondrocyte-like cells using CS/PVA nanofiber scaffolds.

Methods

1. Preparation of different spinning solutions containing different weight fraction form Cs and PVA
2. 1.The electrospinning procedure, with optimized parameters, namely; applied voltage of 25 KV, flow rate of 0.7 ml/h, and a tip-to-collector distance (TCD) of 10 cm
3. 3. Characterization of the CS/PVA were carried out in terms of microstructure and FTIR .
4. Isolation of adipose tissue Mesenchymal stem cells (ADSCs).

➢ ADSCs were obtained from freshly isolated subcutaneous fat from healthy donors (n = 5, age: 22–41) undergoing cesarean section surgery as described previously (Gimble and Guilak 2003).

5. Characterization of ADSCs

➢ The undifferentiated ADSCs were analyzed for Mesenchymal stem cell surface markers using flow cytometry, multi-lineage differentiation potential, and stemness gene markers expression.

6. Cell seeding of scaffolds and culture

The cell seeding of scaffolds was performed in a 6 well plates at density of 1 × 105 cells/well. Human Adipose-derived Stem Cells (hADSCs) were harvested from the cell culture plates with 0.05% trypsin.

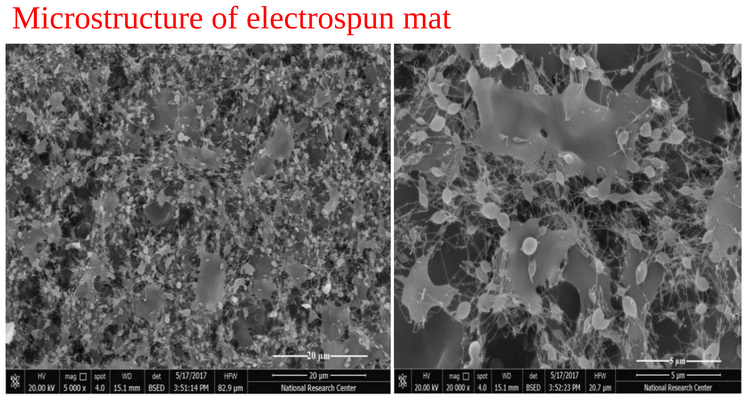
7. Cell viability and proliferation assay

➢ Cell viability on the scaffolds and tissue culture plate was assessed by MTT cell proliferation assay kit

8. Cell adhesion assay
9. Apoptosis assay
10. Chondrogenic differentiation
11. Scanning Electron microscopy (SEM)
12. Reverse transcription quantitative polymerase chain reaction (RT-qPCR)
13. Statistical analysis

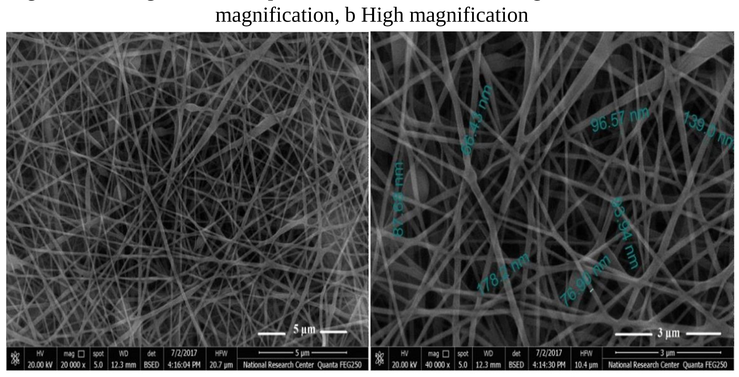
Results

Microstructure of electrospun mat



a b

Fig. 1 SEM images of the as-spun mate of CS/ PVA with weight ratio = 20:80 a Low magnification, b High magnification



a b

Fig. 2 SEM images of the as-spun mat of CS/ PVA with weight ratios equal to 10:90 a at low magnification, b High magnification

FTIR of the Nanofibers

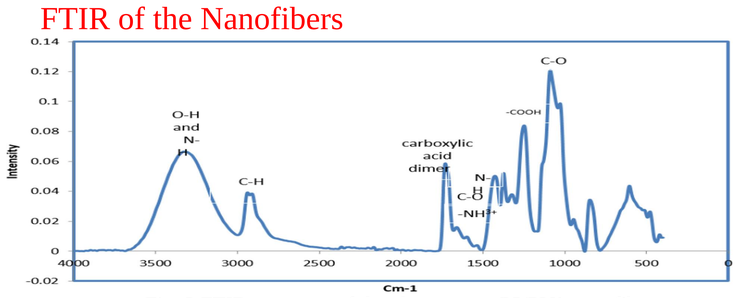


Fig. 3 FTIR spectrum of the electrospun CS/PVA nanofiber mat

Characterization of isolated hADMSCs

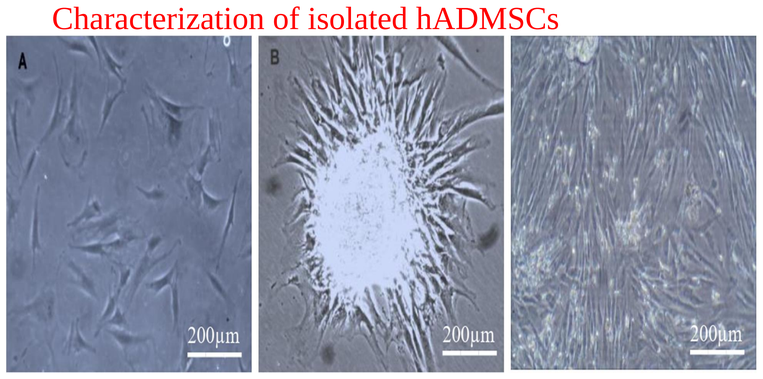


Fig. 4 Morphological characterization of isolated ADSCs, Scale Bar 200 µm. A Representative image of spindle-shaped ADSCs, b Colony forming unit. c 90% confluent at 10th day

Fig. 5 Immunophenotypic expression of mesenchymal stem cell surface marker with flow cytometry analysis of ADSCs showing that cells were positive for (CD73, CD105, CD90, and CD271) and were negative for (CD34, and HLA-DR)

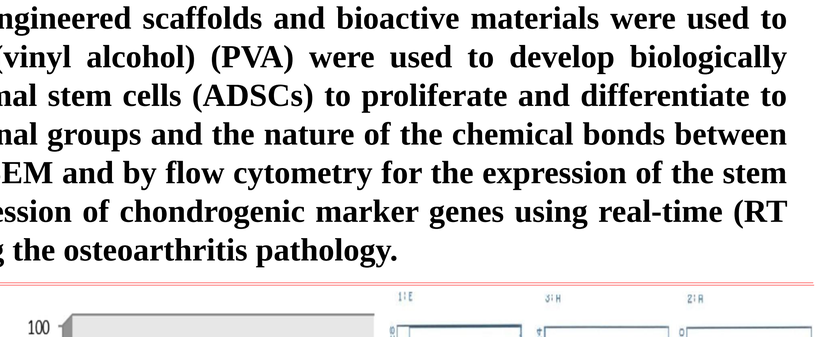


Fig. 6 (A) In vitro multi-linage differentiation of human ADSCs: osteogenic differentiation demonstrated by alizarin red stain in the form of dark orange mineral deposits (a), chondrogenic differentiation was stained positive for glucosaminoglycans by Alcain blue stain (b), specific oil red stain indicated adipogenesis induced lipid droplets observed in red colour (c). Scale bars: 200 µm. (B) reverse transcriptase (RT-PCR) determined ADSCs expression of pluripotent markers Nanog, Oct-4 and Sox-2

Cell adhesion and viability

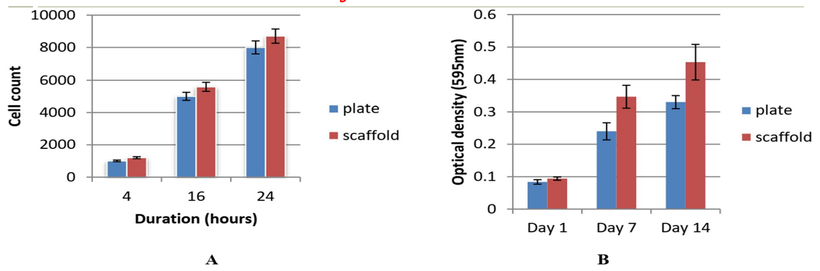


Fig. 7 showing cell viability, proliferation and adhesion. a Cell adhesion assay. b MTT assay results using mean and standard deviation

Cell attachment and differentiation potential

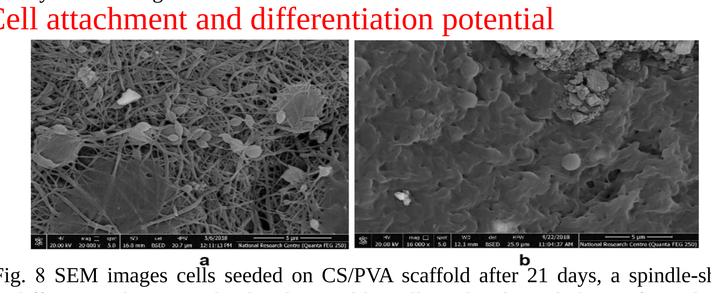


Fig. 8 SEM images cells seeded on CS/PVA scaffold after 21 days, a spindle-shaped undifferentiated ADSCs, b Chondrocyte-like cells with spherical shape after culture in chondrogenesis differentiation medium

Conclusions

Adult mesenchymal stem cells along with biomaterial scaffolds seem to be attractive candidates for regenerating articular cartilage dysfunction, due to chondrogenic differentiation potential, and immunomodulatory characteristics. Current study suggests a significant potential applications for human ADSCs with Cs/PVA nanofibrous scaffolds in improving osteoarthritis pathology. Our future plan is to establish a controlled animal model.