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Original Article

# Comparative analysis of chondrogenesis from cartilage tissue and alginate encapsulated human adipose stem cells

*Tanya Debnath* [a], *Usha Shalini* [a], *Lakshmi K. Kona* [b], *J.V.S. Vidya Sagar* [c], *Suguna Ratnakar Kamaraju* [a], *Sumanlatha Gaddam* [d], *Lakshmi Kiran Chelluri* [a,*]

[a] *Transplant Immunology & Stem Cell Laboratory, Global Hospitals, Lakdi-ka-Pul, Hyderabad, India*
[b] *Department of Bariatric Surgery, Global Hospitals, Hyderabad, India*
[c] *Department of Orthopedics, Aware Global Hospitals, Hyderabad, India*
[d] *Department of Genetics, Osmania University, Hyderabad, India*

ARTICLE INFO

*Article history:*
Received 14 December 2014
Accepted 24 June 2015
Available online 16 July 2015

*Keywords:*
Articular cartilage
Sodium alginate
Chondrogenesis
Extra-cellular matrix (ECM)
Differentiation

ABSTRACT

*Aim:* Alternate strategies to regenerate the damaged tissue require exogenous supply of several chondrocyte implants. There are inherent challenges to optimize an appropriate tissue culture methodology that can aid in enrichment of chondrocytes. The aim of the study was to explore the differentiation potential, expansion and growth kinetics of the human adipose derived stem cells (hADSCs) in alginate microspheres in comparison to chondrogenesis from the cartilage tissue.

*Methods:* Isolated hADSCs and cartilage derived chondrocytes were cultured and characterized. The distribution of stem cells within alginate bead was imaged by scanning electron microscopy (SEM). Encapsulated hADSCs were monitored for their cell viability, cell proliferation and apoptosis via JC-1 staining, MTT assay and Annexin V assays respectively. The alginate cell constructs were analyzed for chondrogenic gene expression by RT-PCR.

*Results:* The chondrocyte pellet culture from cartilage demonstrated lower growth potential as compared to alginate encapsulation. hADSCs were successfully encapsulated within alginate matrix with >80% cell viability. Apoptotic assays provided safety profile for the alginate during cell growth. The up-regulation of cartilage specific genes like TGF-β, collagen type-X, COMP was observed during the entire period of culture.

*Conclusion:* The chondrogenesis in pellet culture from cartilage tissue conserved the chondrocyte phenotype better with rich GAG polysaccharides. However, owing to an enriched chondrocyte requirement, alginate as a scaffold design would aid in the treatment of large focal defects.



* *Corresponding author*. Tel.: +91 040 30244501.
E-mail address: lkiran@globalhospitalsindia.com (L.K. Chelluri).

http://dx.doi.org/10.1016/j.jajs.2015.06.001

## 1. Introduction

The limited ability of articular cartilage to self-repair has led to a wide variety of treatment approaches for focal chondral defects with varying levels of success. Limited proliferative and regenerative capacity of adult chondrocytes and their potential dedifferentiation upon expansion is an impeding factor and hence it is imperative to explore alternate strategies.[1] An alternative therapy for the repair of damaged articular cartilage resides in the tissue engineering approach.[2] Tissue engineering can potentially use cells taken either from the patient (autologous) or from a donor (allogenic). These cells may be mature cells (e.g., chondrocytes) or immature cells (e.g., mesenchymal stem cells). The adult stem cells could be the solution to differentiate into different types of cells, allowing us to regenerate damaged tissues.[3]

Adipose tissue represents a good candidate tissue for obtaining adult stem cells for regenerative therapy because of least ethical implications and increased voluntary donation.[4] Furthermore, the stromal vascular portion of adipose tissue has been reported to contain up to 2% of cells that are able to differentiate into various cell types compared with only 0.002% of cells with this capability in bone marrow.[5]

A wide variety of scaffolds have been used to mimic the extra-cellular matrix of the cartilage tissue *in vitro*.[6] The most common matrix material used is collagen or alginate. Sodium alginate has been widely recognized as a conventional stem cell delivery system for repair of cartilage defects and as a model for 3D culture system.[7]

This innate feature of alginate hydrogel allows a good transfer of gases and nutrients to maintain cell viability, proliferation and differentiation. Three-dimensional systems could potentially provide the improved ratio of surface to volume necessary to cope with the scale of cell expansion required for allogenic tissue engineering applications. Several studies have shown the induction potential of stromal cells to chondrogenic lineage as evidenced by the expression of aggrecan, type II collagen and collagen IX.[8] However, on prolonged culture and therapy these differentiated stromal cells have resulted in the development of fibrocartilage tissue and scar formation. Failure rate of autologous chondrocyte implantation is about 60% due to de-differentiation phenomenon. It is of utmost importance that chondrocyte cell phenotype should be conserved in the development of *in vitro* chondrocyte culture technologies. The present study was designed to compare the biological performance of articular chondrocytes and the alginate matrix incorporating the hADSCs towards chondrogenic lineage. Calcium chloride was chosen for its' better chelating properties as compared to others, based on the previous reports. The study parameters in 3D alginate matrices as temporary physical support for hADSCs included, the cell proliferation, viability, compatibility, chondrocyte gene expression profile during chondrogenic differentiation. Thus, the current study would provide a preliminary insight to design a strategy for encapsulation and differentiation of hADSCs within the alginates paving for further studies in regenerating the injured tissues.

## 2. Materials and methods

Low Glucose–Dulbecco's Modified Eagle Medium, FBS (fetal bovine serum), antibiotics (Penicillin, Streptomycin, Gentamicin, Amphotericin B), enzymes (Trypsin–EDTA, Collagenase) were purchased from Gibco (Life technologies, Switzerland). Chemical and growth factor were of the highest grade and purchased from Sigma–Aldrich (St. Louis, USA). SA (250 kDa; medium viscosity, Sigma–Aldrich (St. Louis, USA)), alcian blue, dexamethasone, ascorbate, and insulin–transferrin–selenium (ITS) were purchased from Sigma–Aldrich (St. Louis, USA). Primers were procured from Bioserve (Beltsville, USA).

Prior written informed consent from the patients was obtained after taking clearance from the Global Hospitals, Institutional Ethical (IEC) Ref no. GMERF/BS/SAC/IEC/IC_SCR2014/01. The samples for the study were collected in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki). Human adipose tissue was collected from morbid obese patients undergoing bariatric surgery. The samples were collected in L-DMEM and pooled for further experimentation to circumvent the age variable. The cartilage scrapes were collected in sterile DMEM/F12 medium supplemented with antibiotics during arthroscopy procedure.

### 2.1. Isolation of chondrocytes from cartilage tissue

The cartilage scrapes of $2 \times 2$ mm bits during arthroscopy procedure were obtained in sterile media supplemented with antibiotics. They were subjected to low speed magnetic stirring at 37 °C for 45 m in 0.2% trypsin. The cells dispensed in the media are re-suspended in 0.2% collagenase solution in a $CO_2$ incubator at 37 °C for 90 m. The digested tissue was filtered using 70 μm cell strainer to separate the chondrocytes from undigested ECM. The cells were cultured as pellet in complete chondrocyte xeno-free media at a density of $5 \times 10^4$ cells/ml in a humid, 5% $CO_2$ incubator at 37 °C. The pellet culture was maintained in a centrifuge tube for 21 days, with the medium replaced at 3 day intervals. The sedimented cells formed spherical aggregates at the bottom of the tube.

### 2.2. Morphological and histological analysis of pellet

Pellets were harvested after 21 days of culturing, fixed in 10% buffered formalin for 2 h, and kept in 70% ethanol overnight. Samples were embedded in paraffin and 5-μm sections were cut. Hematoxylin and Eosin (H&E) staining of paraffin sections was done for evaluation of cell morphology in pellets. Sulfated glycosaminoglycans (GAG) were visualized by staining with 0.5% alcian blue for 10 m.

### 2.3. Cell growth kinetics of chondrocytes

The growth rate of human chondrocytes was measured by seeding initial cell density at $4 \times 10^4$cells/mL. The cells were harvested periodically to check the growth profile of the cells.