ISAKOS

International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine

10th Biennial ISAKOS Congress • June 7-11, 2015 • Lyon, France

Paper #2

Hypoxic Pretreatment Promotes Chondrogenic Capacity of Scaffold-free Tissue Engineered Construct Derived from Synovial Mesenchymal Stem Cells

Yukihiko Yasui, MD, JAPAN
Ryota Chijimatsu, MS, JAPAN
Yu Moriguchi, MD, PhD, JAPAN
Kota Koizumi, MD, JAPAN
Norihiko Sugita, MD, JAPAN
Morito Sakaue, PhD, JAPAN
Akira Myoui, MD,PhD, JAPAN
Hideki Yoshikawa, MD, PhD, JAPAN
Norimasa Nakamura, MD, PhD, JAPAN

Osaka University
Suita, Osaka, JAPAN

Summary:
We have developed a scaffold-free tissue-engineered construct (TEC) and demonstrated its feasibility to cartilage repair. While, it is important to further improve chondrogenic differentiation of the TEC. This study revealed hypoxic generated TEC had higher chondrogenic capacity than the conventional TEC, suggesting the availability of hypoxic preparation of the TEC towards clinical application.

Abstract:
Introduction
Stem cell therapies in cartilage repair have been widely investigated based on poor healing capacity of articular cartilage. We have recently developed a scaffold-free tissue-engineered construct (TEC) composed of synovial mesenchymal stem cells (MSCs) and extracellular matrices synthesized by the cells and demonstrated the feasibility of TEC to facilitate cartilage repair in a first-in-man clinical trial. However, repair cartilage generated by the TEC has been shown to have superficial abnormality morphologically and mechanically in a large animal model. Limited chondrogenic capacity of adult stem cell could be one of the considerable reasons. Therefore, promoting the chondrogenic capacity of the TEC can improve therapeutic outcome. Hypoxia is one of the promising methods to promote chondrogenic differentiation, to maintain stemness, and to escape cellular senescence of stem cells with safety. The purpose of this study was to test the feasibility of hypoxic preparation of the TEC to promote chondrogenesis.

Method
MSCs isolated from human synovium from 3 ACL injured patients were plated (triplicate from each sample) on culture dishes at a density of 4.0 x 105/cm2 in the growth medium with 0.2 mM ascorbic acid 2-phosphate to promote collagen synthesis. MSC isolation, proliferation, and generation of TEC in hypoxic group were under 5% O2 consistently and those in normoxic group under 20% O2. Then, in vitro chondrogenic differentiation capacity of the TEC was tested under 5% O2 for three weeks. In both hypoxic and normoxic group, we evaluated cell proliferation, p16 gene expression (marker for cellular senescence) and ßGAL staining (marker for cellular senescence) of MSCs as well as GAG content, gene expression, histology of the TEC after chondrogenic differentiation.

Result
During early passages of MSCs, there was no apparent difference between normoxic and hypoxic condition. However, MSCs expanded in normoxic condition started to slow proliferation around passage 6-8 while MSCs in

hypoxic condition maintained their growth. Normoxic cultured MSCs at passage 3 and 6 were positive for SA-ßGAL staining whereas hypoxic cultured MSCs were mostly negative. Expression level of p16 gene was 2 times lower($P<0.01$) in hypoxic cultured MSCs.

There were no significant differences in volume and weight of pre-differentiated TEC indicating no suppressive effect of hypoxia on collagen production and generation of TEC.GAG content/weight of chondrogenic differentiated TEC was 1.6 times higher ($P<0.01$) in hypoxic generated TEC. Collagen II gene expression was 2.5 times higher($P<0.05$) in hypoxic generated TEC. Hypoxic generated TEC was more hyaline cartilage-like with stronger safranin O staining after chondrogenic differentiation culture as compared with normoxic prepared TEC.

Discussion

Oxygen tension of peripheral tissue is reported less than 40mmHg which is equivalent for 5.6% O2 in incubator, thus low oxygen condition used (5%) provides physiological cellular microenvironment. Indeed, the present study revealed hypoxic cultured MSCs were free from cellular senescence and hypoxic generated TEC had higher chondrogenic capacity, indicating feasibility of hypoxic pretreatment in tissue engineering. Hypoxic pretreatment is physiologically reasonable, safety and cost effective. Therefore, clinical application of hypoxic pretreatment in stem cell therapy for cartilage repair has possibility to improve therapeutic outcomes.