# 探討第二型膠原蛋白與生長因子對去分化軟骨細胞及中胚層幹原細胞之軟骨化的研究

# The effects of growth factors in combination with type II collagen on the differentiation of dedifferentiated chondrocytes and chondrogenesis of mesenchymal stem cells


## 中文摘要

軟骨細胞是身體中非常特殊的組織。 因為缺乏血管系統的支持，軟骨細胞自我修復的能力極差。 因此，軟骨缺損的修復成為值得研究與討論的議題並且有許多有關軟骨修復的研究結果發表。 在 1994 年，Brittberg 於新英格蘭醫學雜誌發表了以健康軟骨細胞經體外(in vitro)大量培養後，再植回病患缺損部位的論文，開創了應用自體軟骨細胞培養移植(autologous chondrocytes transplantation；ACT)來治療關節缺損的新技術。 但是如何獲得足夠的細胞數量仍是 ACT 技術的關鍵之一；因此必須靠平面培養(monolayer culture)的方式來大量擴增細胞總數。 遺憾的是經過平面擴大數量培養後，軟骨細胞會失去其正常特徵與功能(lose phenotype)，稱之為去分化(dedifferentiation)，而此種軟骨細胞稱為去分化軟骨細胞(dedifferentiated chondrocytes)。 因此，如何讓軟骨細胞在體外大量培養時，仍維持或恢復其正常的型態，成為值得研究的課題。

本實驗利用軟骨細胞外間質所特有的第二型膠原蛋白(type II collagen) 搭配不同的生長因子(Growth factors)，來探討如何以兩者與細胞間的交互作用誘導已去分化之軟骨細胞再表現其正常型態(phenotype)。 本論文將探討調控軟骨細胞生理機能的生長因子和細胞外間質，與軟骨細胞間之交互作用，所產生的細胞生理及功能變化。 希望以接近於體內軟骨生理環境的條件，促使已去分化之軟骨細胞再次表現其所應當表現的正常型態。 並且以第二型膠原蛋白搭配促進中胚層幹細胞軟骨分化的生長因子，使中胚層幹原細胞/中胚層前趨細胞(mesenchymal stem cells / mesenchymal progenitor cells；MSCs / MPCs)，進行軟骨化分化(chondrogenic differentiation)；再與去軟骨化細胞之再軟骨化相比較，以了解幹原細胞分化程度。

我們的結果顯示，軟骨細胞在第二型膠原蛋白的環境下培養，其 Col2a1 mRNA 的表現與醣胺多醣(Glycosaminoglycans; GAGs)含量皆增高。 TGF-β1 可促使軟骨細胞 Col2a1 mRNA 表現上升，與 IGF-I 搭配則更可進一步增進軟骨細胞分泌 GAGs 並促進軟骨細胞 Col2a1 mRNA 的表現。 但是 IGF-I 本身也會促使平面培養的軟骨細胞 Col-I mRNA 的表現上升。 研究結果也顯示，使用第二型膠原蛋白與 TGF-β1 及 IGF-I 搭配可使平面培養的軟骨細胞分泌更大量的 GAGs 並促進 Col2a1 mRNA 表現。 FGF-2 (bFGF)可促使細胞增生，進而達到增殖大量細胞的目的；雖然 bFGF 並不能促進軟骨細胞分泌 GAGs， 但是也不會促進 Col-I mRNA 表現。 因此可用來促進軟骨細胞增生。

綜合以上資料可推測，在平面培養軟骨細胞時加入第二型膠原蛋白、TGF-β1 及 IGF-I 或再加上 FGF-2 具有最佳的成效，可使軟骨細胞在平面培養中維持 Col2a1 mRNA 表現，使軟骨細胞與中胚層幹原細胞產生大量 GAGs 的表現而達到促進細胞分化的目的；同時又因為有 FGF-2 的

加入而不大量抑制細胞增生。 如此可同時達到促使軟骨細胞與中胚層幹原細胞增生與分化的總目標。

## 英文摘要

Articular cartilage is a specific tissue of body. Due to it’s lacking of vessel system supports, the self-renewal ability of it is very poor. Thus, the repair of cartilage defects becomes a topic for study and discussion. Chondrocytes in vivo are surrounded with various extracellular matrices (ECM), including type II collagen. According to Trippel S.B., the formation of articular cartilage defects in osteoarthritis arises from imbalance between catabolism and anabolism in the extracellular matrices (ECM) of cartilage. Recent reports show that exogenous type II collagen fragments regulate metabolic activity of chondrocytes, including maintenance of cell morphology, and increasing synthesis of type II collagen and aggrecans. These suggest that cell- matrix interactions provided signals to regulate cell phenotype. In addition, cytokines and growth factors play a major role in the regulation of articular chondrocytes and mesenchymal stem cell behavior. They regulate chondrocytes to synthesized proper ECM to constructed exact cartilage structure or mesenchymal stem cell to differentiated to proper cell linage.

Chondrocytes cultured in monolayer will gradually change its phenotype to form type I collagen instead of type II collagen, and decrease their the secretion of glycosaminoglycans (GAGs). All of these characteristics termed of de-differentiation. In this study, it was intended to study the combined effects of ECM and growth factors on chondrocytes and mesenchymal stem cells in monolayer culture. TGF-β1, IGF-I, FGF-2 and BMP-2 were choused in collaboration with type II collagen to modulate de-differentiated chondrocytes behavior.

Our result show that chondrocytes cultured on monolayer surrounded by type II collagen can increase Col2a1 mRNA expression and GAG secretion. TGF-β1 promotes Col2a1 mRNA expression of chondrocytes and combined with IGF-I, the GAG secretion and Col2a1 mRNA expression will further raised. Our data also show that chondrocytes cultured on monolayer in the presence of type II collagen plus TGF-β1 and IGF-I increased GAG secretion and Col2a1 mRNA expression. Extra addition of bFGF further promotes cell proliferation without elevating Col-I mRNA expression. Besides, mesenchymal stem cells GAGs secretion promoted by adding dexamethasone, TGF-β1 and type II collagen. Thus, it is suggested that the combination of these agents in culture media for chondrocytes or MSCs on monolayer may promote their chondrogenic expressions in these cells. These may be further applied to tissue engineering of neocartilage.