計畫編號：BM07-00
計畫名稱：螢光基因轉殖動物骨髓間葉幹細胞分化之分子機制及治療潛能研究
計畫主持人：鄭登貴
計畫摘要(中)：

本計劃旨在利用綠色或紅色螢光基因轉殖動物(豬與鼠)之骨髓間葉幹細胞為材料，研究間葉幹細胞分化為成骨細胞(osteocyte; 子計畫一)，肝臟細胞(hepatocyte; 子計畫二)，脂肪細胞(adipocyte;子計畫四) 及軟骨細胞(子計畫五)之分子與後生遺傳(Epigenetic)機制(子計畫七)，並期研發出骨質疏鬆(子計畫一)，肝臟損傷(子計畫一)，心肌梗塞症(子計畫三)，關節軟骨損傷(子計畫五)及脊椎椎間盤退化(子計畫七)之最佳幹細胞治療動物模式。我們的研究團隊已成功的培育出螢光轉殖豬和小鼠(子計畫一)，這些動物的骨髓間葉幹細胞已被初步證實能夠在體外長期培養而仍然保持其螢光物質的表現，因此非常適合利用其螢光特性來追蹤其移植後的去向及分化程度，並有助於將此等移植後之細胞重新分離出，以進行分子與後生遺傳調控之分析(子計畫一及七)。

本計劃之各子計畫間有著非常緊密之聯結與整合：吾等將持續產製紅色及綠色螢光基因轉殖小鼠及豬，並自其骨髓分離間葉幹細胞(子計畫一)，做為後續於體外分化為上述各細胞系(子計畫一至六)及分化前後之基因表現差異性與後生遺傳調控機制(含 DNA 甲基化、組蛋白(Histone)修飾及功能性 RNA 之作用途徑（子計畫七)之探討。第二年將進一步進行骨髓間葉幹細胞之體內移植試驗，探討其對骨質疏鬆小鼠之修補功效(子計畫一)；建立這些螢光標的的間葉幹細胞在體外及體內分化為類肝細胞的方法，及把刺激分化後的類肝細胞由門脈或脾臟注入，並輔以適當的動物前處理來達到最高的肝臟替換比率(子計畫二) ；使用轉殖 HAX-1 基因之骨髓間葉幹細胞修補受損之心臟肌肉，探討用以治療心肌梗塞之可行性（子計畫三）；探討脂肪細胞結合素及其受體在脂肪細胞分化與動脈粥狀硬化形成之功能（子計畫四）；以異體軟骨細胞與骨髓間葉幹細胞修補關節軟骨損傷之成效探討（子計畫五）；評估綠色螢光之椎間盤髓核細胞於自製 Hyaluronan/Alginate hydrogel 基質內之生長效能與治療潛能研究（子計畫六）。吾等亦將針對移植後之細胞進行分子與後生遺傳特性分析，一方面應證研究體外分化過程中分子與後生遺傳調節機制之成

果，此外更可針對移植細胞與本體細胞間之交互作用等重要議題進行深入探討(子計畫七)。這些研究成果將可提供未來發展臨床試驗建立最適宜的治療模式。

計畫摘要(英)：

The purpose of this joint project is to establish the best animal models for stem cell based therapy to treat osteoporosis, liver damage, ischemic heart disease, articular cartilage defects and intervertebral disc degeneration caused lower back pain, and to study the Molecular and Epigenetic regulatory mechanisms governing the differentiation process from mesenchymal stem cells (MSCs) to osteocytes, hepatocytes, adipocyte, chondrocyte and nucleus pulposus cells. To accomplish these goals, we take advantage of the GFP- and RFP-tagged transgenic pigs and mice generating/generated from subproject 1. Pig is a very useful model for regenerative medicine studies because of their suitable body size and similar physiology compare to primates. Preliminary studies demonstrated that the GFP protein is expressed stably after in vitro differentiation of the GFP-tagged MSCs, isolated from GFP-pigs and mice, into various cell lineages including hepatocytes (subproject 2), adipocytes (subproject 4), osteoblast and chondrocytes (subproject 1 and 7) while maintaining the GFP fluorescent signal. These undifferentiated GFP-MSC cells and in vitro differentiated GFP-tagged cells are therefore the best tools for us to study their homing, differentiation activity after transplanting into the RFP-tagged recipient animals. The RFP-recipients will be preconditioned into the osteoporosis model by ovariectomy (subproject 1) or the liver damaged model by allyl alcohol treatment (subproject 2). In addition, the animal models for ischemic or infracted heart disease (subproject 3), the articular cartilage defect (sub- project 5) and the degenerated intervertebral disc (subproject 6) will also be prepared with the RFP- transgenic animals. The advantage of using RFP-tagged recipients includes the clarification of real differentiation from the implanted GFP-tagged MSCs or cell fusion events as demonstrated in other studies. Moreover, the long-term stable expression of the GFP marker also allows us to isolate transplanted cells for epigenetic analysis (subproject 7). As very little is known about the exact

epigenetic mechanism regulating stem cell differentiation, we will have great opportunities in uncovering the critical molecular and epigenetic regulators as well as their target genes from the in vitro differentiation system and/or the post-transplanted GFP cells.

This highly integrated joint program involves experts with backgrounds in Animal Science, Medicine,Biomedical Engineering, Cell and Molecular Biology. Several technical platf orm established in this program will benefit all subprojects. We expect very tight collaboration among all subgroups as illustrated in the flow chart attached. The animal model concerning mesenchymal stem cell based therapy for osteoporosis, various liver diseases, ischemic heart disease, articular cartilage defects and intervertebral disc degeneration caused lower back pain, as well as the underlying molecular and epigenetic regulatory mechanisms being established/studied in this project, will provide significant clinical and academic impact in regenerative medicine.

計畫編號：BM07-01
計畫名稱：再生醫學研究之紅色及綠色螢光小鼠和豬的產製與應用
計畫主持人：吳信志
計畫摘要(中)：

間葉幹細胞可分離自骨膜、骨小樑、脂肪組織、關節液、骨骼肌及脫落之牙齒，其在特定培養環境下具高增殖能力及可分化為多種細胞系特性，由許多臨床前及臨床之研究試驗中已證實間葉幹細胞在再生醫學及組織工程具應用之潛力及治療價值。此外，攜報導基因的間葉幹細胞經移植後之表現穩定性將有助於確認其在體內之功能及分化潛能。分離自表現報導基因之骨髓幹細胞具有相同之表現型式，此策略有助於追蹤其移植及分化後之命運。因此產製表現不同報導基因之轉基因動物將可滿足吾人對此獨特間葉幹細胞之需求。本計畫之擬於第一年產製紅色及綠色螢光基因轉殖小鼠及豬，並自其骨髓分離間葉幹細胞，做為後續於體外分化為不同細胞系及探討分化前後之基因表現差異性之細胞來源。第二年將進一步進行骨髓間葉幹細胞之體內同種異體及異種移植試驗，探討其對骨質疏鬆小鼠之修補功效及免疫排斥耐受性。另將進行源自不同品系小鼠間葉幹細胞之表面抗原差異性，並進一步分離其周邊血液淋巴球進行自體及同種異體之間葉幹細胞與淋巴球混合培養之免疫反應試驗。第三年將以淋巴球混合培養法

探討螢光豬間葉幹細胞之免疫學特性，同種異體 (ICR-C57BL/6) 及異種 (豬-小鼠) 移植一段時間後之存活間葉幹細胞及宿主周邊血液淋巴球將被分離及其淋巴球混合培養之免疫反應試驗亦將被執行。

計畫摘要(英)：

Mesenchymal stem cells (MSCs) have been isolated from bone marrow, periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle and deciduous teeth. They also can be expanded with high efficiency, and induced differentiation into multiple lineages under defined culture conditions. These cells have generated a great deal of interest because of their potential use in regenerative medicine and tissue engineering, and there are some dramatic examples, derived from both pre-clinical and clinical studies, that illustrate their therapeutic value. In addition, consistent expression of reporter gene incorporating into genome of implanted MSCs will facilitate the progress for verifying their functions and differentiation potential in vivo. The bone marrow-derived MSCs isolated from transgenic animal expressing reporter gene possessed the characteristics of identical expression pattern, and this strategy is favorable to track their fate after transplantation and differentiation. Furthermore, the issue of rejection of transplanted MSCs is more considerate. Recent studies have found that MSCs do not elicit alloreactive lymphocyte response due to immune modulations, but only some mechanism have been elucidate. The immunologic properties of MSCs after differentiation and of different species such as swine are still unclear. Hence, the fist year plan to generate transgenic animals expressing different reporter gene (green fluorescence protein (EGFP) and enhanced red fluorescence protein (ERFP) will be able to meet the necessity of unique MSCs for giving rise to difference cell lineage and exploring genes differential expression of pre-differentiation versus post-differentiation. Including transgenic pigs and mice harboring β actin-EGFP or β actin-ERFP transgene, respectively. The second year, try to establish an osteoporosis mouse (C57/BL6, inbreeding strain) model by ovariectomy (OVX), characterize the bone marrow-derived MSCs from EGFP transgenic mouse (ICR, outbreeding strain),stably express GFP, and transplant the allogeneous MSCs into the OVX mice. Following

implantation in the mouse model, MSCs survivability and homing will be determined, and application of allogeneous and/or xenogeneous MSCs for the treatment of the osteoporosis mouse will be conducted, and assessed immune-tolerance of transplanted cells as well. In order to understand whether different strain mice MSCs have different immune surface markers, we want to analyze the immune marker on MSCs of different strain mice (ICR and C57BL/6) by flow cytometric analysis. Furthermore, in order to study the effects of murine MSCs on autogeneic or allogeneic lymphocyte reactivity, murine peripheral blood lymphocytes (PBL) are purified and to be used in mixed lymphocyte reaction (MLR). The third year, the survival of MSCs in an allogeneic (ICR-C57BL/6) and xenogenic (pig-murine) model will be studied. Green fluorescent protein (GFP) transgenic mice MSCs are implanted into different strain mice. After a period of time, the implants were harvested and processed. The PBL of each animal are also collected to measure the in vitro immune responses by MLR. In additions, in order to investigate the feasibility of xenogeneic MSCs implantation, the immunologic properties of porcine MSCs are explored. GFP transgenic porcine MSCs are implanted into mice and the implants will be harvested and processed after a period of time. The PBL of each animal are also performed by MLR to study the immune response in vitro.

計畫編號：BM07-02

計畫名稱：以螢光轉殖動物之間葉幹細胞及肝細胞建立肝組織替換之研究

計畫主持人：李宣書

計畫摘要(中)：

肝臟移植至今仍是治療末期肝病、急性猛爆性肝病、及肝臟為標的器官的先天性代謝疾病的最佳治療方法。但是全世界都面臨一個共通的難題，那就是捐贈肝臟的短缺。以冷凍貯存的肝細胞移植是一個可行的代替方法，但是仍面臨許多問題，例如用來分離細胞的肝臟來源不足、肝細胞不易在體外大量培養、解凍後的肝細胞存活能力不良。

許多研究已證明間葉幹細胞可以在體外大量增殖並保有在體外分化為類肝臟細胞的能力。所以它們成為肝細胞移植的潛在代替者。甚至在一個以人類骨髓細胞移植到烯丙基乙醇傷害的大鼠肝臟的實

驗，發現只有間葉幹細胞部份可以在大鼠肝臟內分化為肝細胞。許多體外的實驗也證明間葉幹細胞可在培養皿內被分化為類似肝臟細胞。

我們的研究團隊已成功的培育出螢光轉殖豬和小鼠，這些動物的骨髓間葉幹細胞將被用來進行本實驗。這些細胞已證實能夠在體外長期培養而仍然保持其螢光物質的表現，因此非常適合利用其螢光特性來追蹤其移植後的去向。在本研究裡，這些細胞將在培養皿內被分化為類肝細胞，然後移植到野生種的同類動物之肝臟，以評估其肝臟替換的能力。

本研究的目的包括建立這些螢光標的的間葉幹細胞在體外及體內分化為類肝細胞的方法。在體外，將以四種方向分化：第一，使用各種組合的生長因子、細胞素、化合物來刺激；第二，與正常或毒物傷害的肝組織共同培養；第三，使用肝組織萃取物來刺激；第四，以肝細胞特異性 transcription factors 轉殖到細胞。在體內，將把刺激分化後的類肝細胞由門脈或脾臟注入，並輔以適當的動物前處理來達到最高的肝臟替換比率。這些研究成果將可提供未來發展臨床試驗建立最適宜的治療模式。

計畫摘要(英)：

Liver transplantation is the treatment of choice for end-stage liver disease, fulminant hepatic failure, and inherited metabolic disorders of hepatic origin. But there is a worldwide problem of shortage in liver donors. Transplantation using cryopreserved hepatocytes may be an alternative. Yet, limited supply of liver organ for hepatocyte isolation, difficulty of in vitro propagation of hepatocytes, and poor recovery of the cells after cryopreservation remain the obstacles for this application.

Mesenchymal stem cells (MSC) have been shown to be extensively expandable in vitro while maintaining their differentiation potential into hepatocytes. Thus they became an appealing alternative in substitution of hepatocytes for transplantation therapy. It has been shown in a xenographic transplantation of human bone marrow cells directly into rat liver damaged by allyl alcohol, only the MSC fraction appeared to give rise to hepatocyte-like progeny. Several in vitro experiments also showed the differentiation potential of cultured MSCs into hepatocyte-like cells.

We have already produced lines of transgenic mice expressing GFP or RFP, and also transgenic pigs expressing GFP. These animals will be

used in this study to yield MSCs from bone marrow. These MSCs were shown stably express GFP as the tag even after long-term culture, thus represent an ideal model for cell fate determination after transplantation into a wild-type animal. These cells will be used to differentiate into hepatocyte-like cells in vitro, and the resulted hepatocye-like cells will be used to transplant into wild-type animals to evaluate their liver repopulation capacity.

The objectives of this project include in vitro differentiation of GFP-tagged MSCs (either from mice or pigs) into hepatocyte-like cells and in vivo transplantation of these cells to repopulate recipients' liver. For the former objective, 4 kinds of induction will be tried, i.e., induction by growth factors/cytokines/chemicals, induction by co-culture of MSCs with normal or injured liver tissues, induction by liver tissue extracts and gene delivery of hepatocyte-enriched transcription factors. For later objective, in vitro differentiated cells will be transplanted through portal vein or spleen to repopulate recipients' liver. Optimal preconditioning of the recipients will be carried out to achieve a higher engraft rate of transplanted cells. This knowledge can be translated to future clinical therapeutic trials.

計畫編號：BM07-03

計畫名稱：使用業經強化 HAX-1 基因表現之骨髓間業幹細胞改善心肌梗塞症之可行性與治療效果探討

計畫主持人：鄭登貴

計畫摘要(中)：

因心臟冠狀動脈阻塞而造成心肌缺氧易使心臟細胞損傷或壞死，每年皆需投入龐大的醫療資源。然而在此方面的相關治療報告至今仍沒有一項具持久及有效的治療方式可資應用。有鑑於此，本計劃希望可以發展出結合骨髓幹細胞及基因治療之系統，除可以長時間保護心肌不受缺氧傷害之外，同時亦可增加心肌血管心生之作用以幫助心臟功能之恢復。間葉幹細胞 (mesenchymal stem cells) 賦予骨髓造血系細胞族群一適當微環境並維持造血系統的穩定狀態，研究證據顯示，骨髓間葉幹細胞確實具有多向分化潛能之事實。同時骨髓間葉幹細胞亦被利用於近年的臨床實驗中，誠屬遺憾者，乃目前與其治療之相關分子調控詳實機制鮮有所知，故於心臟疾病之應用上有所限制。

因此本計畫利用慢病毒(lentivirus) 轉染間質幹細胞，使其大量表現抗細胞凋亡之基因 HAX-1，藉此提高移植幹細胞之存活率以達到最佳的治療效果。職是之故，在本三年期研究計畫中，期望藉由標地基因 HAX-1 於心臟缺氧時，會與半胱天冬蛋白酶 9 (caspase-9)或受磷蛋白(phospholamban)結合進而抑制細胞死亡之特性，以此達到修復受損心肌並恢復其功能之目的。透過本計畫之執行完成，預期成果除包括建立動物疾病模式之外，對其治療之相關分子調控機制將有更詳實深入之瞭解。此等研究成果，除有助於國人在哺乳動物疾病及治療模式探討邁向卓越外，且利用豬做為動物模式，提供進行臨床前細胞治療、基因治療或組織工程用途等相關實驗，奠定穩固之研究基礎。

計畫摘要(英)：

Ischemic injury to the myocardium due to the spectrum of coronary occlusion events has been known as acute coronary syndromes account for a large proportion of all hospital admissions and of all causes of death in western society. Moreover, myocardial infarcts occur predominantly in an older population, and senescent myocardial tissue may differ from that of younger patients. The senescent heart has a higher population of apoptotic cells, and others have shown high circulating levels of C-reactive protein, which when added to cell medium impairs differentiation of endothelium and increases the rate of apoptosis. Senescence does affect the functioning of stem cells, so survival of the engrafted population is also an important factor. Manipulation of various pro-survival genes has also been attempted, as have genes affecting angiogenesis. Accordingly, the principal strategy should be to replace the loss cells to optimize restoration of cardiac contractile function as well as to provide the cardiac environment with anti-apoptotic effect. Stem cells are pluri-potent and have the property of self-renewal as well as multi-lineage differentiation and can therefore regenerate damaged cells. Mesenchymal stem cells (MSCs) are defined as unspecialized cells that have the capacity to renew themselves and display a potential for giving rise to diverse differentiated progenies. Because of their characteristics of highly proliferate ability, easily isolation and multi-potentiality for differentiation. Cell transplantation into ischemic or infracted hearts, typically delivers cells into or adjacent to a poorly perfused segment by

using intramyocardial injection, often results in those injection regions appeared to be infiltrated by macrophages and poor cell viability associated with transplantation has limited the reparative capacity of these cells in vivo. Under these conditions, the majority of transplanted cells died, though the left ventricle function appeared to be better improved. Recent advances genetically engineered MSCs modification technology including the use of recombinant lentiviral that can prolong in vivo and in vitro transgene expression for months. Moreover, our interest target gene is HAX-1, an anti-apoptosis protein and previews evidence indicated that the HAX-1 could associate with caspase-9 or phospholambon and represses of post mitochondrial caspase-9 activation and cell death during ischemia in the heart. Combined bone marrow stem cell and HAX-1 gene therapy for acute ischemic myocardium has not been previously reported in large animal model for pre-clinical therapy. Therefore, in this project, we try to clearly define whether utilizes long-term expression HAX-1-MSCs can repair infracted myocardium. In this 3-year of research project, following efforts will be evaluated:

(1) Purification of pig mesenchymal stem cell (pMSC) and to develop a lenti-virus containing HAX-1 gene construction, which can over expression with pMSC.
(2) To assess the effect of recombinant lentivirus-HAX-1 and modify the stem cell with lentivirus-HAX-1 expression in which to treat infracted heart following ischemia injury to pig through in vivo analysis.

To explore the therapeutic potential and characterize the molecular mechanism of stem cell therapy and gene transfer for myocardial infarction.

計畫編號：BM07-05
計畫名稱：關節軟骨修補之細胞來源研究
計畫主持人：江清泉
計畫摘要(中)：

關節軟骨損傷是目前臨床醫學難以治療的問題。目前學界主流研究方向是採用自體或異體軟骨細胞移植，來產製足以填補軟骨缺損的新軟骨基質。由於這種治療模式牽涉到將植入的軟骨細胞定位在軟骨

損傷處、需要用到適當的可分解生醫材料，所以並不被視為單純的細胞治療、而是一種組織工程。我們研究團隊過去已累積相當多的經驗，有合適的生醫材料和標準手術流程，可以有效處理這個難題。

由於可用來作為移植的自體軟骨細胞非常有限，因此異體軟骨細胞移植一直仍然是學界研究的興趣所在。然而關節軟骨修補後，新生軟骨究竟是完全仰仗植入的細胞、或是可由軟骨下方骨髓的幹細胞幫忙，迄今沒有定論。如果新生軟骨完全仰仗植入的細胞，那麼植入的異體軟骨細胞是否可以存活，也是未知的問題。這些未知是因為無法追蹤新生軟骨中，細胞的來源。

本子計畫，一方面在驗證異體軟骨細胞移植的成功率，另一方面也在探究新生軟骨的細胞來源。我們的假說是：異體軟骨細胞在移植到軟骨缺損處後，可得以在該處存活並產製新生軟骨。實驗的方法是，利用綠色或紅色螢光基因轉殖動物之關節（透明）軟骨細胞作為異體軟骨細胞移植的主體，植入同種的原生豬隻膝關節的軟骨缺損處，並令其在生體內生長。於適當時間後，再觀察軟骨修補的成效、以及新生軟骨內軟骨細胞的性質。如果這些軟骨細胞具有螢光，便可驗證異體軟骨細胞移植用於填補軟骨缺損的可行性。

計畫摘要(英)：

Articular cartilage defect is an unsolved clinical problem. Mainstay of current strategy is the autogenous/ allogenic chondrocyte implantation (ACI) to regenerate cartilage, which serves to fill the defect. Such treatments include a biodegradable biomaterial scaffold to hold the implanted chondrocytes in place, therefore are more than cell therapy and pertain to tissue engineering. Our research team has accumulated plenty of experience on treating cartilage defects with ACI, developed a suitable biomaterial scaffold, and set a standard operating procedure of surgery.

Autogenous chondrocytes have an extremely short of donor resource, therefore allogenic chondrocytes has continued to attract the research interest as a source of graft. Whether the regenerate cartilage is the product of implanted chondrocytes, or from other source such as the subchondral marrow-derived stem cells, remains undetermined. If the regenerate cartilage originates from the implanted cells, the viability of implanted allogenic chondrocytes has not been known, either. These unknowns result from the difficulty to trace the cells after their

implantation to the in vivo sites.

This subproject intends to: (1) verify the viability of implanted chondrocytes after implantation in ACI, and (2) identify the cell source and nature of the regenerate cartilage. Our hypothesis is that the allogenic chondrocyte remain viable and produce extracellular matrix to regenerate cartilage as the repair of chondral defect. We use the chondrocytes harvested from the articular cartilage of "florescent pig" as to repair cartilage defect on native pig of the same species, with ACI. The implanted chondrocytes are allowed to grow in vivo. The repaired site will be retrieved six months after cell implantation, to determine the nature of regenerate cartilage. The fluorescence of the chondrocytes in the regenerate cartilage will indicate the applicability of allogenic chondrocyte implantation to repair cartilage defects.

計畫編號：BM07-06

計畫名稱：表現綠色螢光之椎間盤髓核細胞於自製 Hyaluronan/Alginate Hydrogel 基質中之生長效能與治療潛能研究

計畫主持人：林峰輝

計畫摘要(中)：

目前椎間盤受損的通常放入 cage 作 fusion，甚至在早期受損也都是作脊椎融合手術，過去雖然有用細胞注射入退化中的椎間盤，可是恢復效果不佳。過去五年本實驗室發展出一種新行可注射是的透明質酸/褐藻酸水膠，在室溫時為液態狀，當溫度升至 37C 時，在依分鐘之內變成水膠，類似椎間盤髓核的細胞外基質。本研究將以這種水膠進一步改良後，混合具有綠色螢光基因豬的骨髓幹細胞或 NP 細胞，觀察植入後的細胞是否能增生且發揮正常功能，所使用的水膠細胞載體，是否可以逐漸吸收消化，被新生的細胞外基質取代，恢復受損的椎間盤功能。

本研究將於三年內完成：第一年將自綠色螢光豬分離出髓核細胞，同時增生培養到足夠的細胞數，作為髓核再生組織工程之應用；所使用之材料將進一步改質、分析、鑑定，使其性質更適合髓核組織工程之應用。第二年將建立具有髓核受損之動物模式及鑑定方法，用於第三年之動物實驗；所發展之材料將與細胞培養，觀察細胞與材料之間的作用，培養的 SOP 將建立；第三年將運用於具有髓核受損的實驗豬上，打入綠色螢光豬的 NP 細胞，實際瞭解髓核的恢復情形。

希望研究完成後，能實際運用於椎間盤受損的病人身上。

計畫摘要(英)：

Many researchers are seeking various biologic ways to repair degenerated intervertebral disc. The therapeutic approaches are generally using growth factor injection, gene therapy and cell therapy to stimulate matrix production. However, growth factors can not stay and keep bioactivity in living body for long time. Gene therapy, although reach certain level achievement, is generally troubled the researchers in virus carrier. Cell therapy is sometime failed because injected cells lose part of functions in degenerative NP environment and/or cell migrate to the surroundings. In the study, we are going to look for an adequate biodegradable material to prepare beads as NP cell carrier to culture the cell in 3D condition. It will be used for cell-based tissue engineering on IVD regeneration treatment. Cell carrier can not only provide specific cellular environments and molecules to direct a desired cellular activity; but also a physical support for cell retention.

The developed hyaluronate/alginate hydrogel with NP cell will be prepared to an injectable form with special designed syringe; that will be used to inject the NP-cell-based hydrogel into degenerative IVD. The next is going to elucidate whether NP cells will keep its phenotype to express normal genes once injecting into the degenerate IVD; and how long injected IVD can go last. The transgenic swine with green gene will be applied in the study to prove it. The project will be completed within 3 years: In the first year, NP cell will be isolated from swine bearing with green gene and will expand to enough number for NP tissue engineering. The HA/alginate hydrogel will be further modified with some of functional group to mimic the NP extra-cellular matrix for NP cell seeding. In the second year, NP degenerative model on swine with green gene will be set up for the third animal study. Modified hydrogel will be characterized and analyzed for later use and clarify the relationship/interaction between the NP cells. In the third year, developed hydrogel seeded with NP cell will be injected into the NP degenerative swine to understand the NP cell proliferation and function that will be used to evaluate the NP function back to normal or not.

計畫編號：BM07-07
計畫名稱：誘導骨髓間葉幹細胞分化之分子與後生遺傳調控機制
計畫主持人：林劭品
計畫摘要(中)：

後生遺傳/上位遺傳(Epigenetics)在不影響 DNA 序列的前提下，經由 DNA 甲基化、組蛋白(Histone)修飾及功能性 RNA 之作用途徑，影響特定基因之表現。此等上位遺傳對於基因體之修飾作用即為幹細胞之所以有別於已分化體細胞之差異所在。上位遺傳於各類細胞間不同之印記是具有可逆性的。這種可逆性是謂幹細胞及再生醫學研究之關鍵課題。目前對於後生遺傳調控基因表達之機制所知仍相當有限。本計畫將有系統的探討小鼠與豬之間葉幹細胞於體外及體內誘導分化為肝細胞、脂肪細胞、軟骨及硬骨細胞過程中，後生遺傳層次調控基因表達之機制。

本整合型計劃之其他六個子計畫，正利用綠色或紅色螢光基因轉殖動物(豬與鼠)之骨髓間葉幹細胞或椎間盤髓核細胞為材料，積極建立骨質疏鬆、肝臟損傷、心肌梗塞症、動脈粥狀硬化、關節軟骨損傷及椎間盤退化造成之下背部酸痛的幹細胞治療動物模式。此等螢光基因轉殖動物的骨髓間葉幹細胞已證實能夠在體外長期培養而仍然保持其螢光物質的表現，因此非常適合利用其螢光特性來追蹤其移植後的去向及分化程度，並有助於將此等移植後之細胞重新分離出，已進行分子與後生遺傳調控之分析。

本子計畫將充分利用於體內及體外誘導分化前後之綠色螢光骨髓間葉幹細胞為材料，經由分析誘導分化前後：基因(coding gene)及功能性 RNA（尤其是 microRNA）表達質與量之差別，DNA 甲基化，與各式組蛋白修飾(各個組蛋白不同胺機酸之甲基化及乙醯化)之差異，深入探究間葉幹細胞分化為成骨細胞(osteocyte) 、脂肪細胞、軟骨及肝臟細胞(hepatocyte)之分子與後生遺傳(Epigenetic)調控機制。此等研究之成果，不但有助於建立以幹細胞為中心之肝臟修復與骨質疏鬆治療的動物模式，本計畫更將對後生遺傳機制維持各式幹細胞之多分化潛能及調控幹細胞分化，帶來突破性之瞭解。這些知識勢必顯著提升以幹細胞為中心之再生醫學成功率。

計畫摘要(英)：

Recently it has been demonstrated that the epigenetic modifications that do not change the DNA sequences but change gene activities marks

the differences between stem cells and the differentiated somatic cells. These epigenetic levels of gene expression regulation include DNA methylation and modification of the chromatin packaging proteins, as well as functional small RNAs that regulate gene activity post-transcriptionally. The tight and complicate epigenetic regulatory mechanisms are most likely the key to maintain pluripotency, trigger and control cell-lineage specific differentiation, but very little is known about exactly how these are done. Over the last several years of intensive study about the genetic networks controlling mesenchymal stem cell differentiation, there has been a rough understanding for the key genetic factors involved in this process. However, it is far from fully understood that how these genes are regulated upon induced differentiation.

Taking advantage of the animal models being established in the other subprojects, we will study the detailed Epigenetic regulatory mechanisms during the process of mesenchymal stem cell differentiation towards osteoblast, adipocyte and hepatocyte. In addition, the homing and differentiation of the mesenchymal stem cells after transplanting to preconditioned liver and articular cartilage damaged recipients will also be evaluated. The criteria we will be analyzing includes the changes of overall gene expression and microRNAs expression profile at specific differentiating time point as indicated by lineage and stage specific markers. We will pay particular attention on the epigenetic modifiers (DNA methyltransferases, histone modifying proteins and functional non-coding RNAs), as well as lineage specific signaling transducers and patterning factors. We will also study the DNA methylation and histone modification changes of the target genes upon stem cell differentiation.

From these precious dataset, we shall be able to figure out many previously unidentified epigenetic regulatory pathways upon mesenchymal stem cell (MSC) differentiation towards osteoblast, adipocyte and hepatocyte. We will also have the opportunity to reveal the genetic and epigenetic regulatory network from the initial epigenetic modification to the cascades that trigger stem cells to differentiate into different lineages. These analyses can significantly facilitate the

establishment of the most efficient in vitro differentiation and in vivo transplantation procedures attempted by researchers from subprojects 1, 2, 4 & 5. Moreover, the result from these studies will elucidate the mechanisms behind cell plasticity in general and will therefore provide a big breakthrough and can easily be applied to the fields of nuclear reprogramming, stem cell research and regenerative medicine.