腦皮質細胞在二維及三維培養環境下生長特性之研究

Characterization of cortical neurons outgrowth in 2D/3D culture system

中文摘要

中樞神經系統 (centralnervous system ，CNS) 損傷後的神經再生，一直是神經醫學研究的一個重要課題。長久以來，中樞神經損傷後無法再生的難題，雖然歷經許多神經科學家不斷地研究，仍無法突破此一瓶頸。近年來的研究方向多偏向於細胞替換 (cell replacem ent) 及自體修復 (endogenous repair)，希望藉由植入健康細胞或加入一些生長因子等，來增強其修復能力，但不管是細胞替換或自體修復，尋找適合用於神經細胞培養的材料都是相同的重點，因爲不僅要儘量符合體內環境，還要能讓神經細胞維持正常的分化、生長等功能，所以在本篇論文中，我們利用廣泛用於細胞實驗且具有三維立體空間的洋菜膠、膠原蛋白及纖維蛋白，希望能找出較適合神經細胞生長的材料。

在本篇研究中，我們將胚胎第 17 天的鼠腦皮質細胞以平面及立體的模式，分別培養在洋菜膠、膠原蛋白、纖維蛋白及它們的混和物中，藉由觀察神經細胞的生長情形及存活率，來評估各個材料對於神經細胞的影響。結果發現：在膠原蛋白及纖維蛋白中生長的神經細胞，最長存活時間可長達三個星期以上，且神經纖維的伸展也遠優於洋菜膠，在存活率的測試方面則是纖維蛋白優於膠原蛋白和洋菜膠；但是纖維蛋白在平面培養時有細胞大量聚集和 stress fiber的產生，而膠原蛋白則無。由以上結果證明，膠原蛋白及纖維蛋白可提供良好的環境供神經細胞生長及分化，是相當適合神經細胞生長的材料。

英文摘要

There are many researches about neuron regeneration in the central nervous system (CNS) after injury, but many problems have remained to be solved. At present, there are two main approaches for promoting neuron regeneration: cell replacement and endogenous repair using artificial substrate implants. To improve the ability of regeneration by grafting living cells or adding growth factor into the lesion site, it is important to find a good biomaterial for the in-vitro study of neuron survival. A good biomaterial matrix has to supply normal neuronal differentiation and outgrowth. Our study focussed on two and three-dimensional biomaterials such as agarose, collagen and fibrin glue that expect to be a suitable biomaterial for neuron outgrowth.

Cortical neuron were dissected from E17 rat embryos and cultured in agarose gel, collagen gel, fibrin glue and the mixture of collagen and fibrin. We found that the neuron cultured in collagen gel and fibrin glue have longer period of survival (over 3 weeks), and the neurite extension was better than in agarose gel. The survival rate using MTT and LDH assay, fibrin glue was the most suitable biomaterial for neuron survival among biomaterials examined. In 2D fibrin plating, neuron cells had cell aggregation and stress fibers, but did not find the same result in collagen gel. There is

no difference of neurite extension and survival in the mixture of collagen and fibrin. The results suggest that collagen and fibrin can provide a suitable substrate for neuronal survival and differentiation