Form 3

Abstract of Thesis

Name（MEHDI KHANMOHAMMADI）	
Title	Development of Natural Polysaccharide Hydrogels via Enzyme-mediated Cross-linking as Novel Substrata in Tissue Engineering （酵素反応による架橋形成を介した天然多糖由来の組織工学用新規足場基材ヒドロゲルの開発）

Abstract of Thesis

Hydrogels with the ability to support cells structurally and biochemically have emerged as promising scaffold materials for the fabrication of tissues *in vitro*. Selecting suitable hydrogel materials and methods of manufacturing these matrices can produce tissues with desirable characteristics. This research study describes the synthesis and fabrication of appropriate hydrogel matrices for two-dimensional (2D) and three-dimensional (3D) cell culture systems utilizing biocompatible and biodegradable chitosan- and hyaluronic acid (HA)-derivative hydrogels.

Chapter 1 briefly reviews the background and purpose of the present study, which aims to develop and apply functionalized chitosan- and HA-derivative hydrogels as scaffold materials for cell culture systems. In addition, previous research history is described to identify the problems and solutions that should be considered, and the overall framework of the study is highlighted.

Chapter 2 describes the synthesis and characterization of galactosylated chitosan hydrogel sheets obtained via horseradish peroxidase (HRP)-catalyzed oxidative cross-linking of a phenolic (Ph)-substituted chitosan (chitosan-Ph) for tissue engineering applications. The degree of galactose moieties incorporated into the chitosan hydrogel modulates its physical properties and also the behavior of hepatic cells cultured on the hydrogel.

Chapters 3 and 4 describe the production process of cellularized spherical and single/bundled fiber-shaped tissue constructs obtained using coaxial double-orifice spinnerets through enzymatic cross-linking and degrading of Ph-substituted HA (HA-Ph). The encapsulated cells within the HA-Ph spherical and fiber-shaped hydrogel vehicles maintained high cell viability during extended culture under physiological conditions. The hydrogel vehicle dimensions were controllable by altering the flow velocities of the co-flowing streams and cross-linking reactant concentrations. These hydrogel vehicles were strong enough to maintain their structural integrity in culture media during the incubation period and the encapsulated cells were not released through the hydrogel membrane following hydrogel dissociation. Furthermore, considerable encapsulated cell proliferation was observed within the HA-Ph hydrogel vehicle hollows. Cell adhesion on the HA-Ph hydrogel membrane surface was achieved through surface modification of the HA-Ph hydrogel vehicles with gelatin-Ph. Formation of spherical and filament-like single/bundled tissues covered with a heterogeneous cell layer was confirmed from the remaining spherical and filament-like tissues, respectively, after degradation of the HA-Ph hydrogel membrane by hyaluronidase. The resulting cellular tissue-engineered constructs had the ability to promote tissue regeneration by providing a biomimetic extracellular matrix of native tissue.

Chapter 5 describes covalent cross-linking of low molecular weight HA (LMWHA) as a stimulator for endothelial cell (EC) migration within the gelatin-based hydrogel. LMWHA with Ph-hydroxyl moieties (LMWHA-Ph) cross-linked through the HRP-catalyzed reaction was immobilized within the gelatin-based hydrogel. This approach resulted in activation of ECs with a higher motility response as well as extensive cytoskeleton elongation and spreading, highlighting the potential to utilize the synthesized hydrogel for fabricating dense and vascularized tissues *in vitro*.

様式7

論文審査の結果の要旨及び担当者

氏　名	（MEHDI KHANMOHAMMADI ）	
論文審査担当者	（職）	氏　名
	主査　教授	田谷　正仁
	副査　教授	馬越　大
	副査　教授	境　慎司
	副査　教授	出口　真次

論文審査の結果の要旨

本研究では、生体適合性の高い天然高分子を原料として２次元あるいは３次元構造をもつ細胞培養場を提供するヒドロゲルの開発を行った。まず天然高分子としてキトサンを選び、ガラクトース基とフェノール性水酸基（Ph）を導入したキトサン誘導体を合成した。このキトサン誘導体を、西洋わさび由来ペルオキシダーゼ（HRP）の酵素反応によりPh基間に架橋を形成させてゲル状のシート構造物を作製した。ガラクトース基の導入量とヒドロゲルの物理的特性の相関、およびゲル構造物上における肝臓細胞の増殖や機能発現を評価し、肝組織構築用基材としての有用性を示した。次に、Ph基導入ヒアルロン酸誘導体から、細胞で覆われた球状や繊維状構造物を作製した。作製のための条件をコントロールすることにより、ゲル構造物のサイズなどを容易に規定できることを明らかにするとともに、細胞培養期間中に細胞の漏出を生じさせることなく安定して培養を行うことのできるゲル構造物を得る条件を確立した。さらに、それらのゲル構造物中で細胞を成長させた後に、ヒアルロン酸分解酵素でゲルを分解することで、異種細胞に覆われた球状および繊維状の複合組織体を作製することに成功した。アルロン酸の利用性をさらに広げるため、Ph基を導入した低分子量化したヒアルロン酸誘導体を調製した。そして、HRP酵素反応により、同じくPh基をもつゼラチン誘導体へ導入することで、新規なヒドロゲル材料を開発した。このヒドロゲルに対するヒト血管内皮細胞の挙動を定量的に調べたところ、ゲル内に配置された細胞の遊走性が促進される効果があることが明らかとなり、血管網を含む培養組織体を構築するマトリクス材料として有用であることが実証された。

以上のように、本研究は生体適合性の高い天然高分子の機能や特性を活かしつつ新たな細胞培養基材の設計とその有効性を実証するものであり、博士（工学）の学位論文として価値のあるものと認める。