(Form4-2)

JOINT RESEARCH PROJECT

FINAL REPORT
For Japan-Korea Joint Research Project

AREA	1. Mathematics & Physics (2.) Chemistry & Material Science 3. Biology 4. Informatics & Mechatronics 5. Geo-Science & Space Science 6. Medical Science 7. Humanities & Social Sciences

1. Research Title:

Preparation of biocompatible hydrogels for tissue engineering applications

2. Term of Research: From July, 2009 To June, 2011

3. Total Budget

a. Financial Support by JSPS: Total amount: 2,400 thousand yen

1st Year 900 thousand yen 2nd Year 1,200 thousand yen

3rd Year 300 thousand yen

b. Other Financial Support : Total amount: 0 thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Yasuhiko Iwasaki
Institution / Department Position	Faculty of Chemistry, Materials and Bioengineering, Kansai Univ. / Professor
b. Korean Principal Researcher	
Name	Gilson Khang
Institution / Department Position	Dept. of Polymer Nano Sci. & Tech. Chonbuk National Univ. / Professor

c. List of Japanese-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Nobuyuki Morimoto	Department of Metallurgy, Materials Science, and Materials Processing, Graduate School of Engineering Tohoku, University	Associate Professor

d. List of Korean-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Soon Hee Kim	Department of Polymer/Nano Science and Technology, Chonbuk National University	PhD candidate
Joon Hee Lee	Department of Polymer/Nano Science and Technology, Chonbuk National University	Ms candidate

5. Number of Exchanges during the Final Fiscal Year*

a. from Japan to Korea

*Japanese fiscal year begins April 1.

Name	Home Institution	Duration	Host Institution
For Final Fiscal Year(FY2011) Total: 0 persons		For Final Fiscal Year(FY2011) Total: 0 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 3 persons FY2010: Total 2 persons			

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution
Gilson Khang	Chonbuk National University	2011.5.31–2011.6.3	Kansai University
For Final Fiscal Year(FY2011) Total: 1 persons		For Final Fiscal Year(FY2011) Total: 4 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 0 persons FY2010: Total 0 persons			

6. Objective of Research

The ultimate purpose of tissue engineering is to recover and replace anatomical structures, damages to organs functions, injuries, and loss of tissues or organs. For the past years, regenerative medicine has been used to recover damages to peripheral nerves and spinal cords, to hybridize biological materials, to treat cells, and to develop drug delivery technologies. To optimize nerve regeneration, components such as supporting system, supporting cells, growth factors, and extracellular matrix (ECM) are important. Biological materials must aid in stabilizing the physical status of the body and growing cells into tissues or organs. Also, the supporting system, created to regenerate nerves, must be close to a natural status, appropriate to the physical body, low immunity, biodegradability, absent of toxic, and equipped with appropriate physical properties.

Porcine small intestinal submucosa (SIS) has been widely used to regenerate discs and divide skeletal muscles. Because the small intestine is composed of more than 90% of type I and II collagen in the body, hyaluronic acid, glycoprotein such as fibronectin, and growth factors such as FGF-2, it provides mechanical strength to ECM and controls the function of cells including its adhesion, growth, transfer, division, and so on. Also, SIS is known of to treat more than areas such as the treatment of various types of hernias, skin injuries, sclero, suture, urinary incontinence, pelvic organ prolapse, Peyronie's symptom, and penis plastica. Also, SIS, suitable to process, may be used for various medical purposes such as sheets, tubes, and cylinders.

In addition, it has been confirmed recently that the SIS is transplanted into damaged sciatic nerve which successfully resulted in a restoration of a nerve accompanied by both the restoration of neuraxis and myelin sheath. Also, it has been found out that the SIS is relatively greater in its mechanical solidity than a SIS of a mouse, thus it better suits in the application of tissue engineering.

Olfactory ensheathing cells (OECs) are known for its function as an induction material that motivates the potential for restoration of motility as well as the restoration and recovery of an axon when it is transplanted into a complete amputated spinal cord. In addition, it leads the functional restoration and restoration of a damaged spinal cord and accelerates the protection of a nerve by secreting antineuritic material such as neuropeptide Y. However, OECs only exist in an infinitesimal volume, which make it difficult for incubation in a large volume. Therefore, the research regarding the ways to safely increase the volume of OECs by creating an optimal incubating environment.

Previously, we reported that various scaffolds such as SIS, silk and keratine enhance the attachment and proliferation of cells. Also, we carried out spinal cord regeneration using Schwann cell in SIS sponge and confirmed that SIS revealed an important role for nerve generation. On the basis of our previous results, this research is carried out the possibility for the application of OECs, which is responsible for one of the most important role in a restoration of the central nerve system, in order to measure the proliferation, attachment and behavior of a cell as well as the suitability against the incubation of OECs.

7. Methodology

Preparation of SIS sheet

SIS were isolated by using Badylak method. Sections of porcine jejunum were harvested from market pigs. To separate SIS in porcine jejunum, fat is firstly removed from porcine jejunum, followed by careful washing with water. The porcine jejunum cut in lengths of approximate 10 cm and then washed with a saline solution. SIS was obtained by mechanical removal of the tunica serosa and tunica muscularis. The obtained SIS was washed again with a saline solution. The SIS was prepared by longitudinal cutting in the longitudinal direction, followed by freeze drying using freeze dryer.

Culture of OECs

OECs were isolated from Fisher rats (Japan SLC Inc., Japan). Briefly, the olfactory nerve layer was peeled away from the rest of the olfactory bulb, then washed with PBS and dissociated with 0.1% collagenase and incubated at 37 °C for 30 min. The cells were then suspended on uncoated flasks twice, each for 18 h and 36 h at 37 °C in 5% CO2. After 48 h, the non-adhesive cell suspension was collected and then seeded onto flasks pre- coated with poly-L-lysine (PLL, 0.1 mg/ml).

Cell Attachment

The OECs suspension (approximate 3×104 cells/cm2) was seed on the sheets in a 24-well plate. The cells were incubated overnight to allow for cell attachment. Cell viability was determined by using water-soluble enzyme substrate MTT.

Cell Morphology

To observe cell morphologies the surfaces were washed with PBS and the cells attached on the surfaces were fixed with 2.5% glutaraldehyde in PBS for 24 h at room temperature. After thorough washing with PBS, the cells on the surfaces were dehydrated in ethanol graded series (50, 60, 70, 80, 90, and 100%) for 10 min each and allowed to dry in a clean bench at room temperature. The cell-attached surfaces were gold deposited with plasma sputter (Emscope, Model SC 500K, UK) and examined by SEM with a tilt angle of 45o. The obtained image was analyzed with image analysis program P- SEM (Mirero, Korea).

RT-PCR

In terms of genes, to research the characteristic conservation of OECs in SIS sheet, PLL-coated well plate and the well plate, reverse transcriptase polymerase chain reaction (RT-PCR) technique were performed.

Immunohistochemistry

To examined the adhesion and proliferation is SIS sheet, we performed immunochemical staining.