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Volume B:

Synthesis and Function Control of Biofunctionality Materials

New Functionality Materials

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T. Tsuruta, M. Doyama, M. Seno, Y. Imanishi

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Volume B:
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List of volumes:

Volume A: Optical and Quantum-Structural Properties of Semiconductors

I Atomic-scale control of crystal growth
II Control of localized electronic states
III Creation of new materials with novel optical functions
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Preface

Over two hundred Japanese scientists (most of them are university professors) have just finished their six-year project research, that is, a Priority Area Research Program for "New Functionality Materials: Design, Preparation and Control", which was supported by the Ministry of Education, Science and Culture, Japan, in the fiscal years from 1987 to 1992.

The objective of this priority area research program is to promote researches which will create novel functional materials through fundamental researches, at atomic or molecular level, on the nature of functionality of materials – metals, semiconductors, ceramics, organic polymers, and their composites or hybridized materials. The multidisciplinary composition of the research group was intended to provide opportunities for cross-fertilization of ideas among researchers having different backgrounds to approach their own goals.

This book was edited to present, to the international communities, a comprehensive volume which contains review articles describing results of researches carried out by all the members of this project research program in the second three-year period from 1990 to 1992. The contents of this book are organized by arranging chapters and sections in accordance with the relevant research groups and subgroups:

[A] Optical and Quantum-Structural Properties of Semiconductors:
(1) atomic-scale control of crystal growth; (2) control of localized electronic states; (3) creation of new materials with novel optical functions; (4) quantum structures and new properties; (5) control of material properties for novel optical devices.

[B] Synthesis and Function Control of Biofunctionality Materials:
(1) control of specific response at interface; (2) transduction of biosignals; (3) activation of biosystems; (4) control of cell functions; (5) acceleration of tissue construction.

[C] Synthetic Process and Control of Functionality Materials:
(1) theoretical study on functionality of materials; (2) syntheses of functionality materials using novel processes; (3) reaction design for synthesis of functionality materials; (4) design of functionality of materials with supramolecular structure; (5) design of functionality materials under controlled interfacial reactions.

I believe this book will serve as a valuable asset to scientists in academic, governmental and industrial institutions, especially because they will recognize from this book newly developing research areas of functionality materials which were brought about only through the intensive cooperation between multidisciplinary researchers.

I express my sincere acknowledgement to the Ministry of Education, Science and Culture, Japan, for its support to this project research program. I am grateful also to all the contributors for their fine review articles and to the four editors (M. Doyama, S. Fujita, Y. Imanishi, and M. Seno) for their valuable cooperation.

Teiji Tsuruta
Editor-in-Chief
Tokyo, March, 1993

Introduction

Biofunctionality materials are defined as key materials which support medical engineering and biological engineering, covering biospecific materials and biosimulating materials. Medical engineering enhances human welfare by developing innovative therapy methods such as artificial organ transplantation and signal-responsive drug delivery. Biological engineering also enhances human welfare by developing innovative production methods such as biological production and bioreactor systems. The evolution of both engineering fields becomes possible through the development of key materials in each field. Biospecific and biosimulation materials are vital to the support of medical and biological engineering, respectively. These situations are summarized in Figure 1.

Biospecific materials function at the materials/biological system interface by controlling the specific recognition function of the biological system including proteins, cells, and biological tissues. Typical examples of biospecific materials are the biocompatible materials used for artificial organs.

Biosimulation materials possess exquisite and efficient functionalities similar to the biological system. Artificial enzymes, artificial cells, and artificial muscles are typical examples of biosimulation materials.

Biofunctionality materials are synthesized from the viewpoint of functionality design. In order to create innovative biofunctionality materials, the functionality design should be done by standing ourselves on the atomic and molecular level in an interdisciplinary field without boundary lines between inorganic, organic, and polymer materials fields.

With this understanding in mind, the investigation on synthesis and function control of biofunctionality materials has been supported by Grant-in-Aid for Scientific Research in Priority Areas, Ministry of Education, Science and Culture, Japan, from 1987 to 1993. This national project was executed under cooperation of five subgroups and yielded marvelous success of new ideas, new techniques, and new products in the biofunctionality materials field. These successful results will be described in this volume.

B-1, Control of specific response at interface: In this subgroup, cell response against biosignals through specific receptors and nonspecific cell response toward physico-chemical signals were investigated. Biocomposite materials having the control function of cell response were synthesized. The final goal of the investigations is the development of biocompatible materials, bioreactor materials, and materials for separation and therapy.

B-2, Transduction of biosignals: This subgroup investigated synthesis and function control of molecular materials which accept, transduce, and amplify molecular information of various types. The control system of molecular information was synthesized, which undergoes acceptance, transduction, and amplification of molecular information.

B-3, Activation of biosystems: This subgroup aimed at understanding the molecular mechanism of activation of specific cells such as antibody-secreting cells. On that basis, the development of effective delivery system for bioactivation factors such as drugs, nucleic acids, enzymes, and physiologically active proteins to a specific site of living body was investigated. The delivery to living bodies and a specific cell of bioactivators was succeeded.

B-4, Control of cell functions: This subgroup investigated artificial construction of intracellular organelle and control of cell functions with biointerfaces made of synthetic polymers or synthetic extracellular matrices (ECM). The mechanism of ECM transmitting biological informations through biointerfaces was clarified. On this basis, functionality material systems, which control functionalities of specific cells, were created and constructed.

B-5, Acceleration of tissue construction: This subgroup investigated the development of artificial matrices on which living cells are stably adhered and the construction of cell assemblies possessing higher-order structure which mimics living tissues or organelle. Biolized artificial organs for lever and blood tube were synthesized on the basis of investigations on the interactions between cells, cell and matrix, and cell and cell-growth factors.

Yukio Imanishi
Editor of Volume B

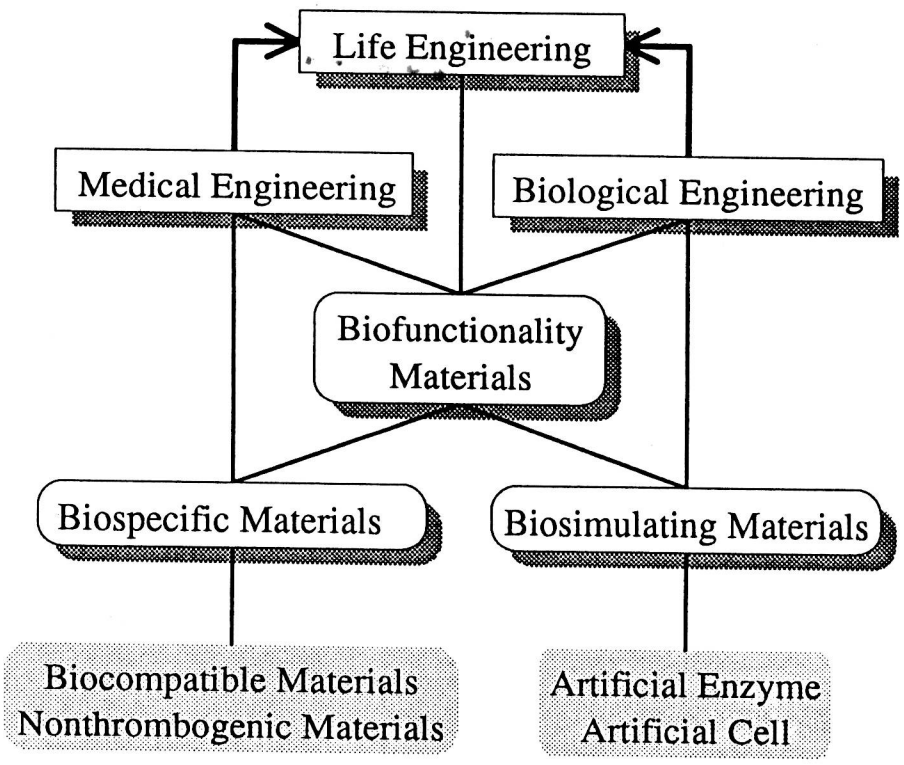

Figure 1. Biofunctionality materials supporting life engineering and human welfare.

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III. Activation of biosystems

IV. Control of cell functions

V. Acceleration of tissue construction

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I. CONTROL OF SPECIFIC RESPONSE AT INTERFACE

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T. Tsuruta, M. Doyama, M. Seno, Y. Imanishi (editors)
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Enhanced growth of anchorage-dependent cells on immobilized cell-growth factor

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The growth of anchorage-dependent cells in serum-free culture media was strongly accelerated on cell-growth factors immobilized on nonbiodegradable polymer membrane. The extent of acceleration was dependent on the nature of cell-growth factors immobilized and the hydrophilicity/hydrophobicity balance of matrix membrane. A synergistic acceleration of cell growth was observed with coimmobilized cell-growth and cell-adhesion factors. The cooperative effect of both kinds of factors on the cell-growth acceleration was interpreted in terms of interreceptor interactions in cell mambrane. These experimental results led us to the synthesis of a chimera protein in which and insulin molecule is connected with a core peptide, RGDS, involved in the active site of cell-adhesion proteins. The chimera protein was found to be more potent than insulin for the cell-growth acceleration. Finally, coimmobilization of a cell-adhesion factor, collagen, with a cell-growth factor, insulin or transferrin, on polyurethane tube brought about greater acceleration of the growth of endothelial cells. The endothelial cells grown on the polyurethane tubes kept a normal cobblestone-like appearance and maintained the ability to secrete prostacyclin for more than 9 months. These experimental results indicate that the present method of controlling cell functions is useful for development of biocompatible materials by *in situ* endothelialization and also for serum-free production of physiologically active substances in bioreactor.

1. INTRODUCTION

Control of cell functions with polymeric compounds is essential in the investigation of artificial-organ materials and cell-engineering materials. In this respect, it has been reported that cell adhesion on to polymer membrane was enhanced by immobilization of cell-adhesion factors such as collagen [1-3], fibronectin [4-6] and vitronectin [7,8], or by immobilization of a tetrapeptide, Arg-Gly-Asp-Ser, which is involved in the active site of cell-adhesion proteins [9]. We have recently reported for the first time that the cell-growth factors immobilized on to nonbiodegradable polymeric membrane accelerated the growth of fibroblast cells more than did the soluble proteins [10]. The discovery that immobilized biosignal proteins control the adhesion and growth of living cells prompted us to investigate comprehensively the effect of immobilized cell-adhesion and cell-growth factors on the adhesion and growth of living cells.

2. ADHESION AND GROWTH OF FIBROBLAST CELLS ON POLY-(METHYL METHACRYLATE) MEMBRANE IMMOBILIZED WITH PROTEINS OF VARIOUS KINDS

The surface of poly(methyl methacrylate) (PMMA) membrane was partially hydrolyzed and the carboxyl groups produced were coupled with various protein

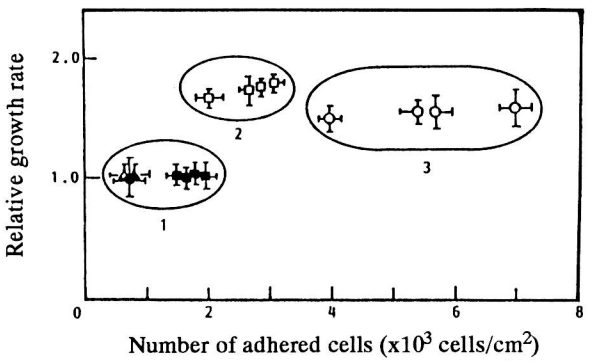

Figure 1. Relationship between adhesion and growth of fibroblasts on protein-immobilized poly(methyl methacrylate) (PMMA) membrane in serum-free Dulbecco's modified Eagle medium (DME). (△), Immobilized albumin; (■), immobilized fibrinogen; (●), immobilized γ-globulin; (□), immobilized insulin; (○), immobilized fibronectin; (▲), control. Bars represent standard deviation (n=6).

molecules with water-soluble carbodiimide (WSC). The immobilized proteins were a cell-growth factor insulin, cell-adhesion factors fibronogen and fibronectin, and serum proteins albumin and γ- globulin. The influence of immobilized proteins on the adhesion and the growth of mouse fibroblast cells STO was investigated in a 0.2 % EDTA/(-)PBS and Dulbecco's modified Eagle minimum essential medium (DME) without serum, respectively and the experimental results are shown in Figure 1. The insulin-immobilized PMMA membrane strongly accelerated the growth and slightly accelerated the adhesion of fibroblast cells. The immobilized fibronectin and fibrinogen enhanced the cell adhesion, and the former also accelerated the cell growth. The immobilized albumin and γ-globulin influenced the adhesion and growth of cells very little.

It is considered that albumin and γ-globulin were ineffective toward fibroblast cells for the absence of specific receptors. Since fibroblast cells possess receptors specific to fibronectin and fibrinogen, cell adhesion and extension are enhanced on the PMMA membrane immobilized with these proteins. Though fibronectin also enhanced cell growth, the effect of fibrinogen on the growth of cells was negligible. In the previous experiment, the acceleration of cell growth by collagen was midway between those by fibronectin and fibrinogen [11]. It is therefore feasible to say that the cell-adhesion activities and the cell-growth activities of cell-adhesion factors depend on the nature of each protein. Since fibronectin shows high activities of cell adhesion and cell growth, the enhanced cell growth by fibronectin should be closely related to its high cell-adhesion activity.

It is very interesting that immobilized insulin enhanced the cell adhesion, whereas free insulin does not do so. Cell adhesion could have been enhanced by aggregation of receptors, which are coordinated by immobilized insulin ligands. This kind of receptor interaction should increase the ligand affinity of receptors [12]. It should be noted in this regard that the mechnism of cell adhesion by insulin is different from that by fibronectin.

3. INTERACTIONS OF FIBROBLAST CELLS WITH INSULIN IMMOBILIZED ON 2-HYDROXYETHYL METHACRYLATE / ETHYL METHACRYLATE COPOLYMER MEMBRANE

In order to investigate the effects of the nature of matrix membrane on which cell-growth factor is immobilized on adhesion and growth of anchorage-dependent cells, experiments were carried out on adhesion and growth of mouse fibroblast cells STO in DME without serum or containing 10 wt% fetal calf serum (FCS) (DME-FCS) in the presence of 2-hydroxyethyl methacrylate (HEMA)/ethyl methacrylate (EMA)

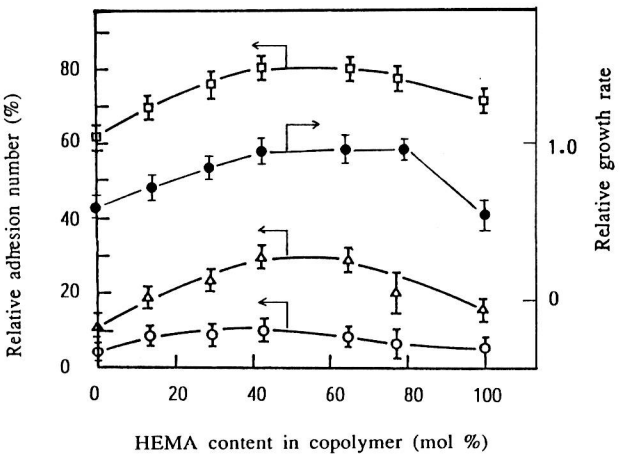

Figure 2. Adhesion after 1 h (○), 4 h (△), and 8 h (□) and relative growth rate (●) of fibroblast cells on HEMA/EMA copolymer membrane in serum-free DME culture medium.

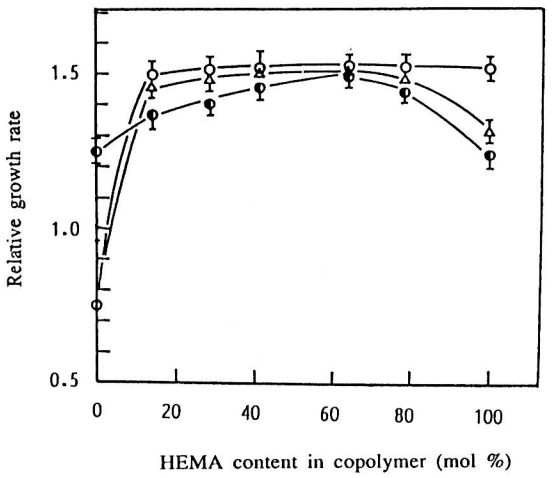

Figure 3. Growth of fibroblast cells on insulin-immobilized HEMA/EMA copolymer membranes in serum-free DME culture medium: (△), immobilized insulin (0.10 μg/cm^2); (○), immobilized insulin (0.30 μg/cm^2); (◐), free insulin (10 μg/ml) added.

copolymer membranes immobilized with insulin. The HEMA/EMA copolymers were obtained by copolymerization of monomer mixture in DMF solution at 60 °C for 2 h. Insulin was immobilized on to the copolymer membranes by the CNBr method [13].

Experimental results are shown in Figures 2 and 3. When the copolymer membrane was without insulin immobilization, the cell adhesion and growth were suppressed on the copolymer membranes containing very high or very low content of HEMA. The same effect of the copolymer composition was observed on the copolymer membrane immobilized with insulin of small amounts (0.10 μg/cm^2). Large amounts of immobilized insulin (0.30 μg/cm^2) remarkably enhanced cell growth and masked the effect based on the copolymer composition. The immobilized insulin enhanced the cell growth more than free insulin.

We have previously reported that the different effects on growth enhancement by immobilized and free insulin depended on the nature of matrix membrane for immobilization, PMMA or poly(ethylene terephthalate) [14,15]. In the present study, the different effects between immobilized and free insulin depended on the composition of matrix membrane. A suitable choice of matrix membrane will develop new possibilities of this novel cell culture technique.

4. ENHANCEMENT OF FIBROBLAST CELL GROWTH ON INSULIN/FIBRONECTIN-COIMMOBILIZED MEMBRANE

As described in the preceding chapters, immobilization of biosignal molecules on nonbiodegradable polymer membranes seems a promising method for synthesis of bioactive materials controlling cell functions. The immobilized cell-growth factors accelerated the growth of fibroblast cells. In

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