Proceeding Book

The 2nd International Joint Symposium on Oral and Dental Sciences

Featuring:

Next Generation of Regenerative Therapies in Dentistry

In Commemoration with

The 64th Anniversary of The Faculty of Dentistry
Universitas Gadjah Mada,
Yogyakarta, Indonesia

Inna Garuda Hotel, Yogyakarta, Indonesia, March 1-3, 2012
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The Indonesian Journal of Dental Research
Faculty of Dentistry, Universitas Gadjah Mada,
Yogyakarta 55281, Indonesia

ISBN:
978-602-9461-16-9
Proceeding Book:

The 2nd International Joint Symposium on Oral and Dental Sciences

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Next Generation of Regenerative Therapies in Dentistry

Program Book and Proceeding Book of the Symposium Can Be Downloaded from:

http://ijm2012.ugm.ac.id
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Address from the Chairperson of the Symposium

It is great pleasure to invite you to “The 2nd International Joint Symposium on Oral and Dental Sciences”. The event is held to commemorate the 64th anniversary of The Faculty of Dentistry, Universitas Gadjah Mada, which has been serving as an academic institution concerning oral and dental sciences to community. This symposium is the continuation of the first fruitful meeting in Bali (2010).

Current clinical technologies, in terms of regenerative medical therapies related to organ transplantation and artificial organ, have been excellent life-saving and life-extending therapies to treat patients who need to reconstitute diseased or devastated organs or tissues as a result of an accident, trauma, and cancer, or to correct congenital structural anomalies. In this context, tissue engineering and DDS (drug delivery system) technology are considered fast growing approaches that play important roles in periodontology, implantology, oral and maxillofacial surgery, and other oral and dental treatments.

We are proudly present world leading scientists and clinicians in the area of tissue engineering and DDS technology, as well as on oral and dental sciences. We will bring together the international community of persons engaged or interested in the field of oral and dental sciences and promotes education and research within the field of oral and dental sciences. We provide a platform where international research colleagues, especially young ones, can share the latest results of research, discuss problems, and envisage a new horizon of oral and dental sciences.

It is a significant platform for the fostering of interactions among scientists, clinicians, and those engaged with funding, regulatory and commercial endeavors. This is a unique opportunity for all stake holders in this exciting field to come together in the beautiful city of Yogyakarta to promote the worldwide advancement of oral and dental sciences; in particular the integration of advanced technologies and clinical needs in our region.

We would like to welcome you in Yogyakarta.

Sincerely,

Dr. drg. Ika Dewi Ana
Chairperson of the Symposium
Address from the Chairperson of The 64th Anniversary of the Faculty of Dentistry, Universitas Gadjah Mada

Dear Symposium Guest Speakers and All Participants,

Welcome to the Symposium which is held as a part of the programs to commemorate The 64th Anniversary of The Faculty of Dentistry, Universitas Gadjah Mada.

Scientific communication in the international level among researchers is important to share knowledge, technology, and to keep in touch with updated progresses in oral and dental sciences. The First International Joint Symposium was held by Universitas Gadjah Mada in collaboration with The University of Tokushima (Japan) and Niigata University (Japan). The first symposium was held in conjunction with “Scientific Meeting on Implant and Aesthetic Dentistry for Next Generation of Dental Therapy” on December 15-18, 2010 in Kuta, Bali, Indonesia. The symposium attracted general practitioners and academicians not only from Indonesia and Japan, but also from Malaysia, Netherlands, Hongkong, and South Korea. Besides having fruitful and productive discussions on the state of the art of oral and dental research, progresses in educational systems among the country involved, participants were able to enjoy dental and medical technology exhibition.

Considering the above driving forces and the uniqueness of the country in relation to researches in dental sciences, the committee of The 64th Anniversary of The Faculty of Dentistry Universitas Gadjah Mada would like to facilitate it by holding The Second International Joint Symposium on Oral and Dental Sciences in Conjunction with Dental Specialist Seminar. This forum is hoped to be a starting point to expand scientific activities and scientific collaborations between the Faculty of Dentistry, Universitas Gadjah Mada, international collaborators, industries (business institutions), alumni, and community (government).

Aside from the above activities, we have been coordinating some events such as: Mini Lecture on Bone Graft (October 25, 2012), National Students Scientific Writing Competition and Dentistry Olympiad (November 21-27, 2012), Alumni Meeting (December 10, 2012) and some other cultural events. By holding the events, we would like to facilitate the co-creation among faculty members and supporting staffs of The Faculty of Dentistry as mentioned in our anniversary theme: Co-creation towards Academic Excellence.

We hope you all to enjoy the programs of the anniversary and enjoy fruitful discussion during the symposium.

Sincerely,

Prof. Dr. drg. Haryo Mustiko Dipoyono, SpProst(K).
Chairperson of The 64th Anniversary Committee
Address from the Dean of The Faculty of Dentistry,
Universitas Gadjah Mada

Dear Honorary Guests and Symposium Participants,

It is our great pleasure to welcome you in The 2nd International Joint Symposium on Oral and Dental Sciences, which is held in commemoration with The 64th Anniversary of The Faculty of Dentistry, Universitas Gadjah Mada.

As mentioned in its Strategic Plans (2008-2012), Universitas Gadjah Mada (UGM) has its vision to be a World Class Research University which is excellent, independent, dignified, inspired by Pancasila, the five-basic principles, and dedicated to the needs and welfare of the nation and the world. The vision is done by UGM mission: To promote excellent teaching-learning opportunities and community service through research, with its special mission on research: To promote excellence in educational activities, research, and community service with the interest of the Indonesian society and to participate in Indonesian socio-cultural building.

Faculty of Dentistry should play significant roles towards the vision of the university. For this, research (scientific activity) indeed is one of important factors towards the vision. This symposium is dedicated to encourage faculty members, clinicians and practitioners, as well as alumni to contribute more to scientific knowledge.

We hope the success of the symposium and the overall programs to commemorate The 64th Anniversary of The Faculty of Dentistry Universitas Gadjah Mada. We do hope the advancement in education, research and community services will be achieved in near future by our synergy.

Sincerely,

Prof. Dr. drg. Iwa Sutardjo RS., SpKGA(K).
Dean of The Faculty of Dentistry
Universitas Gadjah Mada
The 64th Anniversary of the Faculty of Dentistry,
Universitas Gadjah Mada

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IMMOBILIZATION OF BOMBYX MORI'S SERICIN ON POLY(L-LACTIC ACID) FILMS AND ITS EFFECT ON SURFACE HYDROPHILICITY

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Abstract

Poly(L-lactic acid) or PLLA as one of tissue engineering material have demonstrated low cell interaction capability due to its hydrophobicity. Bombyx mori's sericin, a natural polymer protein, contains large amount of polar functional group which has hydrophilic characteristic. The aim of this study was to modified PLLA surface using Bombyx mori's sericin and to investigate its effect on surface hydrophilicity. Three concentration of sericin (2.5, 5, and 10 mg/ml) were applied on PLLA surface modification using carbodiimide chemistry. Surface characterization using Fourier Transform-Infrared Spectroscopy (FTIR) was performed to observe the changes on surface-modified PLLA. The evaluation of surface hydrophilicity was made through water contact angle measurements. FTIR graphs showed that new absorption bands located at 1597 cm⁻¹ has appear assign to the amide bond (N-H) which indicates that sericin was successfully immobilized to PLLA surface by carbodiimide chemistry. The contact angle of PLLA surface was measured to be ±78.38° and decreases to about 42° after modified with sericin. The conclusion was PLLA surface can be modified with Bombyx mori's sericin and surface hydrophilicity was enhanced.

Keywords: Sericin, PLLA, surface hydrophilicity.

Introduction

In the field of biomaterials, the nature of biomaterial surface has been shown to be critical for biocompatibility. A large number of research groups have extensively studied the effect of the surface hydrophilicity on the interactions of cell-substratum since hydrophilicity is one of the most important parameters when biomaterials are design. Poly(L-lactic acid) or PLLA, one of biodegradable polymers, has been an attractive candidate for scaffold material. Significant mechanical strength and slow degradation rate are the key features of PLLA for its popular use as a biomaterial in tissue engineering [1]. On the other hand, as hydrophobic polymer PLLA-based scaffold surface have demonstrated less protein and cell interaction capability. Therefore it cannot effectively facilitate attachment and growth of many types of cells [2], which leads to failed new tissue formation. To overcome those limitations, several approaches to immobilize various biomolecules on PLLA surface have been used to improve the biological functions without changing its bulk properties [3, 4].

Sericin, a natural protein derived from arthropods e.g. silkworm Bombyx mori, has recently been investigated for its activities in the biotechnological field. Sericin protein is highly hydrophylic and made of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl and amino groups and is characterized by high Serine [5]. It can be coated on polymeric biomaterial surface by chemical reaction using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS) as coupling
agents, to enhance functionality in promoting cellular function [6].

In this study, carbodiimide chemistry were used to immobilized Bombyx mori's sercin on PLLA surface and hydrophilicity of the surfaces were investigated.

Materials and Methods

Materials

PLLA (Mw 58.000-160.000) were purchased from Sigma, fresh cocoon of Bombyx mori were kindly donated by PT Tugu Mas Sutra Alam Yogyakarta, Na₂CO₃, cellulose membrane (Sigma), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) purum, ≥97.0% (Aldrich), N-hydroxysuccinimide (NHS) 98% (Aldrich)

Preparation of sercin-modified PLLA films

PLLA film was made by solvent casting method. 1,012g PLLA sample was dissolved in 10 ml of chloroform at room temperature. The solution was transferred to a 96mm-diameter glass plate to form a film by evaporation. Chloroform had been allowed to evaporate for a minimum of 10 minutes and then the film was peeled off and dried in vacuum oven for 24 hours to remove the residual solvent.

Cocoon shells of Bombyx mori were cut into pieces (1x1 cm). One liter of 0.2% sodium carbonate solution containing 40g of the cocoon was boiled for approximately 1 hours and then filtered through a glass microfiber filter in order to remove fibroin and other impurities. The filtrate was then dialyzed using cellulose membrane against deionized water for 2 days by changing the water daily to remove the ions and other impurities and then freeze dried at -60°C for approximately 15 hours. Pure PLLA film was placed into 0.1 M NaOH aqueous solution at 50°C for 1 hour then rinsed successively with 0.1 N HCl and distilled water at room temperature. The –COOH groups of the PLLA surface were preactivated for 1 hour at room temperature in phosphate buffered saline (PBS) solution, containing 1 mg/mL of NHS and 10 mg/mL of EDC. The substrates were then transferred to PBS solution containing 5% sercin with different concentrations (2.5 mg/mL, 5 mg/mL, 10 mg/mL). The reaction was allowed to proceed for 24 hours at 4°C to give the PLLA-sercin surface. After the reaction, the reversibly bound silk sercin was desorbed in copious amounts of PBS for 24 hours at 4°C and then dried.

Surface characterization

PLLA films were characterized by Fourier Transform-Infrared Spectroscopy (FTIR) to confirm the immobilization of sercin on PLLA surface. The evaluation of surface hydrophilicity was made through water contact angle method. Contact angles were measured with the sessile drop method. A 5μl water droplet was placed on PLLA surface and the static contact angle was measured using digital camera (Canon 30D) with macro lens EF 100mm 1:2.8. For each reported contact angle value, four measurements on different areas of the surface were obtained.

Results

Surface characterization by FTIR as seen at figure 1 showed that new absorption bands located at 1597 cm⁻¹ has appear assigned to the amide bond (N-H) which indicated that sercin was immobilized on PLLA surface.

![Figure 1. FTIR spectra of (a) pure PLLA; and (b) sercin-modified PLLA](image)

Table 1. Water contact angles on pure PLLA films and sercin-modified PLLA

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<th>Group</th>
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<tr>
<td>PLLA</td>
<td>78.38±1.07</td>
</tr>
<tr>
<td>PLLA+sercin 2.5mg/ml</td>
<td>42.20±1.01</td>
</tr>
<tr>
<td>PLLA+sercin 5mg/ml</td>
<td>41.70±1.76</td>
</tr>
<tr>
<td>PLLA+sercin 10mg/ml</td>
<td>41.83±1.41</td>
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*Average ± standard deviation
Table 1 shows the differences of water contact angles on the PLLA surfaces before and after modification. Sericin-modified PLLA films were more hydrophilic (significant lower contact angle) than that of control PLLA films (p<0.05).

Discussion

In this study, sericin was covalently immobilized using carbodiimide and NHS as coupling agents in three different concentrations (2.5; 5; 10 mg/ml). To covalently immobilized protein molecules in the chemically inert polymeric biomaterials, reactive groups, in this case carboxyl, was firstly introduced as coupling sites. For PLLA, hydrolysis by simply treating in NaOH solution can be used to produce reactive groups. One problem in hydrolysis process is the molecular weight of polymers will be partially sacrificed, thus the reaction conditions should be well controlled [7].

When films was immersed in NaOH solution, the hydrolysis process produced carboxyl on the PLLA surface. It followed by the activation of the carboxyl group with water soluble carbodiimide. NHS were added to form more stable amide bonds. Final reaction were between the activated carboxyl groups and the amino groups of sericin that produced amide bond (N-H). New absorption bands located at 1597 cm⁻¹ assigned to the amide bond (N-H), indicates that sericin was successfully immobilized to PLLA surface by carbodiimide chemistry.

Contact angle, formed on three-phase line of solid/liquid/gas system, provides a simple and convenient way to evaluate the hydrophilicity of film materials. Measurement of the contact angles on PLLA and sericin-modified PLLA surfaces give an indication of the relative hydrophilicity of these surfaces before and after the modification. Ordinarily, more hydrophilic films have smaller water contact angles. All of sericin-modified PLLA groups demonstrated lower contact angle significantly, implying a great improvement of the hydrophilicity. The contact angle of PLLA surface was measured to be 78.38° and decreases to about 42° after modified with sericin.

PLLA is hydrophobic polymer due to the presence of an extra methyl group in lactic acid [1], while sericin contains a large amount of amino acids with polar functional groups such as hydroxyl, carboxyl, and amino groups [8]. The strongly polar groups of sericin was assumed to give contribution to a more wettable surface on sericin-modified PLLA, as indicated by the decreases of contact angles.

Bombyx mori’s sericin was successfully immobilized onto PLLA surface covalently via carbodiimide chemistry. The data of contact angle demonstrated that sericin had improved the PLLA surface hydrophilicity.

References