Workshop on
New Visions for Biomaterials and Regenerative Medicine

16-17 March 2011
Organized by
Professor David Williams, FREng
Editor-in-Chief, Biomaterials

Sponsored by
Tissue Engineering & Regenerative Medicine International Society (TERMIS)

Biomaterials
Biomaterials (Elsevier)

Sree Chitra Tirunal Institute for Medical Sciences & Technology
Thiruvananthapuram, Kerala
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Professor David Williams, FREng
Editor-in-Chief, Biomaterials
President-elect,
Tissue Engineering & Regenerative Medicine International Society (TERMIS)
Director of International Affairs
Wake Forest Institute of Regenerative Medicine, North Carolina, USA
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Emeritus Professor, University of Liverpool, UK

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Tissue Engineering & Regenerative Medicine International Society (TERMIS)

Biomaterials (Elsevier)

Department of Science & Technology,
Govt. of India, New Delhi

Sree Chitra Tirunal Institute for Medical Sciences & Technology
Thiruvananthapuram, Kerala

Endorsed by

Society for Biomaterials and Artificial Organs, India (SBAOI)
Society for Tissue Engineering and Regenerative Medicine, India (STERMI)
Preface

Professor David Williams has been appointed as a Visiting Professor at the Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, India. He has traveled to, and lectured in, India for the last 30 years. In 2010 he was elected as President-elect of TERMIS and will take up the role of President in 2013, when will have responsibility for global developments in tissue engineering and regenerative medicine. He is also Editor-in-Chief of the journal *Biomaterials*.

Although some very good work is performed in India in biomaterials science and tissue engineering, scientists from India are currently under-represented at International conferences and publish less frequently in the major journals than many competing countries.

It was proposed, therefore that, during his March 2011 visit to Thiruvananthapuram, Professor Williams will organize and deliver a Workshop that will be devoted to instructional presentations on the directions that biomaterials science are currently taking, and to guidance on research and publication strategies that will help Indian scientists in this very competitive area.

With this perspective, a two day workshop is organized by Prof. DF Williams. This workshop is sponsored by Biomaterials journal, Tissue Engineering and Regenerative Medicine International Society, Department of Science and Technology, New Delhi and Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram. This Workshop is endorsed by Society for Biomaterials and Artificial Organs (India) and Society for Tissue Engineering and Regenerative Medicine (India).

On behalf of Prof. David F Williams we welcome you all to this Workshop at SCTIMST, Thiruvananthapuram. The encouragement and support we received from Prof. K. Radhakrishnan, Director, SCTIMST and Dr. G.S. Bhuvaneswar, Head, BMT Wing, has been the inspiration in the development of this workshop. We appreciate the speakers for sparing their time, Mr. Willi Paul and Dr. Rekha MR for overall efforts, institute faculty and staff members of Facility for Advanced Drug Delivery Systems (FADDS) and Biosurface Technology Division for their help in organizing this workshop.

Chandra P. Sharma  
Coordinator NVBRM 2011  
BMT Wing, SCTIMST,  
Thiruvananthapuram
New Visions for Biomaterials and Regenerative Medicine

March 16-17, 2011

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Director, SCTIMST

Advisor
Dr. GS Bhuvaneshwar
Head BMT Wing, SCTIMST

Coordinator
Dr. Chandra P. Sharma
Senior Scientist G & Associate Dean, SCTIMST

Treasurer
Mr. Willi Paul

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Dr. Maya A. Nandakumar  Mr. Sujesh Sreedharan
Dr. Mira Mohanty  Dr. Umashankar PR
Dr. Mohanan PV  Mr. Vinod Kumar V
New Visions for Biomaterials and Regenerative Medicine

Programme

Day 1: March 16, 2011
Session I: 'Publishing in High Impact Factor Journals'

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<td>13:30 – 14:00</td>
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<td>Welcome and Introduction</td>
<td>Prof. K. Radhakrishnan</td>
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<td>14:10 – 15:30</td>
<td>Publishing in Biomaterials</td>
<td>Prof. DF Williams &amp; Ms. Peggy O'Donnell</td>
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<td>15:30 – 16:00</td>
<td>Meet Prof. David Williams</td>
<td>Prof. David Williams</td>
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<td>16:00 – 16:30</td>
<td>Tea</td>
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Day 2: March 17, 2011
Session II: Principles of Regenerative Medicine
Chairperson: Dr. G.S. Bhuvaneshwar

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<tr>
<td>9:00 – 10:00</td>
<td>Silk proteins as biomaterial for tissue engineering and regenerative medicine</td>
<td>Prof. SC Kundu</td>
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<td>10:00 – 10:30</td>
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<td>10:30 – 11:30</td>
<td>Biomaterials in Regenerative Medicine</td>
<td>Prof. DF Williams</td>
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<td>11:30 – 12:30</td>
<td>The Essential Materials Paradigms for Regenerative Medicine</td>
<td>Prof. DF Williams</td>
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<td>12:30 – 13:30</td>
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Session III: 'The Way Forward for Regenerative Medicine and Biomaterials Research in India'
Chairperson: Dr. Mira Mohanty

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<td>13:30 – 14:30</td>
<td>Scaffold free tissue constructs for ocular surface regeneration</td>
<td>Dr. TV Kumary</td>
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<td>14:30 – 15:30</td>
<td>Design of Biomimetic Matrix for in vitro Tissue Regeneration with Adult Stem Cells</td>
<td>Dr. Lissy Krishnan</td>
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<td>15:30 – 16:00</td>
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<tr>
<td>16:00 – 17:00</td>
<td>Stem cells and Ceramics for the repair of bony defects in Orthopaedics</td>
<td>Dr. Annie John</td>
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Publishing in Biomaterials
Professor David Williams, Editor-in-Chief
Peggy O'Donnell, Managing Editor
biomaterials@online.be
India March 2011

Publishing in High Impact Factor Journals
Why publish scientific papers
Why publish in scientific journals
What is an Impact Factor
Who decides what papers are accepted for publishing
The basic rules of scientific writing
Why Publish Scientific Papers

There is no point in carrying out research if the results are never analysed, conclusions properly drawn and appropriately disseminated.

Unless constrained by confidentiality (military, product development, industrial know-how, economic forecasting for commercial reasons), the outputs of research should be subjected to review and comment by other scientists and disseminated as widely as possible.

The best indicators of the scientific quality of individuals are their output of scientific papers - importance of CVs.
The quality of research teams and their corporate institutions are judged primarily by their outputs, including scientific papers, - also patents, strategic reviews etc.

Why Publish in Scientific Journals

The main forms of published outputs are edited books, conference proceedings, magazines and peer-reviewed scientific journals.

Edited books are rarely peer-reviewed and the choice of author and subject usually made before the quality of the contribution is known.

Abstracts of conference proceedings are usually reviewed before acceptance of the paper for the conference, but the full papers are rarely reviewed and the quality is very variable.

Magazines are of variable quality, content determined by editorial policy, commercial considerations and topicality of subjects.

Scientific journals also vary in quality but they should always be peer-reviewed, are subject to independent editorial control and are generally widely available and widely read.
What is an Impact Factor

The value of a journal is judged by the impact which its papers have on society.

It is impossible to assess how many people read any one particular paper, although it is possible with some publishers to know how many times particular papers are downloaded from on-line versions of journals. We can, however, measure exactly how many times readers quote a particular paper in their own subsequent publications.

It is convention in scientific writing for authors to cite any previous work which is directly relevant to their paper, where they have drawn upon the previously published work in support of their work or where they seek to contradict previous data or theory.

Usually at the end of a scientific paper there will be a list of such 'references' to previous work, these generally being known as 'citations'.

The number of citations to a particular paper is a mark of the quality and impact of that paper.

What is an Impact Factor

The impact of a journal on society is judged by the collective impact of its papers.

This is assessed through the Impact Factor of the journal, which is calculated annually by the Institute of Scientific Information, USA, and published each June.

The Impact Factor of a journal for 2010 will be published in June 2011 and is calculated as the total number of citations that had appeared in 2010 to all papers published in that journal during the years 2008 and 2009, divided by the total number of papers published in the journal during those two years.

Impact Factors tend to vary considerably with the broad subject matter of a journal. Many engineering journals have IFs less than 1.0 and an IF of 2-3 is considered quite high. In some areas of biology, IFs are in excess of 20, and some as high as 30. IFs should be normalised to the subject area.
Background

• Established in 1980
• Previous editors, Garth Hastings, Nick Peppas, Bob Langer
• David Williams appointed editor in 1996
• Editorial process, hard copy, Liverpool
• David Williams appointed sole Editor-in-Chief in 2001
• Peggy O'Donnell appointed to manage editorial process from office in Brussels in 2001, migration to electronic submission
• Web submission and review process initiated in 2003
• Journal continues to improve, IF of 7.365 in 2009

Statistics

• Aim is to complete review by 2 months, with most substantially less, but this depends on availability of referees
• Less than 10% take a little longer than 2 months
• Production time has progressively decreased during recent years.
Statistics – Submissions

<table>
<thead>
<tr>
<th>Year</th>
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<tbody>
<tr>
<td>2002</td>
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<tr>
<td>2004</td>
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<td>2006</td>
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<td>2009</td>
<td>2900</td>
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<tr>
<td>2010</td>
<td>3600</td>
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10 per day, every day of the year

Statistics – December 2010

- Average time from submission to first decision: 16 days
- Average time from submission to final decision: 22 days
- Average time for author revision: 5 days
- Average time from acceptance to publication on-line: 20 days
- Average time from acceptance to publication in hard copy: 9.5 weeks
Statistics –Manuscript Origin, 2010

USA 661 (44% accept)
China 598 (24% accept)
South Korea 200 (34% accept)
Japan 149 (41% accept)
Germany 131 (29% accept)
UK99 (26% accept)
France98 (27% accept)
Italy 85 (17% accept)
Singapore 69 (49% accept)
India 119 (8% accept)

Current Procedures

• Submission through EES
• Mandatory Author Declaration
• Editor-in-Chief reads every submission, decides on either rejection (50%) or review process (50%)
• Four referees selected, cascade process (7,000 name – keyword combinations in database)
Current Procedures

- Editor-in-Chief makes decision based on referees reports (1-4) and his own opinion
- A further 25% are rejected at this stage
- Reports sent to corresponding author together with Mandatory Editor’s Requirements
- Authors given limited time (1 week) to revise manuscript
- Importance of proof reading (short time, no new material)

Best Practice

- Content
- Style and length
- Title, authors and key-words
- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- Conclusions
- Acknowledgements
- References
- Figures and Tables
- Revising papers
Best Practice : Content

Original research only – hypothesis driven not just collecting data.
No unsolicited review papers, no short technical notes, no techniques oriented papers.
Criteria of originality, relevance to the aims and scope, substantial and significant new data.
No synthesis and characterisation of materials unless biologically relevant
Generally no in vitro tests on materials that are already in clinical use.
No comparisons of commercial products unless they are of clinical relevance.
No experiments that confirm established facts.
No papers that add incrementally to author’s recent papers.
No sequences of papers (dividing work into separate parts).

Best Practice : Style and length

Read recent issues of the journal to assess acceptable style. Read ‘Writing Papers for Biomaterials’ given in the Editorial Manager home page.
The Editor determines that all papers follow a similar style of presentation and this must be observed. The Managing Editor will send papers back to authors for correction if the style is clearly inappropriate. Continued failure to comply will result in rejection.
The style should be conventional English text, without formats that involve excessive use of notes, bullet points, lists etc.
If English is not the mother tongue of any of the authors, use the services of a translator / scientific writer.
There is no specified length. The manuscript must be substantial but should be focused and concise and not too verbose or repetitive.
Best Practice: Title, Authors and Keywords

**Title**
Concise but informative, avoid multiple parts to titles.
Title should be readily identified by search engines, therefore avoid redundant words or phrases that will not contribute to visibility.
Avoid sentences or definitive statements as titles such as "titanium causes the over-expression of gene x but not gene y".
Avoid 'novel', 'new', innovative or 'unique'.
Do not use uncommon abbreviations or trade names.

**Authors**
Include all authors who significantly contributed to the work, but not any one else (e.g. for political or funding reasons). The number of authors should be consistent with the amount of work done.

**Keywords**
Ideally should not be repetitive of the words in the title; use list provided in the journal where possible, adding new words where essential.

Best Practice: Abstract

The Abstract is often the first thing that will be read – it is available free of charge with most databases and will determine whether the full version will be accessed or not – therefore take great care.

Avoid self-centred claims – 'this is the first paper to', 'we present an entirely novel approach' etc, and focus on facts.

Abstract should be a single paragraph, without sub-headings.

Put the work into perspective but only very briefly. Do not over-elaborate experimental methods, concentrate on the data and its significance.

Do not include references and avoid abbreviations except where they are inevitable or obvious.
Best Practice: Introduction

The best introductions are about two manuscript pages long.
The readership of *Biomaterials* is sophisticated and does not need
lengthy explanations of disease states, demographic trends, the
size of markets etc.
A very brief summary of the background to the work is sufficient,
with citations to the many body of recent work on the relevant
topic. Do not include too many references, but make sure you
cite all the relevant references of the last couple of years.
Explain the rationale of the project and of the methodology used.
Ideally the Introduction should focus on the hypothesis that is
being tested in the experiments.
Do not describe the results obtained and do not speculate on the
significance of your findings in this section.
Explain abbreviations when first used.

Best Practice: Materials and Methods

This should have sufficient detail to allow the reader to repeat the
experiments.
All materials, supplies, equipment etc should be described as completely
as possible, with manufacturers / distributors details.
Arrange the section with a logical number of sub-sections – avoid using too
many sub-sections of a few lines length. Most sub-sections should be
formatted as single paragraphs, with clear succinct titles.
Frequently, some of the methods will have been used by the author in
other recently published manuscripts. This is acceptable, giving a
reference to previous descriptions. Include, if necessary a brief
summary of the technique.
Always include a section describing how the data will be handled
statistically.
This section should be written in the past tense.
Best Practice: Results / Discussion

It is acceptable to have Results and Discussions as separate or combined sections.
Avoid repetition of sections of Introduction or Materials.
The results should mention all figures and tables.
Avoid excessive numbers of sub-headings – again avoid sentences as sub-headings in these sections.
It is better to refer to results in the present tense.
The data should be presented according to the statistical rules set out in the Methods. If the data show no statistical significance this should be clearly stated and not re-interpreted as a ‘trend’.
The Discussion should place the results in the context of the current state of knowledge and how this new data advances that knowledge. It is permissible to speculate on the future significance of the findings, but always within the bounds of reasonableness.

Best Practice: Conclusions

The manuscript must have a separate Conclusions section. This should be a single paragraph summary of the main findings and their immediate significance. This should not be an extended discussion nor should it be speculative. It should not include any references.
It should be factual and not self-congratulatory – no ‘novel, ‘for the first time’ etc. Never end the Conclusions with ‘this work shows that material x is biocompatible and is a promising candidate for application y’.
Best Practice : References

Use the minimum number of references that are necessary to support statements of fact, to describe methodology, or to discuss competing or supporting evidence and theories. Do not use multiple references to support single simple un-contentious issues. Typically a manuscript should have 30-35 references. Follow the correct style precisely – this is given in the Instructions for Authors but is the largest problem we have with non-compliance. Do not use software, such as Reference Manager, without checking that it delivers the modified form of Vancouver that we use.

Be generous with references to the work of competitors and avoid too much self-citation.

Best Practice : Figures and Tables

Use the minimum number of tables and figures to represent the data accurately.

Avoid large charts that give minimal data – bar charts that compare one property for two materials- simple data should just be given in the text.

Avoid excessively large panels of images that do not contribute to the understanding of the data.

Always include statistical data (e.g. confidence limits).

Avoid simplistic schemes of experimental set-ups (e.g. electrospinning, micro-patterning).

Figure captions should be sufficiently long to explain the data, but should not be repetitive of the text.

Note that Elsevier does not publish colour images in the printed version anymore (unless author pays, or work was commissioned by the Editor).

Additional data may be included as 'Supplementary Material' which is included in on-line version but not printed copy.
Best Practice: Revising Papers

The majority of accepted manuscripts have to be revised after the review process.
Carry out the revision process quickly – if not resubmitted in the timeframe given, the paper could be rejected.
Consider all comments made by the referees. Make appropriate changes in the revision. Occasionally the Editor will agree to a rebuttal from the author which conveys a scientific argument (not a personal view) why a change should not be made.
Most requests for revision come with one or more MANDATORY EDITOR’S REQUIREMENTS. These obviously should be followed.
The resubmission should include an explanation of what the author has done to comply with the requested changes.
No changes in authors are allowed at this stage unless with permission of the Editor-in-Chief following a written explanation of why this is necessary and the submission of signed statements from new authors or authors that have been deleted.

Best Practice: Journal Sections

As from January 2011, Sections have been revised. They are:

- Biomaterials Design and Medical Device Performance
- Biomaterials and the Stem Cell Niche
- Biomaterials and Regenerative Medicine
- Biomaterials and Cancer
- Biomaterials at the Nanoscale for Diagnostic Systems
- Biomaterials for the Delivery of Drugs, Genes, Vaccines and Active Molecules

Author recommends section but Editor-in-Chief makes final decision

As from March 2011, will have occasional Debate Sections
Significant Issues

- Communication with the office.
- Referees.
- Multiple submissions.
- Correction of errors after acceptance.
- What to do after a manuscript is rejected.
- Advice on areas of research, alternative publication options.
- Archiving and definitive versions.
Silk Proteins as Biomaterial for Tissue Engineering and Regenerative Medicine

Nandana Bhardwaj, Sunita Nayak and S. C. Kundu*

Recent developments in the field of tissue engineering and regenerative medicine have accelerated the demand for the functional biomaterials which are natural in origin, biodegradable and biocompatible with enhanced mechanical properties. Silk proteins from cocoons and glands of both mulberry silkworms Bombyx mori (Bombycidae, domesticated) and non-mulberry Antheraea mylitta (Saturniidae, wild) are exploited considerably as textile material since decades now finds new applications as natural biopolymer in tissue engineering. Silk fibers are composed of a fibrous protein (fibroin) core and a glue protein (sericin) surrounding it. The major biomedical applications of silk revolve around B. mori fibroin protein. Limited knowledge about the wild non-mulberry tropical tasar silkworm A. mylitta as being endemic to India forms the basis of our study.

Silk proteins based matrices such as 2D films, 3D scaffolds, biopun scaffolds, micro beads, hydrogels, micromolded matrices and nanoparticles are engineered and characterized as biomaterial system for tissue engineering, 3D tumor model, development of immobilization substrates and drug delivery vehicles for controlled release of drugs and bioactive molecules. Further, silk fibroin matrices are used as substrates for stem cell culture, osteogenic, adipogenic and chondrogenic differentiations, subcutaneous fibroblast adhesion and non-differentiation into myofibroblasts. Potential sericin matrices in the form of 3D scaffolds, 2D films and nanoparticles are also fabricated and characterized for various tissue engineering applications and silk sericin from A. mylitta is found to have potential anti-oxidant effect and anti-apoptotic properties.

**INTRODUCTION**

Tissue engineering and regenerative medicine are an up coming multidisciplinary field with greater impact on health-care technology. This area employs non-therapeutic strategy and biomedical technologies, which support and accelerate the regeneration and repair of defective and damaged tissues. Biomaterial technology combining cells with scaffolding materials plays an important role in this field. The scaffolds provide the cells a local environment, enhance and regulate cell proliferation and differentiation. This is very crucial point for the cell-based tissue regeneration. Restoration and maintenance are expected by the natural healing potentials of the body itself.

In this context the importance of silk protein material has increased with its proven potential as a natural biopolymer for tissue engineering and biomedical applications. Silks are highly expressed protein polymer produced by various species of spiders of class Arachnida and silkworms of the order Lepidoptera for various structural roles like web formation, safety lines, egg protection, and cocoon formation (Kaplan et al., 1998; Altman et al., 2003). The fibrous silk protein is synthesized inside the silk glands of these organisms within specialized epithelial cells. These proteins are stored in the middle silk gland and at the end of the fifth instar; they are expelled through the anterior duct and spinneret (Sutherland et al., 2010). The silk fiber obtained from the silkworm cocoons comprises of two major protein components. The fibroin, core protein, contributes 70-80 % of cocoons silkworm. It is a fibrous, glycoprotein linked by disulfide bridges and is composed of heavy (H) chain, Light (L) chain (Zhou et al., 2000; Kikuchi et al., 1992) with hydrophobic nature. Silk fibroin, extracted from silkworms cocoon has found diverse application in the biomedical field as natural biopolymers due to its distinctive biophysical and mechanical properties. These properties include high tensile strength, controlled biodegradability and biocompatibility with low antigenicity and non-inflammatory responses (Altman et al 2003; Vepari and Kaplan 2007). Silk-based biomaterial offers the strongest and toughest natural biopolymer and provides many prospects for its processing, functionalization, and biological integration (Lawrence et al., 2008). The second silk protein sericin forms the outer hydrophilic adhesive coating that unites fibroin and constitutes 20-30% silk cocoons of silkworm (Kundu et al 2008a). Commercially, silk obtained from domesticated mulberry, Bombyx mori and wild non-mulberry; Antheraea mylitta, Antheraea assama and Antheraea pernyi silkworms have been used in the textile industry for its luster and mechanical properties.

Although for centuries, clinically silk has been used as sutures, it is presently appreciated much as biomaterial in various forms like 2D films, 3D scaffolds, hydrogels, nanofibers, membranes, sponges and nanoparticles and is being exploited in vitro and in vivo for cell culture and tissue engineering (Altman et al 2003). Recently tissue engineering based on stem cells and 3D silk fibroin scaffolds has escalated interest in a variety of applications such as skeletal tissue engineering like bone, ligament, and cartilage, connective tissues like skin etc. (Wang et al 2006, Blumiratana et al., 2011). B. mori fibroin has been utilized as a matrix for growth and proliferation studies of various cell types including osteoblast, fibroblast, hepatocyte and keratinocyte (Gotoh et al., 2004; Min et al., 2004; Unger et al., 2004; Chiamenti et al., 2003). However, stronger cell adhesion has been observed on films formed by silk fibroins from A. pernyi, the wild-type
silkworm comparative to those from B. mori domestic silkworms (Minoura et al., 1995). This suggests that due to the presence of tripeptide sequence Arg(R)-Gly (G)-Asp (D), a recognition site for integrin-mediated cell adhesion sequence, exclusively present in wild silkworm’s silk fibroin, increases the cell adhesion property (Pierschbacher and Ruoslahti, 1984). Three-dimensional silk fibroin porous materials have shown human cell-compatibility and have offered their application for the vascularization of the newly formed tissue (Wang et al., 2006) suggesting their use as a 3D model to monitor cell proliferation and migration. The different methods to prepare silk fibroin porous materials are using porogens, gas forming, and freeze-drying, freeze-drying/foaming, and electrospun fibers (Park et al., 2008).

Silk hydrogels formed from regenerated silk fibroin by a sol-gel transition in the presence of acid, ions, or other additives for drug delivery in vitro, in vivo and for cell culture and regenerative medicine purpose as porous matrixes have been investigated by several investigators (see Mandal et al, 2009a). Recently lyophilized silk fibroin hydrogel matrix (lyogel) has been used for the sustained release of monoclonal antibodies (Guziewicz et al., 2011).

Silk fibroin nanoparticles for sustained and long-term release, with minimal to no toxicity to healthy tissues have been investigated. It has been reported that silk fibroin-derived particles due to sustained release augments intracellular uptake and retention of the entrapped drug with the down modulation of more than one pathway (Mathur and Gupta, 2010). Sustained release of growth factor from the silk nanoparticles demonstrates its potential application for growth factor delivery (Kundu et al 2010; Numata and Kaplan 2010).

The work evolved from our laboratory, mainly emphasize on silk proteins (both sericin and fibroin) obtained from Indian origin tropical tasar silkworm Antheraea mylitta. The possibility of using wild non-mulberry silk protein as a biopolymer remains unexplored compared to domesticated mulberry silk protein. One of the main reasons that there has not been any suitable method of extraction of silk protein fibroin from cocoons and silk glands. We have developed a novel and ecofriendly method for dissolution of gland silk protein fibroin of non-mulberry silkworm, A. mylitta. Silk gland fibroin

![Silkworms diagram](image-url)
is found to be optimally soluble in 1% (w/v) anionic surfactant sodium dodecyl sulfate (SDS) because of enhanced dissolution via internalization of hydrophobic amino groups inside a hydrophilic amino acid core in the form of micelles. The new technique is important and unique because it uses a mild surfactant for fibroin dissolution and is advantageous over other previously reported techniques using chaotropic salts (Mandal and Kundu, 2008 a). We have fabricated and utilized different types of matrices like films, scaffolds, hydrogels, and nanoparticles for various tissue engineering applications. Pure silk proteins and their blends with different polymers such as chitosan, carboxy methyl cellulose, gelatin, hydroxyl propyl methyl cellulose (HPMC), polyethylene glycol, lactose have been fabricated and characterized biophysically and biochemically for variety of cell based tissue engineering applications.

We have evaluated the macrophage responses elicited by non-mulberry silkworm Antheraea mylitta fibroin protein and biocompatibility of the silk fibroin (SF) films by adherence of L929 murine fibroblasts as well as compared them with mulberry silkworm Bombyx mori fibroin protein and collagen matrices. Fibroin and collagen matrices have also been subjected to a study on comparative kinetics of adhesion of L929 dermal fibroblasts to help in formulation of dermal coverings or matrices which support a high cellularization potential with dermal cells at the time of wound recovery (Acharya et al., 2008 a).

We have fabricated SF-based scaffolds and films conjugated with lactose by using a cyanuric chloride (CY) linker (Acharya et al., 2008 b). We can adjust the properties of this material to support attachment of fibroblastic cells. At the same time, development of a fibrogenic role of pore size, porosity and interconnectivities in cell proliferation and migration of fibroblasts. Recently, we have fabricated polyelectrolyte complex porous scaffolds of silk fibroin and chitosan and evaluated its suitability for cartilage tissue engineering (Bhardwaj and Kundu, 2011). The effect of blending on stability, degradation, mechanical, antimicrobial properties, cell attachment and adhesion has been studied. The results indicate suitability of these scaffolds for various tissue engineering applications. A. mylitta silk fibroin protein have shown cytocompatibility with wide variety of cell types such as feline fibroblasts, human mesenchymal stem cells, chondrocytes, rat bone marrow stromal cells etc. We have investigated the 3-D scaffolds as substrate for osteogenic and adipogenic differentiation of rat bone marrow cells (Mandal and Kundu, 2009 b and c). The scaffolds have showed mechanically robust and have shown homogenous pore distribution. Histological analysis has shown osteogenic differentiation and adipogenic differentiation within silk scaffolds resulting in extensive mineralization in the form of deposited nodules and by the presence of lipid droplets as observed through intense Alizarin Red S staining and Oil Red O. Real-time PCR studies revealed higher transcript levels for osteogenic and adipogenic genes.

Apart from the cell based tissue engineering applications, silk fibroin matrices such as beads, hydrogels and nanoparticles have been utilized for drug delivery applications. Silk fibroin (SF) and polyacrylamide (PAAm) semi interpenetrating network (semi-IPN) hydrogels have also been fabricated and characterized to investigate their use as a matrix for sustained drug release. The matrices are synthesized by redox reaction using N, N2- Methylenebis (acrylamide) (N2 N2-MBAAm) as crosslinker, ammonium persulfate (APS) as initiator and N, N’, N2- tetramethylenediamine (TEMED) as accelerator. The physico-chemical properties including morphology, stability, swelling, mechanical, rheological and compatibility have also been investigated. Further, the release kinetics of the semi-IPNs are evaluated using two model compounds i.e. trypan blue dye and FITC labeled inulin for its potential use in tissue engineering and drug delivery applications (Mandal et al., 2009a). Silk fibroin beads have been used for controlled and sustained release of two different model compounds i.e. BSA (66 kDa) and FITC–Inulin (3.9 kDa) from predefined porous silk scaffolds in an independent fashion (Mandal and Kundu, 2009 d). The proteins have been selected for their differences in their molecular weights to evaluate the ability of the delivery system to modulate the independent release of the two compounds. We have also investigated silk protein fibroin spherical nanoparticles from both mulberry and non-mulberry silk utilizing
dimethyl sulfoxide as desolating agent (Kundu et al., 2010). The investigation outlines for their morphology, surface properties, conjugation with fluorescence isothiocyanate and the cellular uptake of these nanoparticles by murine squamous cell carcinoma as well as VEGF release from the nanoparticles to explore their use as potential therapeutic drug delivery system.

Surface topology and roughness present powerful cues for cells. The surface can strongly influence cell morphology, adhesion, and proliferation. The mechanisms mediating these phenomena are still unclear. The understanding of cell responses to material surfaces is required in order to design and fabricate next-generation tissue engineering materials. We have investigated the effects of nanoroughness assemblies of silk fibroin protein membranes on cytoskeletal organization, proliferation, and viability using primary rat bone marrow cells (Mandal et al., 2010). The silk fibroin films treated with 50 % ethanol to 100 % ethanol have been used for nanoroughness study.

Recently, we have initiated work using A. mylitta silk fibroin scaffolds for in vitro model system. The model may mimic the in vivo microenvironment and has escalated much interest in cancer research. Similarly A. mylitta silk fibroin protein matrices have been used as potential biomaterial for in vitro tumor modeling using MDA-MB-231 and LNCaP prostate cancer cancer cells (Talukdar et al., 2011). For control we have used B. mori scaffolds, matrigels and tissue culture plates. The cells have attached and proliferated well on A. mylitta scaffolds and films. The cells grown in all 3D cultures have shown more MMP-9 activity indicating a more invasive potential. Yield coefficient of glucose consumed to lactate produced by the rat bone marrow cells are being used for studying the differentiation into chondrocytes in silk fibroin blended scaffolds to observe the effect on cell viability, proliferation, biomechanical properties and biochemical properties of the 3D cartilage construct. The effect of initial cell seeding is very important in cartilage tissue engineering. The effect of different seeding density of chondrocytes is important for the extracellular matrix production, biochemical and biomechanical properties also. Chondrocytes and mesenchymal stem cells are two important sources of cells for cartilage tissue engineering. The rat bone marrow cells are being used for studying the differentiation into chondrocytes in silk fibroin blended scaffolds to observe the effect on cell viability and proliferation and extracellular matrices production. The direct cartogenesis of human embryonic stem cell-derived neural crest cells in non-mulberry silk 3D microenvironment are also being investigated.

Breast cancer commonly spreads to bone at a frequency of approximately 70% in patients having distant metastasis. However, the mechanism of bone metastasis is not well understood. Hence it is very important to have an in vitro model to study the various interactions between breast cancer and bone tissue. Co-culture studies of cancer cells and osteoblast are being attempted to study the interactions between these cells. An appropriate model will not only throw new light on the area but also help in designing newer and effective treatments. Other work are being carried out include fabrication of silk proteins and blended nanoparticles for release of bioactive molecules, sericin based matrices such as films and scaffolds for variety of applications. The pH responsive silk sericin protein hydrogels for drug delivery applications and as biomaterials are also being investigated. The biomaterial database is being created to maintain the huge amount of data and retrieval of information. The database is prepared by the use of MySQL, HTML, PHP and java script. It provides the information related to homologous query sequences against the biomaterials database, based on BLAST search tool and retrieve the useful information from it.

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Medical technology is changing rapidly. Several disease states can now be treated very effectively by implantable devices that restore mechanical and physical functionality, such as replacement of hip joints or restoration of heart rhythms by pacemakers. These techniques, however, are rather limited, and no biological functionality can be restored through the use of inert materials and devices. This paper explores the role of new types of biomaterials within the emerging area of regenerative medicine, where they are able to play a powerful role in persuading the human body to regenerate itself.

INTRODUCTION

We must place the paradigm for materials in regenerative medicine within the context of the objectives of regenerative medicine and the new role for biomaterials in this area. We have been using biomaterials in medical technology for a variety of purposes for well over half a century. Most of these applications have involved implantable medical devices that have been used for the replacement of diseased or damaged tissues or organs, or for the replacement of the function of such organs, or for assisting in the repair of traumatized tissues. The most obvious examples of these are the prostheses for total replacement of joints such as hips and knees that suffer from osteoarthritis, intraocular lenses for patients with cataracts, grafts that bypass atherosclerotic arteries, pacemakers and defibrillators for patients with defective heart rhythms, deep brain stimulators for Parkinson’s disease sufferers, and plates and other devices to repair broken bones.

Clearly, these medical devices have to provide for a specific function, and the materials used for their construction have to meet the specifications for these functions, including some highly specific mechanical and physical properties. In addition, these materials have to remain in the body for protracted periods of time, often for the remaining natural lifetime of the patients, and in doing so must not be damaged by the environment of the body nor cause any significant and harmful effect on that body. The latter two aspects are subsumed within the phenomena of biocompatibility.

As I have argued recently, we have had a fairly good idea of what constitutes biocompatibility under these conditions, even if we have not fully understood all of the mechanisms by which biomaterials interact with the environment of the human body. In the vast majority of circumstances, we require that the biomaterials used in long-term implantable devices should suffer no significant corrosion, degradation or other form of deterioration within the body, nor should they exhibit any form of toxicity or irritation to the tissues and organs of that body. Thus, my current definition of biocompatibility in this context is ‘The biocompatibility of a long term implantable medical device refers to the ability of the device to perform its intended function, with the desired degree of incorporation in the host, without eliciting any undesirable local or systemic effects in that host.’ Accordingly, we now use a small group of thoroughly stable and biologically inert materials, which we may describe as conventional biomaterials, for these devices, including some titanium alloys, platinum group metals and their alloys, cobalt-chromium based alloys, certain forms of carbon, oxide ceramics such as alumina, polytetrafluoroethylene, polyethylene, Polyethylene terephthalate textiles, some acrylic polymers, polyether ether ketone, and silicone elastomers.

THE LIMITATIONS OF CONVENTIONAL MEDICAL DEVICES

So far, so good. With these basic conventional biomaterials, and with some
very well designed and tested products, and good quality surgical procedures, we should get excellent results in the majority of patients; over 90% of patients with total hip or knee prostheses will still have intact, functioning, pain-free, devices 15 or more years later. Similar stories will be found with most types of implantable device.

However, these medical devices are limited in their function; indeed they all perform a physical or mechanical function, such as transmission of mechanical forces, channeling of blood flow, providing electrical signals, allowing light transmission, and so on, but not any biological function. They are replacing aged, diseased, or damaged tissues with synthetic or man-made materials that are dead rather than living. There is, and always will be, a serious limitation to the usefulness of such medical devices. There are many diseases, and many human conditions, that these medical devices cannot help; included here are diabetes, Alzheimer’s disease, liver failure, and cystic fibrosis.

One possible alternative to inert medical devices involves the transplantation of living tissues to the patient from some donor site. This may be achieved in situations where the donor is also the patient, taking a bone or skin graft from some undamaged part of the body and transplanting it, as viable tissue, to an injured part. Alternatively we may use donors who are different to the patient; this may be achieved with a live donor, but is mostly done with those who have died and from whom the required tissue (e.g., corneas) or organs (e.g., heart, kidney) can be quickly and atraumatically removed. A big advantage is that the patient now has a living replacement, and there is not the same limitation of function as that associated with synthetic biomaterials. The main disadvantages are that donor organs and tissues are scarce and that they are likely to induce an immune response in the patient, a phenomenon which has to be treated by the use of immunosuppressants, not without its own problems.

Since the two main methods of tissue replacement—implantable biomaterials and transplanted tissues—both have their limitations, alternative therapies are being sought. It is intuitively obvious that it would be better if we were able to regenerate new tissue rather than try to replace it artificially. The problem with humans, and indeed mammals in general, is that there is very limited ability to regenerate tissues. A few species, such as newts and salamanders, are able to regenerate significant parts of their bodies; mechanisms vary from species to species but usually involve either the retention of stem cells in the adult body that are able to migrate to a site of healing, or the de-differentiation of cells into embryonic-like stem cells, forming a blastema, a mass of proliferating de-differentiated cells capable of forming new tissue. However, the evolution of mammals has left them with very limited capacity to do this. We are able to heal skin to a limited extent, and can regenerate bone in order to heal fractures, but we do not spontaneously regenerate our bodies when they become diseased or damaged. Since we had the capacity to generate tissues during the development of a fetus and in infancy, it would appear that generation of new tissue is not impossible in humans, but rather that the mechanisms have been switched off in adulthood.

Regenerative medicine, in which attempts are made to recapitulate that capacity under controlled conditions, provides one potential alternative to the replacement of diseased and damaged tissue.2,3

BIOMATERIALS IN REGENERATIVE MEDICINE

If regenerative medicine provides a possibility of recreating new tissues instead of replacing them with synthetic structures, it might be concluded that biomaterials have little place in the future of medicine. It is indeed possible that biomaterials will play little or no role in some forms of regenerative medicine. However, if we now consider how the ability to generate new tissue can be switched back on, it will become obvious that biomaterials will probably have a crucial role.

The key to any regenerative process is the cell. If we need to generate new bone, we need the active function of bone-producing cells, the osteoblasts. For cartilage it will be the chondrocytes, for skin the keratinocytes, for nerves the neurons, and so on. These cannot start to generate new tissue spontaneously; they have to be given some signals. In general these have to include both molecular signals and mechanical signals; that is, we need a variety of biochemistries such as growth factors, and mechanical stress to initiate and maintain the cell function. In the case of mechanical stress, the phenomenon mechanotransduction, mediated both by fluid shear stresses and structural stresses within the material, which affect the attachment of integrins on cell surfaces to the material, is very important. Biomaterials will play a significant role in the delivery of these signals. Equally importantly, any

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**TISSUE ENGINEERING: CENTRAL PARADIGM AND VARIATIONS**

**Central Paradigm**
- Cell sourcing
- Cell expansion and manipulation
- Cell seeding and extracellular matrix expression
- Mechanical and molecular signalling
- Implantation of construct
- Full incorporation into host

**Variations on Central Paradigm**
- Cell sourcing: Fully differentiated, embryonic stem cells, adult stem cells, iPS cells, autologous or allogeneic
- Cell expansion, manipulation and seeding: Growth factors, Gene transfer
- Implantation of construct: Preformed scaffold plus cells or injectable self curing gel incorporating cells
- Mechanical and molecular signaling: In vivo
- Extracellular matrix expression: In vivo
- Full development within the host
tissue that is grown in this way has to have shape, and it is biomaterials templates that will give it this shape. We normally describe such a template as a ‘scaffold,’ which is often a degradable porous structure or alternatively, a hydrogel.\textsuperscript{4,5}

Before discussing the specifications for such materials, we must briefly describe the situations in which they may be used. Most of the early attempts to generate tissue in this way involved the techniques of in vitro (or ex vivo) tissue engineering\textsuperscript{6} (see the sidebar). This is concerned with the sourcing of the appropriate cells, expanding the cell colony into a sufficiently large volume, seeding these cells into an appropriate scaffold within some form of in vitro bioreactor, feeding these cells with a relevant cocktail of stimulating molecules and nutrients, perfusing the bioreactor with fluid so that fluid shear stresses provide mechanical stimuli to the cells, harvesting the tissue that forms in the scaffold, and implanting this new construct into the patient. In recent years, it has been recognized that although this is an acceptable concept, and can indeed result in relevant regenerated tissue under some circumstances,\textsuperscript{7,8} it does not necessarily provide the most effective way to do this. Alternatives in which much of the cell signaling occurs within the patient rather than within an external bioreactor have been described\textsuperscript{9} (see the sidebar). Under these circumstances, it may well be only cells and the stimulating molecules are used, perhaps by injection into the patient, without any biomaterial support, in processes known as cell therapy. However, it is becoming clear that the majority of situations will require a scaffold, so now we must turn to the optimal nature of such a material; in other words, as the title of this paper suggests, what are the essential materials paradigms for regenerative medicine.

**MATERIALS SPECIFICATIONS**

We should perhaps start this discussion of what should be the specifications for scaffold materials in regenerative medicine by saying what they should not be. It was noted above that a small group of materials now exists from which biomaterials for implantable medical devices are naturally selected. To the examples mentioned earlier we must add a few materials that have been developed for use in biodegradable devices, in particular, a group of polymers that are used for transient function in the body, such as resorbable surgical sutures. Since all of these materials, both biostable and biodegradable, are used in clinical devices, they have been required to meet certain requirements of regulatory bodies, especially the U.S. Food and Drug Administration (FDA). The tests that the materials have to undergo have been designed to confirm that the materials comply with the conditions of the definition of biocompatibility given before, which essentially means that they do the patient no harm. This implies that it will not interact biologically with the patient. In order to obtain FDA approval for a new biomaterial to be used in an implantable medical device, companies have to spend millions of dollars, and the process may take many years.

It is of no surprise, therefore, that when seeking biomaterials for new tissue engineering scaffolds, most eyes turned toward materials that had prior approval for use in implantable devices in the hope that regulators would concede that if they did no harm in such devices, they would do no harm in these scaffolds. It was largely the synthetic biodegradable polymers used in sutures and similar devices that tissue engineering companies used in their first products. Whilst of course it is essential that these scaffolds do not harm to patients, it is nowhere near sufficient to imply that they must not interact biologically with the tissues that are involved, since that is precisely what we want them to do in their stimulatory role. Thus, prior regulatory approval in other medical technology scenarios is definitely not a proper specification.

Thus a similar definition to that given before for biomaterials in implantable medical devices may derived for these new products, for example “the biocompatibility of a scaffold or matrix for a tissue engineering product refers to the ability to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signaling systems, in order to optimize tissue regeneration, without eliciting any undesirable local or systemic responses in the eventual host.”

Let us consider, therefore, how we can develop better specifications for such materials. We can use as an example a porous polymeric scaffold and we wish to develop specifications for that scaffold in order to generate, de novo, tissue such as a nerve or a blood vessel.

Following the paradigm of ex vivo tissue engineering mentioned before, we start with a cell source. There are several alternatives, primarily including stem cells, which may be embryonic stem cells, induced pluripotent stem cells, amniotic fluid derived stem cells, autologous adult stem cells, or they may be fully differentiated adult cells, such as chondrocytes or cardiomyocytes. The nature of these cells must have some impact on the materials we use for their support, since we require them to be manipulated to varying extents and with varying objectives. It has become quite clear in recent years that the precise environment of stem cells in culture, including the material substrate (often now referred to as the stem cell niche) has a powerful role in determining their performance. Once we have seeded cells into a scaffold, instead of the scaffold material ignoring the cells, we require them to facilitate the desired biological processes, which may mean that the cells should be adherent to their surface, so that control of hydrophilicity and molecular mobility become very important.

The molecular signals and nutrients may be simply added to the cell culture medium, but we may require
greater control, in which case these active molecules may be attached to the materials surfaces or incorporated into the scaffold material for controlled release over time. For example, specific interactions with cell receptors may be induced by the attachment of specific peptide sequences to the material. The delivery of the appropriate mechanical signals requires effective design of the bioreactor, but the stresses have to be converted into biological signals within the cells, in the processes of mechanotransduction, the stress transfer being controlled by elastic modulus of the substrate.

Assuming that the required quality of tissues is being generated, the scaffold material then has to degrade. Its initial presence will have given form to the developing structure and it should have facilitated the signaling as just discussed, but then it is no longer required and should give way to the new tissue. This is not a trivial process, since the degradation of a material implies the generation and release of degradation products. If a polymer degrades, the degradation products may include physical fragments as the material disintegrates, and also smaller molecules such as oligomers, side groups, and monomers. Many of the products of degradation are pro-inflammatory, that is they can initiate an inflammatory reaction, the aggressive cells of which can destroy the tissue that has just been generated. In addition, if the scaffolds are derived from natural polymers or natural tissues, this may be very advantageous, but some of the products may also stimulate an immune response, which is definitely not required.

We can therefore see that the development of material specifications for scaffolds in regenerative medicine is complex, and there may be many competing requirements. The subject is fast moving and there is some data in the world’s literature concerning the physical and biological characteristics of potential biomaterials and the tissues that can be generated within them. These materials include proteins such as collagen, elastin, keratin, fibrin, and silk. They also include polysaccharides such as heparin, chitosan, hyaluronan, and alginate.

They may be biopolymesters, such as one of the polyhydroxybutyric acid group or other polyalkanoates or synthetic polymers such as the aliphatic polyesters, polyethylene glycol, or polyurethanes. Their intrinsic characteristics involve structural and chemical parameters, their materials-science oriented functional properties, and biological functionality. These include features of pore architecture, including pore size, shape, and distribution, elasticity, including moduli and time-dependent deformation, the surface energetics parameters such as hydrophobic–hydrophilic balance and molecular mobility. In addition, the chemical functionality, environmental responsiveness with respect to pH, stress and temperature, the surface topography at micro- and nano-scale, and biodegradation mechanisms such as hydrolysis and enzymatic degradation and the rates of degradation and metabolism of degradation products will all play a role. Biological functionalization may be imparted by, for example, growth factors (e.g., nerve growth factor, proteins such as laminin, peptide motifs, cellular antigens (CD molecules), genes/ transcription factors or oxygen generating species). The tissue generation will be achieved through the activity of cells, including stem cells from a variety of sources induced pluripotent stem cells or adult cells. The attempted tissue generation may take place ex vivo using bioreactors, cell printing, or microfluidics.

As we move away from conventional biomaterials for the purposes of scaffold and matrix design, the need for radically new concepts in biomaterials has become very obvious.

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Scaffold Free Tissue Constructs for Ocular Surface Regeneration

T.V. Kumary

INTRODUCTION

Elements of Tissue engineering for reconstruction of tissues and organs are cells, scaffolds and growth factors/supplements. Although the scaffolds are usually biodegradable, use of scaffold results in the implantation of a foreign material into human body. Moreover, some applications of tissue engineering like skin and cornea do not require the use of scaffold. In order to eliminate the use of scaffolds in constructing three dimensional tissue structures and organs, thermoresponsive, smart culture surfaces have been studied [1–3]. These surfaces can be used for detachment of cell layers with intact cell–cell and cell–extra cellular matrix [4], avoiding the damages caused by enzymatic or mechanical treatment. The reversible thermal switching property of thermoresponsive polymers enable it to undergo phase transition between hydrophilic and hydrophobic stages [5]. Above the lower critical solution temperature (LCST) the polymer will be hydrophobic resulting in the detachment of cells. Culture surface for corneal cell growth should be cytocompatible and support limbal cell culture as regeneration and repair of the corneal epithelium in the eye is ensured by adult stem cells that reside in limbus, a region in the corneoscleral rim (6). Deficiency of stem cells, due to either inherited or acquired reasons, lead to several complications (7) and can result in gradual opacity and blindness. Limbal stem cell transplantation after ex vivo expansion of autologous limbal stem cells (LSC) (8) is ideal for treating limbal stem cell deficiency. The use of 3T3 feeder layer is a prerequisite for the cultivation and expansion of LSC (9). However, this murine feeder layer leaves open the possibility of xenocell contamination of limbal cells. In addition, the cultured cells can take up sialic acid (Neu5Gc) from mouse feeder cells that can raise circulating antibody, leading to in vivo cell killing after transplantation. Moreover, Food and drug administration (FDA, USA), has classified tissues regenerated using 3T3 feeder layer as xenogenic. These facts underscore the demand to develop feeder free culture systems for transplantation purpose.

Objective of this study was to prepare thermoresponsive polymers to act as cell culture substrate and for preparation of in vitro scaffold and feeder free tissue constructs. As scaffold free tissue constructs are ideal for ocular surface regeneration, attempt was made to create scaffold free corneal construct using feeder free culture of limbal stem cells and to assess the efficacy of scaffold free constructs by transplantation to rabbit limbal stem cell deficient model.

METHODOLOGY

Different PIPAAm based polymeric formulations were prepared that can be used to obtain cultured intact corneal epithelial cell sheets which can find application for ocular surface regeneration. Polymeric formulations were characterized physico chemically by different techniques like Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry, Proflometry, Atomic force microscopy. Any material intended for medical application should be safe for the human body or should be proven cytocompatible before in vivo use. The thermoresponsive copolymer coated surfaces were evaluated for cytocompatibility by analyzing its ability in supporting viability, metabolic activity, attachment, spreading and proliferation of fibroblast cells in comparison to tissue culture polystyrene (TCP). Xeno feeder free culture of limbal stem cells was standardized by manipulation of the microenvironment. Thermoresponsiveness or temperature induced cell detachment to form detachable cell layers which is required for generation of scaffold free tissue construct was also looked into. Tissue constructs were analysed for their specific cell characteristics.

RESULTS & DISCUSSION

Different formulations of thermoresponsive polymers showed the specific characteristics upon physicochemical characterization and was cytocompatible (10, 11, 12). The LCST of PNIPAAm can be tuned to specific applications by copolymerization with other comonomer having either higher hydrophilic or hydrophobic nature. Moreover the culture surface supported limbal stem cell growth. A combination of Iscove’s modified Dulbeco’s medium and Panserin 801 resulted in formation of autofeeder layer with maintenance of progenitor characteristics, thus mimicking natural tissue architecture (13). Thermoresponsiveness was confirmed by detachment of tissue construct (14). Generated tissue also...
showed the specific markers of stem cells as well as epithelial cells (15). The specific explant culture system mimicked natural tissue architecture and niche closely by enabling formation of autofeeder layer and thereby eliminating the need of xeno-feeder layers for ex vivo expansion of epithelial progenitor cells. Other sources of stem cells like bone marrow mesenchymal stem cells can also be differentiated to corneal epithelial lineage. This will be specifically useful in the of bilateral limbal stem cell deficiency.

Limbal stem cell deficiency model rabbits showed the main hall marks of LSCD like neo vascularization and corneal opacity. Pre clinical evaluation by transplantation of tissue construct showed very promising results of ocular surface regeneration.

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Once the differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of transplantation-based therapies. The microenvironments in which stem cells reside is termed as “niche” which regulate stem cell fate. Replication of the in vivo niche conditions for in vitro standardization of cell expansion may turn out useful for regenerative therapies. The stem cells and niche may support each other during development and reciprocally signal to maintain each other during adulthood. Simple location of stem cells is not sufficient to define a niche; the niche must have both anatomic and functional dimensions (1). For in vitro culture of adult stem cells it is often difficult to define the niche because the factors present in vivo in the microenvironment and the type of signaling available for cell differentiation and tissue homeostasis is not clearly understood. Fibrin is a good reservoir that binds various ECM components such as adhesive proteins (eg, fibronectin and gelatin) and growth factors (eg, fibroblast growth factor [FGF] and vascular endothelial growth factor [VEGF]), making it a matrix with cell adhesion and signaling potential [2].

The concept of using a fibrin based matrix for growing differentiated endothelial cell was tested before it was modified and used for growing circulating endothelial progenitor cells (EPC) and differentiating it into endothelial cells (EC). We have designed a fibrin composite that maintains endothelial cell culture through several passages without altering its phenotype [3]. In addition, HSVEC isolated from diseased artery was found to grow on the fibrin matrix forming more non thrombogenic phenotype after 2 to 3 passages on designed matrix (4). It was found that addition of more angiogenic and mitogenic growth factors and glycosaminoglycans to the fibrin matrix has promoted deposition of ECM especially the components of basement membrane by EC (5-7). It was shown that the matrix can be coated on various biomaterial surfaces for improved HUVEC attachment, proliferation and survival (8-11).

Depending on the cell that has to be grown, the composition of niche needs to be different. The concept of using specific matrices for differentiation of circulating progenitors from human peripheral blood mononuclear cells (PBMC) has been demonstrated earlier (12,13).

METHODS

Tissue culture polystyrene (TCPS) (NUNC, Rakslide, Denmark) were coated with fibrin composite as described earlier (3) with some modification to create specific matrix for endothelial progenitor cells, smooth muscle cells or neuronal progenitor cells. Morphological analysis of the cells in culture was done periodically. Specific markers were used for identifying cell phenotype.

RESULTS

Use of specifically designed matrices can generate endothelial cells, smooth muscle cells and neuronal cells from unseleced population of PBMC isolated from human blood. The differentiation was demonstrated using specific markers for each cell types. Many of the components of the matrix in all 3 types of niche are common such as fibrin, gelatin, fibronectin and certain growth factors. Cell differentiation was confirmed in each case using specific markers. The designed matrices allowed cell survival and growth in a cell specific manner.

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Design of Biomimetic Matrix for in vitro Tissue Regeneration with Adult Stem Cells

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Stem cells and Ceramics for the Repair of Bony Defects in Orthopaedics

Annie John

In this era of Regenerative Medicine, bio-functional aspects of bioactive ceramics intended for the repair of bony defects will become a relevant technology in Orthopaedic applications. This is because bone defects caused by tumor resection, trauma and skeletal abnormalities still remain a major clinical problem in attaining functional bone after the treatment. Stem cell-based tissue engineering is a promising technology in the effort to create functional tissue of choice. Successful bone tissue engineering relies not only on the differentiation of cells in contact with the implant material but also on the proliferation capacity of the cells to obtain a proper colonization of the 3-D matrix to make a 'hybrid bone' in vitro. In the development of such 'combination products', the utility of incorporating cells have been initiated into ceramic scaffolds.

See Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum, India, is an Institute of National Importance in the field of Biomedical Technology - biomaterials, biomedical devices and Tissue Engineering. Research in the area of bioceramics, is one of the major activities in the Institute where novel bioceramic compounds have been developed in the Bioceramics Laboratory to form a broad range of goal oriented indigenous, cost effective materials for bone grafts in the health care system. The 3-D lattice structure of the bioactive ceramic offers a more appropriate niche where cell-cell/cell-material interactions could be favored and be closer to their in vivo situation counterpart.

In this context, the focus is on the isolation, expansion and characterization of bone marrow-derived mesenchymal stem cells (BMSCs) in vitro and its ability to support, express bone markers and maintain osteogenesis on bioactive ceramic scaffolds in the cell culture system. To create stable but degradable implants for hard tissue regeneration, a material that mimics the mineral phase of bone is the first choice as a substrate. Ceramics have a chemical composition similar to bone and are known to bond with bone. They are pervaded by adequate pores larger than 100 µm for the cells to dwell within and to facilitate oxygen and nutrient supply. For tissue engineering, these highly cell loaded scaffolds are fabricated as implants. The living part demands for an appropriate architecture and above all the material needs to be biocompatible. These cells adhered, attached and displayed a well spread-out cell morphology making focal contacts on the material surface, eventually forming multilayer of aligned cells fabricating a cell sheet-like covering, probably influenced by the physical and chemical stimuli emanated from the underlying 3-D ceramic scaffold. Cells invaded and migrated into the 3-D porous ceramic. Optimal functionality of a tissue depends on its appropriate histological organization and the alignment of cells in the ECM is an essential indicator of tissue integrity to improve the functionality of tissue constructs.

Subsequently, transplantation of the fabricated cell-seeded ceramic scaffolds to segmental bony defects bridged the defect at an early period where healing was uneventful. Implanted 'bio-constructs' encouraged osteoinduction, osteoconduct, osteointegration and biodegradation without fibrous tissue formation. The newly formed bone organized, mineralized and attained the contour of the original bone with time in vivo. In short, the research is dedicated on the possibility of sculpting and designing bio-functional ceramics for the prospect of repair of large segmental defects in clinical situations which is a formidable challenge in orthopaedic reconstructive surgery.

INTRODUCTION

In this era of Regenerative Medicine, bio-functional aspects of bioactive ceramics intended for the repair of bony defects is posed as a relevant technology in Orthopaedic applications. This is because bone defects caused by tumor resection, trauma and other skeletal abnormalities still remain a major clinical problem in attaining functional bone after the treatment. Bone has the remarkable capacity to heal without scar formation, but this regenerative process fails in patients with large bone lesions or impaired wound healing, requiring clinical intervention. Such critical-sized segmental bone defects do not heal spontaneously during the lifespan of the organism (Reichert JC et al., 2009).

Stem cell-based tissue engineering is a promising technology in the effort to create functional tissue of choice. Successful bone tissue engineering relies not only on the differentiation of cells in contact with the implant material but also on the proliferation capacity of the cells to obtain a proper colonization of the 3-D matrix to make a ‘hybrid bone’ in vitro. Thus the triad of tissue engineering consists of (a) 3D scaffold (b) cells and (c) growth factor, each of these can be used independently or in combination. In the development of such ‘combination products’, the utility of incorporating cells have been performed into ceramic scaffolds with and without growth factors. Research in the area of bioceramics, is one of the major activities in the Institute where novel bioceramic compounds have been developed to form a broad range of goal oriented indigenous, cost effective materials for bone grafts in the health care system.

So the focus of the research is on –

1. The isolation, expansion and characterization of bone marrow-derived mesenchymal stem cells in vitro and
2. Its ability to support, express bone markers and maintain osteogenesis on bioactive ceramic scaffolds in the cell culture system and its preclinical translations.
In vitro - Cell culture Procedures

MSCs were isolated from goat bone marrow, expanded, and differentiated in an osteogenic medium and characterized for their osteogenic markers. Scaffolds with 1 x 10⁷ cells/cm² seeded onto them are fabricated as implants and these highly cell loaded scaffolds are used for tissue engineering. Morphological and functional evaluation of such TE constructs was done by SEM and using other biochemical markers.

In vivo – Animal surgical Procedures

Segmental bone defects were created in anaesthetized goats under the approval and guidelines of the IAECC and CPCSEA. This bone loss was replaced with in-house developed bioactive ceramics by a press fit method with the support of internal or external fixators. Bare implants and cell-seeded implants with and without PRP were also used. The implant sites were assessed post-implantation, by radiography and further evaluated by histology of Stevenals Blue and Van-Gieson Picrofuschin stained Polymethyl Methacrylate sections.

RESULTS

In vitro

Bioactive ceramic showed a 3D rough surface topography having pores of 50 – 500 µm size with interconnections. Cells adhered, attached and displayed a well spread-out cell morphology making focal contacts on the material surface, eventually forming multilayer of aligned cells. Cells were cultured on the bioactive ceramic scaffolds to bony defects bridged and healed the defect at an early period where healing was uneventful. The newly formed bone eventually organized, mineralized and attained the contour of the original bone with time in vivo.

DISCUSSION

To create stable but degradable implants for hard tissue regeneration, a material that mimics the mineral phase of bone is the first choice as a substrate. Ceramics (Hydroxyapatite) have a chemical composition similar to bone and bioactive. Preparation of the scaffolds with adequate pores larger than 100 µm for the cells to dwell within and to facilitate oxygen and nutrient supply ensures optimal cell colonization. The 3-D lattice structure of the bioactive ceramic offers a more appropriate niche where cell-cell/cell-material interactions could be favored and be closer to their in vivo counterpart. This satisfies the need for an appropriate architecture at the cellular level and ensures biocompatibility. Optimal functionality of a tissue depends on its appropriate histological organization and the alignment of cells in the ECM is an essential indicator of tissue integrity to improve the functionality of tissue constructs.

Mesenchymal stem cells (MSCs) show extensive capacity for expansion in vitro, so these cells have been considered as candidates for cell therapy. These cells can differentiate into multiple cell lineages including bone, cartilage, muscle, bone marrow stroma, tendon / ligament, fat, dermis, and other connective tissues. MSC population must express CD105, CD73 and CD90 and they themselves secrete a broad spectrum of bioactive macromolecules that are both immunoregulatory and serve to structure a regenerative microenvironment in the arena of tissue injury.

Through the addition of osteogenic supplements including dexamethasone, ascorbic acid and α-glycerophosphate, purified culture-expanded MSCs can be differentiated into osteogenic lineage in vitro, as envisaged by the production of collagen-rich matrix, increased expression of non-collagenous proteins like ALP, osteocalcin and finally by the formation of mineralized matrix (Kern S et al., 2006. (Son E et al., 2007).

Therefore, the current state of art within bone tissue engineering consists of the combination of culture-expanded MSCs with 3D porous biomaterials. Although the implantation of MSC-seeded biomaterials has shown significant increase in skeletal repair (Kadyala et al., 1997, Bruder SP et al., 1998; Li Z et al., 2005) additional in vitro culture methods have been developed that promote osteogenic differentiation of MSCs in vitro in lieu of in vivo incorporation. These cell-loaded ceramics have succeeded in creating bone formation in animal models (Liu G et al., 2008; Nair et al., 2009c; Nair et al., 2009d). The possibility of engineering bone in a goat model has been tested using osteogenic induced bone marrow-derived MSCs and HAsi. The cells were cultured on the bioactive ceramic for 1 week before the transplantation. In the surgical procedure, a 2 cm segmental defect was created in the femur of the goat model and the defect was treated with tissue-engineered HAsi (HASi+C) and HAsi alone. The materials were stabilized with titanium intramedullary nails and screws. In bare HAsi groups, lamellar pattern of bone formation was seen only in the peripheral region, while the mid region was filled with woven bone at 4 months. The mid region was later organized into lamellar pattern at 6 months and a complete bone remodeling at 12 months. Interestingly, in HASi+C groups, a mature lamellar pattern of newly formed bone was observed throughout the defect at 4 months. Thus, whatever the qualities attained in the tissue-engineered groups at 4 months was achievable only at 6 months in bare HAsi groups. This may be due to the combined osteoinductive and osteoconductive effect of HASi+C groups (Nair et al., 2009d; Nair MB et al., 2010). It was observed that the culture of cells on HAsi could not only enhance bone
regeneration, but also mediate material degradation (HASi degraded faster in HAsi+CP groups than HAsi alone groups), which is one of the main problems encountered in the category of tissue-ingrowth. It is hypothesized that osteogenic induced MSCs cultured on HAsi create an ECM that can favor the successive migration of osteoclasts. Among many ECM proteins, osteopontin has an important role in promoting the adhesion of osteoclasts to the scaffold surface through their arginine-glycine-aspartic acid sequence (Mastrogiacomo M et al., 2006). In vitro studies have shown that the expression of osteopontin was significantly enhanced on HAsi than HA (Nair MB et al., 2009b). Thus the combined effects of the characteristics of the material (porosity and silica content) and the ability of cells on HAsi to attract osteoclasts have improved cell-mediated resorption in HAsi+CP groups. These cells can also secrete inductive factors that recruit indigenous mesenchymal stem cells (MSCs) into osteogenic lineage and produce mature bone matrix quickly. Thus the combined contribution from cells on the scaffolds and host-derived MSCs together with biomolecules can advance osteogenesis at the defect site.

The cost of almost all genetically engineered growth factors is prohibitively high, which limits its use for large defects where large amount of growth factors are needed. Platelet-rich plasma is a proven source of many growth factors like platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF-I) (Anitua E et al., 2007). In this study, the platelets adhered and were activated on these materials without the use of triggering agents (Nair et al., 2009). The activation was significantly enhanced on HAsi than HA where the chemical composition, particularly silica may have played an important role (Nemmar A et al., 2005). Nevertheless, HAsi+CP groups showed a better response qualitatively where the lamellar bone was more organized (Nair et al., 2009c). In general, it is understood that PRP does not have any strong osteogenic effect so that filling a defect with PRP alone will not allow osteogenesis to occur in larger defects. Instead, PRP has an osteopromotive effect and can enhance bone regeneration in the presence of osteogenic precursor cells by encouraging their proliferation and differentiation (Schlegel KA et al., 2004). Additionally, MSCs associated with PRP are potent angiogenic inducers proven to release VEGF leading to faster bone regeneration and remodeling (Yamada Y et al., 2004). Kitoh et al., (2007) observed that transplantation of bone marrow cells and PRP shortened treatment period and reduced associated complications by accelerating new bone formation in distraction osteogenesis

CONCLUSION

Interestingly, the research focuses on the possibility of sculpting and designing bio-functional ceramics for the prospect of repair of large segmental defects in clinical situations which is a formidable challenge in orthopaedic reconstructive surgery. Segmental bone defects can result either from acute trauma or chronic infection requiring bone resection or chronic non-unions due to osteoporosis or osteomyelitis, osteogenesis imperfecta or osteosarcoma. The current treatments like autograft, allograft or distraction osteogenesis are associated with several limitations. Therefore the focus is on the new concept called “Tissue engineering” in which 3D porous biodegradable scaffolds are incorporated with cells or growth factors to regenerate tissue within the body. It is apparent from the study that the tissue-engineered groups showed enhanced regeneration compared to the bare material groups in the healing of the bone defect. Although significant results have been achieved, there are challenges that are to be addressed before the strategy can become a reality in the future.

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Brief Biodata

**Professor David F. Williams FREng**

David Williams was trained as a materials scientist at the University of Birmingham, UK (B.Sc. 1965, Ph.D. 1969, D.Sc. 1982). In 1968 he took up a faculty position in the School of Medicine at the University of Liverpool, UK, where he remained for 40 years, writing, researching and teaching on the science of biomaterials. He created the Department of Clinical Engineering in the University and was its Head for 20 years. He was granted a Personal Chair in the University in 1984, then the youngest person in the history of the University to receive such an honour. Towards the end of his career in Liverpool, he became the Pro-Vice-Chancellor (equivalent to Academic Vice-President) of the University, and then won major funding from the British Government to form, in collaboration with the University of Manchester, the UK Centre for Tissue Engineering, of which he became Director. He was instrumental in obtaining over 25 million euro for a European Union Integrated Project, involving 25 partners across Europe, for a systems engineering approach to tissue engineering (STEPS). Professor Williams has extensive experience with medical device and regenerative medicine companies, and significant experience in both patent and product liability litigation. He is President-elect of Tissue Engineering and Regenerative Medicine International Society (TERMIS).

Professor Williams is the Editor-in-Chief of *Biomaterials*, now the leading journal in the field of biomaterials science. During his research career he has published over 30 books, including the first text book in this area, *Implants in Surgery*, and the *Williams Dictionary of Biomaterials*, and around 400 papers. He has presented keynote and plenary lectures at conferences in over 30 countries over the last 30 years. He has received the major awards from the US (Clemson Award 1982, Founders Award 2007), European (George Winter Award, 1996), UK (Presidents Award 2004, Chapman Medal of the Institute of Materials, 2007) and Indian (Sharma Award 2008) societies of biomaterials. He was a scientific adviser to the European Commission and wrote many Opinions on which European laws in health technology and nanotechnology are based. In 1999 he was elected as a Fellow of the Royal Academy of Engineering in recognition of his contributions to engineering in medicine.

Professor Williams left the University of Liverpool, and the UK, at the end of 2007. While retaining the title of Emeritus Professor at Liverpool, he is currently Professor of Biomaterials and Director of International Affairs, Wake Forest Institute of Regenerative Medicine, North Carolina, USA., where he has responsibilities for establishing and managing international collaborations. He is also a Visiting Professor in the Christian Barnard Department of Cardiothoracic Surgery, Cape Town, South Africa, a Visiting Professorial Fellow at the Graduate School of Biomedical Engineering, University of New South Wales, Australia, and a Guest Professor, Tsinghua University, Beijing and Visiting Professor, Shanghai Jiao Tong University, China. He has been appointed as Visiting Professor at Sree Chitra Tirunal Institute for Medical Sciences & Technology. He spends approximately 5 months a year in the USA, 1 in Europe, 2 in South Africa, 2 in Australasia and 2 in Asia.
Ms. Peggy O’Donnell

Peggy O’Donnell is Managing Editor of the journal Biomaterials and had played a significant role in making Biomaterials journal the number one in Materials Science with an impact factor of 7.365 (2009).

Dr. Subhas C Kundu

SC Kundu, Ph.D. is Professor and the formerly Founder-Head of Department of Biotechnology of Indian Institute of Technology (IIT) - Kharagpur since July 1994. Before joining IIT Kharagpur he was also an Assistant Professor to a Full Professor including Head of Department at Manipur University (erstwhile Jawaharlal Nehru University Centre, Imphal, India from June 1976 to June 1994). He teaches Genetics, cell and molecular biology and recombinant DNA technology to under graduates and pre-doctoral students at IIT. Dr. Kundu received his post doctoral training at Institute of Molecular Biology, Moscow; Department of Biology, York University, Canada; Medical University, Lubeck, Germany; Department of Biology & Biochemistry, Brunel University, UK. His main area of interest is molecular genetics and silk biomaterials. He has published over 75 research articles in Journals such as Chromosoma, Experimental Cell Research, Genetical Research, Cytogenetics and Cell Genetics, and Molecular Biology, J. General Virology, Biomaterials, Macromolecular Biosciences, Progress in Polymer Science, Biotech Advances, Soft Matter, Tissue Engineering etc. He is associated with several national and international research project work (like Indo-Australia Biotechnology Fund, Indo-Russia Biotechnology Programme, Indo-US Science and Technology Forum, New Delhi) focusing mainly on silk protein based biomaterials and cell based tissue engineering and regenerative medicine.

Dr. T.V. Kumary

Dr. T.V. Kumary, presently Scientist ‘G & In Charge of the Tissue Culture Laboratory in the BMT wing has a doctorate in Biochemistry from Medical Faculty of University of Kerala. She joined our Institute in 1984. She has set up the Tissue Culture Laboratory for evaluation of cytocompatibility of Materials for Medical Devices. In keeping with the recent elevation of testing laboratories she along with her team has brought the laboratory to international standards by implementing Quality system based on ISO, accredited by the French Agency. In addition to routine testing of materials for cytocompatibility, the morphological, biochemical and functional aspects of cell-material interaction are also carried out in the lab. Dr. Kumary has been a recipient of Leverhulme Trust Fellowship, which she did at University of Liverpool. Her interests today include development of in vitro systems for testing of materials and Tissue Engineering.

Dr. Lissy K. Krishnan

Lissy K. Krishnan started her pre-doctoral research on platelet biology in 1980. She obtained her Ph.D from the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum in Platelet Biochemistry. From 1990 to 1992 she was at the University of
Minnesota, and worked as a Post Doctoral Research Associate. She transferred back to SCTIMST in 1992 and took charge as the Leader of Thrombosis Research Unit in 1995.

She has contributed to product development for clinical use by developing Fibrin Glue and its standardization of viral inactivation to meet EP and WHO requirements. The product is now clinically used. Another biological product under evaluation is anti viper venom antibodies. Her major contribution in tissue engineering is towards development of small diameter vascular grafts. Her approach to use fibrin matrix as an in vitro homing site for human adult stem cells has resulted in differentiation of circulating progenitors into endothelial cells, smooth muscle cells, keratinocytes and neurons to be used for tissue engineering and for regenerative medicine.

She teaches a course on Stem Cells and Regenerative Medicine and blood compatibility of Biomaterials to post graduate students in her Institution and guides graduate students enrolled for both Ph.D and M. Phil programs. She has several peer reviewed publications, text book chapters, conference proceedings and patents to her credit and is active in various academic societies.

**Dr. Annie John**

Annie John Ph.D, Scientist E & in-charge -Transmission Electron Microscopy (TEM) Laboratory, Div. of Implant Biology and the Division of Laboratory Animal Science to date at the Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojapura, Trivandrum -695012, Kerala, India.

Her area of research includes; Biomaterials & Bone Tissue Engineering - Ceramics as bone substitutes and Adult Stem Cell Research in various National & International research projects involving analytical & microscopy studies of cells and tissues and its response to materials in different animal models.

Graduated from University of Kerala; A Postdoctoral Fellow of The Japan Society for the Promotion of Science (JSPS) for foreign research in Japan (Long Term in 1999- 2001 & Short Term in 2006), at Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; A recognized Ph.D guide of the Institute; have attended many National and International Conferences and has several publications to credit.

**Dr. Chandra P. Sharma FBAO, FBSE**

Chandra P. Sharma is Head, Biosurface Technology Division, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram since January 1980. He has been Associate Head, Biomedical Technology Wing, SCTIMST and presently he is Associate Dean, PhD Affairs. He is also Adjunct Professor, Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal University. He is basically a Solid State Physicist from IIT Delhi and received his training in Biomaterials area in the University of Utah (USA) with Prof. D.J. Lyman as a graduate student and in the University of Liverpool, England with Prof. D.F. Williams as a Post Doctoral Research Associate. Dr. Sharma has been awarded
FBSE (Fellow Biomaterials Science & Engineering) by The International Union of Societies for Biomaterials Science & Engineering (IUS-BSE) in 2008 and FBAO (Fellow Biomaterials and Artificial Organs) by Society for Biomaterials & Artificial Organs (India) (SBAOI) in 2011. He has been recognized with various awards and honors such as MRSI medal (1994) and MRSI-ICSC Superconductivity & Materials Science Annual Prize Award 2009 - Materials Research Society of India, Distinguished Scientist award - Society for Biomaterials and Artificial Organs, India (SBAOI) and shares Whitaker and National Science Foundation Award - International Society for Artificial Organs (ISAO) USA. He is the founder of the SBAOI and the Society for Tissue Engineering and Regenerative Medicine, India (STERMI). Dr. Sharma has published over 300 research papers and has processed 32 patents including in Canada, European Union, Japan and USA. He has edited several special issues of International Journals and two books. He is also the Founder editor of Trends in Biomaterials and Artificial Organs (journal published by SBAOI). His basic activities relate understanding of blood/tissue - material interactions at the interface. His laboratory has completed prestigious programmes under NMITLI-CSIR New Delhi on oral delivery of insulin and has been awarded the FADDS project (Facilities for Micro/Nanoparticles based Advanced Drug Delivery Systems) under Drugs and Pharmaceuticals Research Programme, Department of Science & Technology, New Delhi, with a funding of over US$ 1 million. It is being planned to convert FADDS as a National Facility with a funding of about US$ 3 million.
Notes
Workshop on
New Visions for Biomaterials and Regenerative Medicine
16-17 March 2011
Organized by
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Editor-in-Chief, Biomaterials

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