

Melvin A. Shiffman
Alberto Di Giuseppe
Franco Bassetto *Editors*

Stem Cells in Aesthetic Procedures

Art, Science, and
Clinical Techniques

 Springer

Contents

Part I Stem Cell Therapy Overview

- 1 Stem Cell Applications: An Overview** 3
Sandro Eridani

Part II Biochemistry and Physiology of the Stem Cell

- 2 Adipose Cell Precursors: Stem Cells in Medicine, Tissue Engineering, and Reconstructive Surgery** 19
Michael V. Dodson, Min Du, Sandra G. Velleman, Douglas C. McFarland, Melinda Fernyhough-Culver, Shengjuan Wei, Marcio S. Duarte, Zhihua Jiang, and Gary J. Hausman
- 3 Tissue Engineering of Vascularized Adipose Tissue for Soft Tissue Reconstruction** 23
Silvan M. Klein, Jody Vykoukal, Lukas Prantl, and Juergen H. Dolderer
- 4 Surface Antigenic Profiles of Stem Cells from the Human Bone Marrow, Subcutaneous Fat, and Omentum Fat** 41
Indumathi Somasundaram, Radhakrishnan Harikrishnan, Rashmi Mishra, Rajkumar J. Sankaran, and Dhanasekaran Marappagounder
- 5 Human Adipose Tissue as a Source of Multipotent Stem Cells** 67
Andrew I. Li, Akishige Hokugo, Reza Jarrahy, and Patricia A. Zuk
- 6 Adipocytes and Osteoblasts from Human Adipose Tissue Mesenchymal Stem Cells for the Production of Compatible and Safe Biomaterial Crucial in Cosmetic, Reconstructive, and Regenerative Medicine** 85
Toai Cong Tran, Ciro Gargiulo, Thao Duy Huynh, Khanh Hong Thien Bui, Luis Filgueira, and Douglas M. Strong

- 7 Adipogenesis Using Human Adipose Tissue-Derived Cells Impregnated with Basic Fibroblast Growth Factor** 111
Ran Ito
- 8 The Adipose Organ: Morphological Perspectives of Adipose Tissues** 123
Arianna Smorlesi, Andrea Frontini, and Saverio Cinti
- 9 Pericytes: a Ubiquitous Source of Multipotent Adult Tissue Stem Cells** 135
Ludovic Zimmerlin, Tea Soon Park, Vera S. Donnemberg, Elias T. Zambidis, and Albert D. Donnemberg
- 10 Adipose-Derived Stem Cells to Modulate Scar Tissue: From Biological Basis to Clinical Applications** 149
Franco Bassetto, Angelo Sapuppo, Giovanni Filippo Borso, and Vincenzo Vindigni

Part III Stem Cells and Adipose Tissue Survival

- 11 Stem Cells, Mature Adipocytes and Extracellular Matrix: What Does Each Contribute to Fat Graft Survival?** 159
Ali Mojallal, Christo Shipkov, Charlotte Lequeux, Lucas Rifkin, Rod Rohrich, Spencer Brown, and Odile Damour
- 12 Multipotential Aspects of Adipose-Derived Stem Cells and Their Spheroids** 181
Sahil K. Kapur and Adam J. Katz
- 13 The Influences of the Density of ASCs and the Stromal Condensation Rates (SCR) on Volume Maintenance Rates (VMR) in the Fresh Fat Graft** 191
Hyun-Jin Yang and Hee-Young Lee

Part IV Use of Stem Cells in Aesthetic Procedures

- 14 Adipose-Derived Stem and Regenerative Cells as Fillers in Plastic and Reconstructive Surgery** 203
Min Zhu, Douglas M. Arm, and John K. Fraser
- 15 Advantages of the Transplantation of Fat in Plastic and Reconstructive Surgery** 219
Jose Guerrerosantos
- 16 Autologous Fat Transfer: Risk or Benefit?** 229
Norbert Pallua and Bong-Sung Kim
- 17 Adipose Tissue Anatomy** 239
Andrea Sbarbati, Giamaica Conti, Pietro Panettiere, and Dario Bertossi

18 Adipose-Derived Stem and Regenerative Cells: Harvesting, Processing, and Administration	249
Robert J. Troell	
19 Cell, Tissue, and Organ Culture: Coulter Counter Use in the Enumeration of Muscle and Fat Stem Cells	293
Melinda Fernyhough-Culver, Deri L. Helterline-Icenogge, Janet L. Vierck, Rod A. Hill, and Michael V. Dodson	
20 Adipose-Derived Regenerative Cells	299
Todd Malan	
21 Adipose Tissue: From Energy Reservoir to a Source of Cells for Epithelial Tissue Engineering	303
Angelo Trivisonno, Marc Abecassis, Massimo Monti, Gabriele Toietta, and Athmani Bachir	
22 The Role of Adipose Tissue-Derived Stem Cells and of Angiogenesis	327
Norbert Pallua and Bong-Sung Kim	
23 Extensive Characterization of Stem Cells Derived from Skin	335
Giovanni Di Benedetto, Manuela Bottoni, Alessandro Scalise, Stefania Gorbi, Matteo Torresetti, Roberto Di Primio, and Monia Orciani	
24 Current Therapeutic Uses of Adipose-Derived Stem and Regenerative Cells	343
Robert J. Troell	
25 Mesenchymal Stem Cells in Clinical Practice	365
John Flynn and Margo Priestly	
26 Adipose-Derived Stem Cells (ADSCs): Current Findings and Future Perspectives in Structural Facial Fat Grafting	383
Elisa Bolletta, Elisabetta Petrucci, Caterina Tartaglione, and Daniele Bordoni	
27 The Stem Cell-Enhanced Regenerative Facelift	415
Renato P. Calabria	
28 Facial Rejuvenation with Stem Cell Fat Graft: The FAMI™ Procedure	429
Roger E. Amar	
29 Specialized Stem Cell Fat Transfer to Face	441
Lewis J. Obi	
30 Fat Transfer for Face Volume Enhancement	463
Alberto Di Giuseppe, Saverio Cinti, Elisa Bolletta, and Elisabetta Petrucci	

31 Fat Transplantation for Hemifacial Atrophy: In Search for Improved Techniques	493
Cristina Isac and Aurelia Isac	
32 Secondary Correction of Facial Deformities Following Major Resection and Reconstruction: Fat Stem Cell for Restoration of Facial Asymmetries	499
Alberto Bedogni, Giordana Bettini, Andrea Fior, and Giorgia Saia	
33 Ear Lobe Revolumization	515
Nathan Newman	
34 Breast Augmentation and Augmentation of the Tuberos Breast with Adipose Tissue Transfer	519
Matteo Santoli, Luca Negosanti, and Domenico De Fazio	
35 Breast Augmentation with Stem Cell Fat Transfer and VASER™	529
Alberto Di Giuseppe and Dennis Wolf	
36 Fat Grafting Supplemented by Adipose-Derived Stem Cells for Breast Augmentation	557
Hiroshi Mizuno and Hiko Hyakusoku	
37 Breast Augmentation with Stem Cell-Enhanced Fat Transfer: Comparison Between Enhanced and Unenhanced Fat Grafting	563
Alberto Di Giuseppe and Dennis Wolf	
38 Multipotential Aspects of Breast Periprosthetic Capsule Stem Cells	573
Monia Orciani, Elisa Bolletta, Alessandro Scalise, Stefania Gorbi, Raffaella Lazzarini, Matteo Gioacchini and Giovanni Di Benedetto	
39 Tuberos Breast Correction with Stem Cell Fat Transfer	587
Egle Muti and Fabrizio Tomassetti	
40 Stem Cell Fat Transfer for Mastoplasty Using VASER™ Ultrasound and Office-Devised Stem Cell	601
Alberto Di Giuseppe and Dennis Wolf	
41 Adipocyte and Stem Cell Grafting: Impact on Cancer Detection	627
Khalid Almutairi and J. Peter Rubin	
42 Fat Grafting for Deep Inferior Epigastric Perforator Flap Refinements in Breast Reconstruction: The Hybrid Autologous Reconstruction	635
Andrea Spano, Daniele Bordoni, Pierfrancesco Cadenelli, Giuseppe Falco, and Maurizio Bruno Nava	

43	Stem Cell Enhanced Fat Grafting to Buttocks	651
	Nathan Newman	
44	Buttock Recontouring	661
	Domenico De Fazio and Matteo Santoli	
45	Improved Methods of Autologous Fat Transplantation in Correcting Buttock Asymmetry	681
	Cristina Isac and Aurelia Isac	
46	Buttock Biomolding with Autologous Adipose Tissue-Derived Stem Cells	689
	Roberto Blum Andrade	
47	Hand Rejuvenation	703
	Nathan Newman	
48	Hand Rejuvenation with Stem Cell Fat Transfer	709
	Andre Berger	
49	Free Fat Graft for Cosmetic Phalloplasty: Twenty-Year Retrospective	721
	Stephen Xavier Giunta	
50	Stem Cell-Assisted Fat Transfer to the Penis	739
	Marc Abécassis	
51	Vaginal Rejuvenation	743
	Marc Abécassis	
52	Free Fat Transfer in Irradiated Tissue	745
	Franco Bassetto, Angelo Sapuppo, Erica Dalla Venezia, and Leonardo Sartore	
53	The GID: A New Device for Fat Harvesting and Washing in Aesthetic Plastic Surgery	753
	Alberto Di Giuseppe and Diana Ronconi	
 Part V Complications		
54	Complications of Stem Cell-Assisted Fat Transfer	771
	Melvin A. Shiffman	
55	Interactions Between Adipose Stem Cells and Cancer	785
	Christopher Chung and J. Peter Rubin	
 Part VI Commentary		
56	Editor's Commentary	797
	Melvin A. Shiffman	
	Index	801

Adipocytes and Osteoblasts from Human Adipose Tissue Mesenchymal Stem Cells for the Production of Compatible and Safe Biomaterial Crucial in Cosmetic, Reconstructive, and Regenerative Medicine

Toai Cong Tran, Ciro Gargiulo, Thao Duy Huynh,
Khanh Hong Thien Bui, Luis Filgueira,
and Douglas M. Strong

T.C. Tran, M.D., Ph.D (✉)

Biomaterial Research Laboratory, Department of
Histology, Embryology, Genetics and Biotechnology
for Tissue Transplants, Pham Ngoc Thach University
of Medicine, 86/2 Thanh Thai St., 10th Dist.,
Ho Chi Minh City, Viet Nam

Department of Histology – Anapathology,
School of Medicine, Vietnam National
University – Ho Chi Minh City, Quarter 6,
Linh Trung Ward, Ho Chi Minh City,
Thu Duc District, Viet Nam

Department of Histology and Embryology,
Faculty of Medicine, The University of Medicine
and Pharmacy at Ho Chi Minh City,
217 Hong Bang St. 5th Dist.,
Ho Chi Minh City, Viet Nam
e-mail: toaiphd@yahoo.com

C. Gargiulo, Ph.D., M.Sc.
Department of Histology, Embryology,
Genetics and Biotechnology for Tissue Transplants,
Pham Ngoc Thach University of Medicine,
Ho Chi Minh City, Viet Nam

University of Western Australia School of Anatomy
and Human Biology, Perth, Australia
e-mail: kimcosmetic@yahoo.com

T.D. Huynh, M.A.
Department of Histology, Embryology,
Genetics and Biotechnology for Tissue Transplants,
Pham Ngoc Thach University of Medicine,
Ho Chi Minh City, Viet Nam
e-mail: Huynhduythao2005@Yahoo.Com

K.H.T. Bui, M.D., Ph.D
Orthopaedic Department,
University Medical Center,
University of Medicine and Pharmacology,
HCM City, 215–217 Hong Bang St., Dist.5,
HCM City, Viet Nam
e-mail: khanhbui1969@yahoo.com

L. Filgueira, M.D.
School of Anatomy, Physiology and Human Biology,
The University of Western Australia,
35 Stirling Highway,
Perth, WA M309, Australia

Anatomy Unit, Department of Medicine,
University of Fribourg, CH-1700,
Fribourg, Switzerland
e-mail: luis.filgueira@uwa.edu.au,
luis.filgueira@anhb.uwa.edu.au, luis.filgueira@unifr.ch

D.M. Strong, Ph.D
Department of Orthopaedics and Sports Medicine,
University of Washington School of Medicine, 18624
94th Ave West, Edmonds, WA 98020, USA
e-mail: dmichaelstrong@me.com

6.1 Introduction

Several published works have confirmed the possibility to obtain a population of stem cells from adipose tissue that share similar features and morphogenetic profile of MSCs from known sources such as BM, UCB, amniotic fluid, scalp tissue, placenta, and synovial tissue [1–13]. A common pattern of all MSC cultures in vitro is a combination of specific and homogeneous traits including the formation of uniform colonies with a typical fibroblast spindle-like shape, self-renewal capacity, high viability, and multilineage ability such as osteoblasts, adipocytes, keratinocytes, cardiomyocytes, chondrocytes, hepatocytes, and tenocytes [4–6, 14–18]. In general, they do not express definite markers of hematopoietic cell surface phenotype and are positive for integrins and adhesion molecules, positive for matrix receptors, and positive for few very specific markers such as CD13 (APN), CD28, CD166 (ALCAM), CD44, CD73, CD90, CD105 and CD166, vimentin, desmin, Runx2, osterix (OX), and HLA class I [4, 10, 11, 14, 15, 19, 20].

One of the most intriguing traits of MSCs is their immune-modulatory capacity that is of relevant clinical importance as they may be transplanted without the need of HLA matching between donor and recipient which is strictly required in allogeneic adult stem cell procedures, in BM transfusion, or in organ transplants [3, 21–26]. While each MSC subgroup shows an identical immune-phenotypic profile, each set clearly expresses remarkable quantitative and qualitative differences, revealing a sort of heterogeneity linked to a specific genetic set that control different activities within the system [5, 11, 13, 18, 25]. The possibility to obtain MSCs from different sources other than the more classic ones like BM and UCB is of vital importance in the field of regenerative medicine and may provide the opportunity to avoid unnecessary invasive procedures or to explore a better sustainable therapeutic strategy which is based on multiple possible choices considered on a case-by-case basis [13, 18, 26–28]. Adipose tissue, like marrow, is of mesoderm origin; it starts to form during the last trimester of intrauterine life and contains a

variety of stromal cells that group into microvascular endothelial cells, smooth muscle cells, and MSCs that can be isolated by enzymatic digestion and centrifugation [18, 24, 29, 30]. Adipose tissue is to be considered a complex structure that performs on multiple levels as an energy store and as an endocrine organ capable of producing and secreting a large number of molecules including leptin, adiponectin, and resistin which control endothelial functionality and may influence the vascular system [29, 31, 32]. Data from our study, in line with published research, have confirmed that fat tissue compared to BM contains more MSCs. From 1 g of tissue, you may collect $5 \times 10^{4-5}$ MSCs which is 500-folds larger than 1 g of MSCs obtained from BM [24, 33, 34]. hATMSCs, as their respective equivalent, have the ability to differentiate into diverse cell lines such as myoblasts, chondroblasts, cardiomyocytes, hepatocytes, adipocytes, and osteoblasts maintaining a unique plasticity typical of all MSCs [13, 19, 24, 30, 35, 36]. This study, in line with edited data, has demonstrated that hATMSCs, at least in vitro culture, behave in a similar manner and are able to home in a bone-like environment such as sea coral scaffold [10, 19, 27].

In line with other studies and with our previously published work, the authors have tried to establish a method that allows the use of these cells in combination with bio-scaffolds to be used in the reconstruction of hard tissue such as bone and in scaffolds in repairing damaged soft tissue and semihard tissues such as cartilage and intervertebral disk bulbs [10, 37, 38]. Therefore, the first task was to use hATMSCs and adipocytes and osteoblasts seeded onto marine coral *Porites lutea* to generate a hard structure bio-scaffold; the second task was to generate a soft scaffold using a fibrin gel seeded with human adipocyte cells to generate a soft structure scaffold. The fibrin gel has been generated by using autologous patient's blood; from this sample, fibrinogen and thrombin have been isolated and then mixed in unique solution in a ratio of 1:1 [37–40].

However, despite the promising results from preliminary clinical trials, our concern is about the possible contribution of hATMSCs in vivo as an active player in the process of vascular

Regenerative Medicine and Plastic Surgery

Skin and Soft Tissue, Bone,
Cartilage, Muscle, Tendon
and Nerves

Dominik Duscher
Melvin A. Shiffman
Editors

Contents

Part I Skin and Soft Tissue Regeneration

- 1 Induction of the Fetal Scarless Phenotype in Adult Wounds: Impossible? 3**
Michael S. Hu, Mimi R. Borrelli, Michael T. Longaker,
and H. Peter Lorenz
- 2 Scar Treatment and Prevention: Know Thine Enemy. 19**
Elizabeth A. Brett and Dominik Duscher
- 3 Challenges and Opportunities in Drug Delivery
and Wound Healing. 27**
Matthias M. Aitzetmüller, Hans-Günther Machens,
and Dominik Duscher
- 4 Harvesting, Processing, and Injection of Lipoaspirate
for Soft-Tissue Reconstruction: Details Make the Difference. . . 39**
Matthias A. Sauter, Elizabeth A. Brett,
Matthias M. Aitzetmüller, and Dominik Duscher
- 5 Adipose Tissue Complex (ATC): Cellular and Biocellular
Uses of Stem/Stromal Cells and Matrix in Cosmetic Plastic,
Reconstructive Surgery and Regenerative Medicine. 45**
Robert W. Alexander
- 6 Preparation, Characterization, and Clinical Implications
of Human Decellularized Adipose Tissue Extracellular Matrix . . 71**
Derek A. Banyard, Christos Sarantopoulos, Jade Tassey,
Mary Ziegler, Evangelia Chnari, Gregory R. D. Evans,
and Alan D. Widgerow
- 7 Mesenchymal Cells that Support Human Skin Regeneration . . 91**
Joanne K. Gardner, Zalitha Pieterse, and Pritinder Kaur
- 8 Stem Cells and Burn 109**
Anesh Prasai, Amina El Ayadi, David N. Herndon,
and Celeste C. Finnerty

- 9 Skin Tissue Engineering in Severe Burns:
A Review on Its Therapeutic Applications 117**
Alvin Wen Choong Chua, Chairani Fitri Saphira,
and Si Jack Chong
- 10 Skin Substitutes for Burn Wounds 137**
Daniel Popp, Christian Tapking, and Ludwik K. Branski
- 11 Wnt Signaling During Cutaneous Wound Healing 147**
Khosrow Siamak Houschyar, Dominik Duscher,
Susanne Rein, Zeshaan N. Maan, Malcolm P. Chelliah,
Jung Y. Cha, Kristian Weissenberg, and Frank Siemers
- 12 Drug Delivery Advances for the Regeneration of Aged Skin . . . 157**
Daniela Castillo Pérez, Matthias M. Aitzetmüller,
Philipp Neßbach, and Dominik Duscher

Part II Bone Regeneration

- 13 Innovative Scaffold Solution for Bone Regeneration
Made of Beta-Tricalcium Phosphate Granules,
Autologous Fibrin Fold, and Peripheral Blood Stem Cells 167**
Ciro Gargiulo Isacco, Kieu C. D. Nguyen, Andrea Ballini,
Gregorio Paduanelli, Van H. Pham, Sergey K. Aityan,
Melvin Schiffman, Toai C. Tran, Thao D. Huynh,
Luis Filgueira, Vo Van Nhan, Gianna Dipalma,
and Francesco Inchingolo
- 14 Ordinary and Activated Bone Substitutes 181**
Ilya Y. Bozo, R. V. Deev, A. Y. Drobyshhev, and A. A. Isaev
- 15 Absorbable Bone Substitute Materials Based
on Calcium Sulfate as Triggers for Osteoinduction
and Osteoconduction 211**
Dominik Pfföringer and Andreas Obermeier
- 16 Perivascular Progenitor Cells for Bone Regeneration 223**
Carolyn Meyers, Paul Hindle, Winters R. Hardy, Jia Jia Xu,
Noah Yan, Kristen Broderick, Greg Asatrian, Kang Ting,
Chia Soo, Bruno Peault, and Aaron W. James
- 17 Bone Repair and Regeneration Are Regulated
by the Wnt Signaling Pathway 231**
Khosrow Siamak Houschyar, Dominik Duscher,
Zeshaan N. Maan, Malcolm P. Chelliah, Mimi R Borrelli,
Kamran Harati, Christoph Wallner, Susanne Rein,
Christian Tapking, Georg Reumuth, Gerrit Grieb,
Frank Siemers, Marcus Lehnhardt, and Björn Behr

Part III Cartilage Regeneration

- 18 Cartilage Tissue Engineering: Role of Mesenchymal Stem Cells, Growth Factors, and Scaffolds** 249
Mudasir Bashir Gugjoo, Hari Prasad Aithal,
Prakash Kinjavdekar, and Amarpal
- 19 Sox9 Potentiates BMP2-Induced Chondrogenic Differentiation and Inhibits BMP2-Induced Osteogenic Differentiation** 263
Junyi Liao, Ning Hu, Nian Zhou, Chen Zhao, Xi Liang,
Hong Chen, Wei Xu, Cheng Chen, Qiang Cheng,
and Wei Huang
- 20 Stem Cells and Ear Regeneration** 281
Hamid Karimi, Seyed-Abolhassan Emami,
and Ali-Mohammad Karimi

Part IV Muscle and Tendon Regeneration

- 21 Muscle Fiber Regeneration in Long-Term Denervated Muscles: Basics and Clinical Perspectives** 301
Ugo Carraro, Helmut Kern, Sandra Zampieri, Paolo Gargiulo,
Amber Pond, Francesco Piccione, Stefano Masiero,
Franco Bassetto, and Vincenzo Vindigni
- 22 Rejuvenating Stem Cells to Restore Muscle Regeneration in Aging** 311
Eyal Bengal and Maali Odeh
- 23 Silk Fibroin-Decorin Engineered Biologics to Repair Musculofascial Defects** 325
Lina W. Dunne, Nadja Falk, Justin Hubenak,
Tejaswi S. Iyyanki, Vishal Gupta, Qixu Zhang,
Charles E. Butler, and Anshu B. Mathur
- 24 Skeletal Muscle Restoration Following Volumetric Muscle Loss: The Therapeutic Effects of a Biologic Surgical Mesh** 347
Jenna L. Dziki, Jonas Eriksson, and Stephen F. Badylak
- 25 Principles of Tendon Regeneration** 355
Jacinta Leyden, Yukitoshi Kaizawa, and James Chang
- 26 Stem Cells and Tendon Regeneration** 369
Hamid Karimi, Kamal Seyed-Foroontan,
and Ali-Mohammad Karimi
- 27 Cell Therapies for Tendon: Treatments and Regenerative Medicine** 385
Anthony Grognez, Pierre-Arnaud Aeberhard,
Murielle Michetti, Nathalie Hirt-Burri,
Corinne Scaletta, Anthony de Buys Roessingh,
Wassim Raffoul, and Lee Ann Laurent-Applegate

Part V Nerve Regeneration

28	Current Trends and Future Perspectives for Peripheral Nerve Regeneration	411
	Georgios N. Panagopoulos, Panayiotis D. Megaloikonomos, and Andreas F. Mavrogenis	
29	The Regeneration of Peripheral Nerves Depends on Repair Schwann Cells	425
	Kristján R. Jessen and Rhona Mirsky	
30	Adipose-Derived Stem Cells (ASCs) for Peripheral Nerve Regeneration	437
	Mathias Tremp and Daniel D. Kalbermatten	
31	Direct Reprogramming Somatic Cells into Functional Neurons: A New Approach to Engineering Neural Tissue In Vitro and In Vivo	447
	Meghan Robinson, Oliver McKee-Reed, Keiran Letwin, and Stephanie Michelle Willerth	
	Index	463



Innovative Scaffold Solution for Bone Regeneration Made of Beta-Tricalcium Phosphate Granules, Autologous Fibrin Fold, and Peripheral Blood Stem Cells

Ciro Gargiulo Isacco, Kieu C. D. Nguyen, Andrea Ballini, Gregorio Paduanelli, Van H. Pham, Sergey K. Aityan, Melvin Schiffman, Toai C. Tran, Thao D. Huynh, Luis Filgueira, Vo Van Nhan, Gianna Dipalma, and Francesco Inchingolo

13.1 Introduction

Diseases, trauma, and surgical procedures can be the cause of bone paucity and defects. Due to the complexity of bone anatomy and physiology bone

tissue degeneration and diseases can pose a big threat to doctors and physicians. However, modern bone tissue biomedical engineering has been considered as a valid substitute solution for these conditions [1]. Procedures applied to repair defects or

C. G. Isacco (✉) · G. Paduanelli · G. Dipalma
F. Inchingolo
Department of Interdisciplinary Medicine (DIM),
School of Medicine, University of Bari Aldo Moro,
Bari, Italy
e-mail: francesco.inchingolo@uniba.it

K. C. D. Nguyen
Department of Stem Cell Research, HSC
International Clinic, Ho Chi Minh City, Vietnam

A. Ballini
Department of Basic Medical Sciences,
Neurosciences and Sense Organs, University of
Bari Aldo Moro, Bari, Italy

V. H. Pham
Department of Microbiology, Nam Khoa-Bioteck
Microbiology Laboratory and Research Center,
Ho Chi Minh City, Vietnam

Department of Microbiology, Nam Khoa-Bioteck
Microbiology Laboratory and Research Center,
Ho Chi Minh City, VN, USA

S. K. Aityan
Multidisciplinary Research Center, Lincoln
University, Oakland, CA, USA
e-mail: aityan@lincolnuca.edu

M. Schiffman
Tustin, CA, USA

T. C. Tran
Stem Cells, Embryology and Immunity Department,
Pham Ngoc Thach University of Medicine,
Ho Chi Minh City, Vietnam
e-mail: trancongtoai@pnt.edu.vn

T. D. Huynh
Department of Embryology, Genetics and Stem Cells,
Pham Ngoc Thach University of Medicine,
Ho Chi Minh City, Vietnam
e-mail: thao_huynhduy@pnt.edu.vn

L. Filgueira
Faculty of Science and Medicine, University of
Fribourg, Fribourg, Switzerland
e-mail: luis.filgueira@unifr.ch

V. Van Nhan
University of Pharmacy and Medicine,
Ho Chi Minh City, Vietnam

Nha Khoa Nham Tam, Polyclinic and International
Dental Implant Center, Ho Chi Minh City, Vietnam

degeneration need the use of proper biomaterials with the right dimensions and anatomy shape that can fit into the damaged area. The cases of larger and more difficult defects need highly osteogenic scaffolds to promote and improve the bone tissue formation and regeneration [2].

The majority of scaffolds made of ceramics and metals or from polymers showed a weak osteogenic capability. Bone grafts are currently available in many different forms, such as allogeneic/autologous demineralized bone matrix or implants, growth factor-loaded microbeads, or bio-derivate as calcium hydroxyapatite, gels, ceramic derivate, sea corals, and metals [2, 3]. The main target is to reduce the incidence of collateral complications such as rejection, infection and inflammation, and donor-site morbidity and of course reduce the overall costs and related expenses such as frequent hospitalization [3–5]. Breakdowns, such as partial ruptures or complete collapse, are the major issues related to synthetic implants, generally due to quality of the material and subtle autoimmune responses that may also create ideal conditions for bacterial growth, inflammation, and rejection [6–9]. Metal implants, for example, may cause malfunction due to aseptic loosening with specific inflammatory and immune responses to metal-wear particles released during bio-corrosion which intensify the osteolytic activity of osteoclasts at the bone-implant interface, leading to a progressive loss of fixation [8, 9]. Therefore, an optimal biomaterial should possess specific bio-characteristics and qualities that should be biodegradable, tolerable, and safely absorbed by the body. This should happen without causing any kind of damaging event such as an inflammation or an immune reaction, capable of carrying and supporting tissue growth and proliferation, thus allowing bone regeneration [5, 9].

The latest generations of bio-implants have been created with the precise intent of functioning as cell carriers capable of reproducing human bone formation process. The newest generation of these types of scaffolds has been developed with materials that possess specific mechanical and structural properties that are compatible with the anatomical site into which they are to be inserted, with enough volume fraction and high surface area to carry an enough number of cells within the scaffold

and the surrounding host tissues. This allows ingrowth and vascularization [5]. Therefore, the new bio-implants tend to replicate the process of the formation of new bone development or which physiologically takes place after an injury [10].

An inflammatory response takes place after an implantation of a biomaterial as a consequence of host immune response [10]. During this phase monocytes differentiate to tissue macrophages. However, presence of MSCs promotes an immunomodulatory activity on macrophage M1/M2 balance towards M2 commencing a favorable cascade of events where interleukins such as IL-10, IL-4, IL-13, and IL-6 and prostaglandin E2 initiate the first step of the repairing process [11–14]. Bone plays a key role in well functioning of immune system and it is the site that immune cells are created. In fact, autoimmune disorders often induce bone tissue damages and degeneration, an event that has been confirmed by an experiment where macrophage ablation leads to intramembranous bone defect and inhibiting of the healing process [14].

In effect, previous studies have shown that some biomaterials due to high similarity with human tissues are able to trigger physico-chemical signals leading to stem cell differentiation towards diverse cell phenotypes as osteoblasts [15, 16]. Results have shown that biomaterials based on calcium phosphate (CaP), a major constituent of native bone tissue, induce naïve stem cells towards osteogenic differentiation promoting *in vivo* bone tissue formation and augmentation [16, 17].

However, though CaP is quickly absorbed *in vivo*, the process often occurs preceding the formation of new bone tissue that results in an incongruence between the host's new bone and scaffold. Conversely, β -TCP seems to be better compatible as the absorption rate is slower with a steady release of both calcium (Ca^{2+}) and sulfate (SO_4^{2-}) ions [18].

In line with our published study, we can confirm that hPB contains the right amount of different subsets of pluripotent and multipotent stem cells such as MSCs, HSCs, NSCs, and ESCs capable of differentiating into cells of different lineages such as osteoblasts [19]. In this current study, we have noted that part of hPB-SCs were induced to differentiate to active osteoblasts under the direct influence of β -TCP granules within a period of 7–10 days without the need of