Springer Series in Biomaterials Science and Engineering 2

Besim Ben-Nissan *Editor*

Advances in Calcium Phosphate Biomaterials

Springer

2

Springer Series in Biomaterials Science and Engineering

For further volumes:
http://www.springer.com/series/10955

Series Editor

Prof. Min Wang
Department of Mechanical Engineering
The University of Hong Kong
Pokfulam Road, Hong Kong
e-mail: memwang@hku.hk

Aims and Scope

The Springer Series in Biomaterials Science and Engineering addresses the manufacture, structure and properties, and applications of materials that are in contact with biological systems, temporarily or permanently. It deals with many aspects of modern biomaterials, from basic science to clinical applications, as well as host responses. It covers the whole spectrum of biomaterials – polymers, metals, glasses and ceramics, and composites/hybrids – and includes both biological materials (collagen, polysaccharides, biological apatites, etc.) and synthetic materials. The materials can be in different forms: single crystals, polycrystalline materials, particles, fibers/wires, coatings, non-porous materials, porous scaffolds, etc. New and developing areas of biomaterials, such as nano-biomaterials and diagnostic and therapeutic nanodevices, are also focuses in this series. Advanced analytical techniques that are applicable in R & D and theoretical methods and analyses for biomaterials are also important topics. Frontiers in nanomedicine, regenerative medicine and other rapidly advancing areas calling for great explorations are highly relevant.

The Springer Series in Biomaterials Science and Engineering aims to provide critical reviews of important subjects in the field, publish new discoveries and significant progresses that have been made in both biomaterials development and the advancement of principles, theories and designs, and report cutting-edge research and relevant technologies. The individual volumes in the series are thematic. The goal of each volume is to give readers a comprehensive overview of an area where new knowledge has been gained and insights made. Significant topics in the area are dealt with in good depth and future directions are predicted on the basis of current developments. As a collection, the series provides authoritative works to a wide audience in academia, the research community, and industry.

Besim Ben-Nissan
Editor

Advances in Calcium Phosphate Biomaterials

Springer

Editor
Besim Ben-Nissan
Chemistry and Forensic Science
University of Technology
Sydney, NSW, Australia

ISSN 2195-0644 ISSN 2195-0652 (electronic)
ISBN 978-3-642-53979-4 ISBN 978-3-642-53980-0 (eBook)
DOI 10.1007/978-3-642-53980-0
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014936420

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

This book is dedicated to Professor Racquel Z. LeGeros, who has taught us and contributed so much in both research and education for the advancement of calcium phosphates and biomaterials. She will be greatly missed.

Tribute to Racquel

I ran across a simple note when cleaning out our offices from someone I really don't remember. It goes like this:

> Dear Dr Racquel. I really wish to thank you for everything you have done for me to advance my career. You really did not know me, and I had difficulty understanding why you would help me until I got to know you and observed that it was your nature to be kind and helpful to everyone in your path.
>
> John P. LeGeros, Ph.D.

Preface

"Cogito Ergo Sum"
René Descartes 1644

I would like to tell a story that all these years I seldom talked about.

It was on one of those dark sunsets of October 1973 that I found myself wounded in one of those conflicts that unfortunately never ended.

A number of doctors were busy trying to help me and other wounded soldiers under the harsh dangers of the front line. These doctors and following surgeries in hospital helped me to realise how "life was at the edge of a fine line" that separated our existence from death. In reality this happens in many instances throughout human history; on one side, humanity tries to exterminate each other and on the other it tries to save or repair life.

This was my first intimate and personal encounter with the medical field, and I admire those individuals that sacrificed themselves to help. It opened a new thinking in my mind to use my knowledge to help to preserve and improve life. It became a search for solutions to problems within medical science, which opened up new avenues for future endeavours.

After a few months, I found a number of people–at that time–that were the backbones of the "biomedical materials field pioneering research" with their new inventions and ground-breaking research. Dr. Charnley in the UK was one of those orthopaedic surgeons that understood well both biomechanics and materials in addition to his surgical skills. He was an excellent inventive thinker and was experimenting on the total hip joint designs that set the benchmark that we still use today. Professor Weis was working on the first blade-type titanium dental implants in the USA.

On the scientific side of the biomaterials field, Drs. Racquel and John LeGeros were active and initiated the basic chemistry and substitutions of calcium phosphates for bone regeneration and repair. Dr. Larry Hench was a young inventor with unique scientific approach to chemistry and ceramic synthesis to introduce the bioglass and other inventions that motivated generations that followed, and still do. Dr. Samuel Hulbert and his PhD student Dr. Klawitter postulated that porous ceramics can

be used as scaffolds, and produced a list of materials that till today is the golden standard of biocompatible materials. Dr. Aoki pioneered and set the synthesis methods for calcium phosphates that motivated me and others to follow his footsteps and all these giants mentioned in the field. During the late 1970s and the early 1980s, in Asia and specifically in Japan, Drs. Kawahara, Yamamuro, Oonishi and Kokubo and in Europe Klaas de Groot, Ducheyne, Rey and Dacusi and others were experimenting on a range of calcium phosphate bioceramics that further opened new avenues into which many of us built our research.

My motivation and involvement in bioceramics goes back to these researchers and their pioneering research efforts mentioned above. I have admired and have respect for all of the new small steps that many scientists contributed during the years and having motivated us with their work. I was thrilled when Prof. Min Wang proposed that I should edit a book on the "Advances in Calcium Phosphate Biomaterials" for Springer. Immediately, I contacted Prof. Racquel LeGeros to ask her to share with me the pleasure of partially writing and editing this book. Although very busy, she graciously agreed. At the Bioceramics 24 meeting last year in Kyushu, Japan, we prepared the basic structure and the list of authors we aimed to invite to contribute.

We were interested in calcium phosphate-based biomaterials and specifically "apatites" that since the early days have taken a role of passive scaffold for bone regeneration and repair. We were observing that during the last two decades, the concept has changed from passive participation to active involvement to stimulate the body to regenerate and repair the tissue. New-generation calcium phosphate scaffolds are designed to stimulate specific cellular responses in the nanoscale level utilising biogenic additives such as bone morphogenic proteins and stem cells to help release the ionic dissolution products and activate the cells in contact with the biomaterials. With the appropriate microbiological, biochemical and biomechanical stimulation, the cells produce additional growth factors that in turn stimulate generation of growing cells to self-assemble to the required tissues. Taking to account all these factors, we aimed in this book to bring these new concepts, mechanisms and methods by experienced and well-known and young academics, clinicians and researchers to forward their knowledge and expertise on calcium phosphate and related materials and their clinical applications. The general aim was directed not only to cover the fundamentals but also to open new avenues to meet the challenges of the future in research and clinical applications. Both Racquel and I were going to share the responsibility of inviting and co-authoring a few chapters, but it was not meant to be. A few months after sending invitation letters to authors, I received the sad, unexpected and hurting news from Prof. Dacusi that Racquel passed away in France, during her visit, where she usually helped students in their research efforts on calcium phosphates and related materials.

It was a very difficult decision to continue with the book without her guidance and support. However, we felt that this was probably what Racquel would have wanted us to do.

We received 17 chapters, and many others apologised that due to their heavy academic load they could not meet the timeline. This book is therefore their story that covers the advances in calcium phosphate materials from its modern characterisation methods to tissue-biomaterial interactions, from bioglass to biocomposites, from marine structures to drug delivery and from its history to new orthopaedic and maxillofacial applications.

To meet various needs of research, education and clinical applications, each chapter provides clear and fully detailed descriptions, theoretical and experimental issues, discussions and future considerations. This in-depth, practical coverage should also assist the recent graduates and the medical professionals in the calcium phosphate and in general in the biomedical materials field.

Throughout history, science never ceased "advancing", and I trust that this reference book conveys the intensity of this fast "advancing calcium phosphate" field in an enthusiastic way to generate further research and their medical applications to further help the well-being of humans.

Sydney, NSW, Australia Besim Ben-Nissan, Ph.D.

Acknowledgements

First of all, I would like to acknowledge the contributions of Prof. Racquel Z. LeGeros to this book during its inception and for setting the tone. During the last five decades, she has inspired many of us to work and publish in the calcium phosphate field, and she set directions in which future generations will take the field further.

It was her unfortunate departure that left me alone to complete what we started together. To achieve this goal, two individual colleagues have contributed on every step of preparing this book, Dr. Andy H. Choi and Mr. Innocent Jacob Macha; without their hard work, persistence and contribution and visions, this book could not have been completed.

Contents

Contributors

Alexandre A. Barros 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Taipas, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga, Guimarães, Portugal

Besim Ben-Nissan School of Chemistry and Forensic Science, University of Technology, Sydney, NSW, Australia

Faculty of Science, University of Technology, Sydney, NSW, Australia

Chris C. Berndt Industrial Research Institute Swinburne (IRIS), Swinburne University of Technology, Hawthorn, VIC, Australia

Department of Materials Science and Engineering, Stony Brook University, Stony Brook, NY, USA

Luiz E. Bertassoni Biomaterials Science Research Unit, Department of Dental Biomaterials, Faculty of Dentistry, University of Sydney, Surry Hills, NSW, Australia

Andy H. Choi School of Chemistry and Forensic Science, Faculty of Science, University of Technology, Sydney, NSW, Australia

Joshua Chou Faculty of Pharmacy, Research Institute of Pharmaceutical Science, Musashino University, Nishi-Tokyo, Tokyo, Japan

Faculty of Science, University of Technology, Sydney, NSW, Australia

Christèle Combes CIRIMAT-Carnot Institute, University of Toulouse, UMR CNRS-UPS-INPT 5085, ENSIACET, Toulouse, France

Richard C. Conway School of Chemistry and Forensic Science, Faculty of Science, University of Technology, Sydney, NSW, Australia

Guy Daculsi Inserm U791, University of Nantes and Inserm CIC-IT 802, Hospital of Bordeaux LIOAD Inserm Université de Nantes UMR U791, Faculté de Chirurgie Dentaire Pl A. Ricordeau, Nantes cedex, France

Sergey Dorozhkin Kudrinskaja sq. 1-155, Moscow 123242, Russia

Christophe Drouet CIRIMAT-Carnot Institute, University of Toulouse, UMR CNRS-UPS-INPT 5085, ENSIACET, Toulouse, France

Hunida M. Elfallah Biomaterials Science Research Unit, Department of Dental Biomaterials, Faculty of Dentistry, University of Sydney, Surry Hills, NSW, Australia

Borhane Hakim Fellah Oniris, National Veterinary School, Nantes, France

David W. Green School of Chemistry and Forensic Science, University of Technology, Sydney, NSW, Australia

Faculty of Dentistry, The University of Hong Kong, Hong Kong, China

David Grossin CIRIMAT-Carnot Institute, University of Toulouse, UMR CNRS-UPS-INPT 5085, ENSIACET, Toulouse, France

Yoshifumi Hanaoka, M.D. H. Oonishi Memorial Joint Replacement Institute, Tominaga Hospital, Osaka, Japan

Jia Hao Oral Implantology and Regenerative Dental Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Md. Fahad Hasan Industrial Research Institute Swinburne (IRIS), Swinburne University of Technology, Hawthorn, VIC, Australia

Larry L. Hench, Ph.D. Department of Biomedical Engineering, Florida Institute of Technology, Melbourne, FL, USA

Kunio Ishikawa Department of Biomaterials, Faculty of Dental Science, Kyushu University, Fukuoka, Kyushu, Japan

Atsuo Ito Human Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

Ryoko Iwata, Ph.D. Olympus Terumo Biomaterials Corporation, Tokyo, Japan

Ikuo Kawahara, M.D. H. Oonishi Memorial Joint Replacement Institute, Tominaga Hospital, Osaka, Japan

Feza Korkusuz, M.D. Department of Sports Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey

Petek Korkusuz, M.D., Ph.D. Department of Histology and Embryology, Hacettepe University Faculty of Medicine, Ankara, Turkey

Racquel Z. LeGeros Department of Biomaterials and Biomimetics, New York University College of Dentistry, New York, NY, USA

Álvaro J. Leite 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Taipas, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Stony Brook University, Braga, Guimarães, Portugal

Innocent J. Macha School of Chemistry and Forensic Science, Faculty of Science, University of Technology, Sydney, NSW, Australia

João F. Mano 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Taipas, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga, Guimarães, Portugal

Olivier Marsan CIRIMAT-Carnot Institute, University of Toulouse, UMR CNRS-UPS-INPT 5085, ENSIACET, Toulouse, France

Bruce Milthorpe Faculty of Science, University of Technology, Sydney, NSW, Australia

Thomas Miramond LIOAD Inserm Université de Nantes UMR U791, Faculté de Chirurgie Dentaire Pl A. Ricordeau, Nantes cedex, France

Hirotsugu Ohashi, M.D., Ph.D. Department of Orthopaedic Surgery, Saiseikai Nakatsu Hospital, Osaka, Japan

Hironobu Oonishi, M.D., Ph.D. H. Oonishi Memorial Joint Replacement Institute, Tominaga Hospital, Osaka, Japan

Hiroyuki Oonishi Jr., M.D. H. Oonishi Memorial Joint Replacement Institute, Tominaga Hospital, Osaka, Japan

Akiyoshi Osaka Faculty of Engineering, Okayama University, Tsushima, Okayama-shi, Japan

Makoto Otsuka Faculty of Pharmacy, Research Institute of Pharmaceutical Science, Musashino University, Nishi-Tokyo, Tokyo, Japan

Ayako Oyane Nanosystem Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

Ricardo A. Pires 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Taipas, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga, Guimarães, Portugal

Rui L. Reis 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Taipas, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga, Guimarães, Portugal

Christian Rey CIRIMAT-Carnot Institute, University of Toulouse, UMR CNRS-UPS-INPT 5085, ENSIACET, Toulouse, France

Stéphanie Sarda CIRIMAT-Carnot Institute, University of Toulouse, UMR CNRS-UPS-INPT 5085, ENSIACET, Toulouse, France

K.-P. Schmitz Institute for Biomedical Engineering, University of Medicine, University of Rostock, Rostock, Germany

Mahdi Shahmoradi Biomaterials Science Research Unit, Department of Dental Biomaterials, Faculty of Dentistry, University of Sydney, Surry Hills, NSW, Australia

Michael Swain Biomaterials Science Research Unit, Department of Dental Biomaterials, Faculty of Dentistry, University of Sydney, Surry Hills, NSW, Australia

U. Tietz Industrial Research Institute Swinburne (IRIS), Swinburne University of Technology, Hawthorn, VIC, Australia

Muharrem Timuçin, Ph.D. Department of Metallurgical and Materials Engineering, Middle East Technical University, Ankara, Turkey

Xiupeng Wang Human Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

Abbreviations

ACP	Amorphous calcium phosphate
ALP	Alkaline phosphatase
ASC	Embryonic stem cells
BCP	Biphasic calcium phosphate
BMP	Bone morphogenetic protein
CDA	Calcium-deficient apatite
CFA	Carbonate- and fluoride-containing apatite
CHA	Carbonate hydroxyapatite
CPPD	Calcium pyrophosphate dehydrate
DCPD	Dicalcium phosphate dehydrate
ECM	Extracellular matrix
HAp	Hydroxyapatite
HCA	Hydroxyl-carbonate apatite
hMSCs	Human mesenchymal stem cells
IBBC	Interface bioactive bone cement
LPD	Liquid phase deposition
MCPM	Monocalcium phosphate monohydrate
OCP	Octacalcium phosphate
SBF	Simulated body fluid
TCP	Tricalcium phosphate

Chapter 1
Introduction to Synthetic and Biologic Apatites

Racquel Z. LeGeros and Besim Ben-Nissan

Abstract In the early 1970s, bioceramics were employed to perform singular, biologically inert roles, such as to provide parts for bone replacement. The realization that cells and tissues perform many other vital regulatory and metabolic roles has highlighted the limitations of synthetic materials as tissue substitutes. Demands of bioceramics have changed from maintaining an essentially physical function without eliciting a host response to providing a more integrated interaction with the host. This has been accompanied by increasing demands from medical devices to improve the quality of life, as well as extend its duration. Bioceramics especially hydroxyapatite incorporating biologic additives can be used as body interactive materials, helping the body to heal or promoting regeneration of tissues, thus restoring physiological functions. The crystallography and characterization of biologic and synthetic apatites are very complex. This chapter attempts to cover over four decades of research on one of the most intriguing and fascinating fields of research.

Keywords Bioceramic • Biologic apatite • Coralline HAp • Biomimetic • Nanocrystals

R.Z. LeGeros
Department of Biomaterials and Biomimetics, New York University College of Dentistry, New York, NY, USA

B. Ben-Nissan (✉)
School of Chemistry and Forensic Science, Faculty of Science, University of Technology, Broadway, Ultimo, PO BOX 123, Sydney, NSW 2007, Australia
e-mail: Besim.Ben-Nissan@uts.edu.au; b.ben-nissan@uts.edu.au

B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, Springer Series in Biomaterials Science and Engineering 2, DOI 10.1007/978-3-642-53980-0_1,

1.1 Introduction

"Apatite" (Gr, to deceive) was the name given first by Werner in 1788 to describe a group of mineral crystals appearing with various tints (yellow, green, pink, etc.) that were often confused with more precious minerals or gems such as aquamarine, amethyst, topaz, etc. These minerals have the general formula $M_{10}(PO_4)_6X_2$, where M could be one of several metals (usually calcium, Ca), P is most commonly phosphorus (P), and X is commonly hydroxide (OH) or a halogen such as fluorine (F) or chlorine (Cl). Currently, the name "apatite" describes a family of compounds having similar structure (hexagonal system, space group, P63/m) in spite of a wide range of compositions [1, 2]. The unit cell of calcium hydroxyapatite (HAp) contains ten calcium (Ca), six PO_4, and two OH groups, arranged as shown in Fig. 1.1. The OH groups located in the corners of the unit cell are surrounded by two sets of Ca (II) atoms arranged in a triangular patterns at levels $z = 0$ and $z = 1/2$, by two sets of PO_4 tetrahedral also arranged in triangular patterns at levels $z = Y$ and %, and by a hexagonal array of Ca (I) atoms at a distance [3]. Critical to

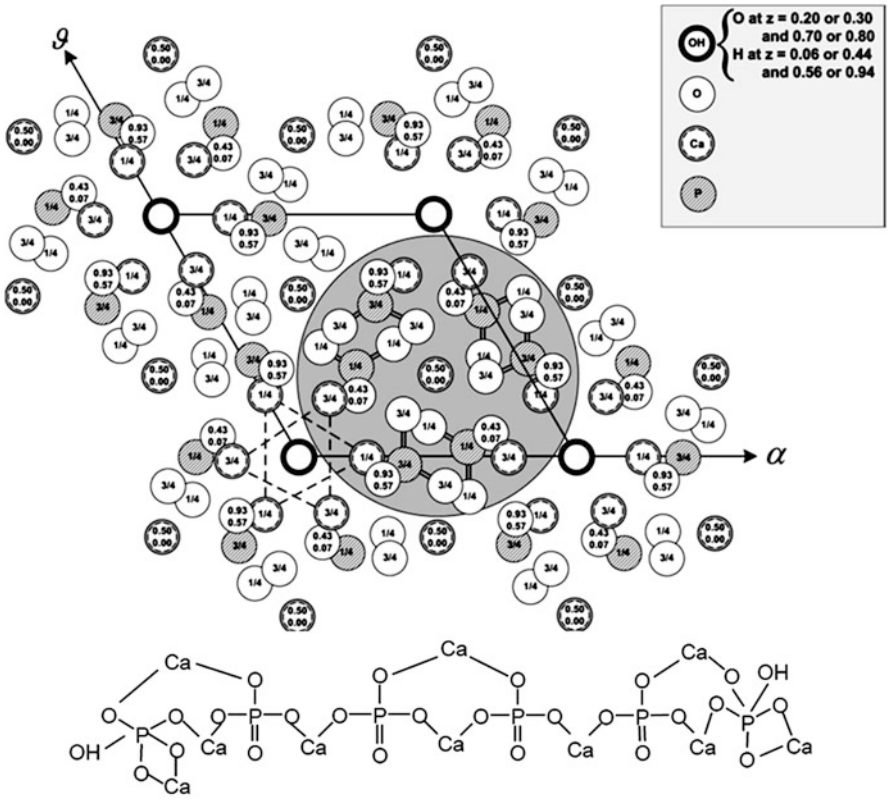

Fig. 1.1 The unit cell and simplified arrangement of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$

the apatite structure is the network of PO_4 groups in tightly packed arrangements [2]. Substitutions in the apatite structure affect lattice parameters (*a*- and *c*-axis dimensions, infrared absorption characteristics, morphology, dissolution properties, and thermal stabilities).

Biologic apatites are the inorganic phases of calcified tissues (teeth and bones). Similarity in composition of calcined bone to the apatite mineral was proposed by Proust and Klaproth in 1788 [4], and similarity in the X-ray diffraction patterns of bone and mineral apatites (HAp and fluorapatite (FA)) and similarity in composition (principally calcium and phosphate ions) led to the conclusion that the inorganic phases of bones and teeth are basically calcium hydroxyapatite [5–7]. Detection of carbonate associated with biologic apatites led to the speculation that these mineral phases are carbonate-containing apatites similar to the minerals dahllite (carbonate-containing apatite) or staffellite (carbonate- and fluoridecontaining apatite) [8]. Studies on synthetic carbonate-substituted apatites demonstrated that carbonate substitution in the apatite structure can proceed in two ways: CO_3-for-OH or type A [9, 10] and CO_3-for-PO_4 or type B, coupled with Na-for-Ca [11, 12], and combined analyses of synthetic and biologic apatites using X-ray diffraction, infrared spectroscopy, and chemical analyses demonstrated that biologic apatites are carbonate apatites approximated by the formula $(Ca,Mg,Na)_{10}(PO_4,CO_3,HPO_4)_6(CO_3,OH)_2$ [12–14]. For example, the following formula $Ca_{8.856}Mg_{0.088}Na_{0.292}K_{0.010}(PO_4)_{5.312}(HPO_4)_{0.280}(CO_3)_{0.407}(OH)_{0.702}Cl_{0.078}(CO_3)_{0.050}$ was proposed to describe the chemical composition of the inorganic part of dental enamel.

Early studies on synthetic apatites and related calcium phosphates were made to gain a better understanding of the structure, composition, and properties of biologic apatites, especially of human enamel apatites. However, studies on synthetic apatites in the last 30 years had focused on their preparation, their applications in medicine and dentistry, and their use as scaffolds for bone and teeth regeneration. Current commercial synthetic calcium phosphate biomaterials classified on the basis of composition include HAp, $Ca_{10}(PO_4)_6(OH)_2$; α- and β-tricalcium phosphates (α-TCP, β-TCP), $Ca_3(PO_4)_2$; and biphasic calcium phosphate (BCP), an intimate mixture of HAp and β-TCP with varying HAp/β-TCP ratios [15–19]. Other commercial HAp biomaterials are derived from biologic materials (e.g., processed human bone, bovine bone derived, hydrothermally converted coral or derived from marine algae) [20–22].

This chapter is a brief review of biologic and synthetic apatites used as biomaterials, updating reviews published earlier by the authors [13, 20, 23, 24].

1.2 Biogenic Apatites

Normal and pathologic calcified tissues are composites of organic and inorganic phases. For bone, dentin, and cementum, the organic phase is principally collagen (about 25 % by weight) with smaller amounts of non-collageneous proteins [25].

On the other hand, the main organic phase in enamel is a non-collageneous protein (amelogenin) comprising about 1 % by weight of the enamel. In normal calcified tissues, such as in teeth and bones, in fish enameloids (teeth or calcified scales of some species), and in some species of shells, only carbonate (carbonate hydroxyapatite, CHA)- or carbonate- and fluoridecontaining apatite (CFA) occurs as the principal inorganic phase [13, 24, 26, 27]. In pathologic calcifications (dental calculus, urinary stones, vascular calcification, and other soft-tissue calcifications), biologic apatite may occur as one of the mineral phases that include other calcium phosphates, e.g., amorphous calcium phosphate (ACP), $Ca_x(PO_4)_y$; dicalcium phosphate dehydrate (DCPD), $CaHPO_4 \cdot 2H_2O$; octacalcium phosphate (OCP), $Ca_8H_2(PO_4)_6 \cdot 5H_2O$; magnesium-substituted tricalcium phosphate (β-TCMP, $(Ca,Mg)_3(PO_4)_2$); and calcium pyrophosphate dihydrate (CPPD), $Ca_2P_2O_7 \cdot 2H_2O$ [24, 28].

1.3 Enamel, Dentin, and Bone Apatite

Enamel, dentin, and bone apatite differ in crystallinity, reflecting crystal size (Fig. 1.2) and concentrations of minor constituents, mainly Mg and CO_3 (Fig. 1.3a, Table 1.1) [13, 24, 29]. Enamel apatite contains the lowest concentrations of these ions and the highest crystallinity (larger crystals) compared to either dentin or bone apatite that shows much lower crystallinity (smaller crystals) or greater concentrations of Mg and CO_3 (Table 1.1). These apatites also differ in solubility, decreasing in the order bone > dentin >> enamel.

These differences in crystallinity (crystal size) and solubility may be attributed to the differences in the concentrations of the minor constituents (e.g., Mg, N, CO_3, HPO_4). Studies on the effect of Mg and CO_3 ions on the properties of synthetic apatites demonstrated that incorporation of these ions independently and

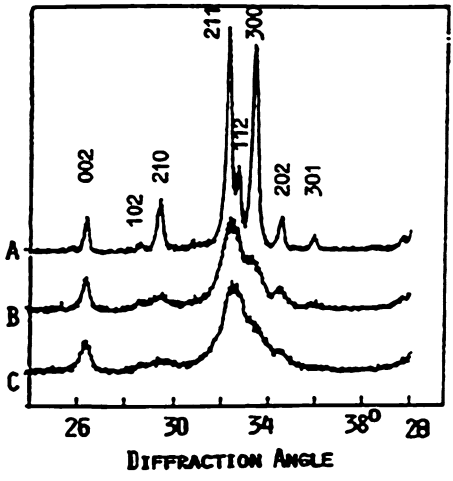

Fig. 1.2 X-ray diffraction profiles of biologic apatites from adult human enamel (**a**), dentin (**b**), and bone (**c**)

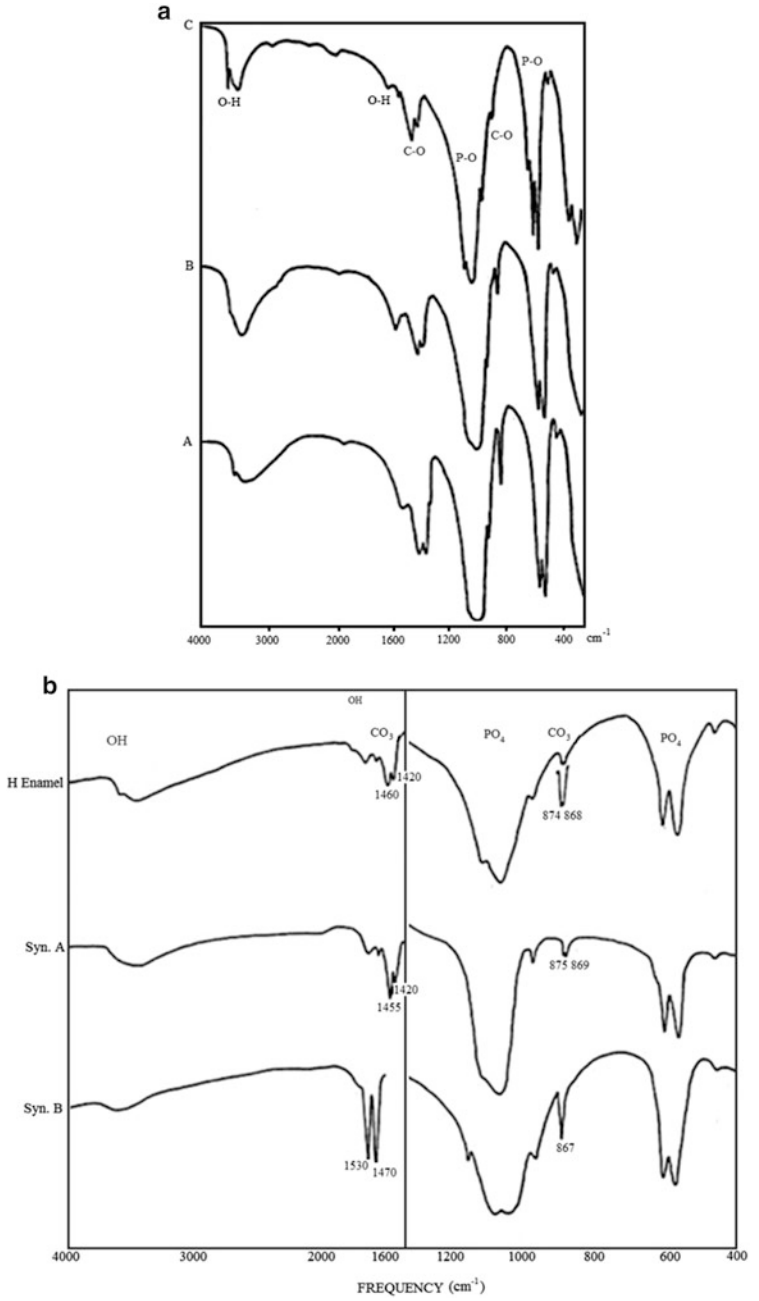

Fig. 1.3 (**a**) FTIR spectra of biologic apatites from adult human enamel, dentin, and bone. Note the higher resolution of the P–O (for PO_4 groups) absorption spectra of enamel (*C*), compared to those of dentin (*B*) and bone (*A*) apatites. (**b**) FTIR spectra comparing the characteristic C–O (for CO_3 groups) absorption bands in human enamel apatite compared to those in synthetic apatite with type A (CO_3-for-OH) substitution (syn A) and with type B (CO_3-for-PO_4) substitution (syn B)

Table 1.1 Composition (%) and physical properties of apatites in adult human enamel, dentin, and bone

	Enamel	Dentin	Bone
Composition			
Calcium, Ca^{2+}	36.5	35.1	34.8
Phosphorus, P	17.7	16.2	15.6
Ca/P (molar)	1.63	1.63	1.71
Sodium, Na^{+}	0.5	0.6	0.9
Magnesium, Mg^{2+}	0.34	1.23	0.72
Potassium, K^{+}	0.06	0.05	0.03
Carbonate, CO_3^{2-}	3.5	5.6	7.4
Fluoride, F^{-}	0.01	0.06	0.03
Chloride, Cl	0.30	0.01	0.13
Pyrophosphate	0.02	0.10	0.07
Total inorganic (mineral)	97.0	70.0	65.0
Total organic	1.5	20.0	25.5
Absorbed H_2O %	1.5	10.0	10.0
Trace elements: Zn, Cu, Fe, Sr, etc.			
Crystallographic properties			
Lattice parameters			
a-axis (+ 0.0003 nm)	0.9441		
c-axis (+ 0.0003 nm)	0.6880		
Crystallite size (nm, avg.)	33 × 3	2 × 0.4	2.5 × 0.3
Crystallinity index, *b*	70–75	33–37	33–37
Ignition products (800 °C)	HAp + β-TCMP	HAp + β-TCMP	HAp
HAp, $Ca_{10}(PO_4)_6(OH)_2$ *a*-axis = 0.9422 nm, *c*-axis = 0.6882 nm Crystallinity index = 100 Composition: Ca, P, OH			

synergistically causes the growth of smaller and more soluble apatite crystals [13, 24, 29–32]. The effects of CO_3 incorporation on apatite crystal size and morphology and on dissolution properties are much more pronounced than that of Mg [13, 24, 32]. Proteins [25] and/or other ions (e.g., pyrophosphate, citrate) [12, 24] may also inhibit the crystal growth of biologic apatites.

Biologic apatites are usually calcium-deficient (i.e., with Ca/P molar ratio less than the stoichiometric value of 1.67 obtained for pure HAp, $Ca_{10}(PO_4)_6(OH)_2$). Calcining above 900 °C of human enamel and dentin apatite results in the loss of the CO_3 constituent and formation of HAp and β-TCMP [13, 24]. Calcining of bone (human or bovine) above 900 °C results in the loss of CO_3 and formation of mostly HAp with small amounts of calcium oxide, CaO [13, 24].

Partial dissolution of biologic apatites and precipitation of other calcium phosphates (DCPD, OCP, β-TCMP) are believed to occur in human enamel and dentin caries, characterized by the dissolution of the tooth mineral (carbonate apatite) by acids produced by oral bacteria [33, 34]. The non-apatitic calcium phosphates

(e.g., DCPD, OCP) may, in turn, transform to apatites by hydrolysis or by dissolution and reprecipitation processes [13, 24, 34].

Biologic apatites have been idealized as calcium HAp, $Ca_{10}(PO_4)_6(OH)_2$ [5–8]. However, the difference in lattice parameters: *a*-axis for enamel, 0.9442 nm vs. 0.9422 nm for HAp. Ca/P stoichiometry (e.g., about 1.63 for enamel or dentin apatite vs. 1.67 for HAp) and the association of other ions, notably magnesium (Mg) and carbonate (CO_3) with biologic apatites (Table 1.1), have caused many years of disagreements and research on the structure and composition of biologic apatites [8, 9, 13, 35].

The nature of CO_3 incorporation in biologic apatites, especially in human enamel apatite, had been a preoccupation of several researchers. The larger *a*-axis dimension of human enamel apatite compared to pure or mineral HAp was first attributed to the CO_3-for-OH substitution (type A) in these apatites [9]. Such type of substitution was observed in synthetic carbonate apatites prepared by diffusing CO_2 into HAp at 1,000 °C under very dry conditions, resulting in an expanded *a*-axis and contracted *c*-axis dimension B compared to pure HAp [9, 10]. However, studies on synthetic apatites prepared at much lower temperatures (60–95 °C) by precipitation or hydrolysis methods showed a partial CO_3-for-PO_4 substitution (type B) coupled with a partial Na-for-Ca substitution, resulting in a contracted *a*-axis and expanded *c*-axis dimension compared to CO_3-free apatites [11–13]. The observed expanded *a*-axis of enamel apatite may be attributed to partial Cl-for-OH substitution [36] and HPO_4-for-PO_4 substitution [37] rather than the CO_3-for-OH substitution in the apatite.

A possible H_3O-for-OH was also offered as a possible cause for the expanded *a*-axis dimension [8]. Additional evidence for the dominant partial CO_3-for-PO_4 substitution in enamel and all biologic apatites is the similarity of the characteristic CO_3 absorption bands between the IR spectra of enamel apatite with those of synthetic apatites exhibiting CO_3-for- PO_4 substitution as shown in Fig. 1.3b [13, 24, 38].

Studies in synthetic systems also showed that F incorporation (F-for-OH substitution) has the following effects: contraction in the *a*-axis dimension and no significant effect on the *c*-axis dimension compared to F-free apatites [13, 24], growth of larger and less soluble apatite crystals [13, 24, 39], and greater structural stability [3]. Such studies elucidated the nature of fluoride (F) incorporation in some biologic apatites (from modern and fossil teeth and bones). For example, the smaller *a*-axis dimension of shark enameloid compared to HAp or human enamel apatite is due to the high fluoride (F) concentration in shark enameloid (Table 1.1) [13, 27, 40]. The therapeutic use of fluoride in dentistry (sealants, topical gels, tablets) was based on the observation of low caries in areas of fluoridated water (1 ppm F) [41]. Fluoride treatment (topically or by the use of fluoridated dentifrices) of enamel and dentin leads to the formation of partially substituted (F, OH) apatite that is more resistant to acid dissolution, i.e., dental caries [29, 33, 34, 39]. Administration of F in the drinking water was shown to increase the crystal size and decrease the extent of dissolution of rat bones [42] and increase crystal thickness of enamel apatite [43]. Fluoride therapy (as NaF) has been recommended for the management of osteoporosis to increase bone density [44, 45].

1.4 Synthetic Apatites

Earlier extensive studies on synthetic apatites were made to gain a better understanding of biologic apatites and their properties. Because biologic apatite was idealized as HAp, most of the studies centered on HAp preparation and evaluation of HAp properties. Studies on synthetic apatites in the last 30 years were motivated by development of calcium phosphate-based biomaterials (principally HAp, TCP, or biphasic) for bone repair, substitution, and augmentation and as scaffolds for tissue engineering in bone and teeth regeneration. The rationale for developing HAp biomaterials was their similarity in composition to the bone mineral.

Synthetic HAp can be made by solid-state reactions or by precipitation or hydrolysis methods and subsequent sintering at high temperatures, usually 1,000 °C and above. Synthetic apatites can also be prepared using hydrothermal [46, 47], microwave [48, 49] or sol-gel [50, 51] methods. Apatite nanocrystals are obtained when prepared by precipitation or hydrolysis at lower temperatures (25–60 °C). Synthetic apatite crystals approximating the size of human enamel apatite may be obtained by precipitation or hydrolysis methods with reaction temperature, 80–95 °C (Fig. 1.4). Apatites may also be prepared in sol-gel systems [52], by electrodeposition [53, 54] or biomimetic precipitation on metallic or polymeric [55, 56] substrates.

Apatites obtained by precipitation involve the reaction of calcium salts (e.g., $CaNO_3$, $Ca(OH)_2$, $CaCl_2$, $Ca(Ac)_2$) and phosphate salts (Na^-, NH^{4-}, or K^- phosphates) [24, 57]. Hydrolysis of non-apatitic calcium phosphates (e.g., ACP,

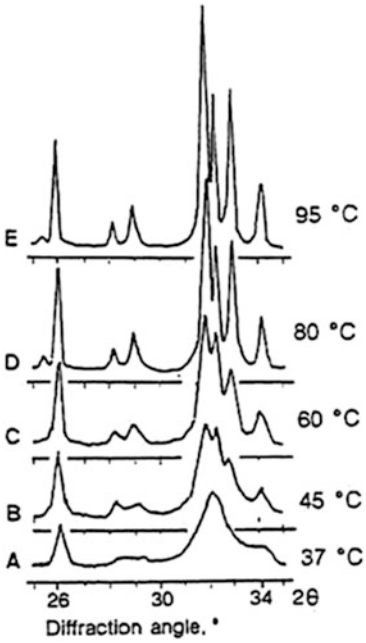

Fig. 1.4 X-ray diffraction profiles of precipitated apatites obtained at different reaction temperatures. The nanoapatite crystals similar to bone apatite are prepared at 37 °C (**a**) or at room temperature. The narrowing of diffraction peaks reflects increased crystallinity; the broader the diffraction peak, the smaller the crystals

DCPD, DCPA, OCP, β-TCP, α-TCP) or calcium compounds (e.g., $CaCO_3$, $CaMg(CO_3)_2$, CaF_2) in solutions containing OH, CO_3, or F results in the formation of apatite, CO^{3-} or F^- containing apatites [24, 32, 36, 58]. Apatites prepared by precipitation or hydrolysis methods when prepared at pH between 5 and 9 are calcium-deficient apatites (CDA), and subsequent sintering results in the formation of BCP [59]. Sintering or firing of synthetic apatites results in an increase in crystal size and decrease in microporosity.

Sintering of HAp at temperatures above 1,200 °C results in thermal decomposition of apatite forming other calcium phosphates such as β-TCP and α-TCP and possibly even mixed with ACP. The combination of calcium phosphates (e.g., ACP, DCPA, DCPD, CDA, α-TCP, β-TCP) with other calcium compounds (CaO, $Ca(OH)_2$, $CaCO_3$), mixed with phosphate solutions or organic acids, results in the formation of apatitic calcium phosphate cements [60, 61].

Studies on synthetic apatites showed that substitutions for Ca, PO_4, or OH ions in the apatite structure result in changes in lattice parameters (Table 1.2) and crystallinity (reflecting crystal size and/or strain) and dissolution properties. For example, CO_3-for-PO_4 coupled with Na-for-Ca substitution has the following effects on apatite properties:

(a) Smaller *a*- and larger *c*-axis dimensions compared to CO_3-free apatites
(b) Change from needlelike to rodlike to platelike with increasing CO_3 incorporation
(c) Lower resolution of the P-O (for PO_4) absorption bands in the IR spectra
(d) Higher solubility
(e) Lower thermal stability [11–13, 24, 31, 32, 38]

Such studies helped explain the contributions of some ions associated with biologic apatites as well as led to the development of some therapies and manufacture of calcium phosphate-based biomaterials.

1.5 Synthetic Apatites as Bone-Substitute Materials

The first successful use of a calcium phosphate reagent in bone repair was reported by Albee in 1920 [62], followed more than 50 years later by the first clinical study by Nery et al. [63] on periodontal bony defects using porous calcium phosphate identified by the authors as "TCP." X-ray diffraction analysis of Nery's material years later revealed that the "TCP" consisted of a mixture of HAp and β-TCP with a β-TCP/HAp ratio of 20/80 [20] and was thus renamed "BCP" [64]. Similarity in composition of the synthetic apatite to biologic apatite was the rationale for the development of calcium-phosphate-based biomaterials for bone repair, substitution, and augmentation and as scaffolds for bone and tooth regeneration. These calcium phosphate bioceramics include HAp, β-TCP, BCP, bovine bone-derived apatites (unsintered and sintered), and coral-transformed apatite (Fig. 1.5). Commercialization of HAp as bone graft materials was largely

Table 1.2 Lattice parameters of mineral and synthetic apatites compared to biologic apatite

Apatite	Substituent	Lattice parameters (+0.0003 nm) *a*-axis	*c*-axis
Mineral			
OH apatite (Holly Springs)	–	0.9422	0.6880
F apatite (Durango, Mexico)	F-for-OH	0.9375	0.6880
Dahllite (Wyoming, USA)	CO_3-for-PO_4	0.9380	0.6885
Staffelite (Staffel, Germany)	CO_3-for-PO_4 and F-for-OH	0.9345	0.6880
Marine phosphorite (USA)	CO_3-for-PO_4 and F-for-OH	0.9322	0.6882
Synthetic (nonaqueous)[a]			
OH apatite	–	0.9422	0.6882
F apatite	F-for-OH	0.9375	0.6880
Cl apatite	Cl-for-OH	0.9646	0.6771
CO_3 apatite	CO_3-for-OH	0.9544	0.6859
Synthetic (aqueous)[b]			
OH apatite	–	0.9438	0.6882
OH apatite	HPO_4-for-PO_4	0.9462	0.6879
F apatite	F-for-OH	0.9382	0.6880
(Cl, OH) apatite	*CI-for-OH	0.9515	0.6858
CO_3-OH apatite	*CO_3-for-PO_4	0.9298	0.6924
CO_3-F apatite	*CO_3-for-PO_4 and F-for-OH	0.9268	0.6924
Sr apatite	Sr-for-Ca	0.9739	0.6913
Pb apatite	Pb-for-Ca	0.9894	0.7422
Ba apatite	Ba-for-Ca	1.0161	0.7722
Biologic apatite			
Human enamel	(CO_3,HPO_4)-for-PO_4, (Na,Mg)-for-Ca, and Cl-for-OH	0.9441	0.6882
Shark enameloid	F-for-OH, Mg-for-Ca, and (CO_3,HPO_4)-for-PO_4	0.9382	0.6880

[a]Prepared at high temperature (1,000 ℃) by solid-state reaction or diffusion [3, 9]
[b]Prepared at 95 °C either by precipitation or by hydrolysis of $CaHPO_4$ in solutions containing the desired substituent [13, 24, 58]

due to the independent efforts of Jarcho [15], deGroot [16], and Aoki [17]. Basic studies on BCP led to its commercialization and popularity as bone graft materials and as scaffolds for tissue engineering [18, 59, 64–69].

Commercial HA biomaterials are usually prepared by precipitation at high pH and subsequent sintering at about 1,000–1,100 °C [15–17]. Coral-derived HAp or coralline HAp is prepared by the hydrothermal reaction of coral ($CaCO_3$) with ammonium phosphate [70]. Bovine bone-derived HAp is prepared by removing the organic phase (resulting in bone apatite) or removing the organic phase and sintering at high temperatures. These different preparations and origin (synthetic vs. biologic) are reflected in the difference in their initial crystallinity reflecting crystal size (Fig. 1.6) and their dissolution rates [71], increasing in the order

HAp << coralline HAp < bovine bone apatite (sintered) << bovine bone apatite (unsintered) HAp << BCP << β-TCP.

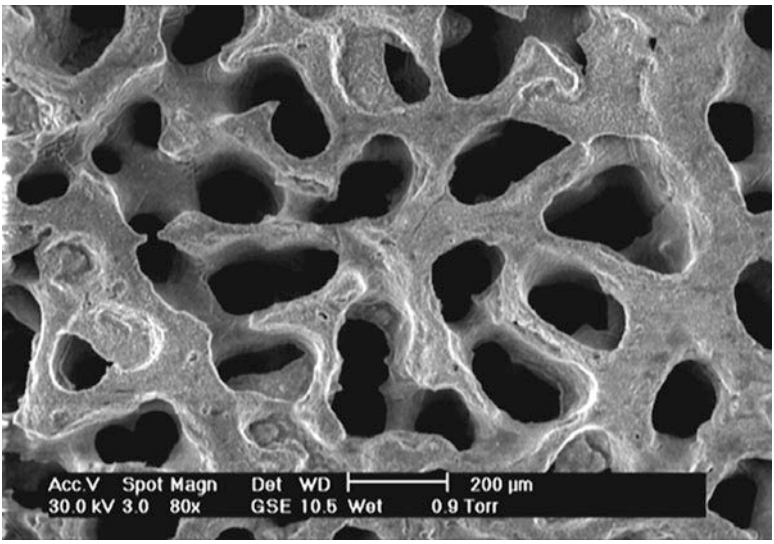

Fig. 1.5 Scanning electron micrograph of coralline apatite

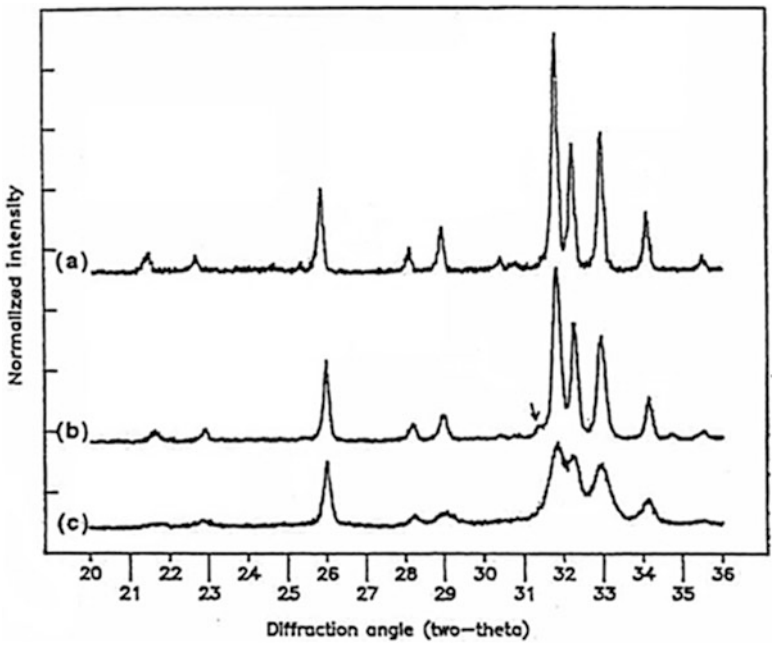

Fig. 1.6 X-ray diffraction profiles of apatite biomaterials (*A*, *B*, *C*). (*A*) Ceramic HAp (Calcitite™), (*B*) coralline HAp (Interpore™), (*C*) unsintered apatite, calcium deficient (Osteogen™). The *arrow* on (*b*) indicates presence of β-TCMP

1.6 Synthetic HAp in Implant Surfaces and Coatings

Porous HAp and related calcium phosphates, in spite of their many desirable properties, are not strong enough to be used in load-bearing areas [15, 16]. The rationale for the development of "HAp"-coated orthopedic and dental implants is to combine the strength of the metal (usually titanium or titanium alloy) and the bioactive properties of HAp and other calcium phosphates. Dense HAp particles are used as the source material for depositing implant coating by the plasma-spray technique [15, 16, 72]. The high temperatures and other variable parameters involved in the plasma-spray process (e.g., velocity of feeding the HAp powder, distance of the gun from the metal substrate) result in the partial transformation of the original HAp into ACP and minor amounts of α-TCP, β-TCP, and tetracalcium phosphate (TTCP, $Ca_4P_2O_9$) [73]. Plasma-sprayed "HAp" coatings have nonhomogenous composition (principally ACP/HAp ratio), varying from the layer closest to the metal substrate to the outermost layer and varying from one manufacturer to another [73]. Alternatives to the plasma-spray method are nanocoating, electrochemical deposition [53, 54], and precipitation or chemical deposition [55, 56], the latter method being also applicable to nonmetallic substrates [56]. These other methods provide homogenous implant coating of the desired composition, e.g., HAp, FA, CHA, and OCP [53–56, 74], and allow coating deposition at much lower temperatures, thus permitting the incorporation of bioactive molecules and growth factors.

During the last decade, HAp or BCP has been used as an abrasive material for grit blasting to roughen the surface and provide a more bioactive surface (compared to alumina or silica abrasive), thus enhancing osseointegration of the implant [75].

1.7 Synthetic HAp in Composites

HAp and related calcium phosphates are used as the inorganic component in composites with natural (e.g., collagen, chitosan) or synthetic (polylactic acid or polylacticglycolic acid, PLA or PLGA, high-molecular-weight polyethylene) polymers [76, 77]. The rationale for developing composite biomaterials is the fact that bone is a composite of a biologic polymer (collagen) and inorganic phase (carbonate apatite).

1.8 Synthetic HAp and BCP as Scaffolds for Tissue Engineering

Several investigators have reported that mesenchymal stem cells (MSC) from bone marrow can be cultured in porous calcium phosphate biomaterials (ceramic HAp, coralline HAp, BCP ceramic) in vitro and implanted as a tissue-engineered material for bone regeneration [78, 79].

1.8.1 Critical Properties of Synthetic HAp and Related Calcium Phosphates

Porosity (interconnecting macroporosity) is an important property of biomaterials to allow bony ingrowth and vascularization [80, 81]. Macroporosity is introduced in HAp and other calcium phosphates by the incorporation of porogens such as naphthalene [82], H_2O_2, or sugar molecules. Biocompatibility of a material is determined in vitro from the cell response (proliferation, attachment, phenotypic expression) to the material. Material surface composition and surface roughness or topography influence cell response [83]. Bioactivity is defined as the property of the material to develop a direct, adherent, and strong bonding and interface with the bone tissue [84, 85]. Bioactivity is demonstrated in vitro and in vivo by the ability of the material to form carbonate apatite on the surface from the simulated body fluid in vitro [56] or biologic fluid in vivo in osseous or non-osseous sites [65, 66, 86, 87].

Osteoconductivity is the property of the material that allows attachment, proliferation, migration, and phenotypic expression of bone cells leading to the formation of new bone in direct opposition to the biomaterial [84]. Osteoinductivity is the property of the material that allows osteoprogenitor cell growth and development for bone formation to occur [88] and is usually determined by the formation of bone in non-bone-forming sites, e.g., under the skin or in the muscle. HAp and related calcium phosphates are generally considered to have all the above properties except osteoinductivity. However, although controversial, it has been reported that with the appropriate composition, geometry, and architecture, osteoinductive properties can be promoted [89, 90].

1.9 Conclusions

The total analysis of the results obtained over four decades of research in this fascinating field of science suggests that with newer scientific tools and with further refinement, we will succeed to address problems relating to the structure, morphology, and analysis of the biologic materials and produce synthetic apatites or composites that will emulate the structure and characteristics of natural soft and hard tissues.

References

1. Naray-Szabo S (1930) The structure of apatite $(CaF)Ca_4(PO_4)_3$. Z Kristallogr 75:387–398
2. Beevers CA, Mcintyre DB (1956) The atomic structure of fluorapatite and its relation to that of tooth and bone mineral. Miner Mater 27:254–259
3. Young RA, Elliott JC (1966) Atomic scale bases for several properties of apatites. Arch Oral Biol 11:699–707

4. Deer WA, Howie RA, Zussman J (1985) An introduction to the rock forming mineral. Longman, Hong Kong, pp 504–509
5. de Jong WF (1926) La Substance Minérale Dans les Os. Rec Trav Chim Pays-Bas 45:445–448
6. Kay M, Young RA, Posner AS (1964) The crystal structure of hydroxyapatite. Nature 204:1050–1052
7. Carlstroem D (1955) X-ray crystallographic studies on apatite and calcified tissues. Acta Radiol Suppl 121:1–59
8. McConnell D (1952) The crystal chemistry of carbonate apatites and their relationship to the composition of calcified tissue. J Dent Res 31:53–63
9. Elliott JC (1964) The crystallographic structure of dental enamel and related apatites. PhD thesis, University of London
10. Bonel G, Montel G (1964) Surune nouvelle apatite carbonatée synthétique. C R Acad Sci Paris 258:923–926
11. Zapanta-LeGeros R (1965) Effect of carbonate on the lattice parameters of apatite. Nature 206:403
12. LeGeros RZ, Trautz OR, LeGeros JP (1961) Apatite crystallites: effect of carbonate on morphology. Science 155:1409–1411
13. LeGeros RZ (1981) Apatites in biological systems. Prog Cryst Growth Character 4:1–45
14. Rey C, Renugopalakrishnan V, Collins B (1991) Fourier transform infrared spectroscopic study of the carbonate ions in bone mineral during aging. Calcif Tissue Int 49:251–258
15. Jarcho M (1981) Calcium phosphate ceramics as hard tissue prosthetics. Clin Orthop Relat Res 157:259–278
16. de Groot K (1983) Bioceramics of calcium phosphates. CRC Press, Boca Raton
17. Aoki H, Kato K, Ogiso M (1971) Studies on the application of apatite to dental materials. J Dent Eng 18:86–89
18. Daculsi G, Passuti N, Martin S, LeGeros RZ (1990) Macroporous biphasic calcium phosphate ceramic for long bone surgery in human and dogs: clinical and histological study. J Biomed Mater Res 4:379–396
19. Metsger DS, Driskell TD, Paulsrud JR (1982) Tricalcium phosphate ceramic–a resorbable bone implant: review and current status. J Am Dent Assoc 105(6):1035–1038
20. LeGeros RZ (1988) Calcium phosphate materials in restorative dentistry: review. Adv Dent Res 2:164–180
21. Schopper C, Moser D, Sabbas A, Lagogiannis G, Spassova E, König F, Donath K, Ewers R (2003) The fluorohydroxyapatite (FHA) FRIOS Algipore is a suitable biomaterial for the reconstruction of severely atrophic human maxillae. Clin Oral Implants Res 14(6):743–749
22. Holmes RE (1979) Bone regeneration within a coralline hydroxyapatite implant. Plast Reconstr Surg 63:626–633
23. Ben-Nissan B, Chai C, Evans L (1995) Crystallographic, and spectroscopic characterization and morphology of biogenic and synthetic apatites. In: Wise DL (ed) Encyclopedic handbook of biomaterials and bioengineering: Part B Applications, vol 1. Marcel Dekker, New York, pp 191–221
24. LeGeros RZ (1991) Calcium phosphates in oral biology and medicine. In: Myers H, Karger S (eds) Monographs in oral sciences, vol 15. Karger, Basel
25. Boskey AL (1985) Overview of cellular elements and macromolecules implicated in the initiation of mineralization. In: Butler WT (ed) The chemistry and biology of mineralized tissues. EBSCO Media, Birmingham, pp 335–343
26. LeGeros RZ, Pan CM, Suga S, Watabe N (1985) Crystallochemical properties of apatites in atremate brachiopod shells. Calcif Tissue lnt 37:651–658
27. LeGeros RZ, Bautista C, Wong JL, LeGeros A, LeGeros JP (1994) Biological apatites in modern and fossil shark teeth and in calcified fish scales. Bull de l'Institut Oceanographique 14:229–236
28. LeGeros RZ (2001) Formation and transformation of calcium phosphates: relevance to vascular calcification. Z Kardiol 90(Suppl 3):116–124

29. LeGeros RZ, Salcae T, Bautista C (1996) Magnesium and carbonate in enamel and synthetic apatites. Adv Dent Res 10:225–231
30. Okazaki M, LeGeros RZ (1992) Crystallographic and chemical properties of Mg-containing apatites before and after suspension in solutions. Magnes Res 5:103–108
31. LeGeros RZ, Tung MS (1983) Chemical stability of carbonate and fluoride containing apatites. Caries Res 17:419–429
32. LeGeros RZ, LeGeros JP, Trautz OR, Klein E (1971) Conversion of monetite, $CaHPO_4$, to carbonate apatite: effect on crystallinity. Adv X-ray Appl 14:57–66
33. Margolis HC, Moreno EC (1985) Kinetic and thermodynamic aspects of enamel demineralization. Caries Res 19:22–35
34. LeGeros RZ (1982) Chemical and crystallographic events in caries. J Dent Res 69:567–574
35. Driessens FCM (1982) In moral aspects of dentistry. In: Myers HM (ed) Monographs in oral sciences, vol 10. Karger, Basel
36. LeGeros RZ (1975) The unit-cell dimensions of human enamel apatite: effect of chloride incorporation. Arch Oral Biol 20:63–71
37. LeGeros RZ, LeGeros JP, Bonel G (1979) Types of 'H_2O' in human enamel and in precipitated apatites. Calcif Tissue Res 26:111–116
38. LeGeros RZ, LeGeros JP, Trautz OR, Klein E (1970) Spectral properties of carbonate in carbonate-containing apatites. Dev Appl Spectrosc 7:3–12
39. Moreno EG, Kresak M, Zahradnik RT (1977) Physicochemical aspects of fluoride-apatite systems relevant to the study of dental caries. Caries Res 11(1):142–171
40. LeGeros RZ, Silverstone LM, Daculsi G, Kerebel LM (1983) In vitro caries-like lesion formation in F-containing tooth enamel. J Dent Res 62:138–144
41. Dean HT, Arnold FA Jr, Elvove E (1942) Domestic water and dental caries. V. Additional studies of the relations of fluoride domestic waters to dental caries experience in 4,425 white children aged 12 to 14 year, of 13 cities in 4 states. Public Health Rep 57:115–125
42. LeGeros RZ, Singer L, Ophaug R, Qujrolgico G, LeGeros JP (1982) The effect of fluoride on the stability of synthetic and biological (bone mineral) apatites. In: Menczel J, Robin GC, Makin M (eds) Osteoporosis. Wiley, New York, pp 327–341
43. Robinson C, Kirkham J (1990) The effect of fluoride on the developing mineralized tissues. J Dent Res 69(Spec No: 685–691):184–185
44. Pak CYC, Sakhaee K, Bell NK, Licata A, Johnson C, Rubine B (1996) Comparison of non-randomized trials with slow-release sodium fluoride with a randomized placebo controlled trial in postmenopausal osteoporosis. J Bone Miner Res 11:160–168
45. Kleerkoper M (1996) Auoride and the skeleton. In: Bilezlkian JP, Raisz LG, Rodan FA (eds) Principles of bone biology. Academic, San Diego, pp 1053–1062
46. Ito A, Nakamura S, Aoki H, Akao M, Traoka K, Tsutsumi S, Qnuma K, Tateishi T (1990) Hydrothermal growth of carbonate containing hydroxyapatite single crystals. J Cryst Growth 163:311–317
47. Hattori T, Iwadate Y (1990) Hydrothermal preparation of calcium hydroxyapatite powders. J Am Ceram Soc 73:1803–1805
48. Pena J, LeGeros RZ, Rohanizadeh R, LeGeros JP (2001) $CaCO_3$-biphasic materials prepared by microwave processing of natural aragonite and calcite. Key Eng Mater 192–195:267–270
49. Lerner E, Sarig S, Azoury R (1991) Enhance maturation of hydroxyapatite from aqueous solutions using microwave irradiation. J Mater Sci Mater Med 2:138–141
50. Milev AS (2002) Chemistry, synthesis and morphological stability of sol-gel derived carbonate substituted platelike hydroxyapatite. PhD thesis, University of Technology, Sydney
51. Deptula A, Lada W, Olczak T, Sarowska S, LeGeros RZ, LeGeros JP (1998) Complex Sol-gel process (CSGP) preparation of calcium phosphate biomaterials (powders, monoliths, fibers). In: Bioceramics, vol II. World Scientific Publishing, Singapore, pp 743–746
52. LeGeros RZ, Morales P (1973) Renal stone crystals grown in gel systems. Invest Urol 11:12–20
53. Vijayragbavan TV, Bensalem A (1994) Electrodeposition of apatite coating on pure titanium and titanium alloys. J Mater Sci Lett 13(24):1782–1785

54. LeGeros JP, Lin S, LeGeros RZ, Legeros JP (2000) Chemically deposited CaP coating on titanium alloy. J Dent Res 79:560–572
55. Rohanizadeh R, LeGeros RZ, Harsono M, Ben-David A (2004) Adherent apatite coating on titanium substrate using chemical deposition. J Biomed Mater Res 72A:428–438
56. Kokubo T (1996) Formation of biologically active bone-like apatite on metals and polymers by a biomimetic process. Thermochim Acta 280–281:479–490
57. Hayek E, Newesely H, Rumpel ML (1963) Pentacalcium monohydroxyorthophosphate. In: Kleinberg J (ed) Inorganic syntheses, vol 7. Wiley, Hoboken, pp 63–69
58. LeGeros RZ, Go P, Vandemaele KH, Quirolgico GB, LeGeros DJ (1980) Transformation of calcium carbonates and calcium phosphates to carbonate apatites: possible mechanism for phosphorite formation. In: Proceedings 2nd international congress on phosphorous compounds, Morocco Institut de Mondial de Phosphate, Boston, 21–25 Apr 1980, pp 41–54
59. LeGeros RZ, Lin S, Rohanizadeh R, Mijares D, LeGeros JP (2003) Biphasic calcium phosphate bioceramics: preparation, properties and applications. J Mater Sci Mater Med 14:201–210
60. Brown WE, Chow LC (1987) A new calcium phosphate water setting cement. In: Brown PW (ed) Cements research progress. American Ceramic Society, Westerville
61. Niwa S, LeGeros RZ (2002) Injectable calcium phosphate cements for repair of bone defects. In: Lewandrowski KU, Wise DL, Trantolo DJ, Gresser JD (eds) Tissue engineering and biodegradable equivalents: scientific and clinical applications. Marcel Dekker Inc., New York, pp 385–500
62. Albee FH (1920) Studies in bone growth: triple calcium phosphate as a stimulus to osteogenesis. Ann Surg 71:32–36
63. Nery EB, Lynch KL, Hirtbe WM, Mueller KH (1978) Preliminary clinical studies of bioceramics in periodontal osseous defects. J Periodontal 49:523–527
64. Ellinger RF, Mery EG, Lynch KL (1986) Histological assessment of periodontal osseous defects following implantation of hydroxyapatite and biphasic calcium phosphate ceramics: a case report. J Periodontics Restor Dent 3:223–233
65. LeGeros RZ, Daculsi G, Nery E, Lynch K, Kerebel B (1988) In vivo transformation of biphasic calcium phosphates of varying β-TCP:HA ratios: ultrastructural characterization. In: Proceedings of the 3rd world biomaterials congress. Business Center for Academic Societies Japan, Tokyo
66. LeGeros RZ, Daculsi D (1997) In vivo transformation of biphasic calcium phosphate ceramics: ultrastructural and physico-chemical characterizations. In: Yamamuro T, Wilson J, Hench LL (eds) Handbook of bioactive ceramics, vol 11. CRC Press, Boca Raton, pp 17–28
67. Daculsi G, Passuti N (1990) Bioactive ceramics fundamental properties and clinical applications: the Osseo-coalescence process. In: Heimcke G, Oonishi H (eds) Bioceramics, vol 2. Butterworth-Heinemann, Cologne, pp 3–10
68. Wykrota LL, Garrido CA, Wykrota FH (1998) Clinical evaluation of biphasic calcium phosphate ceramic used in orthopaedic lesions. In: LeGeros RZ, LeGeros JP (eds) Bioceramics, vol 11. World Scientific Publishing, Singapore, p 64
69. Nery E, LeGeros RZ, Lynch KL, Lee K (1992) Tissue response to biphasic calcium phosphate ceramic with different ratios of HA/β-TCP in periodontal osseous defects. J Periodontol 63:729–735
70. Roy DL, Linnehan SK (1974) Hydroxyapatite formed from coral skeletal carbonate by hydrothermal exchange. Nature 247:220–222
71. LeGeros RZ, Bautista C, LeGeros JP, Vijaragbavan TV, Retino M (1995) Comparative properties of bioactive bone graft materials. In: Sedel L (ed) Bioceramics, vol 8. Pergamon Press, London, pp 1–7
72. Berndt CC, Haddad GN, Farmer AJD, Gross KA (1990) Review: thermal spraying for bioceramic applications. Mater Forum 14:161–173
73. LeGeros RZ, LeGeros JP, Kim Y, Kijkowska R, Zheng R, Bautista C, Wong JL (1995) Calcium phosphates in plasma-sprayed HA coatings. Ceram Trans 48:173–189
74. Lin S, LeGeros RZ, LeGeros JP (2003) Adherent octacalcium phosphate coating on titanium alloy using a modulated electrochemical deposition method. J Biomed Mater Res 66A:8228

75. Salgado T, LeGeros JP, Wang J (1998) Effect of alumina and apatitic abrasives on TI alloy substrate S. In: Ohgushi H (ed) Bioceramics, vol 11. World Scientific Publishing, Singapore, p 686
76. Doyle C (1990) Bioactive composites in orthopaedics. In: Yamamuro T, Hench LL, Hench LL, Wilson-Hench J (eds) Handbook of bioactive ceramics, vol 2. CRC Press, Boca Raton, pp 195–207
77. Okazaki M, Ohmae H, Hino T (1989) Insolubilization of apatite-collagen composites by UV irradiation. Biomaterials 10:564–568
78. Ohgushi H, Caplan AI (1999) Stem cell technology and bioceramics: from cell to gene engineering. J Biomed Mater Res Appl Biomater 48:913–927
79. Toquet J, Rohanizadeh R, Guicheux J, Daculsi G (1999) Osteogenic potential in vitro of human bone marrow cells cultured on macroporous biphasic calcium phosphate ceramic. J Biomed Mater Res 44:98–109
80. LeGeros RZ (2002) Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthopaed Relat Res 395:81–98
81. Klawitter JJ (1979) A basic investigation of bone growth in porous materials. PhD thesis Clemson University, Clemson
82. Hubbard W (1974) Physiological calcium phosphate as orthopaedic implant material. PhD thesis, Marquette University, Milwaukee
83. Harada Y, Want JT, Doppalppudi VA, Willis AA, Goldring SR (1996) Differential effects of different forms of hydroxyapatite and hydroxyapatite tricalcium phosphate particles on human monocyte/macrophages in vitro. J Biomed Mater Res 31:19–26
84. Hench LL (1994) Bioceramics: from concept to clinic. J Am Ceram Soc 74:l487–l1510
85. Osborn JF, Newesely H (1980) The material science of calcium phosphate ceramic. Biomaterials 1:108–111
86. Heogbebaert M, LeGeros RZ, Gineste M, Guilhern M, Bonel G (1988) Physico-chemical characterization of deposits associated with HA ceramics implanted in non-osseous sites. J Biomed Mater Res 22:257–268
87. LeGeros RZ, Daculsi G, Orly I (1991) Substrate surface dissolution and interfacial biological mineralization. In: Davies JE (ed) The BoM biomaterials interface. University of Toronto Press, Toronto, pp 76–88
88. Urist MR (1965) Bone formation by autoinduction. Science 150:893–898
89. Ripamonti U, Ma S, Reddi AH (1992) The critical role of geometry of porous hydroxyapatite delivery system induction of bone by osteogenin, a bone morphogenetic protein. Matrix 12(3):202–212
90. Le Nihouannen D, Daculsi G, Saffarzadeh A, Gauthier O, Delplace S, Pilet P, Layrolle P (2005) Ectopic bone formation by microporous calcium phosphate ceramic particles in sheep muscles. Bone 36(6):1086–1093

Chapter 2
Clinical Applications of Hydroxyapatite in Orthopedics

Hiroyuki Oonishi Jr., Hironobu Oonishi, Hirotsugu Ohashi, Ikuo Kawahara, Yoshifumi Hanaoka, Ryoko Iwata, and Larry L. Hench

Abstract This chapter describes since 1982 the use of porous synthetic hydroxyapatite (HA) granules (0.1 to approximately 1.5 mm) interposed at the cement-bone interface to enhance bone bonding, a surgical procedure labeled interface bioactive bone cement (IBBC). HA granules were smeared on the bone surface just before cementing. Because the HA granules used in IBBC were pure polycrystalline HA, they were scarcely absorbed and their osteoconductive activity can continue indefinitely even after the onset of osteoporosis due to aging and even in conditions of extremely low pathological activity of bone. The appearance rate of radiolucent lines and osteolysis was extremely low even over 30 years when IBBC was used. Since 1986, in an attempt to fill the massive bony defect in the acetabulum at revision surgery of total hip arthroplasty, a mixture of HA granules with a size between 0.9∼1.2 mm and 3.0∼5.0 mm was placed densely and firmly into the bone defects. Bone ingrowth was measured to be over 2.5 cm in full depth and the new bone was very stable. Long-term clinical results over 26 years were excellent. On the weight-bearing area, bone ingrowth over 2.5 cm in full depth can be expected. However, on non-weight-bearing area, bone ingrowth is only 0.5 cm in depth. In large cavities

H. Oonishi Jr., M.D. • H. Oonishi, M.D., Ph.D. (✉) • I. Kawahara, M.D. • Y. Hanaoka, M.D.
H. Oonishi Memorial Joint Replacement Institute, Tominaga Hospital, 1-4-48, Mintatomachi, Naniwa-ku, Osaka 556-0017, Japan
e-mail: oons-h@os.rim.or.jp

H. Ohashi, M.D., Ph.D.
Department of Orthopaedic Surgery, Saiseikai Nakatsu Hospital, 2-10-39, Shibata, Kita-ku, Osaka 530-0012, Japan

R. Iwata, Ph.D.
Olympus Terumo Biomaterials Corporation, Shinjuku Monolith, 3-1, Nishi-Shinjuku 2-chome, Shinjuku-ku, Tokyo 163-0941, Japan

L.L. Hench, Ph.D.
Department of Biomedical Engineering, Florida Institute of Technology, Melbourne, FL 32901, USA

B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, Springer Series in Biomaterials Science and Engineering 2, DOI 10.1007/978-3-642-53980-0_2,