

---

Paul Ducheyne • David Christiansen

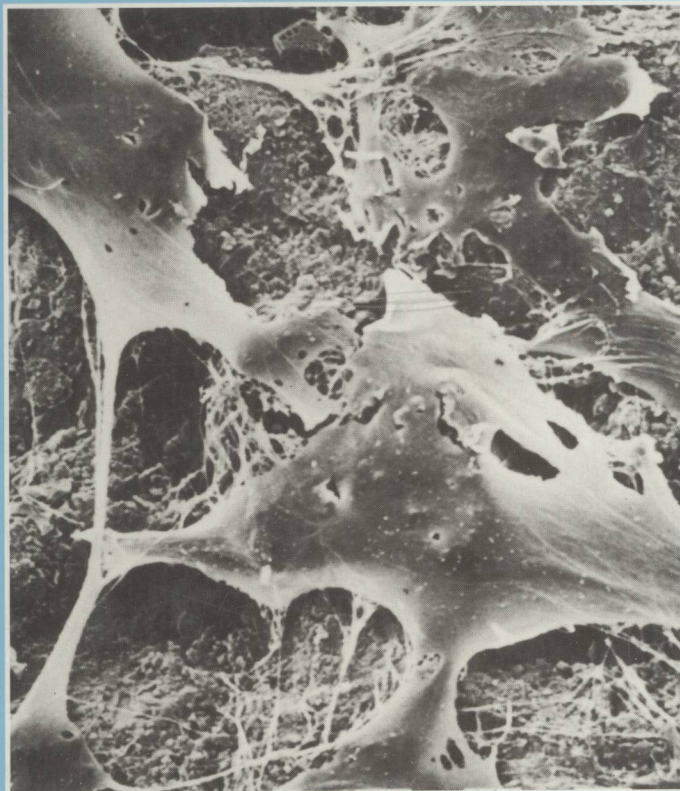
---

# Bioceramics

---

Volume 6

---



---

Philadelphia, USA

---

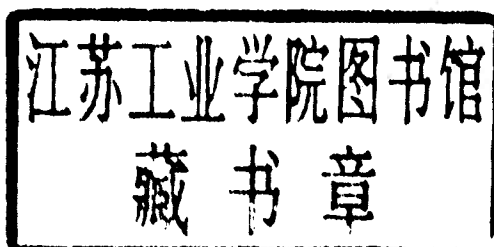
# Bioceramics

Volume 6

*edited by*

Paul Ducheyne  
David Christiansen


University of Pennsylvania, Philadelphia, USA



Pergamon

UTTERWORTH  
EINEMANN

Butterworth-Heinemann Ltd  
Linacre House, Jordan Hill, Oxford OX2 8DP

 A member of the Reed Elsevier group

OXFORD LONDON BOSTON  
MUNICH NEW DELHI SINGAPORE SYDNEY  
TOKYO TORONTO WELLINGTON

First published 1993

© Butterworth-Heinemann Ltd 1993

All rights reserved. No part of this publication may be reproduced in any material form (including photocopying or storing in any medium by electronic means and whether or not transiently or incidentally to some other use of this publication) without the written permission of the copyright holder except in accordance with the provisions of the Copyright, Designs and Patents Act 1988 or under the terms of a licence issued by the Copyright Licensing Agency Ltd, 90 Tottenham Court Road, London, England W1P 9HE. Applications for the copyright holder's written permission to reproduce any part of this publication should be addressed to the publishers.

**British Library Cataloguing in Publication Data**

A catalogue record for this book is available from the British Library

**Library of Congress Cataloguing in Publication Data**

A catalogue record for this book is available from the Library of Congress

ISBN 0 7506 18841

**Cover picture**

The cover shows a scanning electron micrograph of the underside of a porous glass template (cells were seeded on the opposite face). The glass surface was totally covered with cells, calcified nodules (0.5–1.0 µm in diameter), and collagen fibrils. Extensive formation of extracellular matrix and bone-like tissue covered 60% of the template surface. See: A. El-Ghannam, P. Ducheyne and I. Shapiro *A New Bioactive Glass Template for the Synthesis of Bone-like Tissue In Vitro*.

Printed in Great Britain at the University Press, Cambridge

# **Bioceramics**

**Volume 6**

**Proceedings of the 6th International Symposium on  
Ceramics in Medicine**

**Philadelphia, USA, November 1993**

***Edited by***

**Paul Ducheyne**

**David Christiansen**

**The publication of the Proceedings of the  
6th International Symposium on Ceramics in Medicine  
was made possible by the unqualified support of  
Howmedica Inc.**

## Preface

Bioceramics as a science has come of age. Whereas no single class of materials has the requisite properties by itself to lead to the optimal design of medical devices, the progress in recent years in the field of ceramics epitomizes the significant advances that are still possible in designing ever better performing medical devices. When materials were first used in the body, one of the most important properties was inertness. However, the field of biomaterials has come to realize that this goal is neither achievable, nor realistic. All materials, when implanted, elicit a reaction. Thus, it became an important objective to conceive implant materials and implant designs that may cause minimal reactions. A further step was the synthesis of materials with the capacity to evoke the normal reactions in the tissue in which they are implanted. Bioactive ceramics highlight the success reached in these efforts. In bone, osseous tissue growth is enhanced and bonding between the bioactive ceramic and bone is achieved. At this time, yet another phase in the development of biomaterials may have started. Ceramics are synthesized that promote bone-like tissue formation *in vitro*. It is conceivable that for the treatment of larger defects in the bone structure, bone tissue will be formed outside the body with cells extracted from the patient, and subsequently implanted as an immunologically identical tissue. As an illustration, the micrograph on the front cover is from a paper discussing *in vitro* synthesis of bone tissue.

This publication offers an extensive account of the leading edge research in this field of bioceramics. The sections are organized in such a way that they reflect the major lines of ceramics research, as well as focus on the critical properties and the mechanistic analyses of the interactions these materials elicit in tissues. The book includes sections on so called bioinert ceramics. These ceramics such as alumina and zirconia, are chemically very stable materials. They are primarily used in artificial joints and dental implants. Carbon-based ceramics have outstanding blood contact properties, and therefore, are the materials of choice in the construction of heart valves. This volume includes sections covering the current research on the use of bioactive ceramics and glasses as coatings on prostheses to achieve long-lasting fixation of devices.

This book is a compilation of the papers presented at the Sixth International Symposium on Ceramics in Medicine which was organized in Philadelphia in November 1993. This series of annual meetings has been organized around the globe, and it was only the second time that this symposium was held in the United States. The symposium brought together, in an unencumbered and relaxed atmosphere, scientists, manufacturers and regulators contributing to the science and technology of bioceramics. A three-fold format was adopted: contributed presentations covering the latest progress in the science of bioceramics, invited presentations discussing areas of critical importance, and panel discussions on key issues limiting further progress.

A major international meeting is not possible without the financial support to cover some of the expenses incurred in bringing together the leading authorities of a field. It is with sincere appreciation for its significant gift towards the meeting and especially this book, the lasting record of the symposium, that we mention Howmedica, Inc. Rutherford, New Jersey, USA. Howmedica's Chief Technology officer, Dr John Dumbleton, contributed greatly to the concept of some of the sessions. His insights, as well as the suggestions of the other International Advisory Board members, were very useful in developing the program that eventually resulted in this compendium of current research in bioceramics.

The International Advisory Board consisted of: D. Bardos (Memphis, USA), J Bokros (Austin, USA), W. Bonfield (London, UK), J. Dumbleton (Rutherford, USA), U. Gross (Berlin, Germany), G. W. Hastings (London, UK), L. L. Hench (Gainesville, USA), S. F. Hulbert (Terre Haute, USA), T. Kokubo (Kyoto, Japan), H. Oonishi (Osaka, Japan), L. Sedel (Paris, France), R. Tarr (Warsaw, USA), C. van Blitterswijk (Leiden, The Netherlands) and T. Yamamuro (Kyoto, Japan). All abstracts were critically reviewed by at least two members of this committee. By setting the criteria for quality, the International Advisory Board members contributed significantly to this volume.

We are very much indebted to people immediately around us. Were it not for the members of the Center in Bioactive Materials and Tissue Engineering, at the University of Pennsylvania, publication of this book would probably not have been possible. Several of them have also contributed papers to this volume.

Annemie, Ruben and Sarah, your patience and understanding for the long hours without me or your dad is an expression of love I can only begin to think I can return. More than this book you are my goal in life.

**Paul Ducheyne**

**David Christiansen**

## CONTENTS

### Induction of Calcification and Osteogenesis

<b>Influence of Cyclic Axial Stress on the Mineralization of Collagen</b> D. L. Christiansen and P. Ducheyne	3
<b>Ceramic-induced Osteogenesis Following Subcutaneous Implantation of Calcium Phosphates</b> J. M. Toth, K. L. Lynch and D. A. Hackbarth	9
<b>Ectopic Bone Induction in Porous A-W Glass Ceramic Combined with Bone Morphogenetic Protein</b> S. Ijiri, T. Yamamuro, T. Nakamura, S. Kotani, T. Shibuya, K. Notoya and Y. Sato	15
<b>TGF-<math>\beta</math> Enhances Osseointegration <i>In Vivo</i></b> K. D. Chesmel, J. L. Beight, R. H. Rothman and R. S. Taun	21
<b>Molecular Biology as a Tool to Investigate Cell/Material Interaction</b> H. Ohgushi, Y. Dohi, M. Okumura, K. Inoue, S. Tamai and S. Tabata	27

### Fundamentals of Bioactivity

<b>The Kinetics of Bioactive Ceramics Part VI: Silica-Water-Amino Acid Interactions</b> L. L. Hench and J. K. West	35
<b><i>In Vitro</i> and <i>In Vivo</i> Evaluation of Bioactivity of Gel Titania</b> P. Li, I. Kangasniemi and K. de Groot	41
<b>Ultrastructural Analysis of Immersion Induced Modifications of Calcium Phosphate Coatings</b> S. Radin, P. Ducheyne, P. Berthold and S. Decker	47
<b>Calcium Phosphate Depositions in PEO/PBT Copolymers Prior to Implantation</b> M. L. Gaillard, J. R. de Wijn and C. A. van Blitterswijk	53
<b>Kinetics of the <i>In Vitro</i> Surface Transformation of Bioactive Ceramics to Biologically Equivalent Apatite</b> S. Radin and P. Ducheyne	59
<b>The Influence of Proteins on the Surface Modification of Calcium Phosphate Compounds During Immersion in Simulated Body Fluids</b> J. Mei, R. L. Sammons, R. M. Shelton and P. M. Marquis	67
<b>Analysis of Ceramic-Tissue Interface Reaction</b> T. Kokubo	73

## **Bioceramics-Tissue Interfaces: Characterization of Ultrastructural Properties**

R. Z. LeGeros, G. Daculsi, I. Orly, M. Walters and J. P. LeGeros 79

### **Clinical Results versus Experimental Data**

**Moving Ceramics to Clinical Applications: A Clinician-Scientist's View**  
H. B. Skinner 87

**Clinical Results Versus Experimental Data in Orthopaedic Applications of Bioceramics**  
H. Oonishi 93

**Long Term Behaviour of Alumina/Alumina Coupling for T. H. R. Information from Clinical Data and Retrieved Specimen Analysis**  
L. Sedel, R. Nizard, A. Meunier, J. M. Dorlot and J. Witvoet 99

**Development of a Bioactive Ceramic, A-W Glass-Ceramic**  
T. Nakamura and T. Yamamuro 105

### **Bioactivity: Interfaces and Surface Reaction Layers**

**A Comparative Study of Bioactive Glass and Porous Hydroxylapatite Particles in Periodontal Bone Lesions**  
E. J. G. Schepers and P. Pinruethai 113

**Numerical Model of Extracellular Fluid Flow Conditions and Chemical Species Transport Around and Within Porous Bioactive Glass Granules**  
A. J. García and P. Ducheyne 117

**Comparison of Hydroxycarbonate Apatite Layers on Bioactive Glasses with Human Bone**  
I. Rehman, L. L. Hench and W. Bonfield 123

**The Reaction of Bone to Microcrystalline Organoapatite Implants**  
C. M. Müller-Mai, S. I. Stupp, C. Voigt and U. M. Gross 129

**Hydroxyapatite Coatings of Varying Crystallinities: Effect of Coating Resorption and Surgical Fit**  
S. H. Maxian, J. P. Zawadsky and M. G. Dunn 135

### **In Vitro and In Vivo Effects of Bioactive Materials**

**A New Bioactive Glass Template for the Synthesis of a Bone-Like Tissue *In Vitro***  
A. El-Ghannam, P. Ducheyne and I. Shapiro 143

**Attachment of Human Oral Mucosa to Bioactive Glass *In Vitro***  
U. I. Touminen, J. I. Salonen, R. -P. Happonen and A. Yli-Urpo 151



<b>Long Term Bone Growth Behavior Into the Spaces of HAp Granules Packed into Massive Bone Defect Cavity</b> H. Oonishi, S. Kushitani, N. Murata, M. Saito, A. Maruoka, E. Yasukawa, E. Tsuji and F. Sugihara	157
--	-----

### **Processing – Property Relationships**

<b>New Calcium Phosphate Coating Methods</b> R. M. Pilliar and M. J. Filiaggi	165
<b>Morphology and Phase Structure of Nanograde Boneapatite-like Rodshaped Crystals</b> L. Yubao, C. Klein, J. de Wijn, J. Wolke and K. de Groot	173
<b>Preparation and Sintering of Carbonate-Substituted Apatite</b> J. E. Barralet, S. M. Best and W. Bonfield	179
<b>The Influence of Powder Morphology on the Microstructure of Plasma Sprayed Hydroxyapatite Coatings</b> M. P. Taylor, P. Chandler and P. M. Marquis	185
<b>Effect of Reinforcing Glass Composition on Phase Transformation and Crystallographic Parameters in Hydroxyapatite</b> J.C. Knowles, I. Abrahams and W. Bonfield	191

### **Ceramic Heart Valves**

<b>Static Fatigue and Stress Corrosion in Pyrolytic Carbon</b> J. L. Ely and A. D. Haubold	199
<b>Evaluation of Fatigue in Pyrolite® Carbon</b> L. A. Beavan, D. W. James and J. L. Kepner	205
<b>Long-Life Cyclic Fatigue of Pyrolytic Carbon</b> G. Sines and L. Ma	211
<b>Fatigue Crack Growth and Fracture of Pyrolytic Carbon Composites</b> C. B. Gilpin, A. D. Haubold and J. L. Ely	217
<b>Hertzian Fracture in Pyrolite Carbon</b> R. B. More, J. L. Kepner and P. Strzepa	225
<b>Role of Small Cracks in the Structural Integrity of Pyrolytic Carbon Heart-Valve Prostheses</b> R. H. Dauskardt, R. O. Ritchie and A. M. Brendzel	229

### **New Clinical Uses**

<b>Hydroxyapatite Coated External Fixation Pins Versus Uncoated</b> A. Moroni, V. Caja, S. Stea and M. Visentin	239
--	-----

<b>Experimental Study of an Artificial Trachea Made of Polymers Coated with Hydroxyapatite</b>	
K. Suzuki, R. Kobayashi, Y. Yokoyama, Y. Harada and T. Kokubo	245

<b>Open Porous Ceramics for Cell Transplantation Devices: A New Biomaterial - First Results</b>	
K. -L. Eckert, U. Rösler, M. Mathey, J. Mayer, E. Wintermantel and H. Hofmann	251

### **Bioinert and Wear-resistant Materials**

<b>Mechanical Characterisation of a Zirconia Ceramic Used as Implant Material</b>	
B. Calès, Y. Stefani, C. Olagnon and G. Fantozzi	259

<b>Evaluating Sol-Gel Zirconia Thin Films for Implant Applications</b>	
M. J. Filiaggi and R. M. Pilliar	265

<b>Zirconia - A Medical-Grade Material?</b>	
G. Willmann	271

<b>Screwed Conical and Cemented Spherical Al<sub>2</sub>O<sub>3</sub> Acetabular Components: Follow Up, Histology and Autopsy Data</b>	
E. J. Henssge, I. Bos, H. Ritschl, J. Berger and M. Temminghoff	277

<b>Safety Aspects of the Fixation of Ceramic Balls on Metal Stems</b>	
G. Heimke	283

<b>Long-Term Reactions of an Alumina Containing Biocompatible Glass in Human Tibia</b>	
Ö. H. Andersson, A. J. Aho, K. H. Karlsson and A. Yli-Urpo	289

<b>Quantitative Analysis of Wear in Canine Acetabular Cartilage after Al<sub>2</sub>O<sub>3</sub> and CoCrMo Alloy Hemiarthroplasty</b>	
S. Nakagawa, K. Kohashi, K. Hayashi and Y. Sugioka	295

<b>Advantages and Risks of Zirconia Ceramics in Biomedical Applications</b>	
W. Burger and G. Willmann	299

<b>Porous Titanium Felt as a Carrier of Osteogenic Cells</b>	
M. Okumura, H. Ohgushi and P. Ducheyne	305

<b>Theoretical Analysis of the Periotest Percussion Diagnosis of Teeth and Dental Implants</b>	
T. M. Kaneko	311

### **Composites**

<b>Titanium Fiber Reinforced Bioactive Glass Composite Implants</b>	
L. Van Hove, E. Schepers and P. Ducheyne	319

<b>Apatite-Polymer Composites Prepared by Biomimetic Process: Improvement of Adhesion of Apatite to Polymer by Glow-Discharge Treatment</b>	
T. Kokubo, M. Tanahashi, T. Yao, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro	327
<b>Pliable Calcium Phosphate Ceramic Composites</b>	
P. Sharrock	333
<b>Mechanical Properties of a Hydroxyapatite-Polymethylmethacrylate Bone Cement Composite: Hydroxyapatite Embedded on Surface and Throughout Cement Matrix</b>	
R. F. Low, S. F. Hulbert and A. Sogal	339
<b>Post-Operative Carbonate-Apatite Formation in a Polymer Matrix: Characterization and Relation to Bone-Bonding</b>	
A. M. Radder, J. E. Davies, H. Leenders, S. van der Meer and C. A. van Blitterswijk	345
<b>Histological Evaluation of Bioactive Glass Fiber/Polysulfone Composite</b>	
M. Marcolongo, P. Ducheyne, J. Cuckler and W. LaCourse	353

### **Applications of Bioactivity**

<b>Clinical Application of Bioactive Glass Granules of Narrow Size Range on Dental Osseous Lesions</b>	
E. J. G. Schepers, P. Ducheyne and L. Barbier	361
<b>Biochemical and Histochemical Analysis of Bone Marrow Cells Activated by HA Particles</b>	
K. J. Kim, K. Sato, S. Kotake, Y. Katoh and T. Itoh	365
<b>Biochemical Analysis of Bone Formed in Porous Hydroxyapatite and Calcium Carbonate</b>	
K. Inoue, H. Ohgushi, M. Okumura, T. Yoshikawa, S. Tamai and Y. Dohi	371
<b>Bone Formation in Porous Hydroxyapatite Obtained from Human Biopsies</b>	
E. C. Shors and R. E. Holmes	375
<b>Sintering of Carbonated Hydroxyapatite</b>	
Y. Suwa, H. Banno, H. Saito, Y. Doi, T. Koda, M. Adachi and Y. Moriwaki	381

### **Bioactive Glasses**

<b>Immersion Induced Changes of Bioactive Glass Surfaces as Observed With Atomic Force Microscopy</b>	
J. D. Marshall, J. Y. Josefowicz and P. Ducheyne	389

<b>Glass Corrosion Layers on Bioactive Glass Granules of Uniform Size Affect Cellular Function</b> A. M. Gatti, P. Ducheyne, A. Piattelli, E. Schepers, P. Trisi, L. Chiarini and E. Monari	395
<b>The Application of Bioactive Glass Particles of Narrow Size Range as a Filler Material for Bone Lesions: a 24 Month Animal Experiment</b> E. J. G. Schepers and P. Ducheyne	401
<b>Optimization of Rapid Immersion Coating of Bioactive Glass on 316L Stainless Steel</b> J. K. West, G. Gonzales, A. Boschi, G. LaTorre and L. L. Hench	405
<b><u>Hydroxyapatite Coatings</u></b>	
<b>A Method to Test Corrosion Fatigue Behaviour of Ceramic Coated Biomaterials II: Open Circuit Experiments for Assessing Corrosion Fatigue Susceptibility</b> R. L. Reis and F. J. Monteiro	413
<b>Properties of HA-Coatings</b> H.-G. Pfaff, G. Willmann and R. Pöthig	419
<b>A Short Term Follow-up Study of Hydroxyapatite-Coated Total Hip Prostheses</b> S. Miyakawa, K. Ebihara and T. Fukubayashi	425
<b>Bone Ingrowth Analysis and Interface Evaluation of Hydroxyapatite Coated Versus Uncoated Titanium Porous Bone Implants</b> A. Moroni, V. Caja, E. Egger, A. Montanari and E. Y. Chao	431
<b>Fixation of Hydroxylapatite Coated Pyrolytic Carbon and Titanium Alloy Implants</b> C. E. Lord, V. J. Hetherington and S. A. Brown	437
<b>Effect of Crystallinity of Plasma Sprayed Hydroxylapatite Coatings on Microbial Induced Degradation</b> K. Kieswetter, K. Merritt and R. Myers	443
<b>Hydroxyapatite Coating on Titanium-Sprayed Titanium Implant</b> Y. Nakashima, K. Hayashi, T. Inadome and Y. Sugioka	449
<b>Comparison of Bone-Implant Interface Shear Strength of Porous Titanium and Hydroxyapatite-Coated Grooved Titanium</b> K. Hayashi, T. Mashima, T. Inadome and Y. Sugioka	455

## Bone Cement

### **Bioactive Bone Cement - Effect of Amount of Glass Powder on its Bioactivity and Mechanical Properties**

J. Tamura, K. Kawanabe, T. Yamamuro, T. Nakamura, T. Kokubo, S. Yoshihara and T. Shibuya 463

### **A New Apatitic Calcium Phosphate Bone Cement: Preliminary Results**

F. C. M. Driessens, M. G. Boltong, J. A. Planell, O. Bermudez, M. P. Ginebra and E. Fernandez 469

### **Hydroxyapatite Composite Resin as a New Bioactive Bone Cement**

M. Saito, A. Maruoka, T. Mori, N. Sugano, K. Hino and H. Oonishi 475

## Mechanical Aspects of Bioactive Ceramics

### **Effect of *In Vitro* "Aging" on the Mechanical Properties of Plasma Sprayed Hydroxylapatite Coatings on Porous and Non-Porous Titanium Substrates**

J. A. Bearcroft, T. J. Robinson and N. B. Beals 483

### **Micro-Mechanical Testing of Hydroxyapatite Coatings on Carbon Fiber Reinforced Thermoplastics**

S. -W. Ha, J. Mayer and E. Wintermantel 489

### **Analysis of the Mechanical Properties of a Ca/P Nanocomposite**

D. L. Christiansen and F. H. Silver 495

**Author Index** 503

**Subject Index** 507

## Induction of Calcification and Osteogenesis



## **Influence of Cyclic Axial Stress on the Mineralization of Collagen**

D. L. Christiansen and P. Ducheyne

Department of Bioengineering, University of Pennsylvania, Philadelphia PA

### **Abstract:**

The influence of periodic axial strain on the *in vitro*, acellular mineralization of a reconstituted collagen fiber was investigated. Reconstituted collagen fibers were exposed to supersaturated solutions of calcium and phosphate, with and without a 1 Hertz periodic axial strain of 2.5 %, and at four separate solution pH's ranging from acidic to alkaline. Control collagen fibers were incubated in similar solutions minus calcium/phosphate ions, and subjected to both static and periodic strains. The results of mechanical tests indicate that periodic axial stress induces increases in hydrated diameters, and decreases in mechanical properties in both the mineralized and control collagen fibers, attributed to a reduction of inter-fibrillar linkages. Electron microscopy indicate the formation of an embryonic hydroxyapatite layer on the surface of the fiber, as well as calcium or complexes of calcium and phosphate ions binding in the d-period of the collagen fibrils in the interior of the fiber. The study of *in vitro* mineralization of collagen under simulated physiological conditions may lend insight into the mechanism of bone-bonding associated with bioactive ceramics.

### **Introduction:**

Bone represents a dynamic tissue which optimizes its structure in response to mechanical stimuli. This structural optimization is mediated by bone cells which balance the processes of tissue synthesis and resorption in accord with the mechanical forces carried by the tissue (1).

At the molecular level bone tissue can be characterized as a highly ordered composite of proteins and inorganic hydroxyapatite (HA). The bulk of the organic phase is composed of type I collagen molecules aggregated in a periodic axial array, or collagen fibrils, which have a characteristic d-periodic repeating pattern (2). Early electron micrographic evaluation of mineralized tissue clearly indicate a strong association of the inorganic apatite with the d-period of collagen, as well as a strong axial orientation of the mineral with the fibril axis (3). Prior pH dependent studies of reconstituted collagen fiber mineralization (4) have indicated that in a limited sense the collagen fiber serves as a substrate for mineralization. However, the hydroxyapatite formed *in vitro* lacks distinct axial orientation with respect to the collagen fibrillar substructure. As noted, axial mechanical forces are known to influence the cell mediated process of bone formation, with reference to this phenomena it is proposed that periodic axial forces may influence the process of acellular, matrix mediated mineralization. To test this hypothesis, a model system for the *in vitro*, mineralization of a reconstituted collagen fiber subjected to periodic axial strain during mineralization, is presented with preliminary mechanical and ultrastructural evaluations. By simulating a physiological environment during the *in vitro* mineralization of a collagenous matrix, it may be possible to gain insight into the nature of "bone-bonding" seen in hard tissue reconstruction with bioactive ceramics.



## Materials and Methods:

### *Preparation of Reconstituted Type I Collagen Fibers:*

Type I collagen isolated from the reticular dermis (corium) of fresh bovine hides was processed using the technique outlined by Weadock et al. (5). The purity of the isolated collagen was assessed by SDS-PAGE and amino acid analysis and found to be exclusively type I collagen with no evidence of non-collagenous protein contamination.

Reconstituted collagen fibers produced by blending a 1% (w/v) solution of the purified type I collagen and distilled water at pH 2.0 to produce a viscous dispersion. The acid dispersed collagen was then extruded through thin (0.5 mm) polyethylene tubing into a fiber formation buffer (FFB) (6) at 37° C and pH 7.4. The extruded fibers were allowed to equilibrate in the FFB for a 45 minute period, at which point the solution was aspirated and replaced with isopropyl alcohol for at least 4 hours. Finally, the alcohol was replaced with distilled water and the fibers allowed to rehydrate for 10 - 15 minutes then removed from solution and allowed to air dry overnight under tension (6).

### *Fiber Mineralization: Static and Cyclically Strained Fibers during Mineralization.*

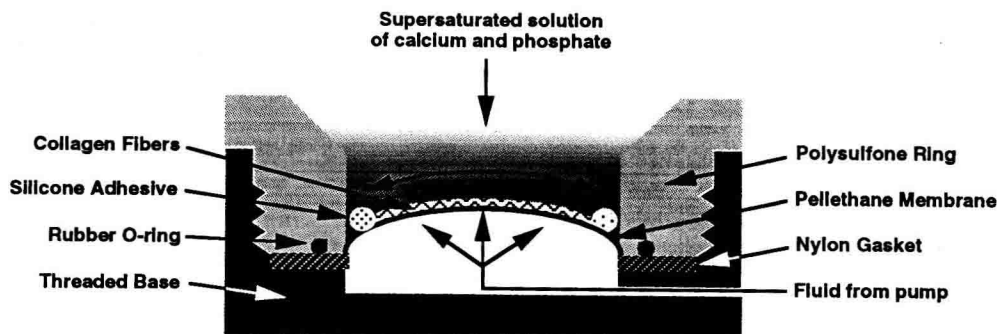
Reconstituted collagen fibers were mineralized by exposure to solutions supersaturated with respect to calcium and phosphate ions for a 48 hour incubation period at room temperature (25° C). During mineralization the fibers were either subjected to periodic axial strain in modified biaxial stressing cells (1), or allowed to mineralize under static conditions. Collagen fibers were also incubated in buffered solutions without calcium phosphate precipitation and also subjected to periodic axial strains or static conditions during incubation.

### *Solution Conditions:*

Two categories of incubating solutions were employed. The first category consisted of 0.05M tris buffered solutions prepared at four initial pH values (pH 5.0, 7.0, 8.0, and 9.0). The second category, comprising the mineralizing solutions, contained 0.1M calcium chloride dihydrate and 0.1M potassium phosphate dibasic in 0.05M tris buffer. The pH was adjusted by the dropwise addition of HCl or KOH to produce four solutions with starting pH's of 5.0, 7.0, 9.0, and 10.0. Each well contained 15 ml of either the mineralizing solution or the incubating solution.

### *Experimental Design:*

Biaxial stressing cells presented by Brighton et al. (1) were modified to subject the reconstituted collagen fibers to periodic axial strain during mineralization (Figure 1).



**Figure 1:** Schematic representation of the adapted biaxial cell stress device modified to provide a periodic axial strain in reconstituted collagen fibers.