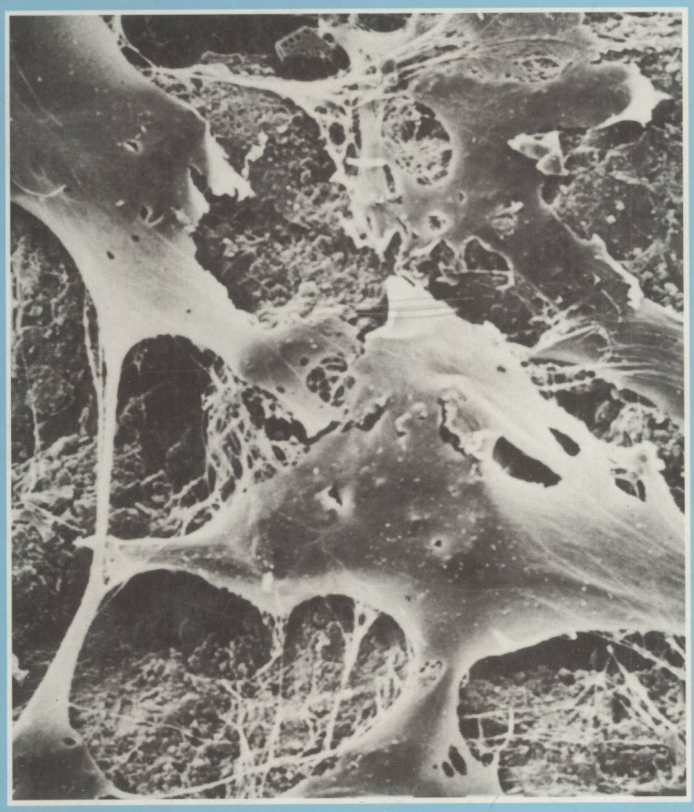
Paul Ducheyne • David Christiansen

Bioceramics

Volume 6



Philadelphia, USA

Bioceramics

Volume 6

edited by

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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*.

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Proceedings of the 6th International Symposium on Ceramics in Medicine

Philadelphia, USA, November 1993

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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

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Influence of Cyclic Axial Stress on the Mineralization of Collagen

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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 a 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).

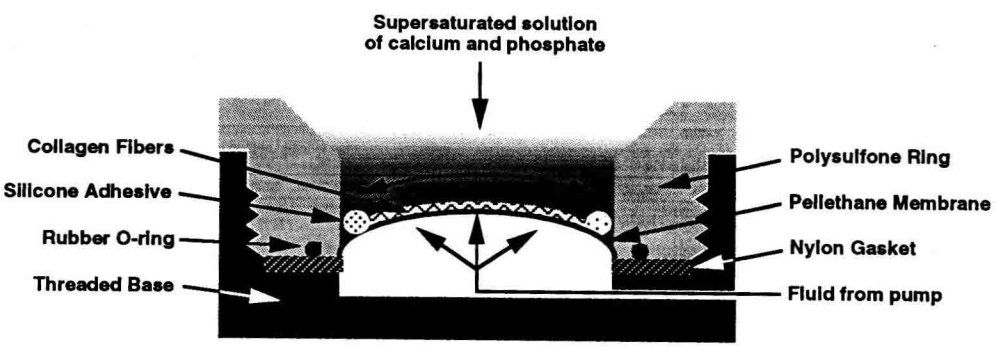

Figure 1: Schematic representation of the adapted biaxial cell stress device modified to provide a periodic axial strain in reconstituted collagen fibers.