J Mater Sci: Mater Med (2008) 19:11–17
DOI 10.1007/s10856-007-0154-x

An open-pored gelatin/hydroxyapatite composite as a potential bone substitute

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Received: 13 January 2006 / Accepted: 29 March 2006 / Published online: 15 August 2007

Abstract Gelatin matrix composites reinforced with fine hydroxyapatite (HA) dispersants were investigated in an exploratory study for their suitability as surgical implants. The criteria for the candidate implant material were that it: (1) be benign, (2) have useful mechanical properties under quasi-in vivo environmental conditions, (3) be dimensionally stable, (4) be sterilizable, (5) be completely assimilable, and (6) exhibit contiguous porosity to encourage invasion by the live host tissue. The synthesis of a composite comprised of a HA particulate reinforcement of a cross-linked gelatin matrix was undertaken to provide preliminary data on its swelling behavior and compressive stiffness that is retained after extended immersion in normal saline solution. A new approach leading to a tailorable, open pore microstructure is described. At a sufficiently high ratio of HA to gelatin the attainable compressive stiffness and the resistance to swelling suggests that this composite system offers potential as a versatile surgical implant material. Suggestions for further studies are offered.

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Introduction

In order to augment the limited availability of live, normal bone for surgical repairs, orthopedic surgeons seek a benign, robust biocompatible substitute material [1, 2] that upon implantation transforms into normal tissue. Although normal bone occurs in various forms, it is comprised of collagen, water, a few noncollagenous proteins and embedded in a predominantly hydroxyapatite (HA), or $Ca_{10}(PO_4)_6(OH)_2$, mineral skeleton. Accordingly many efforts [3–10] that have sought to create a suitable bone substitute have focused on combining HA with collagen. However, considerable effort has also been directed [11–19] at the related system of HA in combination with gelatin. This is the system of interest in the present paper in as much as gelatin per se has many desirable properties from a surgical point of view. However, it has the well-known propensity of absorbing water, causing it to swell, which in turn may affect the behavior of the composite system of HA in a gelatin matrix when in a moist in vivo environment. Hence, the interactions involving swelling, the HA/gelatin ratio, mechanical properties, gelatin cross-linking and various physical factors were examined in considering this composite system as a implant material.

Emphasis was also given to achieving a material having substantial open porosity. This attribute has been widely recognized as enhancing access of live cellular constituents [20, 21] into the implant, thereby accelerating the healing processes. Prior approaches to achieving such porosity include incorporating inert soluble constituents such as salt, into the implant body, followed by their physical or chemical dissolution [22]. Another strategy is to make an open skeleton of HA by partial sintering or by use of chemically modified coral. Such mineral skeletons are subject to brittle fracture [23]. Accordingly we have sought a benign porous composite material that would be robust,

mechanically adequate, and capable of being completely absorbed and remodeled into natural bone.

In summary, the broad scope of this study was (1) to synthesize HA/gelatin composites over a range of compositions having an open porous meso-scale structure, and (2) to determine the mechanical properties and swelling behavior of such composites upon exposure to quasi-vivo conditions. Accordingly the mechanical properties and swelling behavior of the various composites were studied over a range of compositions and porosities. However, these studies did not include in vivo animal implantations nor in vitro behavior towards live cell cultures.

Background

Prior work

The effects of how the structure, size, and concentration of HA amongst other reinforcing particulates affect the compressive strength and compressive modulus of compacts bonded together with cross-linked gelatin were previously studied [24] systematically. The test samples, although incubated under 100% humidity at 37 °C, were otherwise dry. The results indicated that "properties appropriate to a number of orthopedic applications" were feasible with strengths >10 MPa, and moduli >100 MPa. Other investigations were performed with composites reinforced with the closely related tri-calcium-phosphate using gelatin as the matrix [14, 15] cross-linked with formaldehyde or glutaraldehyde. Subsequent evaluation was largely based on the in vivo histological response rather than on the physical response of the composites.

Gelatin and collagen as bone constituents

Gelatin [25–27] closely matches the chemistry of the collagen in natural bone and approximates much of the latter's polymeric architecture and is more conveniently available than is collagen itself. Gelatin consists of a mixture of helical fibril fragments produced by the hydrolysis of collagen to which it remains chemically and structurally closely related and is constituted of some 19 amino acids joined by peptide linkages. Gelatin is widely available as a highly refined material, is an excellent adhesive [11, 28], is mechanically tough, and is readily resorbed by the body. The various grades of gelatin are commonly designated by their "Bloom value" on the basis of the stiffness of the hydrosol of standard gelatin concentration. In general the greater the stiffness the greater the mean molecular weight of the particular gelatin. Gelatin "dissolves" in water as a hydrosol above 37 °C; below that temperature the sol transforms to a rubber-like gel state, even in concentrations [29] as dilute as 2 wt%. This makes possible the convenient synthesis of a microscopically uniform HA/gelatin composite by mixing the sol with an aqueous dispersion of colloidal sized HA followed by removal of the excess moisture. The amino side groups are believed to provide sites for chemical cross-linking between the fibrils, rendering them water insoluble and resistant to swelling. As a class aldehydes, notably formaldehyde and glutaraldehyde, are effective cross-linking agents. Cross-linking produces an increase in stiffness of the hydrogels [29] and renders them somewhat friable as was exploited in the present study.

Hydroxyapatite as a bone constituent

For convenience in preparing the experimental composites for study we considered the mineral component to be adequately modeled by HA. Hydroxyapatite, being the least soluble of the calcium phosphates; bulk HA, while benign, is resistant to assimilation. It can also be sintered to a fully dense mechanically strong body having attributes like those of other ceramics with respect to hardness, abrasion resistance, and stiffness rendering it especially suitable for certain dental applications or joint (socket) repairs. Such fully dense HA bodies are biocompatible but are only very slowly resorbed [30, 31] into the body.

To compensate for this relative inertness, a fine particle size was enhance its being remodeled into normal bone structure. In bovine bone the particle size of the individual HA grains is about 50–100 nm. Phase-pure HA particulates of this size can be produced by precipitation from mixtures of calcium and phosphate salts dissolved in water or out of gelatin hydrosols. However, HA being readily available commercially in the form of a very fine, chemically pure powder, was used in the present study to prepare the composites.

Open structures and materials akin to cancellous trabecular bone [21–23] are often desired for bone repair. One such material, Pro-Osteon (Interpore Cross International, Irvine, CA), available since 1982, is derived from coral in which the porous exoskeleton has been converted into an HA pseudomorph that is capable of being carved or ground into a desired shape. Vitoss (Orthovita, Inc., Malvern, PA), a pure beta-TCP structure of crystallites 70–100 nm in size, is made into sculpable blocks having a porosity of 90%. Neither of these materials is load bearing. Many other HA or HA/carbonated HA blends have been comprehensively characterized [32] and commercially offered in the form of porous granules or carvable blocks.

The HA/gelatin composite system

In as much as gelatin is known to be an excellent bioadhesive and HA to be benign, compatable and readily

assimilatable by live tissue, a combined HA/gelatin composite is expected to be accepted following implantation. There is some concern regarding its structural integrity and property maintenance as a result of differences in the rate of absorption/assimilation of the two constituents. This would require further study. The rate of absorption of HA [3, 30, 31] depends on its density, particle size and "crystallinity"; that of gelatin depends on its grade and/or its chemical or thermal cross-linking treatments. Our intuitive approach has been (1) to make the composition and microscopic structure of the composite as near to that of natural bone as feasible, (2) to produce a network of wide open channels to enhance the cellular remodeling processes, and (3) to adjust the HA/gelatin ratio and cross-linking treatments that optimize the mechanical stiffness and non-swelling behaviors. These conditions were conjectured to promote rapid, controlled recovery from the trauma of the surgery in treating the bone lesion.

HA/gelatin composites considered as filled thermoplastics

Intuitively the gelatin hydrogels particulate-filled gel may be expected to behave analogously to particulate-reinforced thermoplastics such as ordinary talc-filled polyvinylchloride with respect to the dependence of mechanical properties on composition, particulate size, and bonding to the matrix. In thermoplastics the polymeric matrix bonds to the reinforcement phase and can be viewed as filling space "contiguously" with polymeric molecules. However, in gelatin hydrogels much of the space-filling material can be viewed [29] as water confined within protein fibril mesh "cages," although some of the water molecules may also be hydrogen-bonded to the fibrils. The cages expand or collapse in accordance with the amount of occluded water. Hence the matrices derived from gelatin sol are subject to considerable swelling and shrinkage as the water content changes.

Present studies

The "composite–composite" concept

A major goal of this study has been to produce a bone-like composite structure having open porosity on a scale that could invite cellular invasion upon implantation. An imperfect packing of sufficiently coarse granules would produce open spaces between the granules. Under a compressive load the point contact between the individual granules requires the individual granules to have a high compressive strength. The granules but do not need to be fully dense solids, but could in turn be composite materials. A coherent macro-scale body is envisioned by forming strong bridges between the granules. The resultant aggregate structure is envisioned as offering at least a modicum of mechanical strength, stiffness, and shape capability as required for a surgical implant. Such a bonded structure can be produced by wetting the granules with a gelatin hydrosol—followed by drying. The scale and amount of open space remaining after bonding will depend on the size and properties of the composite granules and of the amount and properties of the "glue" used to bridge between the granules. Such a material is defined as a "composite–composite" (C–C). The term "simple composite" will be used to refer to the HA particulate reinforced composite that result from the dispersion of HA particulates throughout an aqueous gelatin sol or gel. The term "composite–composite" or "C–C" refers to a the composite that results when a simple composite is granulated to form coarse entities (granules) which can be bonded together at their points of contact to produce a kind of 2nd generation C–C structure.

Source of constituents

The gelatin, designated as 250B FDA-approved edible grade gelatin, was supplied through the generosity of Mulligan and Higgins Company, Johnstown, NY in the form of coarse, dry granules. This is a porcine-derived material produced by an alkaline lime extraction process. Hydrosols ranging up to 35 w/o gelatin were achieved by dissolution in de-ionized water above 40 °C.

The HA "powder", obtained from ALFA was labeled tri-calcium phosphate, but was determined by us using X-ray diffraction analysis to have the hexagonal hydroxyapatite structure and the composition $Ca_5(PO_4)_3OH$. The as-supplied powder was in the form of an agglomerated "powder," which was broken up by ball-milling in an aqueous medium at a pH of 5 to which a dispersing agent was added. The agglomerates were broken down into smaller aggregates about 0.1–1 microns in diameter. Scanning electron microscopy revealed the aggregates to consist of primary HA tabular crystallites about 100 nm in the largest dimension as seen in Fig. 1.

Fabrication of the HA/gelatin granules

Composites having desired *HA:gelatin(dry)* compositions with weight ratios ranging from *20:2* to *1:2*, were prepared by mixing an aqueous slurry of HA into a gelatin hydrosol containing the predetermined amount of (dry) gelatin. Upon cooling to room temperature a hydrogel resulted that was cross-linked by exposure to formaldehyde vapor or by

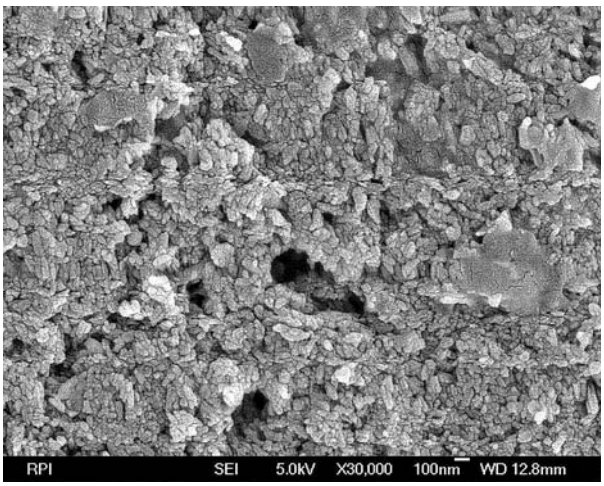

Fig. 1 SEM Micrograph of input HA particulates used to synthesize C–C material

immersion in a 5% glutaraldehyde solution. Moisture was removed by evaporation to the desired water content. The rubbery cross-linked gel of the desired composition was cut into regular shapes for subsequent property determination. This allowed the swelling and compressional stiffness behavior of this basic homogeneous composite material (later to be converted into to C–C granule) to be determined.

Alternatively, the quasi-brittleness of the cross-linked gel allows it to be crumbled into fragments. This facilitates a more rapid moisture removal and conversion into dry sub-millimeter size C–C granules having dry compositions with HA:Gelatin weight ratios that range from 20:2 to 20:8; the weight of HA being held fixed at 20 g.

Fabrication of the C–C test specimens

The C–C granules were minimally moistened with hydrosols containing 5, 10, or 15 wt% gelatin to provide adhesive bridges between the granules. This mass was placed in a steel die, and pressed to 7 GPa to produce 12.9 mm diameter pellets. Upon drying the pellet specimens had open porosity. They were remeasured and used in swelling or compression testing. The pressure affected the density of the granules.

Measurement methodolgies

Swelling/Shrinkage

Swelling and shrinkage are deceptively simple concepts. An early qualitative demonstration of swelling occurred when dry granules formulated to have a HA:gelatin composition corresponding to cortical bone were packed into a cylindrical cavity intended to simulate a surgical implant procedure. Upon wetting the dry-packed compact with a dilute gelatin hydrosol intended to provide intergranular adhesive bridging, surprisingly the compact began to extrude from the cavity. This experience motivated the study of the swelling behavior of HA/gelatin composite as influenced by composition and other factors. Swelling and redrying of the cross-linked composites were found to be essentially reversible processes.

Density determination

Density provides inferential insight into the structure and spatial utilization of the constituents of the composite and offers useful clues for modeling the structure at the meso level. The theoretical densities can be calculated from the weight-base composition and the intrinsic density of the constituents. The fibril density of gelatin is the same as that of collagen which is 1.34 g/cm^3. The crystallographical density of HA is 3.16 g/cm^3. The experimentally measured densities depend strongly on the method of synthesis. The bulk density of the dry simple composites indicates porosity on a microscopic scale within the dry matrix region.

Compressive moduli

Compressive moduli were determined on the same samples as used for the swelling behavior studies. The tests were performed on unconstrained samples tested between the flat faces of a servohydraulic test machine (Instron 4204) at a displacement rate of 1 mm/min. The dimensions of the various samples were measured using micrometers on the thicknesses and vernier caliper on the diameter. They were subjected to an compressive displacement until the applied compressive load versus displacement became linear. The compressive strain was taken to be the ratio of the displacement to the initial thickness and the stress was the load divided by the disk area. The measurements and the testings were repeated after soaking in an aqueous 0.9% sodium chloride solution at 40 °C for the indicated durations. No compressive failures were observed.

Details of the procedures and results

Table 1 compares the dry densities and the swelling behavior of various HA–gelatin simple composites and C–Cs as determined from the physical dimensions of the specimens using a non-contacting traveling microscope. The simple composite samples were cut to be nominally

Table 1 Swelling of HA/gelatin composites

Composition (1)	Theoreticaldry density g/cm^3 (2)	Measured dry density g/cm^3 (3)	Vol_{wet}/Vol_{dry} after 1 day soaking (4)	Vol_{wet}/Vol_{dry} after 2 days soaking (5)	Vol_{wet}/Vol_{dry} after 1 week soaking (6)	Vol_{wet}/Vol_{dry} after 2 weeks soaking (7)
20:2 ng	2.812	1.289	1.155	1.165	1.145	1.16
20:3 ng	2.684	1.347	1.217	1.253	1.273	1.278
20:4 ng	2.577	1.395	1.298	1.380	1.504	1.471
20:8 ng	2.277	1.551	1.653	1.861	2.168	2.218
20:2-5	2.790	1.649	1.138	1.172	1.207	1.207
20:3-5	2.665	1.645	1.400	1.467	1.567	1.567
20:4-5	2.562	1.591	1.394	1.455	1.670	1.667
20:8-5	2.268	1.510	2.094	2.219	2.875	2.938

20:*x* indicates the dry weight ratio of HA:gelatin composite specimens

ng means coherent non-granulated material

circular. The C–C materials were pressed in a cylindrical die at 7 GPa and were uniformly circular and thick.

The four upper-most rows refer to the density and swelling behavior of *as-cast simple* composites having composition ratios ranging from 20:2 to 20:8. Column 2 displays the calculated theoretical densities for the compositions assuming the constituents to be dry and fully dense. Column 3 contains the actual measured densities of these composites over the same compositional range after being dried to 70 °C. The difference between column 3 and column 2 indicates that the visually homogeneous gelatin sol matrix phase is microscopically very porous. The 4 lower rows pertain to *pressed* C–C specimens over the same range of compositions. These display a relatively greater dry bulk density than do the simple ones. This property difference indicates that a squeezing-out of some of the microscopic porosity occurs under pressure which more than compensates for the expected increase in bulk interconnected porosity arising from the imperfect packing of the bonded composite granules. The factors are expected to affect swelling behavior upon exposure to real or simulated moist implantation conditions. These details are expected to be relevant to the biological remodeling or absorbance processes following implantation.

Columns 4–7 relate to their dimensions after immersion in normal saline solution for the indicated times relative to their dry volumes. The volumes was calculated from the measured mean thickness and the mean diameters of the individual specimens.

Table 2 relates the swelling behavior of the pressed C–C composites is only mildly influenced by the concentration of the sol used to bridge and bond between the granules. Thus the swelling behavior of the C–C materials depends primarily on the behavior of the individual granules and not on how they are linked. The least swelling occurs when the gelatin content of the granules is minimized.

Table 3 displays the compressive moduli obtained on the same samples as were used in the swelling behavior studies. In this way exposure to simulated body fluid (normal saline solution) could be surveyed relative to its effect on compressive stiffness. The pressed C–C specimens consistently displayed greater compressive stiffnesses that did the simple ungranulated materials. No compressive failures were observed. The composite displaying the greatest stiffness, both in the dry state, or after up to 2 weeks immersion in normal saline solution, were the C–C material composites having a HA:gelatin weight ratio of 20:2.

Discussion and summary

The above pilot study surveyed a range of microscopically uniform HA/gelatin composites with respect to their likely mechanical performance and geometric stability as a fully absorbable, biocompatible surgical scaffold material. The individual entries shown in the tables were mostly obtained on single specimens so that attention should be directed at trends, rather than individual entries. The data show a strong dependence of the properties on composition and on whether the materials have been soaked in a body-simulating normal saline solution. The least swelling and the greatest stiffness occurred at the greatest (feasible) HA/gelatin ratios. The density measurements show the composites retain considerable porosity at a submicroscopic level not visible in Fig. 1 by SEM inspection at 30,000×. The decrease in the compressive stiffness for most compositions upon imbibing moisture may be related to the significant void content left behind as the gelatin gel dries. Such empty space would provide room into which the hydrated gelatin matrix could expand without imposing a large counter stress on the HA constituent. If so, conventional modeling, in which the gelatin is treated as if it were a space-filling material thermoplastic is inappropriate and requires modification to bring predictions into accord with observed behavior.

Table 2 Swelling of the HA/gelatin composite-composites

Composition	Dry volume (mL)	Vol_{wet}/Vol_{dry} after 1 day	Vol_{wet}/Vol_{dry} after 2 days	Vol_{wet}/Vol_{dry} after 1 week
20:2-5	0.318	1.08	1.10	1.13
20:2-10	0.285	1.12	1.15	1.24
20:2-15	0.327	1.22	1.29	1.48
20:3-5	0.320	1.40	1.40	1.62
20:3-10	0.313	1.35	1.43	1.63
20:3-15	0.310	1.43	1.40	1.85
20:4-5	0.33	1.39	1.46	1.670
20:4-10	0.34	1.35	1.41	1.68
20:4-15	0.34	1.50	1.59	1.88
20:8-5	0.32	2.09	2.22	2.88
20:8-10	0.34	2.03	2.35	–
20:8-15	0.34	2.00	2.35	–

The number following the hyphen denotes the wt% concentration of the gelatin hydrosol used as binding agent in the C–C disks pressed at 7 MPa, i.e., 20:*x*-5 designates specimens made of compacted granules bonded with 5 wt% gelatin hydrosol

Table 3 Compressive moduli of CHOH-crosslinked HA/gelatin disks

Composition	Modulus (MPa) Dry	Modulus (MPa) after 1 day wet	Modulus (MPa) after 2 day wet	Modulus (MPa) after 2 weeks wet
20:2 ng	46.6	47.7	62.1	62.2
20:3 ng	172.3	40.9	42.0	28.3
20:4 ng	147.8	28.9	26.5	17.8
20:8 ng	105.4	13.5	16.4	6.5
20:2-5	308.9	126.6	160.4	127.8
20:3-5	740	59	70.2	68.5
20:4-5	376.0	50.0	50.4	39.6
20:8-5	277.5	36.7	25.6	11.0

Uncertainty estimated to be ±10%

20:*x* indicates a composition having a HA:dry gelatin weight ratio of 20:*x* in the dry condition

20:*x* ng indicates specimen was an intitially non-granulated composite material

20:*x*-5 indicates that the specimen is a die-pressed granular material bonded with 5 wt% gelatin hydrosol

Future work is needed to determine how the meso-scale HA/gelatin composite granules relative to the constituent sub-micron-scale HA crystallites with respect to how the healing process actually proceeds subsequent to implantation. Intuition suggests that the C–C approach should accelerate the repair process. However, there appears to be contra-indicating information [30, 31] indicating an inhibiting absorption effect of decreasing the particle size of HA inclusions in implants as obtained by in vitro studies. What the overall in vivo effect is on the behavior of implanted C–C composites requires experimental investigating beyond the scope of the present study.

Conclusions

The data trends with respect to minimizing the gelatin content relative to that of HA on the compressional moduli and the short term as well as the long term shrinkage behavior offers encouragement that this composite system may evolve into a useful implant material. This was a pilot study and requires further effort to provide a statistically reliable data base. The composite–composite approach appears to offer a practical route for controlling and tailoring porosity. This has been applied to ceramic systems, but the composite gelatin matrix underlying structure may result in a more robust material, a characteristic of some attractiveness to the orthopedic surgeon. While it appears that the constituents offer total absorbability, the relative rates and completeness of the remodeling process may be problematic. However, heat treatment is expected both to facilitate the strength and the stability of the of the gelatin bonds with respect to moisture and biological attack, which may be used to advantage. In conclusion considerable more effort is required to establish the potential of this composite approach.

Acknowledgements W.B. Hillig in particular appreciates the encouragement and useful discussions with osteopathic surgeon Dr. R. Uhl of Albany Medical College, and with his colleagues

Drs. E. Ledet and D. Vashishth. We gratefully acknowledge the help provided by NSF through its REU program in support of S. Murtha and N. Natravali.

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