

Acknowledgments

First and foremost, I'd like to thank my advisors, Ching-Chang Ko and E. Russell Ritenour, for support and encouragement to complete this degree. I owe a great debt of gratitude to the Queen Mary, University of London group, headed by Andy Bushby, and including the great Alan Boyde, Aman Bembey, and Ginger Ferguson. Their data, generously provided, made a significant difference in this work; I am also indebted to them for many long discussions of mineralized tissues. Special thanks to Andy Liu, for performing the original bone indentation tests associated with this project. Thanks also to Warren Oliver, Jenny Hay, and Erik Herbert at MTS Nano, and Oden Warren at Hysitron. Finally, other colleagues, here and elsewhere, with whom I have had invigorating technical discussions on topics more-or-less related to this work include Y.T Cheng, Robert Cook, Eve Donnelly, Ravi Nalla, Wook-Jin Seong, and Mike Swain.

I dedicate this work to my grandparents:

Adriana Gertrude (Jane) Tims Paulson

Willard (Bill) Paulson

Merwin Oyen

Karen (Kay) Kleven Oyen

Abstract

Recent developments in instrumentation have increased the use of contact mechanical testing techniques (“nanoindentation”) for the examination of local mechanical responses. These techniques are ideal for testing tissue biomechanical responses on the scale of the tissue ultrastructure. However, features of the mechanical response of tissues, such as time-dependence and inhomogeneity, add complexity to the analysis of indentation testing. The current work is aimed at promoting understanding of mechanics of biological tissues at the level of the ultrastructure. Engineering materials with known composition and properties are used as model materials for exploring the indentation mechanical behavior of time-dependent and inhomogeneous systems. Modeling techniques, both analytical and computational, are used to understand the fundamental mechanical behavior of biological composite material systems at ultrastructural length-scales. Models of mineralized tissue behavior at small scales are developed for both homogeneous and indentation loading. After establishing the mechanics framework for investigating these material types, indentation experiments are performed on biological materials and mechanical properties are extracted using the models that have been developed. Techniques developed in the current work are used to examine variations in mechanical response of healing bone as a function of distance from the dental implant interface and as a function of healing time since implantation. Relative contributions of mineral content and local mineral network structure on variations in elastic modulus were examined.

Table of Contents

Chapter 1: Introduction	1
1.1 Statement of Problem	2
1.2 Materials Background	6
1.2.1 Engineering Materials and Mechanical Behavior	6
1.2.2 Bone	12
1.2.2.1 Bone Composition	12
1.2.2.2 Bone Hierarchy and Microstructure	15
1.2.2.3 Bone Phase Continuity	18
1.2.2.4 Bone Healing and Remodeling	19
1.2.2.5 Mechanical Behavior of Bone	20
1.2.3 Mineralized Tooth Tissues	23
1.2.3.1 Enamel	24
1.2.3.2 Dentin	25
1.2.3.3 Tooth Replacement via Dental Implants	26
1.3 “Road Map” of this Work	28
Chapter 2: Mechanics Background	30
2.1 Contact Mechanics and Indentation Testing	31
2.1.1 Indentation Testing Advantages	31
2.1.2 Spherical Indenter (Hertzian) Solutions	35
2.1.3 Conical Indenter Solutions	38
2.1.4 Oliver-Pharr Analysis	39
2.1.5 Experiment Types for Indentation Testing	44
2.1.6 Indentation Testing Instruments	45
2.1.6.1 MTS Nanoindenter XP	46
2.1.6.2 Hysitron Triboscope/Triboindenter	48
2.2 Time-Dependent Mechanical Behavior	50
2.2.1 Model-Independent Measures of Time-Dependence	50
2.2.2 Viscoelastic Spring-Dashpot Models	52
2.2.3 Linearly Viscoelastic Behavior	57
2.3 Mechanical Properties of Composite Materials	59
2.3.1 Physical Properties of Composite Materials	59
2.3.2 Elastic Modulus of Composite Materials	61
2.4 Finite Element Analysis	64
2.4.1 Introduction and Discretization	64
2.4.2 Fully 3D Problems	65
2.4.3 Plane Strain Problems	67
2.5 Mechanical Directions for this Work	69

Chapter 3: Elastic-Plastic Indentation Experiments	70
3.1 Elastic-Plastic Indentation Analysis	71
3.1.1 Calibration	71
3.1.1.1 Typical Area Function Calibration	72
3.1.1.2 Frame Compliance Effects in Calibration	75
3.1.2 Compliant Material Calibration Issues	82
3.1.2.1 Calibration Standards for Compliant Materials	83
3.1.2.2 Oliver-Pharr Analysis and the Contact Displacement	85
3.2 Elastic-Plastic Bone Indentation	89
3.2.1 Bone-Dental Implant Study Methods	89
3.2.2 Effect of Instrument and Calibration	91
3.2.3 Effect of Bone Healing Time	94
3.3 Nanoindentation Variability Experiments	98
3.3.1 Homogeneous Controls	98
3.3.2 Biological Composites	101
3.3.3 Summary of Indentation Variability	102
3.4 Property Mapping	105
3.4.1 Mapping the Dentin-Enamel Junction	105
3.4.2 Bone Modulus Mapping	109
3.5 Discussion	120
Chapter 4: Time-Dependent Indentation	122
4.1 Time-Dependent Effects in Indentation Testing	123
4.1.1 Hallmarks of Indentation Creep	123
4.1.2 Indentation Creep in Mineralized Tissues	127
4.2 Viscoelastic Indentation by Radok Correspondence	129
4.2.1 Nonlinearly Viscoelastic Indentation Mechanics	129
4.2.2 Creep Following Ramp Loading	133
4.3 The “VEP” model	138
4.3.1 Model	138
4.3.2 Adaptation of the VEP Model to Routine Testing	141
4.4 Time-Dependent Indentation Experiments on Mineralized Tissues	144
4.4.1 VEP Analysis of Dentin Hydration	144
4.4.2 VEP Analysis of Healing Bone	147
4.4.3 Implications of Viscosity-Modulus Correlations	151
4.4.4 VEP Comparison of Bone and Dentin	154
4.5 Discussion	157

Chapter 5: Modeling Bone as a Two-Phase Composite	158
5.1 Mineralized Tissues as Composite Materials	159
5.1.1 Mineral Volume Fractions	160
5.1.2 Mineral Phase Elastic Properties	162
5.1.3 Organic Phase Elastic Properties	162
5.2 FEA Model for Homogeneous Loading	165
5.3 Anisotropy: Particle Shape and Distribution Variations	169
5.3.1 Anisotropy Model	169
5.3.2 FEA Comparison with Halpin-Tsai	176
5.3.3 Examination of the Stiffening Mechanism in High-AR Composites	179
5.4 Modeling of Interpenetrating Phase Composites	183
5.5 Porosity and Collagen Modulus Effects	186
5.6 Intermediate connectivity	189
5.6.1 Intermediate Connectivity in 1D	190
5.6.2 Intermediate Connectivity in 2D	194
5.7 Discussion	204
Chapter 6: Modeling and Bone Indentation	207
6.1 Scaling Laws for Indentation of Particulate Composites	208
6.2 FEA Model for Inhomogeneous Indentation	211
6.2.1 Model Validation for Indentation Loading	211
6.2.2 Model for Indentation Loading of a Composite	214
6.2.3 Indentation Model Applied to Bone and Dentin	218
6.3 Examination of Bone Indentation Variability	221
6.3.1 Background	221
6.3.2 Conversion to Composite Materials Framework	222
6.3.3 Estimates of Bone Structure from Modulus	228
6.4 Embedded bone	230
6.4.1 Background	230
6.4.2 Analysis	231
6.5 Discussion	237
Chapter 7: Discussion and future work	240
7.1 Discussion	241
7.1.1 Indentation Testing ..	2
7.1.2 Mineralized Tissues as Composite Materials	2
7.2 Implications of Bone Variability	2
7.3 Conclusions	2

Appendix A: Gelatin-Apatite Composites	2
A.1 Biomimetic Composites for Modeling Bone	2
A.1.1 Bone Tissue Engineering	2
A.1.2 Biomimetic Composite Fabrication	2
A.2 Variability in Gelatin-Apatite Composites and Components.	2
A.2.1 Components	2
A.2.2 Composites	2
A.2.3 Materials Variability and Modulus Comparison	2
A.3 Composite Bounds and the Modulus of Gelatin-Apatite Composites	2
Appendix B: Indentation Contact Hardness	2
B.1 Contact Hardness background	2
B.1.1 Vickers Indentation	2
B.1.2 Contact Hardness in Nanoindentation	2
B.2 Elastic-Plastic Contact Hardness	2
B.2.1 Series Model	2
B.2.2 Elastic-Plastic Materials Analysis	2
B.2.2.1 Engineering Materials	2
B.2.2.2 Dentin	2
B.2.2.3 Implications for Bone	2
B.3 Viscous-Elastic-Plastic Contact Hardness	2
B.3.1 Series Model	2
B.3.2 Estimation of Material Time-Constant from Contact Hardness ...	2
B.3.3 Contact Hardness Calculations for Time-Dependent Materials. ...	2
B.3.3.1 PL-1 Polymer	2
B.3.3.2 Bone	2

List of Tables

Table 1-1: Isotropic elastic properties for common engineering materials.....	9
Table 3-1: Means and standard deviations for modulus and hardness of bone.....	96
Table 3-2: Small-depth indentation modulus variability.....	103
Table 3-3: Large-depth indentation modulus for variability studies	104
Table 4-1: PL-1 properties from VEP fits	143
Table 4-2: Mechanical properties from dentin indentation	146
Table 4-3: Averages of parameters for the VEP fits of data shown in Fig. 4-23.....	156
Table 4-4: Average deformation components for the data in Tables 4-1 and 4-3.....	156
Table 5-1: Mineralized tissue mean composition by weight or by mass density.....	161
Table 5-2: Mineralized tissue volume fraction based on data in Table 5-1	161
Table 5-3: Phase fraction calculated for FEA meshes with different particle spacing	166
Table 5-4: Summary of finite element simulations run in the current study	171
Table 5-5: Isotropy or anisotropy in different FEA meshes	172
Table 5-6: 3D Unit cell FEA results for the structures of Figure 5-16.	184
Table 5-7: Comparison of models for compliant collagen or a void phase.	187
Table 5-8: Volume fractions of mineral for different interparticle spacing	196
Table 5-9: Effective modulus results for partially-connected structures	203
Table 6-1: Mean and ranges of mineral fraction and elastic modulus in bone	223
Table 6-2: Indentation moduli and anisotropy ratio for bone	231
Table 7-1: Coefficient of variation for modulus from different analyses	2
Table A-1: Small-depth variability for component and composite materials	2
Table A-2: Large-depth convergence for component and composite materials	2
Table A-3: Gelatin-apatite component and composite moduli	2
Table B-1: Plastic deformation resistance for engineering materials	2
Table B-2: Relative proportions of elastic and plastic deformation	2
Table B-3: Property averages for indentation tests near to and far from the DEJ	2
Table B-4: Plastic deformation resistance calculated for data in Table B-3	2
Table B-5: Time constants and exponential decay constant fits	2
Table B-6: Contact hardness calculated for PL-1 using data in Table 4-1	2

List of Figures

1-1: Uniaxial tension testing geometry	7
1-2: Uniaxial stress-strain curves for three material types.....	8
1-3: Mechanical response of aluminum under tensile loading.....	10
1-4: Time-dependent creep and relaxation responses under fixed loading.....	11
1-5: Viscoelastic stress-strain response sfor constant rate loading at two rates.....	11
1-6: Collagen periodic banding.....	13
1-7: Collagen cross-linking	13
1-8: Apatite crystal structure	14
1-9: Bone mineralization by apatite crystal nucleation and growth	15
1-10: Quantification of the scales of bone hierarchy, after Weiner and Wagner.....	16
1-11: Ultrastructural lengthscales of bone (from nm to mm)	17
1-12: Stress-strain responses for cortical and trabecular bone	20
1-13: Strain-rate effects on bone mechanical stress-strain responses	21
1-14: Stress-strain responses of demineralized bone	22
1-15: Tooth and Jawbone anatomy and relationships.....	23
1-16: The dentin-enamel junction (DEJ)	24
1-17: Collagen network around the dentinal tubules.....	25
1-18: Dental implant anchored into bone	26
1-19: Osseointegration in dental implants and the bone-implant interface	27
2-1: Mechanically tested volume of material for tensile and contact testing	32
2-2: Profile (transverse section) of conical/pyramidal and spherical indenter tips.....	33
2-3: Contact projected area as a function of tip displacement for indenter tips.	33
2-4: Indentation tests performed on three classes of materials two indentation tips.....	34
2-5: Perfectly elastic spherical indentation data for glass.....	36
2-6: Spherical indentation data for purely elastic contact	37
2-7: Spherical indentation data for elastic-plastic contact	38
2-8: Berkovich pyramid indenting a deforming surface, contact depth	39
2-9: Berkovich indentation data for quadratic load-displacement behavior.....	39
2-10: parameters obtained directly from the raw load-displacement data	42
2-11: Reduced modulus as a function of sample plane-strain modulus.....	44
2-12: Common load-time testing inputs for nanoindentation contact mechanical tests ..	45
2-13: Schematic diagram of the MTS Nanoindenter XP.....	47
2-14: Schematic diagram of the Hysitron indentation system	49
2-15: Schematic diagram of Relaxation elastic fraction or total creep displacement.....	51
2-16: Schematic diagram of Hysteresis energy.....	52
2-17: Linear Hookean spring and Newtonian Dashpot mechanical elements.....	53
2-18: Simple combinations of spring and dashpot elements give viscoelastic models ...	54
2-19: Elastic Modulus bounds for modulus mismatch factors of 10, 100, or 1000.	63
2-20: Domain discretization in one dimension (left) and two dimensions (right).....	64
3-1: Elastic modulus versus depth for fused silica using continuous stiffness.....	74
3-2: Plane strain modulus vs depth for polyurethane using continuous stiffness	75
3-3: Peak displacement, stiffness and contact displacement for fused silica	78
3-4: Contact area for fused silica for different values of the frame compliance....	79

3-5: Area function fitting for different assumed values of frame compliance.....	80
3-6: First and second terms of the area function as a function of frame compliance	81
3-7: Modulus and hardness for fused silica with different frame compliance	82
3-8: Plot of the complete area function and the individual component terms	84
3-9: Nanoindentation depth-dependence of the elastic modulus of a polymer.....	86
3-10: Three bone samples harvested from bone along the dental implant interface.....	90
3-11: Modulus vs distance from the bone-implant interface [Chang et al, 2003b].....	91
3-12: Plane strain modulus from the original study and after re-calibration.....	93
3-13: Modulus for the same samples tested with two indentation instruments.....	94
3-14: Modulus versus distance from interface for indentation tests at 1, 2, 4 months.....	95
3-15: Frequency histograms for modulus values at different time points healing.....	97
3-16: Raw indentation responses for fused silica at three peak load levels.....	99
3-17: Elastic modulus for the fused silica indentation tests shown in Figure 3-17.	100
3-18: Elastic modulus data for single crystal mineral apatite	100
3-19: Variability in bone indentation modulus at different peak load levels.....	101
3-20: Variability in indentation modulus of mineralized tooth tissues.....	102
3-21: Cross section of a tooth (3rd molar) illustrating the indented region.....	106
3-22: Composite image of the indented area in a tooth.	106
3-23: Raw indentation traces for tests across the dentin-enamel junction.....	107
3-24: Plane strain elastic modulus map of the dentin-enamel junction.	108
3-25: Plane strain elastic modulus overlay map of the dentin-enamel junction.	108
3-26: Raw indentation load-displacement traces for indentation tests on healing bone..	110
3-27(a): Composite optical image for bone specimen following one month healing	111
3-27(b): Plane strain elastic modulus map for 1-month sample in 3-27(a).....	111
3-27(c): Overlay image of the combined optical and mechanical information	112
3-28(a): Composite optical image for bone specimen following two months healing	113
3-28(b): Plane strain elastic modulus map for 2-month sample in 3-28(a).....	113
3-28(c): Overlay image of the combined optical and mechanical information	114
3-29(a): Composite optical image for bone specimen following four months healing	115
3-29(b): Plane strain elastic modulus map for 4-month sample in 3-29(a).....	115
3-29(c): Overlay image of the combined optical and mechanical information.....	116
3-30: Frequency histograms for modulus values after bone healing 1, 2, 4 months	119
3-31: DEJ modulus map (Figure 3-25) with the scale set to that used for bone maps	121
4-1: Indentation load-displacement and creep displacement-time responses.....	124
4-2: Indentation response at slow rate to illustrate apparently negative stiffness.....	124
4-3: Indentation load-displacement responses for PMMA at three rise times.....	125
4-4: Contact hardness deconvoluted from PMMA indentation at three rise times.....	126
4-5: Residual impressions for PMMA, tested at rise times of 5 s, 50 s, and 500 s.....	126
4-6: Bone creep load-displacement and creep displacement-creep time responses.....	127
4-7: Dry dentin indentation load-displacement responses for tests at three rates	128
4-8: PL-1 polymer indentation ramp-hold creep displacement-time data for four rates...	135
4-9: Data from the load-hold portion of the PL-1 polymer data shown in Fig. 4-8.....	136
4-10: Unloading displacement-time and load-displacement data for PL-1 indentation..	142
4-11: Indentation load-displacement responses for dry and wet dentin	145
4-12: Normalized indentation responses for dry and wet dentin	145
4-13: Displacement-time and load-displacement traces for indentation on bone.	148

4-14: VEP parameters as a function of distance from the bone-implant interface	149
4-15: The range of bone indentation responses obtained in the current study.	150
4-16: Direct dependence of VEP viscosity on modulus.	153
4-17: Two indentation load-displacement traces generated from the VEP model	153
4-18: Load-displacement traces for dry bone and dentin, tested in the same conditions..	155
5-1: Elastic modulus bounds and mineralized tissue composite behavior	159
5-2: Stress-strain plot for four levels of collagen organization [An et al, 2004].....	164
5-3: Finite element model of two-phase composite, loaded homogeneously	165
5-4: Effective elastic modulus computed for FE tensile loading of composites	168
5-5: Geometric identifiers for finite element models.	170
5-6: Effective modulus as a function of mineral volume fraction for square particles.....	172
5-7: Composite elastic modulus in anisotropic particle composites.....	174
5-8: Anisotropy ratio as a function of particle aspect ratio	175
5-9: Results from FEA and Halpin-Tsai empirical expressions for whiskers.....	177
5-10: Calculated effective modulus for a random orientation composite	178
5-11: Effective elastic modulus as a function of particle “length fraction”	180
5-12: Staggered particle model of Jager and Fratzl [2000] and Gao et al [2003]	181
5-13: Comparison of composite bounds and anisotropic finite element results	182
5-14: 3D finite element model geometries for varied phase continuity.....	184
5-15: 3D rendering of 2-phase IPC FE model with one phase void	187
5-16: Effect of compliant phase modulus on Hashin-Shtrikman bounds	188
5-17: Schematic illustration of bounds and corresponding structures	189
5-18: Interrupted fiber model for examining partially connected composite materials	191
5-19: Modulus values for interrupted fiber model and the Voigt-Reuss bounds	192
5-20: Illustration of the parameters used to estimate connectivity from modulus.	193
5-21: Connectivity fraction calculated from geometry and estimated from modulus	194
5-22: Three-component model with matrix, particles, and inter-particle connectors.....	195
5-23: Schematic illustration of connectivity indices C_i	196
5-24: Composite modulus results for four configurations shown above in Fig. 5-25.	197
5-25: Schematic illustration of intermediate values of the connectivity indices C_x	198
5-26: Intermediate connectivity for the removal of single linkages or whole rows	198
5-27 Variation in the modulus with mineral fraction for different connectivity.	199
5-28: Calculated connectivity fraction for the data shown in Figure 5-28.....	200
5-29: Variation in the composite modulus with mineral phase connectivity	201
5-30: Partially-connected composites arranged in all possible configurations.	202
5-31: Proposed structural model of bone based on the observed mineral geometry	206
6-1: 2D and 3D unit cells for a single circular or spherical particle.....	208
6-2: Particles displaced by a Berkovich indenter as a function of displacement.....	210
6-3: The contact width equivalence for Berkovich and flat punch indentation.....	212
6-4: Schematic illustration of indentation model boundary conditions	212
6-5: Pressure profile from flat punch indentation FE model of a homogeneous solid	213
6-6: Comparison of tensile and indentation finite element results	215
6-7: Centering of flat punch “indent” over particle or matrix.....	216
6-8: Line load-displacement responses from FEA flat punch indentation.....	216
6-9: Effective elastic modulus from indentation FEA models for composite materials....	217

6-10: Effective modulus for different indent locations at increasing indent depth	218
6-11: Comparison of bone indentation data with indentation finite element model	219
6-12: Comparison of dentin indentation data with indentation finite element model	220
6-13: Indentation modulus versus qBSE mineralization [Ferguson et al, 2003]	222
6-14: Indentation elastic modulus versus mineral fraction for subchondral bone.....	224
6-15: Modulus data averaged for volume fraction in increments of 0.01 and line fit.....	225
6-16: Average bone modulus data with the Hashin-Shtrikman bounds	226
6-17: Histograms of observed modulus values for mineral fractions of 0.43 to 0.49	227
6-18: Mean and range of calculated connectivity fraction for subchondral bone	229
6-19: Data from Figure 5-8 compared with the measured anisotropy ratio for bone.....	232
6-20: Structures representing a composite with connected or disconnected mineral	233
6-21: Hashin-Shtrikman modulus bounds for a composite of PMMA and apatite	234
6-22: Illustration of structures with different mineral phase connectivity	239
7-1: Elastic modulus map of a portion of bone-implant interface	2
7-2: Schematic diagram of an axisymmetric bone-implant finite element model	2
7-3: Assignment of modulus values in heterogeneous FEA model of bone	2
7-4: Shear strain map for FEA model of bone assuming bone homogeneity	2
7-5: Shear strain map for FEA model of bone assuming bone heterogeneity	2
7-6: Shear strain map for FEA model of bone assuming bone heterogeneity	2
A-1: Variability in elastic modulus values for gelatin and polycrystalline apatite	2
A-2: Indentation responses for small load levels on a gelatin-apatite composite	2
A-3: Variability in modulus values with peak load for gelatin-apatite composites.....	2
A-4: Variability in modulus values for a gelatin-apatite composite	2
A-5: Gelatin-apatite composites have modulus values closest to the lower bounds.....	2
B-1: Vickers Indentation schematic illustration	2
B-2: Illustration of elastic recovery in a transverse section of indentation impression....	2
B-3: Vickers indentation residual impression in hydroxyapatite and composite C	2
B-4: Contact hardness for hydroxyapatite and gelatin-apatite composite C	2
B-5: Property gradients with distance from the dentin enamel junction	2
B-6: Calculated contact hardness versus plane strain modulus	2
B-7: Calculated contact hardness versus rise time for four values of viscosity	2
B-8: Oliver-Pharr contact hardness as a function of rise time for polymers	2
B-9: Modulus, plastic deformation resistance and contact hardness relationships	2