

# Contents

|   |    |
|---|----|
| <b>1 Collagen: Structure and Mechanics, an Introduction</b> .....                               | 1  |
| P. Fratzl   |    |
| 1.1 Collagen-Based Tissues .....  | 1  |
| 1.2 Basic Mechanical Parameters .....   | 4  |
| 1.2.1 Stress and Strain .....   | 4  |
| 1.2.2 Elastic and Viscoelastic Behavior .....   | 5  |
| 1.2.3 Stiffness, Strength and Toughness .....   | 7  |
| 1.3 Mechanical Properties of Collagen-Based Tissues .....                                       | 9  |
| 1.4 Hierarchical Structure of Collagen-Based Tissues .....                                      | 10 |
| References .....  | 12 |
| <b>2 Collagen Diversity, Synthesis and Assembly</b> .....                                       | 15 |
| D.J.S. Hulmes   |    |
| 2.1 Introduction .....  | 15 |
| 2.2 Fibrillar Collagens .....   | 16 |
| 2.3 Non-fibrillar Collagens .....   | 19 |
| 2.3.1 Basement Membrane and Associated Collagens .....  | 19 |
| 2.3.2 Collagen VI .....   | 20 |
| 2.3.3 Collagens VIII and X .....  | 21 |
| 2.3.4 FACITs .....  | 21 |
| 2.3.5 Other Collagens and Collagen-Like Proteins .....  | 22 |
| 2.4 Collagen Biosynthesis .....   | 22 |
| 2.4.1 Post-translational Modifications of Polypeptide Chains .....                              | 23 |
| 2.4.2 Chain Association and Triple-Helix Formation .....  | 26 |
| 2.4.3 Intracellular Transport and Secretion .....   | 28 |
| 2.4.4 Procollagen Processing .....  | 29 |
| 2.4.5 Covalent Cross-Linking .....  | 30 |
| 2.5 Assembly of Fibrillar Collagens .....   | 31 |
| 2.5.1 Reconstitution of Fibrils In Vitro .....  | 31 |
| 2.5.2 Fibril Formation De Novo from Procollagen .....   | 33 |
| 2.5.3 Heterotypic Fibril Assembly .....   | 34 |
| 2.5.4 Interactions with Proteoglycans and Other Components<br>of the Extracellular Matrix ..... | 35 |
| 2.5.5 Cell Interactions and Long-Range Order .....  | 38 |

|          |   |           |
|----------|---|-----------|
| 2.6      | Assembly of Collagen-Like Peptides .....  | 39        |
| 2.7      | Conclusions .....   | 41        |
|          | References .....  | 41        |
| <b>3</b> | <b>Collagen Fibrillar Structure and Hierarchies .....</b>   | <b>49</b> |
|          | T.J. Wess   |           |
| 3.1      | Introduction and Background .....   | 50        |
| 3.2      | The Fibril-Forming Collagens .....  | 51        |
| 3.3      | Molecular Composition of Type I Collagen-Rich<br>Fibrillar Structures .....   | 52        |
| 3.4      | Molecular Composition of Type II Collagen-Rich<br>Fibrillar Structures .....  | 53        |
| 3.5      | Collagen Molecular Packing in Fibrils .....   | 53        |
| 3.6      | Lateral Packing and Molecular Connectivities .....  | 58        |
| 3.7      | Evidence of Subfibrillar Structures .....   | 59        |
| 3.8      | Order and Disorder in the Collagen Fibril .....   | 60        |
| 3.9      | Partition of Structure in the Collagen Fibril .....   | 60        |
| 3.10     | Molecular Kinking .....   | 62        |
| 3.11     | The Fibril Surface and Interface Properties .....   | 63        |
| 3.12     | Factors Involved in Fibril Growth and Size .....  | 67        |
| 3.13     | Distribution of Fibril Diameter and Length .....  | 68        |
| 3.14     | Suprafibrillar Architectures .....  | 70        |
| 3.15     | Relationships with Mechanical Properties<br>of Collagen-Rich Tissues .....  | 72        |
|          | References .....  | 74        |
| <b>4</b> | <b>Restraining Cross-Links Responsible for the Mechanical Properties<br/>of Collagen Fibers: Natural and Artificial .....</b> | <b>81</b> |
|          | N.C. Avery and A.J. Bailey  |           |
| 4.1      | Introduction .....  | 82        |
| 4.2      | Enzyme Cross-Linking (Lysyl Oxidase) .....  | 83        |
| 4.2.1    | Immature Tissues .....  | 83        |
| 4.2.2    | Mature Tissues .....  | 84        |
| 4.2.3    | Changing Cross-Link Profiles of Different Tissues .....   | 87        |
| 4.2.4    | Importance of Lysine Hydroxylation .....  | 89        |
| 4.2.5    | Cross-Linking and Tissue Adaptation<br>to Mechanical Force .....  | 90        |
| 4.2.6    | Determination of the Cross-Links .....  | 90        |
| 4.2.7    | Non-enzymic Cross-Linking (Glycation) .....   | 91        |
| 4.2.8    | Unusual Cross-Linking Mechanisms in Native<br>Collagen .....  | 96        |
| 4.2.9    | Stabilization by Chemical Cross-Linking<br>for Bioengineering Tissues .....   | 99        |
| 4.2.10   | Mechanisms of Some Common Chemical<br>Cross-Link Reactions .....  | 100       |

|          |  |            |
|----------|--|------------|
| 4.2.11   | Location of Enzymic, Glycation and Chemical Cross-Links . . . . .  | 103        |
| 4.2.12   | Mechanism of Increased Denaturation Temperature by Cross-Linking . . . . .   | 104        |
| 4.3      | Future Prospects . . . . .   | 105        |
|          | References . . . . .   | 105        |
| <b>5</b> | <b>Damage and Fatigue . . . . .</b>  | <b>111</b> |
|          | R.F. Ker   |            |
| 5.1      | Introduction . . . . .   | 111        |
| 5.2      | Cracks . . . . .   | 113        |
| 5.2.1    | The Griffith Crack: Material Resistance, Energy Release Rate and Stress Intensity Factor . . . . .                         | 113        |
| 5.2.2    | Tough Materials . . . . .  | 117        |
| 5.2.3    | The <i>R</i> -Curve . . . . .  | 118        |
| 5.2.4    | The <i>J</i> -Integral . . . . .   | 119        |
| 5.3      | Fatigue Cracks . . . . .   | 119        |
| 5.4      | Creep and Fatigue in Tendon and Bone . . . . .   | 121        |
| 5.4.1    | Creep . . . . .  | 121        |
| 5.4.2    | Time-to-Rupture as a Function of Stress . . . . .  | 122        |
| 5.4.3    | Cyclic Loads Compared to a Constant Load: Times-to-Rupture . . . . .   | 123        |
| 5.5      | Crack Stopping in Bone and Tendon . . . . .  | 125        |
| 5.5.1    | Bone . . . . .   | 125        |
| 5.5.2    | Tendon . . . . .   | 126        |
| 5.6      | Biological Aspects: Evolution, Growth and Adaptation . . . . .   | 127        |
| 5.6.1    | Tendons . . . . .  | 127        |
| 5.6.2    | Bones . . . . .  | 128        |
| 5.6.3    | Other Materials . . . . .  | 128        |
| 5.6.4    | Generalizations . . . . .  | 129        |
|          | References . . . . .   | 129        |
| <b>6</b> | <b>Viscoelasticity, Energy Storage and Transmission and Dissipation by Extracellular Matrices in Vertebrates . . . . .</b> | <b>133</b> |
|          | F.H. Silver and W.J. Landis  |            |
| 6.1      | Introduction . . . . .   | 133        |
| 6.1.1    | Concept of Energy Storage, Transmission and Dissipation . . . . .  | 134        |
| 6.1.2    | Molecular Basis of Energy Storage and Dissipation . . . . .  | 136        |
| 6.1.3    | Viscoelastic Behavior of Tendon . . . . .  | 139        |
| 6.1.4    | Viscoelasticity of Self-Assembled Type I Collagen Fibers . . . . .   | 140        |
| 6.1.5    | Viscoelasticity of Skin . . . . .  | 140        |
| 6.1.6    | Viscoelastic Behavior of Cartilage . . . . .   | 142        |
| 6.1.7    | Viscoelastic Behavior of Vessel Wall . . . . .   | 143        |

|          |  |            |
|----------|--|------------|
| 6.1.8    | Determination of Elastic and Viscous Properties<br>of Mineralized Tendon and Type I Collagen . . . . . | 143        |
| 6.1.9    | Effects of Strain Rate and Cyclic Loading . . . . .  | 144        |
| 6.2      | Concept of Mechanochemical Transduction and Changes<br>in Tissue Metabolism and Aging . . . . .        | 145        |
| 6.3      | Relationship Between Viscoelasticity and Hierarchical Structure . .                                    | 147        |
| 6.3.1    | Aligned Collagen Networks and Mechanical Models<br>of Tendon . . . . .                                 | 148        |
| 6.3.2    | Mechanical Models of Orientable ECMs . . . . .   | 150        |
| 6.3.3    | Mechanical Models of ECMs Comprised<br>Only of Collagen Fibers . . . . .                               | 150        |
| 6.3.4    | Mechanical Models of Composite ECMs Containing<br>More Than Collagen Fibers . . . . .                  | 151        |
| 6.4      | Conclusions . . . . .  | 152        |
|          | References . . . . .   | 153        |
| <b>7</b> | <b>Nanoscale Deformation Mechanisms in Collagen</b> . . . . .  | <b>155</b> |
|          | H.S. Gupta   |            |
| 7.1      | Introduction . . . . .   | 155        |
| 7.2      | Deformation at the Fiber Bundle Level . . . . .  | 156        |
| 7.3      | Fibrillar and Molecular Deformation Mechanisms . . . . .   | 158        |
| 7.4      | Mineralized Collagen Deformation . . . . .   | 165        |
| 7.5      | Conclusion . . . . .   | 169        |
|          | References . . . . .   | 170        |
| <b>8</b> | <b>Hierarchical Nanomechanics of Collagen Fibrils:<br/>Atomistic and Molecular Modeling</b> . . . . .  | <b>175</b> |
|          | M.J. Buehler   |            |
| 8.1      | Introduction . . . . .   | 175        |
| 8.1.1    | Deformation and Fracture: An Introduction . . . . .  | 177        |
| 8.1.2    | Collagen Structure – From Atoms to Tissue . . . . .  | 178        |
| 8.1.3    | Outline of This Chapter . . . . .  | 180        |
| 8.2      | Numerical Simulation Techniques and Theoretical Framework . . .  | 180        |
| 8.2.1    | Multi-scale Modeling of Deformation and Failure . . . . .  | 181        |
| 8.2.2    | Basics of Atomistic Modeling . . . . .   | 182        |
| 8.2.3    | Large-Scale Parallelized Computing . . . . .   | 183        |
| 8.2.4    | Analysis and Visualization . . . . .   | 184        |
| 8.2.5    | Complementary Experimental Methods . . . . .   | 185        |
| 8.2.6    | Summary . . . . .  | 185        |
| 8.3      | Deformation and Fracture of Single Tropocollagen Molecules . . .                                       | 185        |
| 8.3.1    | Atomistic Model . . . . .  | 186        |
| 8.3.2    | Tensile and Compressive Loading . . . . .  | 190        |
| 8.3.3    | Bending a Single Tropocollagen Molecule . . . . .  | 195        |
| 8.3.4    | Shearing Two Tropocollagen Molecules . . . . .   | 196        |
| 8.3.5    | Development of a Mesoscopic, Molecular Model . . . . .   | 197        |

|          |  |            |
|----------|--|------------|
| 8.3.6    | Validation of Mesoscale Model in Tensile Deformation . . .   | 202        |
| 8.3.7    | Stretching an Ultra-long Tropocollagen Molecule:<br>Mesoscale Modeling . . . . .                         | 202        |
| 8.3.8    | Discussion and Conclusion . . . . .  | 203        |
| 8.4      | Deformation and Fracture of Collagen Fibrils . . . . .   | 205        |
| 8.4.1    | Model Geometry and Molecular Simulation Approach . . .   | 205        |
| 8.4.2    | Size-Dependent Properties: Effects of Molecular Length . .   | 206        |
| 8.4.3    | Effect of Cross-Link Densities . . . . .   | 214        |
| 8.5      | Nanomechanics of Mineralized Collagen Fibrils: Molecular<br>Mechanics of Nascent Bone . . . . .          | 221        |
| 8.5.1    | Introduction . . . . .   | 222        |
| 8.5.2    | Molecular Model . . . . .  | 224        |
| 8.5.3    | Computational Results: Elastic, Plastic Regime<br>and Fracture . . . . .                                 | 225        |
| 8.5.4    | Discussion . . . . .   | 230        |
| 8.5.5    | Conclusion . . . . .   | 232        |
| 8.6      | Structure–Property Relationships in Biological Protein Materials . .                                     | 233        |
| 8.6.1    | Cross-Scale Interactions: Fracture Mechanisms<br>in Collagenous Tissue . . . . .                         | 234        |
| 8.6.2    | The Significance of Hierarchical Features . . . . .  | 237        |
| 8.6.3    | Universality Versus Diversity . . . . .  | 237        |
| 8.7      | Discussion and Conclusion . . . . .  | 239        |
|          | References . . . . .   | 240        |
| <b>9</b> | <b>Mechanical Adaptation and Tissue Remodeling . . . . .</b>   | <b>249</b> |
|          | M. Kjær and S.P. Magnusson   |            |
| 9.1      | Introduction . . . . .   | 249        |
| 9.2      | Collagen Adaptation to Loading – Biochemical Approaches . . . . .  | 250        |
| 9.2.1    | Dynamics of Collagen Metabolism in Human Tendon<br>and Skeletal Muscle with Mechanical Loading . . . . . | 250        |
| 9.2.2    | Regulatory Factors for Collagen Adaptation to Exercise . . .   | 252        |
| 9.2.3    | Interplay Between Collagen-Rich Matrix and Contracting<br>Skeletal Muscle . . . . .                      | 254        |
| 9.2.4    | Role for Stem Cells in Tendon Adaptation and Healing . . .   | 255        |
| 9.3      | Mechanical Properties of Human Tendon, In Vivo . . . . .   | 256        |
| 9.3.1    | Tendon Hypertrophy . . . . .   | 256        |
| 9.3.2    | Regional Differences in Cross-Sectional Area . . . . .   | 257        |
| 9.3.3    | The Ultrasonography Method . . . . .   | 258        |
| 9.3.4    | Human Aponeurosis Shear, In Vivo . . . . .   | 258        |
| 9.3.5    | Mechanical Properties of Individual Human<br>Tendon Fascicles . . . . .                                  | 260        |
| 9.3.6    | Force Transmission Between Human Tendon Fascicles . . .  | 261        |
|          | References . . . . .   | 263        |

**10 Tendons and Ligaments: Structure, Mechanical Behavior and Biological Function** . . . . . 269

A.A. Biewener

10.1 Introduction . . . . . 270

10.2 Tendon–Ligament Force Transmission and Weight Savings . . . . . 271

10.3 Tendon and Ligament Compliance, Resilience and Functional Stress Limits . . . . . 275

10.4 Tendon Elastic Energy Savings During Locomotion . . . . . 280

10.5 Role of Tendon Elasticity in Jumping and Acceleration . . . . . 281

References . . . . . 282

**11 Collagen in Arterial Walls: Biomechanical Aspects** . . . . . 285

G.A. Holzapfel

11.1 Introduction . . . . . 285

11.2 Structure of the Arterial Wall . . . . . 286

    11.2.1 Intima . . . . . 287

    11.2.2 Media . . . . . 288

    11.2.3 Adventitia . . . . . 289

11.3 Typical Biomechanical Behavior of the Arterial Wall . . . . . 290

    11.3.1 Layer-Specific Mechanical Properties of Human Arteries . . 292

11.4 Structural Quantification of Collagen Fibers in Arterial Walls . . . . . 296

    11.4.1 Polarized Light Microscopy . . . . . 296

    11.4.2 Small-Angle X-Ray Scattering . . . . . 298

    11.4.3 Computer Vision Analysis . . . . . 300

11.5 Models for the Elastic Response of Arterial Walls . . . . . 302

    11.5.1 The Basic Building Block for a Structural Model . . . . . 302

    11.5.2 A Structural Model for Arterial Layers . . . . . 304

    11.5.3 Arterial Models Considering Fiber Dispersion . . . . . 306

11.6 Collagen Fiber Remodeling in Arterial Walls . . . . . 311

References . . . . . 319

**12 The Extracellular Matrix of Skeletal and Cardiac Muscle** . . . . . 325

P.P. Purslow

12.1 Introduction . . . . . 326

12.2 General Structure of IMCT . . . . . 327

    12.2.1 Striated Muscle: Gross Morphology of Intramuscular Connective Tissue . . . . . 327

12.3 Composition of the Perimysium and Endomysium . . . . . 331

12.4 The Amount, Composition and Architecture of Endomysium and Perimysium Vary Between Different Striated Muscles . . . . . 332

12.5 The Orientation of Collagen Fibers in Perimysium and Endomysium Changes with Muscle Length . . . . . 334

12.6 Mechanical Properties of the Perimysium: Models and Measurements . . . . . 337

12.7 Mechanical Properties of the Endomysium: Models and Measurements . . . . . 338

12.8 Mechanical Roles In Vivo for Perimysium and Endomysium  
in Striated Muscles ..... 339  
12.8.1 Endomysial Role in Force Transmission ..... 339  
12.8.2 Myofascial Force Transmission ..... 342  
12.8.3 Perimysium: Coordination of Shape Change  
on Muscle Contraction ..... 343  
12.9 Connective Tissue Networks Within Cardiac Muscle ..... 344  
12.9.1 The Structure of the Cardiac Wall ..... 344  
12.9.2 Structure and Arrangement of ECM Within  
the Myocardium ..... 346  
12.10 Mechanical Roles for ECM in the Myocardium ..... 348  
12.11 Heart Valves are Special ECM Structures ..... 351  
12.12 Conclusions ..... 353  
References ..... 353

**13 The Cornea and Sclera ..... 359**  
K.M. Meek

13.1 Introduction ..... 360  
13.1.1 Macroscopic Structure ..... 360  
13.1.2 Microscopic Structure ..... 361  
13.1.3 Nanoscopic Structure ..... 363  
13.1.4 Composition of the Corneal Stroma ..... 365  
13.2 The Basis of Corneal Shape – Collagen Lamella Organization .... 366  
13.2.1 X-ray Scattering Used to Determine Lamellar  
Organization in the Cornea ..... 368  
13.2.2 Corneal Biomechanics ..... 373  
13.2.3 Corneal Ectasia ..... 376  
13.3 The Basis of Corneal Transparency – Collagen  
Fibril Organization ..... 378  
13.3.1 Transparency in the Normal Cornea ..... 378  
13.3.2 Light Scattering in Swollen Corneas ..... 381  
13.4 Artificial Corneal Constructs ..... 384  
13.5 The Sclera ..... 387  
13.5.1 Scleral Structure ..... 387  
13.5.2 Scleral Composition ..... 388  
13.5.3 Scleral Biomechanics and the Development  
of Myopia ..... 389  
13.6 Conclusion ..... 392  
References ..... 393

**14 Collagen and the Mechanical Properties of Bone and Calcified  
Cartilage ..... 397**  
J. Currey

14.1 Introduction ..... 397  
14.2 Structure of Bone ..... 397  
14.3 Mechanical Properties of Compact Bone ..... 400

14.4 Mechanical Properties of Cancellous (Trabecular) Bone . . . . . 404

14.5 Collagen–Mineral Interactions and the Effect of Different  
Collagen/Mineral Ratios on the Mechanical Properties of Bone . . . 405

14.6 The Effect of Remodeling on Mechanical Properties . . . . . 411

14.7 A Natural Experiment . . . . . 412

14.8 The Effect of Differences of Collagen on Bone’s  
Mechanical Properties . . . . . 413

    14.8.1 Aging . . . . . 413

    14.8.2 Osteoporosis and Osteoarthritis . . . . . 414

    14.8.3 Osteogenesis Imperfecta . . . . . 414

14.9 Calcified Cartilage . . . . . 415

References . . . . . 417

**15 Dentin . . . . . 421**

P. Zaslansky

15.1 Introduction . . . . . 421

15.2 Composition and Main Features . . . . . 423

15.3 Functions of Dentin . . . . . 423

15.4 Dentin as a Material . . . . . 428

    15.4.1 Average Materials Properties of Dentin . . . . . 429

    15.4.2 Variation of Properties as a Design Concept – Elastic  
    Properties . . . . . 430

    15.4.3 Anisotropic Failure Properties . . . . . 432

15.5 Dentin Microstructure as the Basis for Mechanical Properties . . . . 434

    15.5.1 Root Dentin . . . . . 435

    15.5.2 Crown Dentin . . . . . 436

    15.5.3 Variations of “Normal” Dentin Structure . . . . . 438

    15.5.4 “Abnormal” Forms of Dentin . . . . . 439

15.6 Conclusion . . . . . 441

References . . . . . 442

**16 Genetic Collagen Diseases: Influence of Collagen Mutations  
on Structure and Mechanical Behavior . . . . . 447**

R.D. Blank and A.L. Boskey

16.1 Introduction . . . . . 447

16.2 Osteogenesis Imperfecta . . . . . 448

    16.2.1 General Description – OI Types . . . . . 448

    16.2.2 Genotype/Phenotype . . . . . 448

    16.2.3 OI Phenocopies in Mice and Men . . . . . 458

16.3 Other Fibrillar Collagen Mutations that Affect Tissue Structure  
and Mechanical Behavior . . . . . 461

16.4 Discussion . . . . . 463

16.5 Conclusion . . . . . 464

References . . . . . 464



**17 Biomimetic Collagen Tissues: Collagenous Tissue Engineering and Other Applications** . . . . . 475  
 E.A. Sander and V.H. Barocas

17.1 Introduction . . . . . 475

17.2 Synthesis and Culture of Collagen Gels . . . . . 476

    17.2.1 Collagen Self-Assembly in Solution . . . . . 476

    17.2.2 FPCL/Tissue Equivalent (TE) . . . . . 476

    17.2.3 Entrapment of Cells/Compaction . . . . . 477

    17.2.4 Generation of Fiber Alignment . . . . . 479

    17.2.5 Free Floating or Constrained . . . . . 479

    17.2.6 Collagen and Cell Concentration . . . . . 480

    17.2.7 Matrix Synthesis and Cross-Link Formation . . . . . 481

    17.2.8 Conclusions . . . . . 482

17.3 Mechanical Properties of Collagen Gels and TEs . . . . . 482

    17.3.1 Shear . . . . . 483

    17.3.2 Extension . . . . . 485

    17.3.3 Compression . . . . . 488

    17.3.4 Effect of Gelation Conditions on Properties of Gels . . . . . 489

    17.3.5 Conclusions . . . . . 489

17.4 Applications . . . . . 489

    17.4.1 Control of TE Properties . . . . . 490

    17.4.2 Boundary Conditions – Free Surfaces  
         and Mechanical Constraints . . . . . 491

    17.4.3 Mechanical Stimulation for Improved Alignment  
         and Matrix Composition . . . . . 493

    17.4.4 Medium Additives for Improved TE Properties . . . . . 495

    17.4.5 Limitations and Future Directions . . . . . 496

References . . . . . 496

**Index** . . . . . 505