Bio-inspired tailored hydroxyapatite-based powder composites for dental applications

https://escholarship.org/uc/item/9862m5rz

Lin, Yen-Shan

2012

Peer reviewed|Thesis/dissertation
Bio-inspired Tailored Hydroxyapatite-based Powder Composites for Dental Applications

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Engineering Sciences (Mechanical and Aerospace Engineering) by Yen-Shan Lin

Committee in charge:

University of California, San Diego

Professor Marc A. Meyers, Co-Chair
Professor David J. Benson
Professor Joanna McKittrick
Professor Sungho Jin

San Diego State University

Professor Eugene A. Olevsky, Chair
Professor Satchi Venkataraman

2012
The Dissertation of Yen-Shan Lin is approved, and it is acceptable in quality and from for publication on microfilm and electronically:

________________________________________

________________________________________

________________________________________

________________________________________

________________________________________

Co-Chair

________________________________________

Chair

University of California, San Diego
San Diego State University

2012
DEDICATION

Dedicated to My Parents
TABLE OF CONTENTS

Signature page………………………………………………………………………………iii
Dedication..............................................................................................................iv
Table of Contents....................................................................................................v
List of Figures........................................................................................................viii
List of Tables..........................................................................................................xiv
Acknowledgements.............................................................................................xv
Vita........................................................................................................................xvii
Abstract.................................................................................................................xix

Chapter 1 Introduction........................................................................................1

1.1 Literature survey on the mechanical properties and microstructure of natural dental materials........................................................................................................1
  1.1.1 Tubule orientation.....................................................................................7
  1.1.2 Tubule density.........................................................................................9
  1.1.3 Effects of age on the mechanical behavior of human dentin.................11

1.2 Literature survey on the processing of HAP-based bio-composites.............15
  1.2.1 Processing of pure hydroxyapatite (HAP).............................................17
  1.2.2 Processing of HAP-based composites.....................................................22
  1.2.3 Biocompatibility of HAP........................................................................27

Chapter 2 Research incentives and objectives..................................................28

  2.1 Research incentives ...................................................................................28
  2.2 Research objectives ....................................................................................31
  2.3 Research tasks.............................................................................................32
  2.4 Expected engineering and scientific novelties..........................................35

Chapter 3 Characterization of animal and human teeth: mechanical properties
and microstructure .......................................................................................................37

3.1 Characterization of animal and human teeth: Experimental setup and sample preparation ........................................................................................................37

3.2 Characterization of animal human teeth: Results and Discussion.........................41
  3.2.1 Micro-indentation, Nano-indentation tests and SEM characterization........41
  3.2.2 Element line mapping ..................................................................................55
  3.2.3 Compression tests ........................................................................................57
  3.2.4 Microstructure of demineralized and deproteinated human dentin ..........64
  3.2.5 Microstructure characterization of teeth morphology..................................66
  3.2.6 Mechanical testing of the Arapaima Gigas scale .........................................70
    3.2.6.1 Arapaima Gigas scale: Tensile testing...................................................72
    3.2.6.2 Arapaima Gigas scale: X-ray diffraction (XRD).................................75
    3.2.6.3 Arapaima Gigas scale: Micro-indentation test ....................................75
    3.2.6.4 Arapaima Gigas scale: Tensile test results ..........................................75
    3.2.6.5 Arapaima Gigas scale: Morphology and fracture surface inspection....86
    3.2.6.6 Arapaima Gigas scale: the micro-indentation tests .............................93
    3.2.6.7 Arapaima Gigas scale: Nanoindentation .............................................95
    3.2.6.8 Arapaima Gigas scale: X-ray diffraction (XRD).................................101
    3.2.6.9 Arapaima Gigas scale: Fourier Transform infrared spectroscopy (FTIR)102
    3.2.6.10 Piranha tooth vs. Arapaima scale (penetration test) .........................104
    3.2.6.11 Arapaima Gigas scale: SEM characterization of demineralized scale 107

3.3 Natural dental materials’ characterization: Conclusion.......................................108

Chapter 4 Spark-Plasma Sintering of Hydroxyapatite-based Composites .......110

4.1 Material systems of HAP-based composites and green specimen preparation ...110

4.2 Consolidation of HAP-CNT composites .............................................................114
  4.2.1 Consolidation of pure HAP powder: Conventional sintering ....................114
  4.2.2 Consolidation of pure HAP powder: Spark-Plasma sintering ...................116

4.3 Characterization of the consolidated HAP-CNT composites: Results ............119
  4.3.1 HAP and HAP-CNT specimens processed by conventional sintering......119
  4.3.2 HAP and HAP-CNT specimens processed by SPS: density and microstructure 125
4.3.2.1 Processed pure HAP specimens ........................................................... 125
4.3.2.2 Processed calcinated HAP specimens ............................................... 133
4.3.2.3 Processed HAP-CNT specimens based on acid-treated powders ..... 136
4.3.2.4 Processed HAP-CNT specimens based on ultrasonicated and freeze-dried powders 137
4.3.3 HAP and HAP-CNT specimens processed by SPS: Tensile rupture testing (TRS) ........................................................................................................ 145
4.3.4 HAP and HAP-CNT specimens processed by SPS: Vickers macro hardness tests ........................................................................................................ 147
4.3.5 HAP and HAP-CNT specimens processed by SPS: Nanoindentation tests 149
4.3.6 Compression tests on HAP and CNT-HAP composites produced by FPSPS 152

4.4 Comparison of HAP specimens sintered by conventional sintering and by Free Pressureless Spark Plasma Sintering (FPSPS) ........................................................ 156
4.4.1 Experimental process ........................................................................... 156
4.4.2 Measurement of relative density ............................................................. 158
4.4.3 Mechanical tests .................................................................................. 161
4.4.3.1 Micro-indentation ............................................................................ 162
4.4.3.2 Compression test ............................................................................ 166

4.5 Fabrication of bio-inspired HAP-CNT composites .................................... 170
4.5.1 Functionally graded HAP-CNT composites .......................................... 170
4.5.2 HAP-based composites with special microstructure topology ............. 175
4.5.2.1 Concept of freeze drying process and experimental setup ............... 177
4.5.2.2 Micro-channel specimen sintering at 1000°C .................................. 181
4.5.2.3 Micro-channel specimen sintering at 1300°C .................................. 189
4.5.2.3.1 Mechanical tests ................................................................. 190
4.5.2.3.2 Compression tests in different directions ................................. 201
4.5.3 Fabrication of a complex-shape dental implant prototype ................... 202
4.5.3.3 Fabrication of a complex-shape dental implant prototype: Conventional SPS 205
4.5.3.4 Fabrication of a complex-shape dental implant prototype: Free pressureless spark plasma sintering (FPSPS) ................................................................. 206

Chapter 5 Conclusions ................................................................................ 211
References .................................................................................................. 215
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Illustration of the human dentin structure showing the microstructure of enamel and dentin</td>
</tr>
<tr>
<td>1.2(a)</td>
<td>The SEM image of demineralized dentin showing the collagen fibers; (b) The AFM image of collagen fiber illustrates the typical 67-nm periodicity</td>
</tr>
<tr>
<td>1.3(a)</td>
<td>The SEM image fracture of DEJ; (b) The AFM image in DEJ showing hydroxyapatite crystals</td>
</tr>
<tr>
<td>1.4</td>
<td>Vickers hardness and toughness measurement profiles across the DEJ from enamel to dentin</td>
</tr>
<tr>
<td>1.5(a,b)</td>
<td>SEM images of arrested cracks showing the uncracked ligament bridging</td>
</tr>
<tr>
<td>1.6</td>
<td>Fracture toughness (R-curve) for young and aged dentin.</td>
</tr>
<tr>
<td>1.7(a)</td>
<td>SEM images of (a) Young dentin tubule; (b) Aged dentin tubule</td>
</tr>
<tr>
<td>1.8(a)</td>
<td>SEM images of Crack path in (a) aged dentin (b) young dentin</td>
</tr>
<tr>
<td>1.9</td>
<td>XRD pattern of hydroxyapatite by SPS at temperatures of 900°C, 950°C, 1000°C, 1100°C</td>
</tr>
<tr>
<td>1.10</td>
<td>SEM image of polished surface showing the crack deflection and crack bridging mechanism in a Ti$_3$SiC$_2$-HAP composite</td>
</tr>
<tr>
<td>2.1</td>
<td>Flow chart of the research work</td>
</tr>
<tr>
<td>3.1</td>
<td>Microhardness value of various kinds of animal dentin and enamel</td>
</tr>
<tr>
<td>3.2(a)</td>
<td>SEM images of cross section of (a) shark tooth; (b,c) Mako shark showing the functionally graded structure between dentin and enamel</td>
</tr>
<tr>
<td>3.3(a)</td>
<td>Microhardness values of human dentin; (b) SEM of indentation site</td>
</tr>
<tr>
<td>3.4(a)</td>
<td>Tubule structure in outer region showing higher tubule density; (b) tubule structure in inner region showing lower tubule density</td>
</tr>
<tr>
<td>3.5(a)</td>
<td>Optical micrograph of cross section of piranha tooth; (b) Reduced modulus map; (c) nano-hardness of cross section in the piranha tooth; (d), (e) higher resolution reduced modulus and nano-hardness at DEJ region</td>
</tr>
<tr>
<td>3.6(a)</td>
<td>Optical micrograph of cross section of great white shark tooth; (b) Reduced modulus map; (c) nano-hardness of cross section in the great white shark tooth; (d), (e) higher resolution reduced modulus and nano-hardness at DEJ region</td>
</tr>
<tr>
<td>3.7</td>
<td>Comparison of load –displacement curves of shark enameloid and dentin tested in dry and hydrated conditions</td>
</tr>
<tr>
<td>3.8</td>
<td>SEM image of a cross-section of a great white shark tooth and element line mapping plot</td>
</tr>
</tbody>
</table>
Figure 3.9 SEM image of a cross-section of a human tooth and element line mapping plot. ................................................................. 57
Figure 3.10 (a) The stress-strain curve for dry shark dentin; (b) the stress-strain curve for wet shark dentin. ................................................................. 59
Figure 3.11 (a) Weibull modulus distribution of dry shark dentin under compression test; (b) Weibull modulus distribution of wet shark dentin under compression test. .......... 59
Figure 3.12 The stress-strain curve of compression test in human dentin in (a) longitudinal direction and; (b) transverse direction. ................................................................. 62
Figure 3.13 The stress-strain curve of deproteinated dentin ................................................................. 62
Figure 3.14 SEM images of fracture surface of human dentin in longitudinal direction . 63
Figure 3.15 SEM images of fracture surface of human dentin in transverse direction. ... 63
Figure 3.16 SEM images of top view of the demineralized dentin ................................................... 64
Figure 3.17 SEM images of side view of the demineralized dentin tubule channel ....... 65
Figure 3.18 (a-d) SEM images of deproteinated human dentin ................................................................. 66
Figure 3.19 SEM images of (a) tip of the piranha tooth; (b) the edge of the serration edge. ........................................................................................................... 68
Figure 3.20 (a,b) SEM images of serrated edge on the piranha tooth showing the serration size of 15 µm. ........................................................................................................... 68
Figure 3.21 The schematic drawing of the guillotine-like biting mechanism of the piranha. ........................................................................................................... 69
Figure 3.22 (a) A photograph of the macro structure of great white shark tooth; (b-d) SEM images of microstructure on the edge of the great white shark tooth showing the serrated edge with the serration dimension of 250 µm. ........................................... 70
Figure 3.23 Hierarchical structure of Arapaima Gigas scale. Arapaima scales are composed of plywood structure with collagen fibers. ................................................................. 72
Figure 3.24 (a)Instron testing machine; (b) fixture for tensile testing. ................................................... 74
Figure 3.25 Typical stress-strain curves for dry conditions. ................................................................. 79
Figure 3.26 Typical tensile stress-strain curves for wet conditions. ................................................... 79
Figure 3.27 Tensile stress-strain curves for scale as a function of strain rate varying form $10^{-4}$ to $10^{-2}$ sec$^{-1}$ ........................................................................................................ 80
Figure 3.28 Elastic modulus of different biological materials plotted as a function of strain rate ........................................................................................................ 83
Figure 3.29 (a) Elastic modulus and (b) ultimate tensile stress of Arapaima scales plotted as a function of strain rate ........................................................................................................ 84
Figure 3.30 (a) Photograph of Arapaima scale showing the arrangement of the scale; (b)
Optical micrograph showing the exposed part and embedded part with ridges structure..................................................................................................................... 86

Figure 3.31 SEM micrographs showing the surface of scale: (a) junction of embedded and exposed area; (b) exposed part; (c) embedded part with ridge structure .............. 88

Figure 3.32 Optical micrograph showing the different orientation of collagen fibers in different layers. ............................................................................................................... 89

Figure 3.33 SEM micrographs of cross-section of scale (a) showing the laminate structure with thickness of 50 µm (b) showing the collagen fiber orient in the same direction in the same layer............................................................................................................ 90

Figure 3.34 SEM micrographs of fracture surface after tensile test: (a) overview of the fracture surface; (b) the collagen fiber network; (c),(d) twisted collagen fiber after tensile test.................................................................................................................. 92

Figure 3.35 Optical micrograph of cross section of scale showing the external and internal layers......................................................................................................................... 93

Figure 3.36 Plot of hardness values across the cross section of the scale. .................. 94

Figure 3.37 Typical load-unload curves for nanoindentation showing higher penetration depth in internal region than that in external region at max load. ..................... 96

Figure 3.38 The elastic modulus after nanoindentation in external and internal regions. 97

Figure 3.39 The nano hardness value in external and internal layers. ......................... 98

Figure 3.40 (a) BSE image of the cross section showing the site for EDS; (b) EDS results showing the higher calcium concentration in external layer......................................................... 99

Figure 3.41 (a) Element mapping of calcium; (b) element mapping of phosphate. ...... 99

Figure 3.42 (a) SEM image of cross section of scale; (b) plot of X-ray intensity vs. relative position.................................................................................................................... 100

Figure 3.43 XRD pattern of (a) internal layer of the scale and (b) external layer of the scale compared to hydroxyapatite peaks (JCPDS file 00-001-1008) ...................... 102

Figure 3.44 Infrared spectrum of Arapaima scale showing amide I, II, and III bands from type I collagen fibers.......................................................... 103

Figure 3.45 The force-displacement curve of penetration test on embedded region of scale showing the force required to penetrate the scale is about 30 N. ...................... 105

Figure 3.46 The force-displacement curve of penetration test on exposed part of Arapaima scale showing the shatter of the piranha tooth.......................................................... 106

Figure 3.47 SEM micrographs of demineralized scale showing collagen fiber with typical periodicity.............................................................................................................. 107

Figure 4.1 (a, b) SEM micrograph of hydroxyapatite raw powder showing the particle size
of 0.09 µm. .......................................................................................................................... 111
Figure 4.2 Cold press and Archimedes method device .................................................. 115
Figure 4.3 Photograph of the spark-plasma sintering machine ........................................... 118
Figure 4.4 Schematic drawing of SPS machine showing the graphite die, graphite punches, spacers, and electrode. ........................................................................................................ 119
Figure 4.5 Plot of relative density vs. sintering temperature showing the relative density increase as the maximum sintering temperature increase ........................................ 121
Figure 4.6 Hardness values of HAP and CNT-HAP composite vs sintering temperature. ................................................................................................................................. 122
Figure 4.7 (a-c) SEM micrographs of CNT-HAP composite fracture surface showing pores due to the dissociation of CNT at high temperature; (d-f) SEM micrographs of HAP fracture surface showing a fully dense structure ........................................................................ 123
Figure 4.8 Plot of grain size vs. sintering temperature showing the grain growth when the sintering temperature increases ........................................................................ 125
Figure 4.9 The relative density over time evolution at SPS temperature of 700°C ...... 127
Figure 4.10 The relative density over time evolution at SPS temperature of 800°C ...... 128
Figure 4.11 The microhardness of HAP at different SPS temperature ranged from 700 to 900°C .................................................................................................................................................................................. 129
Figure 4.12 (a, b) SEM micrographs of fracture surface of HAP produced by spark-plasma sintering temperature at 800°C .................................................................................. 130
Figure 4.13 (a, b) SEM micrographs of fracture surface of HAP produced by spark-plasma sintering temperature at 900°C .................................................................................................. 131
Figure 4.14 Plot of grain size vs. SPS temperature showing both CNT-HAP composite and HAP grain growth as the SPS temperature increases. ........................................... 132
Figure 4.15 XRD pattern of HAP powder at different calcination temperature showing that the crystallinity becomes obvious as the calcination temperature increases .......... 134
Figure 4.16 Microhardness vs. different calcination temperature for specimens produced at 900°C by spark plasma sintering ........................................................................................................ 136
Figure 4.17 SEM micrographs of CNT-HAP composite with acid treated CNT showing the agglomeration of CNT ........................................................................................................ 137
Figure 4.18 The hardness value of different vol% CNT-HAP composite vs maximum SPS temperature ................................................................................................................................. 139
Figure 4.19 Microhardness vs. different vol% of CNT in HAP-based composites sintered at 900°C ........................................................................................................................................................................... 140
Figure 4.20 SEM images of an indentation site of 2vol%CNT-HAP composite sintered at
900°C (a) Typical indentation site; (b) higher magnification of indentation site; (c, d) micrographs showing nanotubes between the crack path................................. 141
Figure 4.21 SEM micrograph of fracture surface of 1vol%CNT-HAP composite sintered at 900°C after tensile rupture test (a) low magnification; (b) high magnification showing the CNT embedded in the HAP matrix................................................................. 144
Figure 4.22 XRD pattern of 1vol%CNT-HAP composite showing same peaks as pure HAP ........................................................................................................ 145
Figure 4.23 The drawing of the tensile rupture strength (TRS) testing design showing the indenter and TRS fixture.......................................................... 146
Figure 4.24 Plot of elastic modulus of HAP and CNT-HAP composites sintered at 900°C by nanoindentation......................................................................... 150
Figure 4.25 Plot of nano-hardness value of HAP and CNT-HAP composite sintered at 900°C by nanoindentation................................................................. 151
Figure 4.26 Typical load-unload curve of HAP and CNT-HAP composites obtained from nanoindentation.......................................................... 152
Figure 4.27 Typical stress-strain curves of HAP and 4vol% CNT-HAP composites... 154
Figure 4.28 SEM images of fracture surface of CNT-HAP composite sintered at 1300°C by FPSPS after compression tests: (a) pure HAP; (b) 2 vol%CNT-HAP composite; (c) 6 vol% CNT-HAP composite; (d) higher magnification showing the CNT with the diameter of 50 nm .......................................................... 155
Figure 4.29 The comparison of relative densities of free pressureless SPS and conventional sintering as a function of sintering temperatures .................. 159
Figure 4.30 A plot of sintering temperature and shrinkage curve vs. time............. 161
Figure 4.31 The comparison of micro-hardness of HAP prepared by FPSPS and...... 164
Figure 4.32 Micro-indentation site of specimen prepared by FPSPS......................... 165
Figure 4.33 Micro-indentation site of specimen prepared by conventional sintering .. 165
Figure 4.34 The stress-strain curves of HAP sintered by conventional sintering at..... 167
Figure 4.35 The stress-strain curves of HAP sintered by FPSPS at different sintering 168
Figure 4.36 SEM images of fracture surface after compression test: (a) conventional.. 170
Figure 4.37 (a) the overview of the entire FGM composites(b)pure HAP layer(c)2 vol%CNT-HAP layer(d)4 vol%CNT-HAP layer ......................................................... 174
Figure 4.38 SEM micrographs of cross-sections of the functionally graded structure: (a) showing the layer of pure HAP; (b) the layer of 2 vol% CNT-HAP; (c) the layer of 4 vol% CNT-HAP................................................................. 174
Figure 4.39 The schematics of directional freezing showing that the ice crystals grow.
from the bottom to top and the hydroxyapatite particles are excluded from the ice to form the micro-channel structure

Figure 4.40 The picture of setup for freezing the hydroxyapatite slurry

Figure 4.41 (a),(b) Green specimen; (c),(d) channels in the specimen obtained from 15 vol% initial slurry concentration; (e),(f) channels in the specimen obtained from 20 vol% initial slurry concentration; (g),(h) channels in the specimen obtained from 25 vol% initial slurry concentration

Figure 4.42 (a) Plot of compressive modulus vs. initial vol% slurry concentration for specimens processed by conventional sintering and free pressureless spark plasma sintering at 1000ºC; (b) Plot of compressive strength vs. initial vol% slurry concentration for specimens processed by conventional sintering and free pressureless spark plasma sintering at 1000ºC

Figure 4.43 Comparison of X-ray diffraction pattern of HAP processed by different sintering processes showing that there is no phase change via different processes

Figure 4.44 Plot of relative density and porosity vs. initial vol% slurry showing that higher relative density and lower porosity results from higher initial vol% slurry

Figure 4.45 (a) Plot of compressive strength vs. initial vol% slurry; (b) plot of compressive strength vs. porosity

Figure 4.46 Compression fracture surface for specimens processed from different initial vol% slurries

Figure 4.47 Compression stress-strain curves of the specimens produced from two different initial vol% slurries

Figure 4.48 Plot of relative density and porosity vs. initial vol% slurry of specimen by conventional sintering showing that higher relative density and lower porosity result for the specimens produced from higher initial vol% slurry

Figure 4.49 Compression stress-strain curves for specimens produced from 20 vol% initial slurry by conventional sintering at 1300ºC

Figure 4.50 Stress-strain curve of the compression test with loading direction perpendicular to channels

Figure 4.51 3D image of the prototype of dental implant

Figure 4.52 Design of the net-shape punch with tooth-like geometry

Figure 4.53 The sketch of FPSPS setup

Figure 4.54 (a-c) SEM micrograph of complex shape dental prototype by FPSPS; (d-f) SME micrograph of complex shape dental prototype by SPS
LIST OF TABLES

Table 1.1 The comparison of young and aged dentin ....................................................... 15
Table 3.1 The micro-hardness value of enamel and dentin from different animals .......... 44
Table 3.2 Serration size of different animals .................................................................... 67
Table 3.3 The comparison of parameters in all the other biological materials including rat-tail tendon, bovine cortical bone, horn, human femur bone, arapaima scale. ..... 83
Table 3.4 The comparison of energy absorption in dry and wet scale.............................. 85
Table 3.5 Summary of nanohardness and elastic modulus in external and internal layers 98
Table 4.1 Temperature profile of HAP powder processing by spark-plasma sintering .. 126
Table 4.2 Vickers hardness values of HAP and CNT-HAP composites......................... 148
Table 4.3 Mechanical properties of CNT-HAP composite and HAP specimens sintered at 900° C measured by nanoindentation. ................................................................. 150
ACKNOWLEDGEMENTS

I would like to acknowledge Professor Eugene A. Olevsky and Marc A. Meyers for their invaluable guidance and support throughout my graduate study. I also deeply appreciate my committee members, Professor Sungho Jin, Joanna McKittrick, David J. Benson, and Satchi Venkataraman for their valuable help and advice on my thesis. I would also like to thank all of my lab mates in my group. Without their suggestion and assistance during my graduate study period, I will not be able to finish my PhD degree.

Chapter 3, in part, is a reprint of the material as it appears in “Mechanical properties and the laminate structure of Arapaima gigas scales”, in Journal of Mechanical Behavior and Biomedical Materials, 2011, v.4, p1145-1156, Lin, Yen-Shan; Olevsky, Eugene A.; and Meyers, Marc A. The dissertation author was the primary investigator and author of this paper.

Chapter 4, in part, has been submitted for publication of the material as it may appear in “Micro-channel hydroxyapatite components by sequential freeze drying and free pressureless spark plasma sintering”, in Advanced in Applied Ceramics, 2011, Lin, Yen-Shan; Meyers, Marc A.; and Olevsky, Eugene A. The dissertation author was the primary investigator and author of this paper.

This research is financial support by National Science Foundation, Division of
Material Research, Biomaterials (DMR 0510138).
VITA

2003    Bachelor in Physics, National Tsing Hua University, Hsinchu, Taiwan
2006    Master in Physics, National Tsing Hua University, Hsinchu, Taiwan
2012    Doctor of Philosophy in Engineering Science (Mechanical and Aerospace engineering), University of California, San Diego and San Diego State University

Field OF STUDY

Major: Mechanical Engineering
LIST OF PUBLICATIONS


Y. S. Lin, M. A. Meyers, and E. A. Olevsky. “Micro-channeled hydroxyapatite components by sequential freeze drying and free pressureless spark-plasma sintering”, Advance in Applied Ceramics. Accepted


ABSTRACT OF THE DISSERTATION

BIO-INSPIRED TAILORED HYDROXYAPATITE-BASED POWDER COMPOSITES FOR DENTAL APPLICATIONS

By

Yen-Shan Lin

Doctor of Philosophy in Engineering Sciences (Mechanical and Aerospace Engineering)

University of California, San Diego, 2012
San Diego State University, 2012

Professor Eugene A. Olevsky, Chair
Professor Marc A. Meyers, Co-Chair

This study includes two interconnected parts. The first part describes the characterization of the natural materials including the teeth of predator and preys’ aspects and Arapaima gigas scale. Based on the first part, several special features presented in the natural materials were investigated. In the second part, the special procedures of the fabrication of tailored hydroxyapatite (HAP) based composites by the novel consolidation technique of spark plasma sintering are developed. These procedures are bio-inspired by
the findings of the first part of the study.

A natural tooth is composed of the external layer, hard enamel which has high degree of mineralization and the internal region - tough dentin which has collagen fibrils and carbonated apatite mineral. The main feature in the dentin is the tubule structure. Arapaima gigas scales serve as armor-like materials to protect Arapaima gigas from being bitten by piranha. The scales have a laminate structure with the hard external layer and soft internal layers. Besides the hydroxyapatite mineral, the main building block of the scale is collagen fibers. The structure of teeth and scale were characterized using scanning electron microscopy and optical microscopy. The chemical compound was analyzed by X-ray diffraction, Fourier transform infrared spectroscopy and Energy-dispersive X-ray spectroscopy. Mechanical tests (microindentation, nanoindentation, tensile and compression tests) were performed to investigate the mechanical properties of those biological materials. The penetration test of piranha teeth on the arapaima scale was conducted to prove that the scale is stronger than the piranha tooth.

In order to fabricate artificial dental materials inspired by the investigated structure of the natural teeth, spark plasma sintering involving high heating rates, shorter sintering
times and low sintering temperatures was utilized to fabricate hydroxyapatite composites. Hydroxyapatite is widely used in biomedical applications, but its low mechanical strength limits its applications in heavy loaded implants. Carbon nanotube (CNT)-HAP composites were fabricated by spark plasma sintering and indicated that the addition of CNT can improve the mechanical strength of pure HAP. Unaxial freeze drying process was utilized to create a micro channel structure in the hydroxyapatite components. To maintain the porous structure of the tailored hydroxyapatite green specimens, a newly developed sintering process, Free Pressureless Spark Plasma Sintering (FPSPS), was used to consolidate the tailored hydroxyapatite without applying pressure. The comparison of the outcome of the treatment of hydroxyapatite components by conventional sintering and free pressureless spark plasma sintering was conducted showing that FPSPS can provide a better approach to consolidate the material with higher micro hardness.
Chapter 1 Introduction

1.1 Literature survey on the mechanical properties and microstructure of natural dental materials

After hundreds of thousands of years of revolution, biological materials evolve continuously to optimize their mechanical properties. It is surprising that many biological materials, produced by nature under ambient temperature and pressure, can achieve higher mechanical properties than those components fabricated from the same materials under specific process conditions, which often involved elevated temperatures and externally applied stresses of a significant magnitude. The reasons that cause the amazing mechanical properties of biological materials are not only the components themselves but also how they are organized in a particular structure. Biological composites comprise organic and inorganic components which are ordered to achieve optimized mechanical properties. For example, bird beak exhibits foam structure to achieve lightweight property for flying. Gecko has large amount of fibers with nanometer size on its foot to provide adhesive force for climbing on the wall due to the van der Waals forces.

Teeth are the most mineralized tissues in a human body, is essential to the biting mechanism. Teeth include an internal region called dentin, which is tougher, an external layer called enamel, which is harder, and the central part, which is a sensitive pulp. The
The interfacial part between the enamel and dentin is the transitional region named dentin-enamel junction - a region where mechanical properties change dramatically. Enamel, which is comprised of approximately 96% mineral, 1% protein and 3% water by weight, is the most highly mineralized tissue of the human body. Enamel acts as a protective enclosure because its high mineral content results in the high hardness value (3-6 GPa), high elastic modulus (70-120 GPa) and overall brittle properties.[1] Dentin is a hydrated composite composed of 30 vol% type I collagen fibrils, 25 vol% fluid, and 45 vol% nanocrystalline carbonate apatite mineral.[1,2] The main feature of dentin is the tubule structure. The dentin serves as an elastic foundation of the hard enamel which can arrest and stop the crack propagation from the enamel to the dentin. The tubules are embedded in the dentin consisting of a collagen matrix reinforced with hydroxyapatite. A cylindrical cuff with high mineral content based on peritubular dentin surrounds each tubule. Between those peritubular dentin-surrounded tubules, there is intertubular dentin which contains collagen fibrils and hydroxyapatite crystals. The diameters of the tubules are about 1um. In contrast to the dentin tubule structure, there are no tubules in enamel. The main components of enamel are hydroxyapatite rods with the size of 5 um which weave one another.
Figure 1.1 includes the structure of a human tooth showing dentin, enamel and pulp. The SEM images of dentin exhibit the main characteristic tubule structure. [1]
Figure 1.2 [2] is the SEM image of demineralized dentin showing that the collagen fibrils randomly orientate on the plane perpendicular to the tubule.

![Image](a)

Figure 1.2 (a) The SEM image of demineralized dentin showing the collagen fibers; (b) The AFM image of collagen fiber illustrates the typical 67-nm periodicity. [3]

Figure 1.2 (b) is the AFM image of dentin specimen showing the characteristic type I collagen – the periodic 67-nm hole and overlap zone.[3] The human enamel exhibits the rise of toughness from $0.67 \text{ MPa} \sqrt{\text{m}}$ in outer enamel to $3.39 \text{ MPa} \sqrt{\text{m}}$ in inner enamel. The toughening mechanisms such as crack bridging, crack deflection and microcracking result in the rise of crack growth resistance.[4]

The dentin-enamel junction, which unites two dissimilar calcified tissues, the hard enamel and tough dentin, can be considered a functionally graded zone. Because of the
mismatch of elastic modulus in dentin-enamel junction, the crack can be arrested to avoid the failure of the entire tooth.\[5\] Several mechanical properties of dentin-enamel junction (DEJ) have been reported.\[6,7\] The DEJ exhibits a scallop shape with a size of 42 µm in molar (Figure 1.3 (a)). The AFM image shows the apatite crystal structure. The thickness of the DEJ is about 10 µm. \[8,9\]

![Figure 1.3](image)

(a) The SEM image fracture of DEJ; (b) The AFM image in DEJ showing hydroxyapatite crystals.\[9\]

Imbeni et al \[14\] measured Vickers hardness and indentation toughness and found a significant difference in the hardness value between enamel and dentin. Figure 1.4 shows that the hardness value of enamel, which is about 4 GPa, is much higher than the dentin hardness, which is 0.5 GPa. The SEM image (Figure 1.5) shows that the crack propagates less than 10 µm after passing the dentin-enamel junction. Several uncracked ligaments can be observed behind the crack tip, which is an indication of a toughening
mechanism in dentin.

Figure 1.4 Vickers hardness and toughness measurement profiles across the DEJ from enamel to dentin [6]

Figure 1.5 (a,b) SEM images of arrested cracks showing the uncracked ligament bridging [6]

Many mechanical properties of human dentin have been investigated such as compression strength, ultimate tensile strength, and flexure strength. Those mechanical properties can be affected by many factors including hydration in dentin, [10,11] age of dentin, tubule density, and tubule orientation.
1.1.1 Tubule orientation

The main feature of the dentin structure, the tubule structure, has a significant influence on the strength of human dentin. The mechanical properties of dentin vary a lot due to the anisotropic structure of dentin depending on the tubule orientation. Several papers [12,13,14,15,16,17] have discussed how the tubules’ orientation influences the mechanical properties including fatigue, fracture toughness, and ultimate tensile strength. The network of the tubules starts from the central pulp and propagates to the dentin-enamel junction in a radiate geometry causing an anisotropic structure, which affects the mechanical strength. Because tubules do not take a straight path in dentin, it is very difficult to accurately define the orientation of a tubule before testing. However, most results indicate that the mechanical strength is higher in the loading direction perpendicular to the tubule axis than in the one parallel to the tubule axis. Reprogel et al.[12] conducted the quasi-static four point flexure and flexural fatigue test and found that the dentin with tubule parallel to the loading direction exhibits higher flexural strength than the dentin with tubule perpendicular to the loading direction. Their results show that the flexural strength of the structure with the parallel orientation is 160 MPa which is higher than that of the structure with the perpendicular orientation. The fracture energy of the structure with the parallel orientation is 1.9 MPa, which is more than three
times higher than that of the structure with the perpendicular orientation, 0.5 MPa. The fatigue strengths in the parallel and perpendicular orientations under $10^7$ cycles are 44 MPa and 24 MPa, respectively. Several other mechanical strength investigations also identified the significance of the tubule orientation. Carvalho et al. [13] measured the ultimate tensile strength (UTS) of the parallel and perpendicular orientation and the results showed that the tensile loading direction parallel to the tubule and the loading direction perpendicular to the tubule render UTS of 57.6 MPa and 80 MPa, respectively. Lertchirakarn et al. [14] conducted also the ultimate tensile strength measurement on root dentin for tensile loading parallel and perpendicular to the tubule and showed the strength values of 42.0 MPa and 52.9 MPa, respectively. According to all of the results indicating that the tensile loading direction perpendicular to the tubule axis exhibits higher ultimate tensile strength (UTS), it is believed that the collagen fibrils oriented on the plane perpendicular to the tubule play an important role in the mechanical strength of dentin. In the tensile direction perpendicular to the tubule (parallel to the collagen fibrils orientation plane), we need to apply more stress to pull and tear the collagen fibrils. On the other hand, in the tensile direction parallel to the tubule (perpendicular to the collagen fibrils orientation plane), smaller stress is needed to fracture the dentin.
The effect of the tubule orientation has been studied not only on human dentin but also on an elephant tusk. The elephant tusks have almost the same tubule microstructure as human dentin and the only difference is that the tubules exhibit elliptical shape and the peritubular cuff is much smaller than tubules in human dentin. Nalla et al. [15] used the compact tensile method to measure the fracture toughness of the tusks and the results showed that the average fracture toughness values in notch directions perpendicular and parallel to the tubule axis were 1.6 MPa\sqrt{m} and 2.6 MPa\sqrt{m}, respectively.

1.1.2 Tubule density

The non-uniform distribution of the tubules in the dentin results in the variation of the density of tubules per unit area in different positions in the dentin. The reason of the non-uniform distribution of the tubule is related to the radial path of the tubule. Indeed, the tubule density is higher in the deep regions than in the superficial regions which are close to the dentin–enamel junction. Besides the fact that the tubule density varies with different depths in the dentin, it also decreases with the increasing distance from the pulp at the same depth. Garberoglio et al. [18] reported that the tubule density in the region close to the pulp, in the middle region and in the peripheral region are 45000/mm, 29500/mm, and 20000/mm, respectively.
Since the tubules cannot provide any strength due to their hollow channels, some mechanical properties such as hardness and elastic modulus of dentin are functions of tubule density which varies for different locations in the dentin. [19, 20] Angker et al. [19] measured the micro-hardness value and elastic modulus of the dentin extracted from different regions of dentin and reported that both micro-hardness value and elastic modulus increased with the distance decrease from the dentin-enamel junction. The average micro-hardness values of regions located within 300 μm from the dentin-enamel junction and at 500 μm from the pulp are 0.91 GPa and 0.52 GPa, respectively. The elastic modulus of the regions located within 300 μm from the dentin-enamel junction and at 500 μm to pulp are 16.91 GPa and 11.59 GPa, respectively. The SEM images of the microstructure of dentin cross-section indicate that both the diameter and density of tubules are smaller in the region close to dentin-enamel junction than in the deep dentin region.

Marcelo et al. [20] measured the ultimate tensile strength of dentin with different depth. The ultimate tensile strength values in superficial, middle, and deep dentin were 60.6 MPa, 48.7 MPa, and 33.9 MPa, respectively. All of the results prove that the tubule density is an essential factor to determine the mechanical strength of dentin.
1.1.3 Effects of age on the mechanical behavior of human dentin

The age of human dentin is another factor that can significantly change the mechanical strength of dentin. As age increases, the dentin tubule can become completely occluded because of the filling of carbonated apatite. Toto et al. [22] found that young teeth (10-20 years) contain more water than old teeth (over 50 years). Dehydration causes the reduction of toughness of dentin. Reprogel et al. [23] conducted an experimental study on the influence of age on the mechanical behavior of human dentin. Young (17-30 years) and old (50-80 years) dentin were chosen to be subjected to quasi-static four-point flexure and four-point fatigue test. The SEM images (Figure 1.7) of both the young and old dentin tubule show that the big difference is in the thickness of the peritubular cuff. The young dentin tubule with a thin peritubular cuff exhibits a large lumen diameter. On the contrary, the old dentin tubule with much thicker peritubular dentin cuff has a very small lumen diameter. The results showed that the fatigue strength of young dentin was higher than that of the old dentin at all cyclic stress. For example, at $10^7$ cycles, the fatigue strength of young dentin is 44 MPa and that of the old dentin is 23 MPa. The different microstructure of young and old tubule influences the crack propagation.

The fracture toughness of human dentin has been studied and showed that it ranges from 1 to $2 \text{ MPa } \sqrt{m}$. Koester et al. [25] conducted an experiment to investigate
how the age influences the fracture toughness. They use a fraction of a filled tubule which represents the main difference between young and old dentin as a controlling parameter of fracture toughness. The number fractions of the filled tubule for young and aged dentin are 0.04 and 0.2-0.87, respectively. Three point bending test method was used to determine the fracture toughness. The fracture toughness R-cure (Figure 1.6) below shows that both the young and aged dentin cracks initiate at $1 \text{ MPa}\sqrt{m}$, however; after the extension of 100 $\mu$m the fracture toughness difference increases. Crack growth toughness, defined as the slope of the resistance curve decreases with the increase of the fraction of the filled tubule. Arola et al. [26] reported that the fracture toughness decreases as the dentin age increases. And they also concluded that the microstructure dominates the toughening mechanism. For instance, the microcracking of tubules and inelastic deformation of collagen matrix are the main toughening mechanisms in young dentin. On the other hand, these microstructures reduction with aging results in the deterioration of the fracture toughness in aged dentin.
The crack propagation mechanism is also different in young and aged dentin. Since the major difference between young dentin and aged dentin is the number fraction of filled and unfilled tubules, the crack propagation mechanism is also different as related to the filled and unfilled tubules. Both the microcracking and microbranching are usually initiated by an unfilled tubule which means that the microcracking and microbranching dominate in young dentin due to its high fraction of unfilled tubule. However, these two
kinds of fracture barely can be seen in the aged dentin because there are fewer unfilled
tubules in the aged dentin compared with the young dentin.

From the environmental SEM images (Figure 1.8) of the crack propagation, the
 crack propagates via different routes in the young and the aged dentin due to the unfilled
and filled tubules. The thickness of the peritubular dentin cuff becomes larger due to the
deposition of carbonate apatite. In young dentin, when a crack encounters an unfilled
tubule with a thin peritubular dentin cuff, the crack will penetrate directly in the tubule
which is a prevalent situation in young dentin. But in aged dentin, when a crack interacts
with a filled tubule with a much thicker and high-mineralized dentin cuff, the crack will
propagate around the interface instead of through the filled tubule. The main reason is the
different mechanical properties between the thicker peritubular dentin and the dentin
matrix.
Figure 1.8 SEM images of Crack path in (a) aged dentin (b) young dentin. The following table1.1 is a summary of the significant differences between young and aged dentin. [26]

<table>
<thead>
<tr>
<th>Unfilled tubules</th>
<th>Filled tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>More microcracking and microbranching tubules</td>
<td>Fewer microcracks and microbranching tubules due to few unfilled tubules</td>
</tr>
<tr>
<td>Straight crack deflection</td>
<td>Less straight crack path</td>
</tr>
<tr>
<td>Crack bridge forms between tubules</td>
<td>Crack bridge is formed by a filled tubule itself</td>
</tr>
</tbody>
</table>

1.2 Literature survey on the processing of HAP-based bio-composites

Hydroxyapatite (HAP, Ca_{10}(PO_4)_6(OH)_2) is the main mineral component contained in bones and teeth. Because its chemical and biological properties are similar to natural mineral in bones and teeth, it is widely used for medical applications including
amputated bone replacements, and coatings on prosthetic implants to improve the bone growth. [27,28]

Hydroxyapatite powder has melting point of 1680°C and Ca/P ratio of 1.67 which is similar to Ca/P ratio of natural bone. Hydroxyapatite is a hydrated phase which decomposes to tricalcium phosphates, TCP at about 1200-1450°C. [29, 30] Hydroxyapatite structure is retained if the dehydroxylation occurs below the critical point of 1300°C. But irreversible dehydroxylation above the critical point results in the decomposition of hydroxyapatite into TCP. The transformation of beta-TCP to alpha-TCP results in the decreasing of the mechanical properties due to the molecular volume increase.

Hydroxyapatite is a promising material for biomaterial applications due to its similar structure to bone showing great biocompatibility with human body. In addition, hydroxyapatite also has osseoconductive properties which are favorable for osseointegration with a host bone. However, its applications are limited due to its low mechanical strength. Therefore, pure hydroxyapatite cannot be used for heavy-loaded implants.
1.2.1 Processing of pure hydroxyapatite (HAP)

Traditionally, tough and strong ceramics are produced by sintering processes. There are many kinds of sintering processes for example, conventional sintering [31,32], microwave sintering [33,34,35,36,37], and an emerging high speed powder consolidation technique of spark-plasma sintering (SPS), which is also known as field assisted sintering (FAST) or pulse electric current sintering (PECS). Spark plasma sintering is applying a DC current to a processed material directly through the powder specimen and/or through the graphite die. Spark plasma sintering has many advantages over conventional sintering such as high heating rate, high sintering temperature, short period of sintering time. Thereby spark plasma sintering offers a promising alternative to produce novel materials with unique properties.

Hydroxyapatite can be sintered up to full density at a certain temperature range (1000-1200°C) by conventional or specialized sintering techniques. However the initial characteristics of the powder, like particle size, particle distribution and morphology play an essential role in the final sintering result. Therefore the powder processing before sintering is very important. The wide particle size distribution and irregular particle shape result in non-uniform microstructures, which may seriously affect the final component’s mechanical properties. [38,39,40,41]
Powders preparation methods before sintering can improve and control the initial powder condition. Those include calcinations increasing the crystallinity, as well as rehydration and mechanical milling decreasing particle size. Hon et al. [42] studied how the calcination temperature affects the sintering of hydroxyapatite. Calcination can not only increase the crystallinity, but it can also shift the particle size distribution. Calcination above 800°C delays the initiation of sintering. Among all the calcination temperatures under 1000°C, the highest bending strength was obtained at sintering temperature of 1250°C.

The main problems in the conventional sintering of hydroxyapatite are extreme grain growth and thermal decomposition above the critical temperature. Spark plasma sintering with high heating rate, which can prevent the extreme grain growth, provides a good approach to consolidate hydroxyapatite. Dense hydroxyapatite compacts have been fabricated by spark plasma sintering. Boey et al. [43] report that the initial particle size and holding time influence the final relative density. The relative density increases with the increase of the holding time and the decrease of particle size. The initial powder with larger particle size needs longer holding time to achieve the full density compared to powders with small particle sizes. Under the same SPS conditions, the smaller initial
particle size enables the higher final relative density. The experimental results [45] also show that it only takes 20 minutes to obtain the highest relative density at the sintering temperature of 950°C which is near the theoretical density for un-calcinated powders. However, for conventional sintering, it takes more than 3 hours to reach the same relative density at sintering temperatures of 1100-1300°C [38]. Thus spark plasma sintering does provide an approach to consolidate the hydroxylapatite to 99.9% relative density at lower temperatures and shorter sintering duration.

Different spark plasma sintering parameters including sintering temperature and sintering pressure can influence the final physical properties of hydroxylapatite sintered specimen such as relative density, grain size and mechanical properties. Okano et al. [44] found that the relative density increased from 92.5% at 800°C to 98.9% at 1000°C under the sintering pressure of 22.3 MPa. Under the same sintering temperature of 800°C, the relative density increases from 92.5% to 94.0% and 98.9% with the sintering pressure increase from 22.3 MPa to 44.6 MPa and 66.9 MPa, respectively. The results also show that the influence of the consolidation pressure becomes smaller when SPS is conducted at higher temperatures. The grain size increases with the increase of the applied pressure at the same sintering temperature. The difference becomes larger as the sintering
temperature increases. The mechanical properties of spark plasma sintered hydroxyapatite are not always enhanced with the increase of the sintering temperature. Gu et al. [45] and Okano et al [44] both found that several mechanical properties of sintering hydroxyapatite achieve their maximum value at the sintering temperature of 950°C. According to Okano et al. [44] results, the mechanical properties of hydroxyapatite can achieve their maximum level at a critical sintering temperature under a certain sintering pressure. The flexural strength and Young’s modulus of hydroxyapatite under sintering pressure of 44.6 MPa can achieve their maximum value at 950°C which is 131.5 MPa and 75.6, respectively. Both flexural strength and Young’s modulus drop at higher temperature of 1000°C. However, under sintering pressure of 66.9 MPa, the maximum value occurs at 850°C rather than at 950°C, indicating that the temperature needed to achieve the maximum strength value decreased as the applied pressure increases. Gu et al. [45] measurements of relative density and mechanical properties of hydroxyapatite including Knoop hardness, Young’s modulus and fracture toughness under different sintering temperatures also show that all the maximum values are obtained at sintering temperature of 950°C. The tricalcium phosphate peaks which appear in the XRD (Figure 1.9) at sintering temperature of 1000 and 1100°C prove that the
relative density decrease slightly above 950°C is due to the decomposition of hydroxyapatite into tricalcium phosphate. The tricalcium phosphate has density of 3.0g/cm³ which is lower than that of hydroxyapatite.

Figure 1.9 XRD pattern of hydroxyapatite by SPS at temperatures of 900°C, 950°C, 1000°C, 1100°C. [45]

A considerable level of transparency has been found in sintered hydroxyapatite ceramics. Roy et al. [46] fabricated the transparent hydroxyapatite ceramics by both conventional and microwave sintering at ambient pressure. They concluded that density, purity and grain size are the main factors that can influence the transparency of ceramics. Grain boundaries in ceramics are the main sources of scattering light resulting in opaque properties. Therefore a powder with high purity, with smaller and uniform particle sizes
can achieve transparency. With different powder preparation, the processed materials can be both transparent and opaque. Hydrothermally synthesized hydroxyapatite powder exhibits uniform grain size, high purity and good crystallinity, therefore it can achieve transparency. Besides conventional sintering and microwave sintering, Moriyoshi et al. [47] fabricated transparent hydroxyapatite with high crystal orientation and relative density of 99.7% by spark plasma sintering. In order to increase crystallinity, hydroxyapatite powder was calcinated at 800°C for 3 hours. The sample was sintered at 1200°C maximum temperature with the heating rate of 50°C/min under 50 MPa of uniaxial pressure in vacuum. The transmission spectrum of the hydroxyapatite specimen with 1mm thickness exhibited over 70% above the wavelength of 700 nm. Okano et al. [44] concluded that the transparency of sintered specimen gradually improved with the increasing sintering temperature.

Since the hydroxyapatite is widely used as a hard bio-tissue replacement in implants, and in orthopedic applications, its biocompatibility and the improvement of the processed hydroxyapatite mechanical properties become main issues of concern.

1.2.2 Processing of HAP-based composites

Although hydroxyapatite has excellent biocompatibility, its poor mechanical
properties limit its utilization in independently loaded implants. Therefore, the improvement of the mechanical properties of hydroxyapatite is of paramount importance for its reliable applications. One of the solutions to enhance the mechanical properties of HAP is to employ reinforced HAP-based composites comprising a second phase such as a metal or ceramic powder and/or polymers. Several hydroxyapatite-based composites with second phase reinforcement including Ti$_3$SiC$_2$, carbon nanotube, and zirconia have been fabricated and indicated the enhanced mechanical strength. [48-60]

Ti$_3$SiC$_2$, an interesting material, has been chosen to be incorporated into an HAP matrix because it exhibits both ceramic and metallic properties. Ti$_3$SiC$_2$ has excellent electric and thermal conductivity, low Vickers hardness (~4 GPa), high fracture toughness (K1c=6-11 MPa M$^{1/2}$). Therefore adding Ti$_3$SiC$_2$ in HAP matrix can make the resulting HAP based composite more easily to machine to the different shape and size needed for implants compared with pure HAP. [48,49] Furthermore, Ti$_3$SiC$_2$ has good biocompatibility and high corrosion resistance in NaCl solutions, which make it a very good candidate for biomaterial applications. Most mechanical properties of the Ti$_3$SiC$_2$/HAP composite enhance with the increase of Ti$_3$SiC$_2$ content except Vickers hardness. Fang et al. [50] reported that the bending strength of the Ti$_3$SiC$_2$ composite
with 50vol% addition of Ti₃SiC₂ is 252 MPa which is higher than that of pure HAP (85 MPa). Fracture toughness can also be improved by 50vol% addition of T₃iSiC₂ up to 3.9 MPa m¹⁄², which is about five times higher than that of the pure HAP. The fracture surface SEM image (Figure 1.10) also shows the presence of crack deflection and crack bridging occurring in the T₃iSiC₂/HAP composite, which is the main fracture toughness-enhancing mechanism. Because the T₃iSiC₂ has lower Vickers hardness than pure HAP its addition results in the decrease of the Vickers hardness with the increase of the T₃iSiC₂ content. However, the elastic modulus increases with the addition of T₃iSiC₂ due to the higher elastic modulus of Ti₃SiC₂ (320 GPa) than that of HAP (102 GPa).

Figure 1.10 SEM image of polished surface showing the crack deflection and crack bridging mechanism in a Ti₃SiC2-HAP composite.
Yttria stabilized zirconia is another material which was used to create HAP-based composites to improve the mechanical properties of pure hydroxyapatite. The zirconia-hydroxyapatite composites were fabricated by spark-plasma sintering.[51,52] Miao et al. [53] successfully fabricated an HAP-YSZ composite for potential medical applications via spark plasma sintering at 1200°C and obtained the material with higher microhardness (9 GPa), Young’s modulus (160 GPa) and bending strength (200 GPa) than those of pure HAP. Besides adding another phase to improve the mechanical properties of hydroxyapatite, manipulating the structure of the composite can also achieve better mechanical properties. Miao et al [54] successfully fabricated the functionally graded hydroxyapatite /yttria stabilized tetragonal zirconia composite by spark plasma sintering. Both the bending strength and Knoop hardness exhibited higher values than those of pure hydroxyapatite. The Young’s modulus and Knoop hardness also increased with the increase of Yttria stabilized zirconia content.

Carbon nanotubes (CNTs) is one of the most attractive materials to reinforce the brittle hydroxyapatite ceramics due to its remarkable mechanical properties including exceptional tensile strength, high stiffness, and significant resistance to bending. Several papers have been published about CNT-reinforced HAP composites fabricated by
conventional sintering [55] and spark plasma sintering. [56] Zhao et al. [57] conducted the sintering of a MWCNTs-HAP composite in different atmospheres including air, Ar and vacuum. Both the bending strength and fracture toughness of the CNT-HAP composite sintered in Ar and vacuum were higher than those of pure HAP sintered in air at 1100°C. Furthermore, the CNT-HAP composite sintered in Ar exhibited loose interface junction between CNT and HAP grain and a large number of pores resulting in lower mechanical strength compared with the CNT-HAP composite sintered in vacuum.

The fabrication of CNT-reinforced HAP composites through spark plasma sintering has been conducted also by other authors. [58,60] One of the biggest problems is to achieve a uniform dispersion of carbon nanotubes in HAP powder. Carbon nanotubes with excellent mechanical strength can reinforce the ceramic matrix composites; however, the agglomeration of the carbon nanotubes due to their high surface area and high aspect ratios can cause stress concentrations and defects resulting in weakening of the processed composites. Chen et al. [58] synthesized 2vol% of CNT reinforced HAP by using SPS and reported that the hardness (6.86 GPa) and elastic modulus (131 GPa) values measured by nanoindentation are higher than those obtained in pure HAP by Gau et al. [45]
Zhao et al. [57] and Chen et al. [58] used the mechanical dispersion in ethanol to mix the CNT and HAP powders. However, sodium dodecylsulphate (SDS) surfactant can be used as a potentially better dispersant of CNTs. Song et al. [59] used SDS solution with ultrasonic agitation to disperse CNT and Al₂O₃. Lee et al. [60] used SDS as the dispersant not only in dispersing pure CNT prior to the mixing but also during ball milling-based mixing. They reported that the fracture toughness of the CNT-HAP composite (1.27 MPa m¹/²) at 1100°C is higher than that of the monolithic HAP (0.98 MPa m¹/²) prepared at the same temperature. The evolution of the mechanical properties of the carbon nanotube-reinforced HAP composites with different concentrations of carbon nanotubes have not been investigated and remains an important opened area of research.

1.2.3 Biocompatibility of HAP

The bioactivity of sintered hydroxyapatite is very essential to its application for hard bio-tissue replacements. Hydroxyapatite-coated implants have shown better osteoconductivity. However, the weakness between the interface of the coated hydroxyapatite and the substrate causes long term failure of the hydroxyapatite-coated implants. Therefore the consolidation of hydroxyapatite bulk implants and their
bioactivity become very important. Besides the advantages of the short sintering time and low sintering temperatures, Gu et al.[61] Cheang et al.[62], Nakahira et al.[63] Shen et al. [64] found also that spark plasma sintering can fabricate more bioactive hydroxyapatite samples than those made by conventional hot-pressing. After immersion in the simulated body fluid, there are large bone-like HAP crystals growing on the surface of hydroxyapatite sintered by SPS. However, hydroxyapatite consolidated by conventional hot-pressing sintering shows fewer bone-like HAP crystals. This indicates that spark plasma sintering can provide a good approach to consolidate hydroxyapatite powders with higher bioactivity.
Chapter 2 Research incentives and objectives

2.1 Research incentives

Based on the conducted literature survey described in the previous sections, one can conclude that:

- The natural dental materials including both human and animal dental materials optimized their structure over long period of time to improve their mechanical properties.

- The mechanical properties of the dentin can be influenced by some major factors addressed in the previous sections including the tubule orientation, tubule density, and the age of dentin. The mechanical strength of dentin is higher in the direction perpendicular to tubule axis; it is higher for lower tubule density, and it is higher for young dentin.

- The analyzed natural materials manifest specially designed structures with the presence of self-organized textures and functionally graded areas. The observed examples include graded structures of a tooth with tough dentin inside, hard enamel outside, and dentin enamel junctions.

- The developed artificial bio-materials until recently rarely employed bio-inspired
combinations of hard and soft structure components.

- Ti$_3$SiC$_2$, ZrO$_2$, and CNTs have been used as a second phase in HAP creating HAP-composites to reinforce the low mechanical properties of pure HAP. However only few papers were dedicated to CNT reinforced HAP composites, and no research has been conducted on the influence of CNT concentrations on the properties of the processed composites.

In order to fabricate a bio-composite, which has similar structure and properties compared to natural dental materials, thorough investigations of the structure and mechanical properties of biological materials are very important. For example, typical natural teeth consist of hard enamel outside, tough dentin inside, and a dentin enamel junction, which is a functionally graded structure. In normal cases, the interfaces between the two materials with dissimilar mechanical properties are the stress concentration zones where failure usually occurs. However, the functionally graded structure of the dentin enamel junction provides a solution to overcome the failure. It can successfully transfer an applied load from enamel to dentin and inhibit the crack propagation through the entire tooth volume.

The stable junction between host tissue and implants is very important to the
reduction of the mismatch between two materials, which is an essential issue in implant applications. Based on the natural structure, a functionally graded material is a solution to solve this problem. A functionally graded material can provide a better interface between two different materials. The applied load can be gradually transferred from the replacement material to the host. Besides the applied load, the thermal expansion can gradually change through the junction due to the graded structure.

The tubule structure in dentin renders also a novel idea of creating an implant with similar channel-like structures. The micro-channels in the implant can provide an innovative route for biomedical applications such as drug delivery components, body fluid penetration, and bone cell growth. [65] Better cell growth in the implant may strengthen the junction between the natural tissue and the implant which should result in lower failure probabilities.

2.2 Research objectives

Based on the conclusions of the literature survey (see section 2.1), the research objectives of the present study are:

- Characterization of the natural dental (animal and human) materials; the analysis of their microstructure and microstructure-effectuated mechanical properties.
The development of bio-inspired HAP-CNT composites with tailored micro-structures based on the findings obtained through the characterization of the natural dental (animal and human) materials.

Net-shape processing of a dental implant prototype using spark-plasma sintering.

Thereby, based on the characterization of the natural materials, the fabrication of a bio-inspired composite is the main objective of the present research. Special structures should include porous, functionally graded, and channel-like arrangements. The obtained results are planned to be extrapolated to the net-shape processing by spark-plasma sintering of a dental implant prototype.

2.3 Research tasks

To achieve the formulated research objectives, the following tasks had to be conducted as follows:

1. Understanding the structure of the natural biological (dental) materials: Before creating a biomimetic composite, it is necessary to understand the structure, composition and mechanical properties of the respective natural biological materials. This first step of the study includes the characterization of human dentin, shark teeth, Arapaima gigas scale. The following analyses are conducted: microhardness testing,
nanoindentation testing, tensile and compression testing, optical microscope, SEM inspection, X-ray diffraction, Fourier transform infrared spectroscopy. This task results in the formulation of microstructure-design principles which are utilized in the next (bio-inspired composite fabrication) task.

2. Study of green specimen manufacturing: dispersion of CNTs in HAP powder: Since CNTs tend to agglomerate due to their high aspect ratio their uniform dispersion is an independent task addressed both through mechanical and chemical approaches. Ball milling, ultrasonication, freeze drying, and usage of dispersants are the methodologies investigated within this task.

3. Spark plasma sintering of the green CNT-HAP specimens: investigation of the impact of SPS parameters including sintering temperature, holding time, and applied pressure, comparison with conventional sintering. Exploring the processing of tailored CNT-HAP structures, including channel-porous and functionally-graded structures.

4. Net shape processing of a complex-shape dental implant. This task will include the design of a specially shaped die-punch SPS setup and its utilization in the SPS net-shape fabrication tests.
Figure 2.1 shows the flow chart of the research work including the research objectives and tasks. Figure 2.1 includes two main parts of the project: (1) characterization of the structure of natural dental materials; (2) Fabrication of bio-inspired tailored HAP based composites.

Understanding of the natural materials provides a general concept on a possible structure of synthesized bio-mimetic materials. The characterization of a natural material includes the analysis of the structure and mechanical properties. The fabrication part includes the two major components. The first component includes the optimized reinforcement of HAP by adding the CNT as a second phase. To consolidate the CNT-HAP composite, it is essential to find out the best condition for processing by manipulating the SPS conditions and optimizing the dispersion method to achieve higher mechanical properties of the composite. The second component is the development of complex shape CNT-HAp composites with bio-inspired tailored structures including a micro-channel material for drug delivery and a functionally graded structure for a dental implant application.
2.4 Expected engineering and scientific novelties

There are two major parts in the present study: the characterization of the natural biological material and the fabrication of bio-inspired composites through spark-plasma sintering. The scientific and engineering novelties of the conducted studies are summarized as follows:

1. Both animal and human dental materials have been characterized. The characterization results suggest the benefits of functionally graded design in the analyzed structures.

2. The CNT-reinforcement HAP composites with different CNT concentration have been successfully consolidated by SPS.

3. The demonstrated capability of SPS of the consolidation of functionally graded
HAP-CNT composites.

4. The potential of SPS and free-pressureless SPS for the net-shape consolidation of complex-shape parts has been demonstrated.
Chapter 3 Characterization of animal and human teeth: mechanical properties and microstructure

In this chapter, the characterization of mechanical properties and the microstructure of several animal and human dental structures through micro-indentation, compression test and SEM are described. In order to fabricate a biomimetic material, it is necessary to understand the structure and mechanical properties of a design-inspiring natural material. Micro-indentation and compression test were performed on the shark and human dentin. Several mechanical tests such as tensile test, micro-indentation and structure investigation were performed also on the Arapaima Gigas scale, a fish from South America.

3.1 Characterization of animal and human teeth: Experimental setup and sample preparation

For the micro-indentation tests, the samples were cut from piranha, gator, great white shark, Mako shark, Rabbit and Rat transversely to expose the cross-section of the teeth and then were kept in ambient dry conditions. The samples for both micro and nano indentation test were mounted in the epoxy and then grinded with 600X, 1200X, 2400X, 4000X sand paper followed by polishing with Al₂O₃ solution with Al₂O₃ particle size of 5
µm and 0.5 µm.

Micro-indentation was performed across the cross-section area of the sample starting from the enamel region towards the internal dentin and then towards the other side of the external enamel region with a load of 500N. A LECO M-400H1 hardness testing machine equipped with a Vickers indenter was used. All indentation points were separated from each other with a distance more than five times of the size of the indentation site to reduce the error measurement. Nano-indentation testing was performed on the cross-section area of both piranha and great white shark teeth by using TI-950 TriboIndenter (Hysitron Inc., Minneapolis, MN, USA). A diamond fluid cell Berkovich probe was utilized to perform tests on both ambient (dry) and hydrated condition on piranha and great white shark teeth. Samples were immersed in a DPBS solution during hydrated testing, with a minimum of 2 hours of hydration time prior to testing. Tests were conducted in load-controlled feedback mode up to various peak forces. A load function consisting of a five-second loading to peak force segment, followed by a five second hold segment, and a one-second unloading segment was finally used. Optical microscope integrated into the nanoindentation system was used to identify the testing regions in relatively large area. For smaller area, Scanning Probe Microscopy (SPM),
which utilize the nanoindentation tip as a probe was used to obtain pre-test in-situ SPM images. In-situ SPM images can provide significant and detailed information on the sample surface morphology to identify the test regions. Nanoindentation hardness and the reduced modulus were calculated based on the well-established Oliver-Pharr method for nanoindentation testing. [66]

The specimens for the compression test were sectioned from human molar teeth and from shark teeth by a diamond saw into a cubic shape (1mmx1mmx1mm). The enamel of the human teeth was removed by a diamond saw and the shark teeth enamel was grinded off with 180X sand paper. Twenty samples of shark dentin were prepared for two different conditions: wet and dry. Compression tests were performed by using a universal testing machine (Instron 3367 Dual Column Testing Systems, Instron Inc., Norwood, MA, USA) with a 30 kN load cell. Cubic samples were tested under a strain rate of $10^{-3}$ sec$^{-1}$, which is equivalent to the crosshead speed of 0.06 mm/min. The tested samples were preserved in Hank’s Balanced Saline Solution (HBSS) (Mediatech Inc., VA, USA) before the test.

Besides conducting the compression tests on a normal human dentin, the compression test were conducted also on the deproteinized dentin. We assumed that the
main two components in human dentin were mineral and protein components. Two processes, deproteination and demineralization were used to remove either the protein content or the mineral content from the dentin. Deproteination was conducted by using sodium hypochlorite (NaOCl) with 2.6 wt% diluted mild bleach. [67] The sodium hypochlorite was changed daily until deproteination was finished. The total deprotenination time was about 7-10 days depending on the size of the specimen. The deproteination was complete when the mass of the sample stopped decreasing. The demineralization was carried out by immersing the machined cubic samples in hydrochloric acid (HCl) with concentration of 0.6 N diluted from 1 N hydrochloric acid. The hydrochloric acid solution was changed 12 hours on the first day and then change daily for 5 days to ensure the demineralization process was in action. To confirm that the samples were fully demineralized, the mass of samples was measured before demineralization and then daily before changing solution. The demineralization process was complete when the mass of the cubic samples stopped decreasing.

Both the microstructures of demineralized and deproteinated sample were investigated by SEM. Since the shape of the demineralized sample changed after removing from the 0.6 N HCL solution, it could not represent the original demineralized
Critical point drying, a method of dehydrating biological material prior to the examination in the scanning electron microscope, can remove the liquid without directly passing through a liquid-gas transition. The demineralized samples were immersed into different concentration of ethanol starting from 20% to 100% with a 20% increment and 20 minutes soaking time for each concentration.

For the microstructure characterization, the fracture surface after compression test and morphology of the animal teeth was characterized by a field emission SEM (FEI-XL 30, FEI Company, Oregon, USA). The samples were attached to the aluminum sample holders and coated with gold in a sputter coating machine.

3.2 Characterization of animal human teeth: Results and Discussion

3.2.1 Micro-indentation, Nano-indentation tests and SEM characterization

Micro-indentation has been done on human teeth and the results show that there is a significant difference in the hardness values of dentin, which is about 1 GPa and of enamel, which is about 4 GPa. The micro-indentation has been conducted on several species of animals’ teeth to see if the hardness values exhibited the same trend as the human teeth. The analyzed tissues included teeth of great white shark, piranha, rat, rabbit, mako, alligator, elephant tusk and hippo. The micro-indentation was performed across the cross section of the tooth starting from the one-side enamel towards the middle dentin.
and then towards the other side enamel. Table 13.1 shows the average hardness value of
dentin and enamel in different species of animals. The enamel hardness values range
from 1.2 to 1.56 GPa and the dentin hardness values range from 0.31-0.55 GPa. The
plotted normalized distance vs hardness value (Figure 3.1) indicates a dramatic drop in
the hardness value when the indentation passes through the dentin-enamel junction. The
results show that the hardness value of rat dentin is the highest among all the conducted
tests and is of 0.55 GPa. The hardness value of human dentin and enamel are ~0.4 GPa
and 1.6 GPa, respectively which are lower than the previously reported results
(enamel:4.6 GPa, dentin: 0.76 GPa) [68] but are still of the same order.
Figure 3.1 Microhardness value of various kinds of animal dentin and enamel.

From the SEM and optical microscope observation, we can clearly see the two regions corresponding to dentin and to enamel components of the tooth. The left SEM image (Figure 3.2) is the cross section of the shark tooth which clearly shows the functionally graded structure. The white thin region is the outside enamel layer and the gray part is dentin with tubules randomly embedded in the dentin matrix. The line between these two regions is a dentin-enamel junction. And the central part of the image is the hollow region of the great white shark teeth. Figure 3.2 is the SEM image of the
cross section of the mako shark tooth which also exhibits the functionally graded structure. The fully white region is the enamel and the inside gray part is the dentin. The region between enamel and dentin shows the overlap pattern which consists of web structure of enamel and dentin filled in the space between the web lines corresponding to the transition region.

Table 3.1 The micro-hardness value of enamel and dentin from different animals

<table>
<thead>
<tr>
<th></th>
<th>Enamel (GPa)</th>
<th>Dentin (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>1.70±0.08</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>Great white shark</td>
<td>1.56±0.19</td>
<td>0.31±0.07</td>
</tr>
<tr>
<td>Mako</td>
<td>1.30±0.20</td>
<td>0.36±0.04</td>
</tr>
<tr>
<td>Piranha</td>
<td>1.36±0.22</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.26±0.15</td>
<td>0.42±0.08</td>
</tr>
<tr>
<td>Rat</td>
<td>1.20±0.08</td>
<td>0.55±0.01</td>
</tr>
<tr>
<td>Alligator</td>
<td>1.45±0.10</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td>Tusk</td>
<td>1.52±0.04</td>
<td>0.36±0.06</td>
</tr>
<tr>
<td>Hippo</td>
<td>1.67±0.09</td>
<td>0.32±0.05</td>
</tr>
</tbody>
</table>
Figure 3.2 SEM images of cross section of (a) shark tooth; (b,c) Mako shark showing the functionally graded structure between dentin and enamel.

The hardness value is affected by the tubule density in human dentin. The hardness values vary between different cross-sections cut from different depth of dentin. The radial distribution of tubule in dentin results in the different tubule density which influences the mechanical properties of dentin examined in different locations. Not only the depths but also the position in the same cross-section may change the hardness values.
due to the non-uniformly distributed tubules. The results shown in Figure 3.3 are the micro-hardness values measured in two different positions but in the same cross-section of the human dentin. The red dots were measured in the region which is close to the center of the dentin (inner parts) and the black dots were measured in the region which is closer to the edge (outer parts). The results exhibit higher hardness value in the inner parts than in the outer parts of the dentin. The BSE images provide the evidence that tubule density is a function of hardness. Figure 3.3 clearly shows the indentation site with the size of 50 by 50 µm. At low magnification we cannot distinguish considerably different tubule densities in the central and the outer parts of the dentin. However, under a higher magnification (Figure 3.4), we can see that the number of tubules per unit area in inner part of dentin (44444/ mm²) is much larger than that of the outer region of dentin (24000/ mm²). The outer parts of dentin have the lower tubule density which results in the higher hardness values. On the contrary, the inner region of the dentin has the higher tubule density resulting in the lower hardness values. The BSE images also show that the diameter of the tubule is about 2-3 µm.
Figure 3.3 (a) Microhardness values of human dentin; (b) SEM of indentation site.
Figure 3.4 (a) Tubule structure in outer region showing higher tubule density; (b) tubule structure in inner region showing lower tubule density.

Nano-indentation was performed on both great white shark and piranha teeth.

Figure 3.5 shows the optical micrograph of the indentation area and the distribution map
of nanohardness and reduced modulus within the cross-section of a piranha tooth. The map clearly shows the higher nano-hardness and reduced modulus in enameloid than in dentin. Further zoom-in nano-hardness and reduced modulus at DEJ with more condensed grid of indents (Figure 3.5 (d,e)) was performed to investigate the mechanical change in the junction. The enameloid of the piranha tooth has a mean nano-hardness of $4.12 \pm 0.9$ GPa and a mean reduced modulus of $86.49 \pm 15.9$ GPa, while dentin has a mean nano-hardness of $0.75 \pm 0.3$ GPa and reduced modulus of $22.98 \pm 6.0$ GPa in ambient condition, respectively. Since the hydration plays an important role in mechanical properties of biological materials, the piranha tooth was further tested in hydrated condition in order to understand the effect of hydration on the mechanical properties of dentin and enameloid. In hydrated conditions, the nano-hardness and reduced modulus of enameloid are $3.05 \pm 0.4$ GPa and $81.92 \pm 8.4$ GPa, respectively. The values are lower than those tested in dry conditions. In hydrated conditions, dentin has nano-hardness of $0.22 \pm 0.04$ GPa and reduced modulus of $12.38 \pm 1.6$ GPa which are reduced more significantly than in enameloid. The enameloid shows less hydration dependence compared with dentin because enameloid is a highly mineralized tissue with a small organic component and water. Therefore, the hydration effect on the mechanical
properties of dentin is more significant than on those of enameloid. Compared with hardness measured from microindentation, the nano-hardness values are higher than micro-hardness values. This is due to the size effect reported by Yao et al. [69]
Figure 3.5 (a) Optical micrograph of cross section of piranha tooth; (b) Reduced modulus map; (c) nano-hardness of cross section in the piranha tooth; (d),(e) higher resolution reduced modulus and nano-hardness at DEJ region.

Figure 3.6 (a) is the cross-section optical image of the great white shark along with the nano-hardness and reduced modulus maps. A higher resolution of reduced modulus
and nano-hardness maps at zoom-in area on the DEJ shown in Figure 3.6 (d,e). The shark enameloid has nano-hardness of $4.13 \pm 1.1$ GPa and reduced modulus of $84.35 \pm 19.9$ GPa in dry condition. The nano-hardness and reduced modulus of shark dentin are $0.68 \pm 0.2$ GPa and $20.38 \pm 5.6$ GPa, respectively. Similar to the piranha, the enameloid has higher mechanical properties than dentin. Both the shark enameloid and dentin were tested under hydrated condition. Figure 3.7 shows the comparison of the load-displacement curve of shark enameloid and dentin tested in dry and hydrated conditions. The difference between dry and hydrated conditions in dentin is more obvious than that in enamel, which is same as in the piranha tooth. The hydration effect on the mechanical properties of dentin is more significant than the one in enameloid due to the higher organic component in dentin. When tested in hydrated condition, the nano-hardness and reduced modulus reduce only to $2.62 \pm 0.9$ GPa and $77.2 \pm 14.8$ GPa, respectively. However, the nano-hardness and reduced modulus of dentin drop dramatically down to $0.21 \pm 0.1$ GPa $8.66 \pm 3.1$ GPa, respectively.
Figure 3.6 (a) Optical micrograph of cross section of great white shark tooth; (b) Reduced modulus map; (c) nano-hardness of cross section in the great white shark tooth; (d), (e) higher resolution reduced modulus and nano-hardness at DEJ region
Whitemack et al. [70] measured the nano-hardness and reduced modulus of tiger shark and bonnethead shark. They reported that the nano-hardness and reduced modulus of tiger shark enameloid are $3.2 \pm 0.2$ GPa and $72.61 \pm 4.73$ GPa, those of bonnethead shark enameloid are $3.53 \pm 0.3$ GPa and $68.88 \pm 1.5$ GPa, respectively. The nano-hardness and reduced modulus of tiger shark dentin are $1.21 \pm 0.16$ GPa and $28.44 \pm 2.21$ GPa, those of bonnethead shark dentin are $0.97 \pm 0.07$ GPa and $22.49 \pm 1.72$ GPa, respectively.
Both nano-hardness and reduced modulus of these two types of shark tooth are in agreement with those of the great white shark tooth.

### 3.2.2 Element line mapping

The micro-indentation shows that the hardness values are higher in the enamel than in the dentin. The different amount of respective elements in enamel and dentin should be responsible for this different hardness values. Therefore, element line mapping was conducted from the dentin to the enamel through the dentin-enamel junction in the great white shark and human tooth materials (Figure 3.8, 3.9). The left-hand side is the SEM images of the spot for which the element line mapping was conducted. The elements present in most biological materials such as oxygen, carbon, and sodium were found and their contents are almost the same in dentin and enamel region. However, the content of calcium and phosphorus which are the main components in the tooth material are higher in enameloid than in dentin. These results clarify why the hardness value is higher in the enamel than in the dentin. The deep drop in hardness represents the gap in properties of the enamel and dentin.
Figure 3.8 SEM image of a cross-section of a great white shark tooth and element line mapping plot.
3.2.3 Compression tests

Craig *et al.* have studied the compressive properties of enamel and dental cements. [71] The elastic and mechanical properties of human dentin have been studied by several groups who reported that the average compressive strength of human dentin was about 36100 to 37200 Psi which corresponds to 248 to 256 MPa.[72,73,74] But the effects of tubule orientation on the compressive strength of human dentin have not been studied in detail.
The compression tests were performed for great white shark teeth and human teeth. The great white shark teeth were tested under dry and wet conditions. Both the dry and wet condition results are shown in Figure 3.10. From the plot, the dry conditions provide the higher Young’s modulus value than that obtained under wet conditions. The average compressive strength in dry condition is also higher than that obtained under wet conditions. However, the compressive strength varies a lot between 100 and 250 MPa from specimen to specimen. The reason is that the tubule orientation in dentin is not uniformly parallel or perpendicular to the loading direction, which results in the deviation of the compressive strength and of the elastic modulus.

The fracture phenomenon is different for wet and dry conditions. In dry conditions, the curve shows the elastic behavior at the beginning and then fracture behavior. However, in wet conditions, the curves also indicate the elastic compression initially, which is then followed by the plastic behavior. For dry conditions, the shark dentin exhibits more brittle material properties.

Figure 3.11 represents the Weibull modulus plots for the ultimate compressive strength in dry and wet conditions. The ultimate compressive strength in wet conditions is below 150 MPa. On the contrary, the ultimate compressive strength in dry conditions is
above 150 MPa and approaches 230 MPa. From the Weibull plot (Figure 3.11), the wet conditions provide a narrower distribution than that provided by dry conditions.

Figure 3.10 (a) The stress-strain curve for dry shark dentin; (b) the stress-strain curve for wet shark dentin.

Figure 3.11 (a) Weibull modulus distribution of dry shark dentin under compression test; (b) Weibull modulus distribution of wet shark dentin under compression test.

Many studies [12-17] on the impact of the tubule orientation on the mechanical properties of human dentin have been carried out including analyses of ultra tensile strength [13,16], and fracture toughness flexural fatigue strength. However, only few
studies discuss how the tubule orientation influences the compressive strength. According to the literature [14,16] it is believed that the collagen fibrils randomly oriented on the plane perpendicular to the tubule play an important role in changing the mechanical properties under different loading directions. The tubule orientation may also significantly influence the compressive strength when subjected to different loading directions. Therefore, the compression test was performed in two loading directions with respect to the tubule axis. One was the compressive direction parallel to the tubule and the other was the compressive direction perpendicular to the tubule.

Human dentin became very flexible and soft after demineralization. The shape of the sample also twisted and deformed a lot. In this condition, the demineralized samples were not suitable for the compression tests. However, after deproteinization, the samples remained cubic and could be subjected to the compression tests. The deproteinized samples were very fragile. The compression test results (Figure 3.12) indicate that there is no much difference between the parallel and perpendicular groups of loaded samples. The compressive strength of human dentin is between 250 to 350 MPa. The compressive strength of deproteinated dentin is much lower than that of the untreated one. The mineral and protein phase are both important for the enhancement of the mechanical
properties of dentin. The matrix of combined mineral and protein phases in dentin performs better in terms of its mechanical properties than a pure protein phase.

The stress-strain curves for the compression tests under different compression directions are shown in Figure 3.12. The Young’s moduli of the group with the longitudinal direction and the transverse direction are of 7.7 GPa and 6.3 GPa, respectively. The compressive strength is also higher in the longitudinal direction (about 340 MPa) than in the transverse direction (about 270 MPa). The reason that the longitudinal case is higher in both compressive strength and Young’s modulus than the transverse case may be attributed to the collagen fibril orientation in the plane perpendicular to the tubule axis. Since the sample underwent a uniaxial split, for the longitudinal case, the fracture surface occurs between the tubules, which need more stress to pull apart the collagen fibril. On the other hand, for the transverse case, the fracture surface appears across the tubules and between the collagen fibril planes which provides less stress requirement to undergo the uniaxial split.
The stress-strain curves shown in Figure 3.13 are the results of the deproteinated dentin loaded by the longitudinal compression. Compared with the untreated dentin longitudinal compression, both the Young’s modulus and compressive strength decrease from 7680 to 4152 MPa and 250-350 MPa to 65-75 MPa, respectively. The difference is about 46% and 74% decrease in Young’s modulus and compressive strength.

The fracture surfaces of both the longitudinal and transverse groups are characterized by using SEM. SEM images (Figure 3.14) are the fracture surfaces
corresponding to the uniaxial split under a loading direction parallel to the tubule axis showing the channel structure which is consistent with the loading direction. The collagen fibrils are randomly orientated around the tubule. The collagen fibrils which point out to the paper are those pulled out and fractured. The bottom two SEM images, (Figure 3.15) are the fracture surfaces of the transverse case. The black holes are the tubules with the loading direction perpendicular to the tubule axis.

Figure 3.14 SEM images of fracture surface of human dentin in longitudinal direction

Figure 3.15 SEM images of fracture surface of human dentin in transverse direction.
3.2.4 Microstructure of demineralized and deproteinated human dentin

Assuming that the two main components in human dentin are mineral and protein, components, the demineralization and deproteinization are the approaches to investigate the structure of dentin with only protein or mineral phase by removing the mineral and protein components, respectively. The structures of the demineralized and the deproteinated human dentin were investigated. Figure 3.16 and Figure 3.17 represent the SEM micrographs of the demineralized human dentin from lower to higher magnification showing the characteristic tubule structure. All the minerals were removed from the sample, the remaining components are collagen. From the tubule holes and the section view of the channel, the tubules were surrounded by the layers composed by collagen fibers with random orientation within their plane. These collagen fibers should affect the mechanical properties of human dentin.

![Figure 3.16 SEM images of top view of the demineralized dentin](image)
Figure 3.17 SEM images of side view of the demineralized dentin tubule channel

Figure 3.18 (a-d) represent the SEM micrographs of the deproteinated human dentin structure. All the collagens were removed and only the mineral remains in the dentin. The small mineral rods with diameter of 10-20 µm and length less than 100 µm are randomly distributed between tubules.
3.2.5 Microstructure characterization of teeth morphology

The piranha (Serasalmus manueli) is a member of the family of omnivorous freshwater fish living in South American rivers and famous for their sharp teeth and voracious appetite. Both the top and bottom teeth exhibit triangular shape and align on the jaw which looks like a curved saw. Moreover, using an SEM analysis, we found that each of the piranha teeth has its own tiny serrated edge. Serration is a very usual shape which can be observed in many kinds of carnivorous animals’ teeth. Not only small fish like piranha but also other larger animals, for example, great white shark are equipped
with serrated edges on their teeth. Several ancient or extinct animals have already
evolved to have serration edges on their teeth, for instance, the saber-teeth tiger and
megalodon, the biggest fish on earth. The biting force can be concentrated on each top
point of the serration to enhance the cutting efficiency compared with the smooth edge
tooth. The SEM images (Figure 3.19, Figure 3.20, Figure 3.22) show the microstructure
of the piranha teeth and of the great white shark teeth. Figure 3.19 (a) is an overview of a
whole piranha tooth with some wear on the edge and on the tooth tip. Figure 3.19 (b) is
the side view of the tooth edge. Figure 3.19 (c,d) is the top view of the piranha tooth
clearly indicating that the tooth is equipped with a serrated edge. Base on the SEM image,
the serration size of the piranha tooth is about 10 -15 µm.

<table>
<thead>
<tr>
<th>Table 2.2 Serration size of different animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serration size (µm)</td>
</tr>
<tr>
<td>Trannosauroïd dinosaurs</td>
</tr>
<tr>
<td>Great white shark</td>
</tr>
<tr>
<td>Piranha</td>
</tr>
</tbody>
</table>
Figure 3.19 SEM images of (a) tip of the piranha tooth; (b) the edge of the serration edge.

Figure 3.20 (a,b) SEM images of serrated edge on the piranha tooth showing the serration size of 15 \( \mu \text{m} \).

The drawing shown in Figure 3.21 is the biting mechanism of piranha. The stress applied on the prey also includes a significant shear component due to the tooth’s triangular shape and due to the serrated edge of the tooth. The prey caught in the teeth is severed in a guillotine-like confinement of teeth.
Figure 3.21 The schematic drawing of the guillotine-like biting mechanism of the piranha.

The great white shark (Carcharodon carcharias) is another example of a serrated edge tooth. Figure 3.22 shows the hierarchical structure of the great white shark jaw-similar tooth -its micro-serrations. The serration size of the great white shark teeth is about 300 µm. The geometry of the serration edge of the great white shark teeth is a cycloid shape.
3.2.6 Mechanical testing of the Arapaima Gigas scale

Arapaima Gigas is a South American tropical freshwater fish. It is one of the largest freshwater fish in the world which can reach length of at least 2 m. It is interesting that Arapaima cannot be eaten by the piranha. The mechanical properties and microstructure of the Arapaima scale must play an important role in protecting its body. The main components in fish scale has been identified as type I collagen fiber and
organization of the collagen also has been investigated. [76,77,78] In the present study, several mechanical tests involving Arapaima gigas have been conducted including the tensile test, micro-indentation, and the microstructure observation using optical microscope and SEM.

Figure 3.23 shows the hierarchical structure of the Arapaima Gigas scale. Scales overlap to cover the entire Arapaima Gigas. The sketch of the cross section view indicates that the scale is composed of two distinct layers. The top ridged part is the external layer with a thickness of 600 µm, which is highly mineralized and the button part is the internal layer with a thickness of 1000 µm. The internal layers are composed of collagen bundles with different orientation in different layers to form the plywood structure. The diameter of the collagen bundle is 1 µm and the fundamental unit is the collagen fibril with diameter of 100 nm.
Figure 3.23 Hierarchical structure of Arapaima Gigas scale. Arapaima scales are composed of plywood structure with collagen fibers.

### 3.2.6.1 Arapaima Gigas scale: Tensile testing

Because the Arapaima Gigas live in fresh water, the mechanical properties of the Arapaima Gigas scale must be influenced by the moisture contained in the samples. The tested samples were subdivided into two groups: one in dry conditions and the other in wet conditions. The specimens were air-dried for the dry group. For the wet conditions, the Arapaima gigas scales were immersed in distill water for 4 days before testing. There are two regions in the whole Arapaima Gigas scale, one is the dark part exposed to water and another one is the white part. The specimens were cut from the white part of the Arapaima Gigas fish scale with the thickness of about 0.85 mm to 1.05 mm. We used the
laser cutting machine to cut the Arapaima Gigas fish scale into a dog-bone shape with the length of 25.4 mm and the width of 6.35 mm, the gage length of 6.35 mm and the gage width of 2.29 mm.

The tensile tests were performed on a specially designed fixture connected to the INSTRON tensile testing machine (Figure 3.24 (a)). The fixture (Figure 3.24 (b)) included two symmetric upper and lower grips and the track. The two grips were confined and guided by the track. Both grips were machined into the dog-bone shape concave geometry same as the sample for the insertion of the specimen. The lower grip was fixed to the track and connected to the tensile test machine. The upper grip was movable along the track and attached to the universal testing machine through a long steel bar. A universal testing machine (Instron 3346 Single Column Testing System, Instron, MA, USA) equipped with a 500N load cell was used for the tensile test.
The mass of eight small pieces of specimens for both wet and air-dried condition were measured individually and then placed in a furnace at 105°C for 2 and half days. After having been dried, the mass of all the sixteen samples was measured. The hydration concentration of air-dried and wet groups was calculated from the original mass by subtracting the dried mass and then dividing by the original mass. For our experiment, the air-dried group had an average 16.15% water content and the wet group had on average 30.34% water content. The wet conditions provided about twice of water content compared to the air-dried condition water contents.

In most of the biological materials, strain rate is usually an effective parameter influencing their mechanical properties. Therefore, we also conducted tensile test at
different tensile strain rate which ranged from $10^{-2}\text{s}^{-1}$ to $10^{-4}\text{s}^{-1}$ to see how the mechanical behavior changed with respect to different strain rates. Figure 3.27 is the tensile stress-strain curve under different strain rate showing that both the tensile strengths and Young’s moduli increase with the increase of the strain rate.

**3.2.6.2 Arapaima Gigas scale: X-ray diffraction (XRD)**

The powder for X-ray diffraction analysis was collected by sawing the Arapaima Gigas scale. A Rigaku MiniFlex™ II benchtop XRD system (Rigaku Company, Texas, USA) with the radiation source of CuKα was used in XRD analysis. The wavelength of the radiation source was 0.154 nm. The scan started from 2theta of 20 degree to 60 degree with step size of 0.01 degrees at a rate of 1 degree/min.

**3.2.6.3 Arapaima Gigas scale: Micro-indentation test**

We conducted the micro-indentation on the external and internal layers on a cross-section of the scale by using the same indentation machine as the one we used for the teeth. The load we used was 50 N and hardness values were obtained by averaging several indentation measurements on the cross-section.

**3.2.6.4 Arapaima Gigas scale: Tensile test results**

The stress-strain curve obtained for tensile tests with different hydration contents is shown in Figure 3.25 and Figure 3.26. Five samples have been tested for each condition.
The strain rate we used in the test was $10^{-4}$ which corresponded to the cross-head velocity of 0.381 mm/min. The obtained stress-strain curves indicate that the scale behaves like a laminated composite material. Several curves show some peaks which represent the consecutive breakage of each layer. The combination of pulling out collagen fibers in a layer and sliding between layers is a fracture mechanism in these laminated composite materials. The Young’s modulus measured for dry conditions is 1226.58 MPa whereas the Young’s modulus measured for wet conditions is 118.03 MPa. The Young’s modulus measured for dry conditions is more than ten times higher than that measured for the wet conditions. The ultimate tensile strength for the dry conditions is approximately 50 MPa. However, the ultimate tensile strength for the wet conditions only ranges between 15-30 MPa. Torres et al. [79] reported that the maximum tensile strengths for dry and wet conditions were 53.86 and 22.26 MPa, respectively; the Young’s modulus measured for dry and wet conditions were 1.38 and 0.83 GPa. Ikoma et al. [80] also measured the tensile strength of Pagrus major fish scales. They reported that the Young’s modulus of Pagrus major fish scale was 2.2 GPa and the tensile strength was 93 MPa. The maximum tensile strain was 4.7%. However, compared to our results, the maximum tensile strain of Arapaima scales was about 5% in dry conditions and 30-40% in wet conditions both
larger than the cited results.\cite{38,39} To measure the hydration content, both the mass of air-dried and wet samples were measured. Then they were baked in a furnace at 105°C to remove the water. The hydration contents for the air-dried and wet conditions were 16.15% and 30.34%, respectively. A considerable difference of the mechanical properties including Young’s modulus and ultimate tensile strength of bio-materials measured under dry and hydrated conditions prove that the hydration significantly changes the mechanical properties of the biological materials. Taking Arapaima scale for example, the Young’s modulus measured under dry condition (1226 MPa) is about 10 times of that under hydrated condition (118 MPa) and the ultimate tensile strength under dry conditions (50 MPa) is higher than that under hydrated conditions (15-30 MPa) as well. One of the main components in the scale is collagen fibers. When a sample is under hydrated conditions, the water acts like a lubricant between the collagen fibers, which softens the scale and results in the decrease of both ultimate tensile strength and elastic modulus.

Besides the affect of the hydration on the mechanical properties of scales, the mechanical response of a scale also depends on the strain rate. As the loading velocity increases, both the elastic modulus and the ultimate tensile stress also increase. Figure
3.27 shows the stress-strain curve for different strain rate ranges of $10^{-2}$ s$^{-1}$, $10^{-3}$ s$^{-1}$, $10^{-4}$ s$^{-1}$.

The results show that the Young’s moduli of the Arapaima scale are 213.4 MPa, 82.7 MPa and 42.2 MPa at strain rates of $10^{-2}$ s$^{-1}$, $10^{-3}$ s$^{-1}$ and $10^{-4}$ s$^{-1}$, respectively, which demonstrates that the mechanical properties of the Arapaima scale are strain rate dependent. The ultimate tensile strength also increases with the increase of the strain rate. These kinds of strain-rate dependent results also have been reported for tests conducted on bones in tension. Crowninshield et al. [81] and Wright et al. [82] carried out tensile tests for a wide range of strain rates on compact bones and concluded that there was a linear relationship between the ultimate strength and the elastic modulus and the logarithm of the strain rate. Both the ultimate strength and the elastic modulus increased with the increase of the strain rate. Currey et al. [83] also reported the same results on the tensile strength and the elastic modulus increase with the strain rate increase under quasi-static conditions.
Figure 3.25 Typical stress-strain curves for dry conditions.

Figure 3.26 Typical tensile stress-strain curves for wet conditions.
Figure 3.27 Tensile stress-strain curves for scale as a function of strain rate varying from $10^{-4}$ to $10^{-2}$ sec$^{-1}$

Figure 3.29 (a,b) show the elastic modulus and the ultimate tensile stress for different strain rates indicating that both the ultimate tensile stress and the Young’s modulus increase with the increasing strain rate. Polymeric materials have strong sensitivity to strain rate. Collagen plays a primary role in the strain rate sensitivity of biological materials. The Ramberg-Osgood equation is commonly used to describe the elastic modulus dependent on strain rate. The original Ramberg-Osgood equation is
shown below:

\[ \varepsilon = \frac{1}{c} \sigma (\dot{\varepsilon})^{-d} + a \left( \sigma^n \right)^b \]  

(1)

where \( \varepsilon \) is the strain, \( \dot{\varepsilon} \) is the strain rate, \( \sigma \) is the stress and \( a, b, c, d \) and \( n \) are constants.

The second term in this equation describes the plastic part of a stress-strain curve. However, since we only consider physiological levels of stress in biological material, the second term can be negligible. Therefore the Ramberg-Osgood equation can be reduced to the following equation:

\[ E = C (\dot{\varepsilon})^d \]  

(2)

where \( E \) is the elastic modulus, \( \dot{\varepsilon} \) is the strain rate, \( C \) and \( d \) are empiric parameters.

After applying the Ramberg-Osgood equation, we obtain the following result:

\[ E = 1499.2 (\dot{\varepsilon})^{0.26725} \]

The experimental parameters \( C \) and \( d \) are 1.5 GPa and 0.26, respectively. Some other biological materials such as antler in hydrated conditions have the experimental parameter \( C \) and \( d \) of 3.47 GPa and 0.05; bovine cortical bone has \( C \) and \( d \) value of 12 GPa and 0.018, and human cranium has \( C \) and \( d \) value of 15 GPa and 0.057. \[84\] The experimental parameter, \( d \), which describes the materials’ sensitivity to strain rate, in Arapaima gigas scale (0.26) is an order higher than that in bone (0.057) and in antler...
The elastic modulus in most of biological materials varies as a function of strain rate primary due to the collagen. The higher strain rate dependency of the Arapaima gigas scale than that of the bone can be attributed to the much lower mineral content (30wt%) in Arapaima gigas scale than in the bone (65wt%).[85]

Figure 3.28 shows a comparison of different biological materials with Ramberg-Osgood equation fitting. All of the results show the elastic modulus increase with increasing strain rate. Among those results, the Arapaima scale has the highest parameter d, which is related to the strain rate sensitivity. The high degree of hydration in scale (~30%) plays an important role to make the hydrated scale much more strain-rate sensitive than other biological materials. Table 3.3 summarizes the strain rate sensitivity parameter d and C for different biological materials as determined by other groups. [86,87,88,89,90,91,92]
Table 3.3 The comparison of parameters in all the other biological materials including rat-tail tendon, bovine cortical bone, horn, human femur bone, arapaima scale.

<table>
<thead>
<tr>
<th>Material</th>
<th>d</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat-tail tendon</td>
<td>0.1042</td>
<td>927</td>
</tr>
<tr>
<td>Bovine cortical bone</td>
<td>0.13</td>
<td>9112</td>
</tr>
<tr>
<td>Horn (longitudinal)</td>
<td>0.19</td>
<td>2760</td>
</tr>
<tr>
<td>Horn (transverse)</td>
<td>0.12</td>
<td>5216</td>
</tr>
<tr>
<td>Human femur bone</td>
<td>0.06</td>
<td>22545</td>
</tr>
<tr>
<td>Arapaima scale</td>
<td>0.26</td>
<td>1499</td>
</tr>
</tbody>
</table>

Figure 3.28 Elastic modulus of different biological materials plotted as a function of strain rate.
To describe the strain rate sensitivity of the strength, Arruda and Boyce used the following equation:

\[ \tau_{AP} = \tau_0 \left( \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \right)^m \] (3)

Where \( \tau_{AP} \) is the applied stress, \( \dot{\varepsilon} \) is the strain rate, \( m \) is the strain-rate sensitivity and \( \tau_0 \) and \( \dot{\varepsilon}_0 \) are the material parameters. Applying the Arruda-Boyce equation [93], the tensile strength as a function of strain rate can be written as:

\[ \tau_{AP} = 153.48 \left( \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \right)^{0.3464} \] (4)

Figure 3.29 (a) Elastic modulus and (b) ultimate tensile stress of Arapaima scales plotted as a function of strain rate
The energy absorption is also obtained by calculating the area under the strain–stress curve. The average energy absorption of wet scale is 4.48 J/m³ which is more than two times of that of the dry scale, which is 1.19 J/m³. This indicates that the wet scales exhibit viscoelastic behavior which requires more energy to break them. However, dry scales have higher elastic modulus but lower energy absorption showing a less pronounced viscoelastic behavior.

Table 3.4 The comparison of energy absorption in dry and wet scale.

<table>
<thead>
<tr>
<th></th>
<th>Energy absorption (J/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry scale</td>
<td>1.19+0.4</td>
</tr>
<tr>
<td>Wet scale</td>
<td>4.48+1.6</td>
</tr>
</tbody>
</table>
3.2.6.5 Arapaima Gigas scale: Morphology and fracture surface inspection

Figure 3.30 (a) is a photograph showing how the fish scales arrange. Using optical microscopy, the vein-like structure appears on the fish scale. The veins overlap partially and the white thin part is the internal part which is not exposed to water directly and is covered by the black thick part. The black thicker part is the external part which is mainly used for protection. The outer highly mineralized layer is also much thicker in the external region than internally. The SEM images of the junction of the external and internal parts show a different morphology in these two regions (Figure 3.31 (a)).

Figure 3.30 (a) Photograph of Arapaima scale showing the arrangement of the scale; (b) Optical micrograph showing the exposed part and embedded part with ridges structure.
Figure 3.30 continued

The Arapaima Gigas scale is composed of several layers we can clearly see the laminate structure from the optical micro-graph of a scale cross-section (Figure 3.35). From the cross-section perspective, the layer can be subdivided into two parts: the external layers and the internal layers. The cross-section cut was perpendicular to the moving direction of the Arapaima Gigas. The serration of the external layer cross-section indicates a groove-like surface along the scale. From the surface view, the fish scale can also be divided into two regions, the exposed part and the embedded part. The exposed part is black, thicker and rougher, whereas the embedded part is white, thinner and smoother. Both the exposed and the embedded parts have a ridge structure. The bigger ridges structure with a rough surface can be visually observed in the exposed part. On the
contrary, the ridge structure of the smooth surface is smaller and is not obvious in the embedded part. However, under the SEM inspection several small granule-like extrusions are distributed all over the fish scale.

The SEM images (Figure 3.31) indicate that the ridges site is smaller in the embedded part than that in the exposed part. The ridge structure has a certain orientation along the partial circle contour around the focus point at the junction of the internal and external parts.

Figure 3.31 SEM micrographs showing the surface of scale: (a) junction of embedded and exposed area; (b) exposed part; (c) embedded part with ridge structure.
The optical image (Figure 3.32) shows a typical fracture surface fractured by hand showing a laminate structure. The patterns on the image indicate that the collagen fiber bundle is oriented in different directions within different layers.

Figure 3.32 Optical micrograph showing the different orientation of collagen fibers in different layers.

Figure 3.33 (a, b) are the SEM images of different magnification of a polished cross-section of Arapaima scale exhibiting the lamellae structure. The internal layers of Arapaima scale arranged in both orthogonal and double twisted plywood patterns. The thickness of each layer is approximately 50-100 µm. Because of the dehydratation, cracks appear in each individual layer.
Figure 3.33 SEM micrographs of cross-section of scale (a) showing the laminate structure with thickness of 50 µm (b) showing the collagen fiber orient in the same direction in the same layer.
Due to the laminate composite structure, the fracture surface exhibits not only the breakage of the collagen fibrils but also the debonding of the laminae. The fish scales are mainly composed of type I collagen fibers and calcium-deficient hydroxyapatite which is also found in bones and teeth. The collagen fibers with the same orientation are parallel to each other to form the single layer of the scale. And several layers are tightly packed together with 90 degree difference in the orientation between each two layers to form the plywood structure which is a very common aligned structure in biological materials such as bones and some fish scales.[94] Figure 3.34 (a) is an overview of the fracture surface showing the debonding between multiple layers with different collagen orientation. At higher magnification, the aligned collagen fibrils were distorted as a result of the application of the tensile force. Figure 3.34 (c, d) clearly shows that the alignment of a single layer is orthogonal to the other adjacent layer.
Figure 3.34 SEM micrographs of fracture surface after tensile test: (a) overview of the fracture surface; (b) the collagen fiber network; (c),(d) twisted collagen fiber after tensile test.

Figure 3.35 is the optical microscope images of an Arapaima scale cross section. Two distinct layers, the external and internal, were shown in the images. The internal layer is also subdivided into several thinner layers.
3.2.6.6 Arapaima Gigas scale: the micro-indentation tests

The micro-indentation test results (Figure 3.36) show that the external layer is harder than the internal layers. The hardness value of the internal layer is 244.8 MPa and that of the external layer is 678.55 MPa which is more than two times higher than the hardness of the internal layer. Electron-dispersive spectrum (EDS) and EDS-mapping analyses were performed for both regions of the scale to analyze the element content and their distribution across the sample. Two spots, the external and internal layers were chosen for EDS. The EDS results show that the external layer has more calcium content.
than the internal layers. The main elements in the bone, calcium and phosphate have been mapped. In the mapping graph, the red dots represent the calcium element and the blue dots represent the phosphate element (Figure 3.41 (a, b)) which indicates that both calcium and phosphate contents decrease from the external layer to the internal layers. The higher hardness value in the external layer may be attributed to the higher calcium content in the external layer.

Figure 3.36 Plot of hardness values across the cross section of the scale.
3.2.6.7 Arapaima Gigas scale: Nanoindentation

Nanoindentation was conducted on the cross section of the scale with the multi-layer structure. Berkovich tip was used to measure the indent resistance including the elastic modulus and hardness through the different layers composing the individual scale. The load was applied at a rate of 50 µN/sec up to the maximum value of 500 µN on the cross-section of the scale and then unloaded.

Figure 3.37 illustrates the load and the depth curve for the nano-indentation; it shows the load and unload curves for the two different indentation sites: the external and internal regions of the scale. The red and black curves are the typical load and unload curves for the external region and the remaining three curves correspond to the internal layers. The unload curve does not follow the same path as the load curve. At the maximum load of 500 µN, the penetration depth of the external and internal regions is approximately 100 nm and 200 nm, respectively. The results show that at the same load value the internal region of the scale exhibit the higher penetration depth than the external region, which is consistent with the high mineral content in the external layer.
Figure 3.37 Typical load-unload curves for nanoindentation showing higher penetration depth in internal region than that in external region at max load.

The two distinct layers have significantly different mechanical properties. Both the hardness and the elastic modulus decrease from the external to internal layers. Nanoindentations were carried out in several sublayers in internal regions and the results from these sublayers do not show much difference. The nanohardness values obtained from the nanoindentation in external and internal layers are 2 GPa and 0.6 GPa,
respectively (Figure 3.39). The reduced elastic modulus \( E_r \) calculated from the stiffness determined by the slope of the unload curve of the external layer (46.8 GPa) is about 2.5 times of that of the internal layer (16.9 GPa) (Figure 3.38). All of the results indicate that the harder external layer protects the fish from sharp biting and puncture and the less hard multiple internal layers have a function of applied load energy dissipation.

![Graph showing elastic modulus comparison between external and internal regions](image)

Figure 3.38 The elastic modulus after nanoindentation in external and internal regions.
Figure 3.39 The nano hardness value in external and internal layers.

Table 3.5 Summary of nanohardness and elastic modulus in external and internal layers

<table>
<thead>
<tr>
<th></th>
<th>External</th>
<th>Internal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanohardness (GPa)</td>
<td>2.0 ± 0.4</td>
<td>0.6 ± 0.08</td>
</tr>
<tr>
<td>Elastic modulus (GPa)</td>
<td>46.8 ± 8.9</td>
<td>16.7 ± 4.0</td>
</tr>
</tbody>
</table>
Figure 3.40 (a) BSE image of the cross section showing the site for EDS; (b) EDS results showing the higher calcium concentration in external layer.

Figure 3.41 (a) Element mapping of calcium; (b) element mapping of phosphate.

Besides the element mapping and the EDS analysis, a line scan was also conducted throughout the cross section of the scale from the internal region to the external region to confirm the different element concentration in these two distinct layers. The SEM image in Figure 3.42 (a) is a cross section of the scale showing the internal layers and an external layer with cracks inside where the line element scan was carried out. Carbon,
oxygen, sodium, calcium and phosphorous which are the common elements in biological materials were also found in the scale. The plot of X-ray intensity versus the coordinate position, Figure 3.42 (b), indicates that sodium, carbon and oxygen concentrations have no difference across the scale. However, calcium and phosphorous concentrations are higher in the external layer than those in the internal layers. These results directly indicate that the high calcium and phosphorous concentrations render the high mechanical properties such as high hardness and nano-hardness value in the external region of the scale. The drop of intensity is due to the crack or gap between the layers, which block the signal from being absorbed by the detector.

Figure 3.42 (a) SEM image of cross section of scale;(b) plot of X-ray intensity vs. relative position
3.2.6.8 Arapaima Gigas scale: X-ray diffraction (XRD)

XRD was conducted on the polished external and internal surfaces. The two X-ray diffraction (XRD) patterns of external and internal surfaces of the Arapaima Gigas are shown in Figure 3.43. The black pattern is the internal surface and the red pattern is the external surface. The XRD peaks of the external region are more obvious than the peaks of the internal region, which can be attributed to the high degree of mineralization and crystallization. On the contrary, the peaks for the internal region are not as obvious as those for the external surface showing less crystallized material. The 2–theta scan starts from 20 degree to 60 degree. The peaks were found at 2 theta equal to 25.96°, 31.9°, 39.85°, 47.2°, 49.38°, and 53.28° which corresponds to d spacing of 0.343, 0.281, 0.226, 0.193, 0.185, and 0.172 nm according to the Bragg’s law. The results are similar to Torres et al.79 who reported that the peaks at 25.8°, 31.8°, 39.6°, 47.2°, 49.3°, 53.1° correspond to d spacing of 0.345, 0.281, 0.227, 0.192, 0.184 and 0.172 nm. The breadth of the peaks of the XRD pattern indicates that the scale has low crystallinity.
3.2.6.9 Arapaima Gigas scale: Fourier Transform infrared spectroscopy (FTIR)

FTIR is a technique used to identify the compounds and determine sample composition based on the specific resonant frequency absorbed by the molecules. FTIR can be also conducted on the scale to verify whether the scale consists of the type I collagen. The FTIR spectra (Figure 3.44) showed some absorption peaks between wave
numbers 600 to 2000 cm$^{-1}$. These peaks are 1637, 1547, and 1239 which corresponds to Amide I, Amide II, and Amide III of type I collagen, respectively. In addition, 869, 1401, and 1450 peaks were also observed which agrees well with the previously reported data [72]. These peaks are attributed to the presence of carbonate anions. These results indicate that the fish scale consists of organic (type I collagen) and inorganic components (carbonate anions substitute apatite).

Figure 3.44 Infrared spectrum of Arapaima scale showing amide I, II, and III bands from type I collagen fibers.
3.2.6.10  Piranha tooth vs. Arapaima scale (penetration test)

Piranha and the Arapaima can co-exist in the Amazon River. A penetration experiment was designed to test the relative strength of piranha tooth and of Arapaima scales.

A Piranha tooth was attached to an upper punch connected to INSTRON test machine through a small cylindrical steel rod. And the Arapaima scale was glued onto a 10 mm thick rubber and then fixed to the lower punch connected to the machine. The penetration test was carried out on the two regions of the scale: exposed black region and the embedded white region. The scales were in wet condition under the test. A cross head speed of 0.06 mm/min was used in this experiment.

The results show that the Piranha tooth can successfully penetrate into both the exposed dark and embedded white regions. Figure 3.45 shows the force-displacement curve of the penetration into the embedded white portion and a simple sketch of the experimental setup. The force needed to penetrate the embedded white part is approximately 30 N. The three pictures besides the force displacement curve are the initial, middle and final stages of the penetration.
Figure 3.45 The force-displacement curve of penetration test on embedded region of scale showing the force required to penetrate the scale is about 30 N.

Figure 3.46 shows the force displacement curve for the penetration into the exposed black part including a typical broken piranha tooth after loading. The drop points in the curve indicated by the arrows are the fracture locations of the piranha teeth.

Both the slope of the load displacement curve in the exposed black region is higher than in the embedded part and the force required to penetrate the exposed black portion is higher than the force needed to penetrate the embedded white part, which can be attributed to the higher mineral content and larger thickness of the exposed region.
Although Piranha teeth penetrated the two regions of the scale, they were either broken during the penetration or fractured after being brought back to their original position. Three high speed camera pictures exhibit the penetration of the tooth into the scale and the shatter of the tooth after removing it from the scale. It is likely that the tooth was seriously damaged before full penetration resulted in the fracture after retrieving it from the scale.

Figure 3.46 The force-displacement curve of penetration test on exposed part of Arapaima scale showing the shatter of the piranha tooth.
3.2.6.11 Arapaima Gigas scale: SEM characterization of demineralized scale

The samples were characterized by SEM to investigate the structure of the Arapaimas scale after demineralization (Figure 3.47). Figure 3.47 are SEM micrographs of demineralized Arapaimas scales from macro to nano size. Collagen fibers, the main components of the scale exhibit 67-nm periodic structure after removing the mineral from the sample.

Figure 3.47 SEM micrographs of demineralized scale showing collagen fiber with typical periodicity.
3.3 Natural dental materials’ characterization: Conclusion

The conducted mechanical and microstructure characterization revealed a number of material design principles, which can be utilized in the manufacturing of bio-implants. One of such principles is the functionally graded structure organization (Figure 3.2) Our analysis clearly showed that this type of structure possesses higher resistance to crack propagation.

Another principle is the presence of special embedded structure elements such as oriented tubules (Figure 3.14) and fibrils (Figure 3.34), which increase the material strength and fracture toughness and at the same time can be used for other functional goals, such as material (liquids) transport through the dental structure. These material design ideas are utilized in the next part of this research project dedicated to the manufacturing of composite dental implants by spark-plasma sintering.
Chapter 3, in part, is a reprint of the material as it appears in “Mechanical properties and the laminate structure of *Arapaima gigas* scales”, in Journal of Mechanical Behavior and Biomedical Materials, 2011,v.4, p1145-1156, Lin, Yen-Shan; Olevsky, Eugene A.; and Meyers, Marc A. The dissertation author was the primary investigator and author of this paper.
Chapter 4 Spark-Plasma Sintering of Hydroxyapatite-based Composites

4.1 Material systems of HAP-based composites and green specimen preparation

Hydroxyapatite powders and multi walled carbon nanotube powders were chosen to be used in the HAP-based composite. Hydroxyapatite powders have good biocompatibility and usually utilized in biomedical applications. Carbon nanotubes drew people’s attention due to their remarkable mechanical properties, also CNTs is a novel material to reinforce HAP composite.

Hydroxyapatite powders were purchased from Alfa Aesar and carbon nanotube powders purchased from Cheap Tube Inc. The melting point of HAP is 1670°C and the density is 3.14 g/cm³. The inner diameter and out diameter of the carbon nanotube are 20-30 nm and 5-10 nm, respectively. The density of the CNT powder is 2.1 g/cm³ and the length of the CNT is 10-30 µm. The specific area of the MWNTs is 110 m²/g.

The initial particle size of the sintering powder is very important. The initially unknown particle size of the hydroxyapatite powder purchased from Alfa Aesar was characterized by SEM. Figure 4.1 (a,b) shows the HAP particle as received from the commercial company at low and high magnifications. HAP powders tend to agglomerate
and the average particle size is about 0.09 µm.

Figure 4.1 (a, b) SEM micrograph of hydroxyapatite raw powder showing the particle size of 0.09 µm.
The dies with outer diameter of 30mm and inner diameter 10.4 mm or 15.4 mm were used in this project. For the 15.4 mm die, the total specimen volume is $0.5 \times 3.14 \times (0.75)^2 = 0.883125 \text{cm}^3$; after calculation, the amount of the 2vol% CNT and the 98vol% HAP we used are determined to be 0.03709g and 2.7175g, respectively. The mixed powders were placed into the beaker with 80 ml isopropyl alcohol. The beaker was placed into the ultrasonication bath to ultrasonicate for 30 minutes accompanied by hand mechanical stirring. Then the solution was subjected to ball milling with three clorina grinding balls for 2 days.

Next, the slurry underwent a freeze drying process. Freeze drying is a dehydration process applied to a frozen material under reduced pressure to sublime the water directly from the material without passing through a liquid phase. Freeze drying can provide a good approach removing the liquid form the well-dispersed powder slurry to generate more homogenously mixed powder.

In order to avoid the powder sedimentation, the container with the slurry was put into a liquid nitrogen bath for quick freezing. It was important not to introduce too much slurry in the container because quick freezing of a large amount of slurry is difficult which may result in the failure of the freeze drying. The container was then introduced in
the freeze drying device. The temperature of the freeze drying should be -50°C and the proper pressure is less than 20 Pa.

Besides using the ball milling to mix the HAP and CNT powders, a powerful high ultrasonicator, Branson model 400 L series was also used to disperse HAP and CNT powders. Different volume percentages of CNT were prepared to investigate how the mechanical properties change with respect to the variation of CNT concentration. Three different volume concentrations of carbon nanotube were prepared by the high ultrasonication followed by freeze-drying. The HAP and CNT powders were placed into the 50 ml beaker and 25 ml of de-ionalized water was used as dispersion. After initial mechanical stirring, the ultrasonication device with an ultrasonication tip was inserted into the HAP-CNT solution to disperse the two phase powder by vibrating. It took three minutes to mix the powder and produce a well mixed slurry.

The CNT and HAP slurry was poured into a plastic container and liquid nitrogen was used to freeze the slurry. After 20 min freezing, the plastic container with the solid HAP and CNT mixture was introduced in the freeze drying device for one day to sublimate the water from the slurry and preserve its well-mixed condition.

The mixed powders were inserted into the 15.4 mm die with a carbon paper wrap
between the die and powders and pre-pressed by the cold pressing before spark-plasma sintering (SPS) or conventional sintering processes. Prior to SPS processing, two round carbon papers were also placed on the top and bottom sides of the specimen before inserting the punches. The main purpose of inserting the carbon paper was making easier the ejection of the consolidated specimen.

### 4.2 Consolidation of HAP-CNT composites

CNT-HAP composites with various volume fractions of CNT were processed by spark-plasma sintering and, for the purpose of comparison, by the means of the conventional sintering too.

#### 4.2.1 Consolidation of pure HAP powder: Conventional sintering

1g of HAP powder was placed into a steel die with outer diameter of 38.1 mm and inner diameter of 12.5 mm. The steel punch was inserted into the steel die to compress the loose powder. The W40 lubricant was sprayed on the die walls and the space between the punch and the steel die to reduce the friction between the walls and the punch. Then the whole set was pressed by the cold pressing in a rigid die with a pressure of up to 7000 pounds (253 MPa). And the pressure was held at 253 MPa for 3 minutes to produce a cylindrical green specimen. The green specimen density was measured by assessing the
specimen’s dimensions and mass. The HAP green specimens were sintered at different maximum sintering temperatures of 1000°C, 1100°C, and 1200°C.

Tube furnace (Thermolyne 79300) was used to sinter the HAP powders with flushing atmosphere of N₂. After loading the specimen into the tube, the N₂ was added into the tube for 30 min to flush the air out of the tube with the flowing rate of 600. The ascending heating rate was 10 °C/min up to the target maximum temperature and the holding time was 3 hours followed by 10 °C/min cooling to room temperature.

Relative density measurement, micro-hardness, and microstructure analysis were conducted for the obtained sintered HAP specimens. Relative density was measured by Archimedes method. All of the specimens were grinded with 180 sand paper and then their relative density was measured again based on the geometric calculations coupled
with mass conservation principle. In order to have a polished surface to measure the hardness of the specimens, the specimens were grinded with a series of sand papers 180, 500, 1200, 2400, 4000 and polished by two Al₂O₃ solutions with two different particle sizes of 0.3 and 0.05 µm.

Micro-indentation was conducted on the HAP specimens with 500N load. Ten indentations were carried out on each specimen to get the average hardness value. The space between the indentation points was more than 5 times of the indentation size to prevent the interference.

4.2.2 Consolidation of pure HAP powder: Spark-Plasma sintering

Spark plasma sintering is an emerging sintering technology (see review of the literature on SPS of hydroxyapatite powders in section 1.2.1). Spark plasma sintering provides many advantages over conventional sintering including high heating rates, the possibility of sintering at lower temperatures, and grain size retention. The high heating rate of the spark plasma sintering enables reaching the target maximum temperature in a short period of time, which allows the reduction of the grain growth. For conventional sintering, it usually takes several hours to sinter specimens to full density. However, it only takes several minutes to achieve fully dense materials in SPS.
The spark plasma sintering machine utilized in the present project was Dr. SINTER® LAB Series model SPS-511S/515S. Maximum pulse direct current output 1000A can be generated in the device between the two electrodes passing through the sample to produce Joule heat. The machine is capable of producing 50 kN force and maximum current can reach up to 1500A.

Figure 4.3 is the photograph of the spark plasma sintering machine and Figure 4.4 is the sketch of the spark plasma sintering system showing the component of the spark-plasma sintering device. Spark plasma sintering utilizes the on-off direct current power supply to generate current going through the conductive punches and the die, and through the specimen, if it is conductive. The SPS machine maintains the temperature regime in accordance with the temperature cyclogram assigned by a user. The powders or green specimens are placed into the graphite die enclosed with two graphite punches. The whole set is placed between the electrode plates which are capable of applying pressure through the spacers and placed in the vacuum chamber with the inner diameter of 200 mm. If the powder is conductive, the current will go through the sample itself as well as through the graphite die and punches. But for a non-conductive specimen material, the current will not pass through the sample. The white arrows in the sketch (Figure 4.4)
show the path of the current. Both the graphite die and the conductive sample are heated up by the Joule heat generated by the electric current. The experiment can be conducted in vacuum or inert gas such as Argon depending on the sintering temperature and on the specimen’s material.

Figure 4.3 Photograph of the spark-plasma sintering machine.
4.3 Characterization of the consolidated HAP-CNT composites: Results

4.3.1 HAP and HAP-CNT specimens processed by conventional sintering

The following results include the micro-hardness and final relative density measurements. The fracture surfaces of the specimens were investigated by SEM. Figure 4.5 shows the final relative density of HAP specimens versus different sintering
temperatures. The relative density increases with the increase of the sintering temperature (Figure 4.5). The highest relative density of 95.5% is obtained at the maximum sintering temperature of $1200\,^\circ\text{C}$ among the three other maximum temperatures of $1100\,^\circ\text{C}$ and $1000\,^\circ\text{C}$. Both the HAP and CNT-HAP had similar green density (45-50%) before sintering, however, the final after processing relative density of HAP with the addition of 1vol% CNT represented by the red dots is much lower than the final relative density of the processed pure HAP specimens. Because CNTs are unstable and dissociated under such a high temperature affecting the specimens for a long period of time under conditions of conventional sintering, the CNT-HAP composite has more defects and voids, which results in a porous structure with lower relative density. There is a 24% relative density difference between HAP and CNT-HAP-based specimens obtained at $1200\,^\circ\text{C}$ maximum sintering temperature.
Figure 4.5 Plot of relative density vs. sintering temperature showing the relative density increase as the maximum sintering temperature increase.

The micro-indentation was carried out on the samples obtained under different sintering temperatures (Figure 4.6). Both the hardness values of HAP and 1%CNT-HAP composites increase with the increasing sintering temperature. The hardness values change from 174 GPa for maximum sintering temperature of 1000°C to 460 GPa for maximum sintering temperature of 1200°C for pure HAP specimens. The fact that the HAP specimens without the addition of CNTs exhibit much smaller hardness values than HAP results from the defects which are caused by the thermal disassociation of CNTs. Therefore, the addition of CNTs does not necessarily reinforce the HAP matrix but can also impede the consolidation of CNT-HAP powders under the slow conventional
sintering conditions. The average hardness values of the processed CNT-HAP specimens are 25, 48, and 73 GPa at sintering temperature of 1000°C, 1100°C, and 1200°C, respectively.

Figure 4.6 Hardness values of HAP and CNT-HAP composite vs. sintering temperature.

Microstructure and average grain size of the HAP specimens and specimens based on HAP with CNT addition were investigated by the SEM of their fracture surfaces. Figure 4.7 are the SEM images of the fracture surface of HAP specimens and specimens based on HAP with CNT addition. The left part are the SEM images of the specimens based on HAP with CNTs showing the porous structure due to the thermal disassociation
of CNTs and the right hand side are the pure HAP specimens’ SEM images showing much denser microstructures.

Figure 4.7 (a-c) SEM micrographs of CNT-HAP composite fracture surface showing pores due to the dissociation of CNT at high temperature; (d-f) SEM micrographs of HAP fracture surface showing a fully dense structure.
The grain sizes of the HAP at different sintering temperature ranged from 1000 to 1200°C are measured by averaging ten random grains from the fracture SEM images. The grain size of HAP sintered at 1000, 1100 and 1200°C are 0.145 µm, 0.325 µm and 0.82 µm, respectively. Figure 4.8 is the plot of grain size vs sintering temperature showing that the grains grow rapidly as the sintering temperature increases from 1100°C to 1200°C. The significant grain growth from 1100°C to 1200°C apparently results from the disappearance of the grain boundary pore pinning mechanism. The relative density at 1200°C is about 95.5% which exceeded the critical relative density when pore pinning mechanism diminishes. Chang et al. [95] reported the considerable grain growth from 410 nm to 765 nm when the sintering temperature increased from 1100°C to 1150°C. However the relative density only increased from 97.74% to 99.03%. Their result is similar to what we observed the significant grain growth occurred above 1100°C.
Figure 4.8 Plot of grain size vs. sintering temperature showing the grain growth when the sintering temperature increases.

4.3.2 HAP and HAP-CNT specimens processed by SPS: density and microstructure

4.3.2.1 Processed pure HAP specimens

Pure HAP specimens were sintered by spark plasma sintering at different maximum temperatures ranging from 700°C to 900°C. The processing temperature regimes are shown in Table 4.1. Temperature was ramped up to 700°C with heating rate of 100 °C/min and then the heating rate decreased to 50 °C/min to reach 800°C or 900°C. The holding time for the maximum temperature was 5 mins followed by the cooling with 50°C/min rate down to 600°C. After 600°C, the specimen cools with natural cooling rate.
Table 4.1 Temperature profile of HAP powder processing by spark-plasma sintering

<table>
<thead>
<tr>
<th>Sintering temperature</th>
<th>100C/min</th>
<th>50C/min</th>
<th>Holding 5mins</th>
<th>Cooling 50C/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>700C</td>
<td>0-700C</td>
<td>700C</td>
<td>600C</td>
<td></td>
</tr>
<tr>
<td>800C</td>
<td>0-700C</td>
<td>700-800C</td>
<td>800C</td>
<td>600C</td>
</tr>
<tr>
<td>900C</td>
<td>0-700C</td>
<td>700-900C</td>
<td>900C</td>
<td>600C</td>
</tr>
</tbody>
</table>

Archimedes method was used to calculate the density. Specimens sintered at 700°C and 800°C had almost the same relative density (approximately 98.9% and 98.6%, respectively). But for the sintering temperature of 900°C, the relative density slightly decreased to 97.8%.

Another way to determine the relative density is based on the Z-axis displacement recorded by the SPS machine. Figure 4.9 and Figure 4.10 show the relative density vs. time plots for spark-plasma sintering of hydroxyapatite at 700°C and 800°C. The relative density was calculated taking into consideration the thermal expansion of the graphite die and punches. The plot indicates substantial densification happening at about 650°C and then vanishing at about 700°C. After the significant densification, the Z-axis position does not change during the holding time apparently due to the already reached fully dense state of the specimen. When the system starts to cool, the Z-axis position continues to change due to the thermal contraction. According to the calculations based on the
Z-axis change, both sintering temperature profiles lead to fully dense specimens. The relative density of the specimens processed at sintering maximum temperatures of 700°C and 800°C are 99% and 98%, respectively.

Figure 4.9 The relative density over time evolution at SPS temperature of 700°C.
Figure 4.10 The relative density over time evolution at SPS temperature of 800°C.

Micro-indentation was conducted for the specimens obtained under three different sintering maximum temperatures. 500 N load was used to indent the specimens. The average hardness value of the specimens obtained under sintering maximum temperatures of 700°C, 800°C and 900°C are 4.3 GPa, 4.1 GPa and 3.9 GPa, respectively. The results show that the hardness value decreases as the sintering maximum temperature
increases. The relative densities of 98.9%, 98.5%, and 97.8% correspond to sintering temperatures of 700°C, 800°C and 900°C.

Figure 4.11 The microhardness of HAP at different SPS temperature ranged from 700 to 900°C.

According to the literature\textsuperscript{45}, the best mechanical properties of HAP specimens can be obtained at the sintering maximum temperature of 950°C. However, our results do not show the highest hardness values for the specimens processed at the sintering maximum temperature of 950°C. Figure 4.12 (a,b) and Figure 4.13 (a,b) show a fracture surface of
the HAP specimen sintered at 800°C and 900°C. At sintering maximum temperature of 900°C, the size and shape of the grains of the processed specimens vary a lot. On the other hand, the grain size and shape of the processed specimens sintered at 800°C are uniform. At 800°C sintering maximum temperature, the grain shape of the processed specimens is regular and close to a spherical geometry. However, at 900°C, the grain shape of the processed specimens is irregular and of a more faceted shape. Both the SEM images (Figure 4.12 (a) and Figure 4.13(a)) show some pores inside the material. Base on the SEM image, HAP powder sintered at 700 and 800°C has much smaller grain size than the powder sintered at 900°C. The grain growth occurs when the sintering temperature is at or above 900°C.

![SEM images](image1.png)

Figure 4.12 (a, b) SEM micrographs of fracture surface of HAP produced by spark-plasma sintering temperature at 800°C
Figure 4.13 (a, b) SEM micrographs of fracture surface of HAP produced by spak-plasma sintering temperature at 900°C.

The grain size versus SPS temperature was plotted (Figure 4.14) showing the grain growth. HAP sintered at 700 and 800°C via SPS exhibit the same grain size which is around 0.1 µm. However the grain size increases to 0.69 µm at SPS temperature of 900°C. The addition of CNT in HAP sintering at 900°C reduces the grain size from 0.69 µm to 0.41 µm which is a way to prevent undesirable grain growth during sintering.
Figure 4.14 Plot of grain size vs. SPS temperature showing both CNT-HAP composite and HAP grain growth as the SPS temperature increases.

Most commercial CNTs are synthesized by CVD which has the advantage of producing a large amount of CNTs with low cost. However, CNTs produced by CVD have more impurities for example amorphous carbon, carbon nanoparticles and catalyzer particles. These impurities can affect the dispersion of carbon nanotubes within other matrix powder. Feng et al. [96] reported that CNTs treated with the reflux within the nitric acid can purify and disperse the tangled CNTs in aluminum powders.
4.3.2.2 Processed calcinated HAP specimens

A calcinated HAP powder under different calcination temperature was also consolidated by the process of spark plasma sintering. HAP powder was calcinated at 700°C, 800°C, 900°C, 1000°C and 1100°C with heating rate of 5 °C/min up to a target temperature and with holding time of 4 hours. XRD was conducted for the SPS-processed HAP specimens whose powder was treated at different calcinations temperatures. The XRD pattern shown in Figure 4.15 indicates that no TCP transformation occurs with the increase of the calcination temperature. With the increased calcination temperature, the intensity of the diffraction peaks increases showing the increase in crystallinity.
Figure 4.15 XRD pattern of HAP powder at different calcination temperature showing that the crystallinity becomes obvious as the calcination temperature increases.

The relative density-time evolution curves show that the HAP powder with higher calcination temperature have the densification starting at the higher temperatures. For example, HAP powder calcinated at 1100°C could not be sintered to fully dense state below 900°C. However the full density is achievable when sintering to 1200°C.

Figure 4.16 shows the results of the micro-hardness measurement for the specimens made from powders treated at different calcination temperature. All the calcinated HAP
powders were spark plasma sintered at 900°C. The hardness value of the specimens made from powders calcinated at 800°C, 900°C, and 1000°C are 340, 354, and 387 MPa, respectively. Therefore, the hardness of the specimen gradually increased with the increase of the calcination temperature of the HAP powder. Hon et al. [37] reported that four point bending strength also show higher value for higher calcination temperatures. The highest 4-point bending strength values were obtained at conventional sintering temperature at 1250°C and for as-received, 700°C, 800°C, and 900°C calcinated HAP powders with 40.5, 42.4, 49.6 and 54.8 MPa, respectively. The calcination temperature can improve the mechanical properties of the sintered HAP.
4.3.2.3 Processed HAP-CNT specimens based on acid-treated powders

Acid treatment was applied to the multiwalled commercial CNTs. 69.8% weight concentration of Nitric acid was diluted to 50 and 25 %. Different amounts of CNTs were purified to fabricate CNT-HAP composites with different volume fractions (0.5%, 1%, and 3%) of CNTs. A certain amount of CNTs was placed in the beaker and mechanical stirring with nitric acid was conducted for 10 min. The powder was then immersed in the nitric acid for 8 hours to purify the CNTs. The acid-treated CNTs were mixed with HAP powder and then subjected to the spark plasma sintering. The SEM images (Figure 4.17)
show the different acid treated CNTs concentrations at sintering temperatures of 700°C and 900°C. One can observe a significant grain growth for the sintering temperature of 700°C to 900°C. And the CNTs agglomeration is noticeable.

Figure 4.17 SEM micrographs of CNT-HAP composite with acid treated CNT showing the agglomeration of CNT.

4.3.2.4 Processed HAP-CNT specimens based on ultrasonicated and freeze-dried powders

Different volume concentrations of the CNTs (1 vol%, 2 vol%, and 4 vol%) were mixed with HAP powder to fabricate HAP-based composites by high intensity
ultrasonication, freeze drying process, and SPS. The results show (Figure 4.18) that some mechanical properties of CNT-HAP composites decrease with the increased amount of CNTs. The hardness value for composites with 1 volume % CNT is 3.7 GPa which is higher than that of composites with 4 vol % CNT (3.2 GPa) processed at maximum SPS temperature of 800°C. As the maximum SPS temperature increases, the hardness of the composite with 1 vol% CNT (3.05 GPa) is still higher than that of with 4 vol% CNT (2.99 GPa). However, the difference between hardness values of composites with 1% and 4% CNT concentrations processed at SPS maximum temperature of 900°C is smaller than that for the same composites processed at 800°C. The difference decreases from 0.5 GPa to 0.06 GPa which indicates that the CNT concentration does not significantly influence the hardness of the composites at higher sintering temperatures. However, the hardness of 2 vol% CNT-HAP composite enhances when the maximum sintering temperature increases from 700°C to 800°C.
Figure 4.18 The hardness value of different vol% CNT-HAP composite vs maximum SPS temperature

Micro-indentation with a load of 500 N was conducted on CNT-HAP composites with CNT concentrations ranging from 0 to 4 vol% produced by SPS at maximum temperature of 900°C. Figure 4.19 shows the hardness values as a function of CNT concentration indicating that the hardness values increase with the increase of the CNT concentration from 0 to 2 vol%. The hardness values of pure HAP, 1 vol% CNT-HAP, 2 vol% CNT-HAP are 3.50, 3.81, and 4.26 GPa, respectively. The hardness value starts to drop to 3.61 GPa when the CNT concentration reaches 4 vol%. Therefore the increase of CNT concentration does not always enhance the mechanical properties of the CNT-HAP composites. This result indicates that there should be a critical concentration of CNT
which achieves the highest hardness value of the CNT-HAP composites. Our experiments indicated that the critical CNT-HAP concentration is 2 vol%. Li et al. [97] also reported that the nanoindentation hardness of HAP-CNT composites increase with the increasing addition of CNT. But to critical CNT concentration, 5wt%, the hardness starts to drop. Therefore a small addition of CNT can enhance the hardness of HAP.

![Graph showing microhardness vs. different vol% of CNT in HAP-based composites sintered at 900°C](image)

**Figure 4.19** Microhardness vs. different vol% of CNT in HAP-based composites sintered at 900°C

The SEM images in Figure 4.20 are the indentation site of the 2 vol% CNT-HAP composite. Figure 4.20(a) is the typical indentation site after micro indentation with a load of 500 N showing the Vickers indentation square geometry. We can see that the main four cracks propagate outward from the edge of the square. At higher magnification, the carbon nanotubes can be clearly observed not only in the square but also in the crack
gaps in the CNT-HAP composite. Figure 4.20 (c), (d) are the images, which focus on the crack path showing that the carbon nanotubes with a diameter of 50nm between the crack edges have the potential to bridge the crack. The crack bridge is one of the common toughening mechanisms in materials especially in biological materials. Carbon nanotubes in the CNT-HAP composites should play the same role as the collagen toughening biological materials.

Figure 4.20 SEM images of an indentation site of 2vol%CNT-HAP composite sintered at 900°C (a) Typical indentation site; (b) higher magnification of indentation site; (c, d) micrographs showing nanotubes between the crack path.
Figure 4.20 continued
Figure 4.20 continued

SEM images (Figure 4.21(a, b)) are the fracture surface after tensile rupture test.

SEM image of lower magnification shows the different geometry of the grain and small white dots are the CNTs. At higher magnification, CNTs with the diameter of 30-40 nm are distributed in the HAP matrix.
Figure 4.21 SEM micrograph of fracture surface of 1 vol% CNT-HAP composite sintered at 900°C after tensile rupture test (a) low magnification; (b) high magnification showing the CNT embedded in the HAP matrix.

Figure 4.22 is the XRD pattern of 1 vol% CNT-HAP composite. These peaks are typical hydroxyapatite diffraction peaks and the addition of CNTs does not change the XRD patterns. The XRD results indicate that the measurement is not sensitive enough to
detect CNTs.

Figure 4.22 XRD pattern of 1vol%CNT-HAP composite showing same peaks as pure HAP

4.3.3 HAP and HAP-CNT specimens processed by SPS: Tensile rupture testing (TRS)

Tensile rupture test [98] was used to measure the rupture strength of cylindrical compacted specimens. The disk-shaped samples were placed on the cylindrical steel fixture (Figure 4.23) which had a 15.2 mm diameter hole and fractured by a tungsten carbide ball-shaped indenter attached to the compression machine. Figure 4.23 is the picture of the tensile rupture strength test setup.
The following is the equation used for calculating the tensile rupture strength for our cylindrical geometry of specimen [99]:

\[
\sigma_{\text{max}} = \frac{P}{h^2} \left[ (1 + \nu) \left( 0.485 \log \frac{a}{h} + 0.52 \right) + 0.48 \right]
\]  

where \( P \) is the maximum force at the fracture, \( h \) is the thickness of the specimen, \( \nu \) is the Poisson’s ratio, \( a \) is the radius of the cylindrical sample and \( \sigma_{\text{max}} \) is the tensile rupture strength.

The tensile rupture strength of 1% CNT-HAP composite exhibits higher value than that of 4% CNT CNT-HAP composite obtained at SPS maximum temperature of 800°C.
However, the 4%CNT_HAP composite processed at 900°C shows higher tensile rupture strength than 1%CNT-HAP composite. The tensile rupture strength of both 1% and 4% CNT-HAP composites drops below 80 MPa at 900°C SPS temperature.

### 4.3.4 HAP and HAP-CNT specimens processed by SPS: Vickers macro hardness tests

Vickers hardness test was also conducted on the HAP-based composites. The test load was 20 kgf. The Vickers hardness was determined by the ratio of force and the indentation area, F/A. The Vickers hardness value can be calculated using the following equation:

\[
HV = \frac{F}{A} = \frac{F}{0.5d^2} = \frac{1.854F}{d^2} \sin\left(\frac{136}{2}\right) \tag{6}
\]

where F is the applied force, A is the projected surface area of the indentation and d is the average diagonal length of the square made by the indenter. Six positions on each sample were randomly chosen for macro-indentation. The results show that the Vickers hardness slightly increases from 320 to 340 kgf/m² after we add 2vol% of CNT. The hardness also enhances from 340 to 399kgf/m² when we increase the sintering temperature from 700 to 800°C in the 2 vol% CNT-HAP composite.
Table 4.2 Vickers hardness values of HAP and CNT-HAP composites

<table>
<thead>
<tr>
<th></th>
<th>d (mm)</th>
<th>HV(kgf/ m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP 700C</td>
<td>339.5</td>
<td>320</td>
</tr>
<tr>
<td>2%CNT 700C</td>
<td>330</td>
<td>340</td>
</tr>
<tr>
<td>2%CNT 800C</td>
<td>309</td>
<td>388</td>
</tr>
</tbody>
</table>

As we increase the sintering temperature, the grain growth becomes very obvious. However, based on the SEM images taken under the same magnification and for the specimens obtained under the same SPS temperature of 1200°C CNT-HAP composites have much smaller grains than pure HAP compacts which is an indication of the CNT capability to inhibit grain growth at higher sintering temperatures. The fracture surface of the 2 vol% CNT-HAP composite shows some nanotubes with the diameter of 10 nm distributed in the HAP matrix (Figure 4.21). Some pores also can be seen in the HAP matrix. On the contrary, the microstructure of pure HAP specimen made from powder subjected to ball milling or heat treatment exhibits less porous structure, with the grain size, however, which is much larger than that of the 2 vol%CNT-HAP composite. We can also see a considerable grain growth when we increase the sintering temperature from 900°C to 1200°C. The micro-hardness value of CNT-HAP composite is less than 1 GPa,
however, the hardness value of the consolidated pure HAP is about 3 GPa which is higher than that of CNT-HAP composite. The more porous structure in CNT-HAP results in the lower mechanical properties.

4.3.5 HAP and HAP-CNT specimens processed by SPS: Nanoindentation tests

Nanoindentation was carried out to compare the mechanical properties of spark-plasma sintered at 900°C CNT-HAP composites with different amounts of CNTs. AFM was used to investigate the morphology of the polished surface and find the most flat site for nanoindentation (due to the roughness of samples). The roughness of samples may cause the inaccuracy of the measurements. The maximum load for the nanoindentation was 1000 µN with a loading rate of 50 µN/min. The reduced elastic modulus and hardness value of 2 vol% CNT-HAP specimens, 0.5 vol% CNT-HAP specimens, and pure HAP specimens were measured. Table 4.3 summarizes the results of the measurement of the reduced elastic modulus and hardness. Both the reduced elastic modulus and hardness increase with the increased concentration of CNT. The hardness and elastic modulus values of 0.5 vol% CNT-HAP are only slightly different from those of the pure HAP specimens; however, for the 2 vol% CNT-HAP specimens there is a significant improvement in the elastic modulus and hardness. The elastic modulus and
hardness increase from 67.7 to 125.67 GPa and from 6.59 to 9.2 GPa, respectively.

Table 4.3 Mechanical properties of CNT-HAP composite and HAP specimens sintered at 900°C measured by nanoindentation.

<table>
<thead>
<tr>
<th></th>
<th>HAP</th>
<th>0.5vol%CNT-HAP</th>
<th>2vol%CNT-HAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic modulus(GPa)</td>
<td>67.7</td>
<td>71.43</td>
<td>125.67</td>
</tr>
<tr>
<td>Hardness(GPa)</td>
<td>6.59</td>
<td>7.86</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Figure 4.24 Plot of elastic modulus of HAP and CNT-HAP composites sintered at 900°C by nanoindentation.
Figure 4.25 Plot of nano-hardness value of HAP and CNT-HAP composite sintered at 900°C by nanoindentation.

Figure 4.26 shows a typical load-unload curve for nanoindentation indicating that the unload curves do not follow the same path as the loading curve. At the maximum load of 1000 µN, 2vol%CNT-HAP specimens have smaller indentation depth (~72 nm) than that of pure HAP specimens (~98 nm), which indicates that the addition of CNT reinforces the composite to resist the indentation.
4.3.6 Compression tests on HAP and CNT-HAP composites produced by FPSPS

Figure 4.27 shows the results in the form of the stress-strain curves of the compression tests conducted for the HAP, 2vol% CNT-HAP and 6vol%CNT-HAP composites. The compressive strength of 2vol% and 6vol%CNT-HAP composite are found to be 143.9 MPa and 131.6 MPa, respectively. The compressive strength of the both CNT-HAP composites are higher than that of the pure HAP specimens (which is
determined to be of 70.6 MPa. However the Young’s modulus of 2 vol% and 6 vol% CNT-HAP composites is found to be about 6961 MPa which is smaller than that of the pure HAP specimens (8221 MPa). The lower Young’s modulus of the CNT composite may be attributed to the more porous structure in the more difficult-to-densify composite (due to the higher CNT concentration). The densities of the green specimens after pre-pressing by the SPS machine may be different in pure HAP and CNT-HAP composites. Also some air may still remain in the gaps between the CNT and HAP particles, which may result in the lower Young’s modulus. Based on the stress-strain curve, the CNT-HAP composites show about three times higher compressive strain compared to the pure HAP material.

The results included in Figure 4.27 show that at the highest compressive stress, the stress-strain curve for pure HAP material drops immediately, which indicates brittle properties of the HAP ceramics. On the contrary, at the highest compressive stress, CNT-HAP composites show some fluctuation of the stress-strain response, which means that the presence of CNTs increases the material toughness and postpones the fracture.
Figure 4.27 Typical stress-strain curves of HAP and 4vol% CNT-HAP composites obtained in compression test.

Figure 4.28 are the SEM images of the fracture surface of pure HAP and CNT-HAP composites after compression tests. The CNTs are distributed uniformly in the HAP matrix and the diameter of the CNTs is about 50nm. The microstructure shows that the pure HAP has much more dense structure than that of the CNT-HAP composite and the relative density decreases with the increase of the CNT concentration.
Figure 4.28 SEM images of fracture surface of CNt-HAP composite sintered at 1300°C by FPSPS after compression tests: (a) pure HAP; (b) 2 vol% CNT-HAP composite; (c) 6 vol% CNT-HAP composite; (d) higher magnification showing the CNT with the diameter of 50 nm
4.4 Comparison of HAP specimens sintered by conventional sintering and by Free Pressureless Spark Plasma Sintering (FPSPS)

4.4.1 Experimental process

Hydroxyapatite powder materials have been sintered by several kinds of sintering processes such as conventional sintering, microwave sintering, and spark plasma sintering. However HAP has not been sintered by free pressureless spark plasma sintering before (FPSPS). The following part is comparing the properties of the HAP specimens consolidated by free pressureless spark plasma sintering and by conventional sintering. Both conventional sintering and free pressureless spark plasma sintering, a modified SPS process, do not apply pressure on the sample during sintering. The main difference between these two approaches is the heating rate. The conventional sintering can only reach the heating rate of no more than 10-25 °C/min; however, free pressureless spark plasma sintering has the capability of sintering materials at a high heating rate up to 300-400 °C/min. The advantage of the high heating rates is a possible prevention of the grain growth during sintering process.

0.9 g of hydroxyapatite powders were used for each specimen. The hydroxyapatite powders were placed in a graphite die with a diameter of 10 mm and pre-pressed by the SPS machine with a pressure of 60 MPa holding for 10 minutes. Then the pressed green
specimen with a diameter of 10 mm and 8 mm height was injected from the graphite die by the cold pressing device. For the FPSPS process, the green specimen with a 50% relative density was placed in the Al₂O₃ crucible and another Al₂O₃ crucible capped the specimen. The Al₂O₃ crucibles covered the entire specimen to isolate it from the graphite die and to reduce the chemical reaction between HAP green specimen and graphite tooling during sintering process at high temperature. Because the maximum sintering temperature increases above 1200°C, instead of using a thermo-couple, the pyrometer [CHINO, Portable Radiation Thermometer] was used to measure the temperature during the process. The graphite die was wrapped by two layers of carbon felts to reduce the error in temperature reading resulting from the graphite tooling radiation. The heating rate was 100 °C/min to 1200°C, 50 °C/min to 1300°C holding at 1300°C for 30 minutes and followed by cooling rate of 50 °C/min to 600°C. For the conventional sintering, the green specimens were sintered by the Theta dilatometer with tube furnace with a capability of maximum sintering temperature of 1600°C in air. The temperature profile for the conventional sintering was 5 °C/min to different maximum temperatures, holding at maximum temperatures for 30 minutes followed by cooling with the rate of 10 °C/min.

Because of the limited capability of the Theta dilatometer (conventionally sintering
the specimens), the maximum heating rate and the cooling rate are both 10 C/min which are much lower than those of FPSPS processing. To make the two sintering processes more consistent, both specimens were sintered without applying pressure and were held at the maximum temperature for 30 minutes. Three different maximum sintering temperatures, 1200°C, 1250°C, and 1300°C were explored by utilizing both FPSPS and conventional sintering. The different conditions in these two approaches were the sintering atmosphere and the heating rate. The heating rate of the FPSPS was 100 °C/min which was higher than that of the conventional sintering, 10 °C/min. The sintering atmosphere of the conventional sintering was air and FPSPS was carried out in vacuum.

4.4.2 Measurement of relative density

The relative densities were measured by Archemedes’ method. Figure 4.29 is the plot of relative densities of the specimens obtained by both FPSPS and conventional sintering vs. the maximum sintering temperature. The relative densities in both sintering processes increase with the increase of the sintering temperature. The relative densities of the specimens sintered by FPSPS at 1200, 1250 and 1300°C are 97.57%, 97.76%, and 99.45%, respectively. Compared with FPSPS, the specimens prepared by conventional sintering have lower relative densities which were 89.85%, 94.63%, and 97.27%,
respectively. The highest relative density was obtained at the sintering temperature of 1300°C by FPSPS (99.45%). The difference between the relative densities decreases as the sintering temperature increases which indicates that the improvement of the relative density by FPSPS is more effective at low sintering temperatures than at high sintering temperatures. The plot does not indicate a significant difference in relative densities at the sintering temperature of 1200°C and 1250°C by FPSPS until 1300°C is reached. However, there is a significant increase of the relative densities obtained at 1200°C, 1250°C and 1300°C in the specimens processed by conventional sintering.

Figure 4.29 The comparison of relative densities of free pressureless SPS and conventional sintering as a function of sintering temperatures
Figure 4.30 is the plot of temperature and shrinkage vs. time. The shrinkage curves show that the HAP powders start to consolidate significantly at about 800°C. Specimens sintered at temperature 1300°C shrink more than those at 1200 and 1250°C. The shrinkage rate decrease drastically when the temperature reaches the maximum sintering temperature. The specimens continue to densify with a much lower densification rate during dwelling time compared with shrinkage during heating ramp.
4.4.3 Mechanical tests

Several mechanical tests were conducted on the specimens prepared by FPSPS and by conventional sintering including micro-indentation test and compression test.
4.4.3.1 Micro-indentation

The cylindrical specimen with a diameter of 6 mm and height of 7 mm was obtained. One side of the specimen was grinded with series of sand paper from 800, 1500, 2400 and 4000 and polished with Al₂O₃ solution down to 0.3 µm. Micro-indentation was performed on the specimens with a load of 500 N by micro hardness test machine equipped with Vickers indenter. 10 spots with a distance of five times of the indenter size between every two points were tested on each specimen to obtain average hardness values.

The results of the testing in terms of the hardness value vs. different sintering temperature are shown in Figure 4.31 for the specimens processed by both FPSPS and conventional sintering. The micro-hardness values increase with the increase of the sintering temperature in the specimens processed by both FPSPS and conventional sintering. The results also indicate that the FPSPS process renders better mechanical properties than the conventional sintering. The average hardness values of the specimens prepared by FPSPS at 1200°C, 1250°C and 1300°C are 4.21 GPa, 4.35 GPa, and 4.71 GPa, respectively; by conventional sintering are 2.84 GPa, 3.69 GPa, and 4.13 GPa, respectively. Jarcho et al. [100] reported that the Knoop hardness of dense polycrystalline HAP sintered in air was 4.8 GPa which is in agreement with our results. The highest
hardness value of 4.71 GPa was obtained for the specimen processed by FPSPS at the sintering temperature of 1300°C. Although the highest hardness value (4.13 GPa) of the specimen processed by conventional sintering occurs at the sintering temperature of 1300°C, it is still lower than the lowest hardness value (4.21 GPa) of the specimen processed by FPSPS at 1200°C. Therefore, the FPSPS provides a better route to consolidate the HAP. Similar to the relative density, the difference in hardness values of the specimens processed at 1200°C is larger than for the specimens processed at 1300°C, which means that at lower sintering temperatures, FPSPS can improve the mechanical strength of HAP more significantly than at the higher sintering temperatures.
Figure 4.31  The comparison of micro-hardness of HAP prepared by FPSPS and conventional sintering vs. different sintering temperature.

The following SEM images are the micro-indentation site of FPSPS (Figure 4.32) and conventional sintering (Figure 4.33) showing the square geometry of the indenter.

We can also observe that the cracks propagate from the four corner points of the square.

The crack length of the sample consolidated by FPSPS is smaller than that prepared by
conventional sintering.

Figure 4.32 Micro-indentation site of specimen prepared by FPSPS

Figure 4.33 Micro-indentation site of specimen prepared by conventional sintering
4.4.3.2 Compression test

Compression tests were performed on the specimen prepared by both FPSPS and conventional sintering with different sintering temperatures of 1200, 1250, and 1300°C. The compression tests were conducted at a strain rate of $10^{-3} \text{s}^{-1}$ which corresponded to a cross head speed of 0.02-0.03 mm/min depending on the initial height of the samples. Figure 4.34 is the compressive stress-strain curve for the specimens prepared by conventional sintering. The curves exhibit traditional brittle properties of ceramic materials. The compressive stress increases linearly, and drops immediately after it reaches the maximum compressive strength. Moreover, the compressive strength increases with the increase of the sintering temperature from 85 MPa, 211 MPa, to 251 MPa at sintering temperature of 1200, 1250, to 1300°C, respectively. The modulus also increases from 10 GPa at sintering temperature of 1200°C to 23 GPa at 1250°C. However, modulus does not change much between temperatures 1250 and 1300°C.

Figure 4.35 is the compressive stress-strain curve of the specimens prepared by FPSPS. The stress-strain curve show typical brittle properties of ceramic. The results show that the compressive strengths are lower than those obtained under conventional sintering. The compressive strengths for 1200°C, 1250°C and 1300°C are 70 MPa, 116 MPa, and 150 MPa, respectively, which increase also with the increase of the sintering
Figure 4.34  The stress-strain curves of HAP sintered by conventional sintering at different sintering temperature.
The compressive strength of the samples sintered by FPSPS is not as high as one of the samples prepared by conventional sintering possibly due to the difference in the total sintering time. Although the maximum sintering temperature and the dwelling time are the same, the total sintering time of conventional sintering is 160 minutes which is longer than the total time of FPSPS (48 minutes). The difference total sintering time may
result in the higher compressive strength of the specimens prepared by conventional sintering compared to the compressive strength of the specimens produced by FPSPS.

Figure 4.36 are the SEM images of the fracture surface of the specimen prepared by both FPSPS and conventional sintering. At maximum sintering temperature of 1200°C, the micrographs of the specimen consolidated by conventional sintering have more pores than the one of the specimen produced by FPSPS. The different amount of pores should correspond to the higher relative density and lower porosity of the specimen produced by FPSPS. However, at maximum sintering temperature of 1300°C, the difference in amount of pores between conventional sintering and FPSPS decreases.
Figure 4.36 SEM images of fracture surface after compression test: (a) conventional sintering at 1200°C; (b) FPSPS at 1200°C; (c) conventional sintering at 1300°C; (d) FPSPS at 1300°C

4.5 Fabrication of bio-inspired HAP-CNT composites

4.5.1 Functionally graded HAP-CNT composites

Functionally graded structure is a characteristic feature in natural materials.

Application of the FGM concept to manufacture artificial materials is an essential
approach for bio-inspired materials. [101,102,103] It is difficult to combine irreconcilable properties of materials such as hardness and toughness in the same component. However, a concept of functionally graded materials provides an approach to overcome this challenge.

Functionally graded materials (FGM) are the materials which have gradual variation of composition or structure throughout their volume. The first concept of FGM originated in Japan for the thermal barrier material capable of withstanding a high surface temperature of 2000K and with 1000K temperature gradient across the junction with less than 10mm thickness [104]. Functionally graded material can also reduce the thermal mismatch. Our analysis of the structure of natural dental materials (see section3.2.1) indicated that functionally graded organization is broadly utilized by nature in both animal and human dental tissues. Because of the graded structure, functionally graded materials can be successfully utilized in joining two materials with different mechanical properties. For example, they can be applied in the junction between an implant and a human bone. Thereby FGM can connect the two materials and transfer gradually the impact load from the implant side to the hard tissue side.

Spark plasma sintering can also provide a venue to fabricate FGM. In this project,
the possibility of the fabrication of the functionally graded HAP-CNT composites (where CNTs are utilized as the analogies of fibrils observed in the natural dental tissues see section 3.2.2.) is explored.

Pure HAP powder and 2 vol%, 4 vol% of CNT-HAP mixed powder were used for the processing. Both 2 vol% and 4 vol% of CNT-HAP mixed powders were prepared by the ultrasonication and freeze drying process. The graphite paper was inserted between the compact powder and graphite die, and between the compact powder and two punches. Firstly, 0.8g HAP powder was placed into a graphite die with a diameter of 15.4 mm and then cold pressed to 50 MPa using the SPS machine. Secondly, 0.6g of 2 vol%CNT-HAP and 4vol%CNT-HAP mixed powders were inserted in the graphite die on top of the HAP powder respectively and then cold pressed again to 50 MPa. The functionally quasi-graded (layered) material has a total of three layers with gradually increasing CNT concentration from 0vol% to 4vol%. The whole set underwent spark plasma sintering with applied pressure of 50 MPa. The temperature profile was 100 °C/min from ambient temperature to 700°C, 50 °C/min to 900°C, holding at 900°C for 5 min then 100 °C/min cooling down to 600°C.

The specimen was successfully consolidated with one side rich in CNT and the
other side with less CNTs. Figure 4.37 is the optical micrograph of the cross-section of the functionally graded material showing the clear three different layers with increasing CNT concentration from 0 to 4 vol%. The right hand side which is the 0vol% CNT (pure HAP) is very different from the other two portion of the sample. However, the 2 vol%CNT and 4 vol%CNT layers are not distinguishable even at higher magnification under microscope because of the small concentrations of CNT. Figure 4.38 (a)-(c) are the SEM images showing the three different layers in the functionally graded material. Figure 4.38 (a) is the pure HAP (0 vol% CNT) phase showing irregular grains with no CNT inside. Figure 4.38 (b) and Figure 4.38 (c) are 2 vol% CNT and 4 vol% CNT, respectively. The white dots in Figure 4.38 (b) and (c) represent the CNTs. The images reveal that the CNTs are distributed in the HAP matrix. Figure 4.38 (c) exhibits more CNTs in the HAP matrix than shown in Figure 4.38 (b).
Figure 4.37 (a) the overview of the entire FGM composites (b) pure HAP layer (c) 2 vol% CNT-HAP layer (d) 4 vol% CNT-HAP layer

Figure 4-38 (a)

Figure 4.38 SEM micrographs of cross-sections of the functionally graded structure: (a) showing the layer of pure HAP; (b) the layer of 2 vol% CNT-HAP; (c) the layer of 4 vol% CNT-HAP.
4.5.2 HAP-based composites with special microstructure topology

The final stage of the project includes also the fabrication of HAP-based composites with oriented porosity (see Figure 2.1). The porous structure in HAP-based composites can be used as a different application depending on the shape and the size of the pores. One of the popular approaches to construct the porous structure in the green specimen is freeze-casting. Several kinds of porous hydroxyapatite
composite have been fabricated such as channel-like porosity [110], graded porous hydroxyapatit [111] and hydroxyapatite/polymer composite scaffolds.[112,113] Porous structure composites have been successfully fabricated by spark plasma sintering[114,115] and the freeze drying process has been proved as a novel technique to create composite with aligned pores.[116,117,118,119] The pore architecture in alginate scaffolds which can affect the compressive strength can be tailored by controlling the freezing regime during fabrication.[120] Deville et al. [121] successfully fabricated hydroxyapatite scaffolds for bone tissue engineering. They used the freeze drying process to create the channel structure and conventional sintering to consolidate the green compact. They concluded that the size of the pores can be controlled by the initial slurry concentration and freezing rate of the slurries, compressive strength increases with the increase of the cooling rate and the maximum compressive strength they could achieve was 145 MPa for 47% porosity. Instead of using conventional sintering, FPSPS with high heating rate should provide an alternative approach to consolidate the green specimens without destroying the micro-channel structure. Therefore, the combination of the freeze drying and FPSPS may provide a feasible approach to consolidate the powder with a micro-channel structure.
According to the literature survey and the characterization of the natural tooth, the main feature of the dentin structure in natural tooth is a tubule structure. These tubules with an average diameter of 1um are essential for nutrition transportation. Aligned porous structures are usually observed in natural materials and also have potential applications in drug delivery and tissue engineering. For materials used for hip implants or bone replacement, the porous structures are beneficial for osteointegration between the replacement material and bone. [122 123 124] In order to fabricate the aligned pore structure in the HAP for dental implant applications, directional freeze drying is a cheap and easy approach to prepare a green specimen. To maintain the micro channel structure in the green specimen, the free pressureless spark plasma sintering [125] was implemented instead of regular pressure-assisted SPS.

4.5.2.1 Concept of freeze drying process and experimental setup

Figure 4.39 shows the directional freeze drying. The slurry was placed on the metal immersed in liquid nitrogen which resulted in ice crystals growing from the bottom of the slurry volume, where the cooled metal plate was located, to the top of the slurry volume. The HAP powders (blue small particles) are excluded from the ice and aggregate between those growing icicles. After freeze drying process conducted at low pressure
(0.6 mPa) and temperature (-50°C), the ice will sublimated and only the HAP powders were left to construct the micro-channel structure.

Figure 4.39 The schematics of directional freezing showing that the ice crystals grow from the bottom to top and the hydroxyapatite particles are excluded from the ice to form the micro-channel structure.

The green specimen preparation can be divided into three parts. The first part is uniformly mixing the HAP powder with the solvent and additives. The particle agglomeration can be eliminated by ball milling or ultrasonic vibration. The second part is freezing the slurry with the design showing in Figure 4.40. The slurry was placed into the cylindrical Al₂O₃ or rubber tube in direct contact with the copper rod with the other side immersed into liquid nitrogen. The ice crystals grow from the bottom of the slurry volume to the top. The final part is the freeze drying process which is a technique to form
a micro-channel structure by sublimating the ice from the green specimen directly. After the whole slurry was frozen, it was removed from the mold and subjected to the freeze drying process with low temperature and pressure environment.

Figure 4.40 The picture of setup for freezing the hydroxyapatite slurry.

Different concentrations of HAP slurry, 15vol%, 20vol% and 25vol% were prepared to investigate how the slurry concentration changes the micro structure and mechanical strength of the samples.

For the 20 vol% concentration of HAP slurry, 23g of HAP powders with density of 3.14 g/cm³ were placed into the 30 ml deionized water. Commercial dispersant (Duraman D-3005, Rohm and Hass, USA) was added into the HAP slurry. The slurry underwent
ball milling process for 10 hours to uniformly disperse the HAP powders in the solution before freezing.

The homogeneous slurry was poured into the Al₂O₃ or rubber tubing connected to a copper rod or an Al plate for freezing. After 5 to 10 minutes of freezing, the solid green specimens were carefully taken out from the rubber tubing and subjected to the freeze drying in a unit with a temperature of -50°C and a pressure 0.6mPa for 1 day. The ice crystals were sublimated by passing through the triple point without passing the state of liquid phase. The dried samples were sintered by conventional sintering and free pressureless spark plasma sintering for comparison. For the conventional sintering, the samples were sintered in a furnace at 1000°C with holding time of 3 hours. The heating rate was 10 °C/min and cooling rate is 10 °C/min. For the FPSPS process, the fragile green specimens were placed into a graphite die with 15.4 mm in diameter and two T-shape punches replaced the regular SPS punches for the FPSPS process. The heating rate was 100 °C/min to 800°C, followed by 50 °C/min to 1000°C and holding at 1000°C for 1 hour. The cooling rate was 50 °C/min down to 600°C.

In order to characterize the micro-channel structure in the green specimen and the sintered specimen, acrylic slurry was used to infiltrate into the specimen for shape
maintenance. The specimens were attached to the bottom of the plastic mold with glue and the solution with ratio of 2.4 g acrylic resin and 4 drops of hardening was poured into the plastic mold to infiltrate into the micro-channels in the specimen. Acrylic solution is thinner than the regular epoxy, which is more beneficial for infiltration. The whole mold including the acrylic solution and specimen was put in a vacuum system for 10 minutes to degas the air from the micro-channels inside the specimen. After one day of hardening and drying, the mounted specimens were cut into half. The cross section of a specimen was grinded with series of sand papers with grid of 400, 1200, 2400 and 4000, and then polished with 0.3 μm Al₂O₃ solution.

4.5.2.2 Micro-channel specimen sintering at 1000°C

Scanning electron microscope with backscattering mode was carried out on the cross section of the polished specimen showing the micro-channel structure in the specimen. The BSE images (Figure 4.41) are the cross section view of the green specimen and the sintered specimens with different slurry concentrations. The first two SEM images are the green specimen after freeze drying process showing the micro-channel structure. The long black regions represent a micro-channel infiltrated by the acrylic material due to its light atomic weight under BSE mode. On the contrary, the
lighter parts are the channel walls which are the HAP powder with higher atomic weight.

The freeze drying process excludes the HAP powders from the ice to form the channel structure. Some agglomeration of the HAP powders can be seen on the micro-channel wall. We can see that the diameter of the micro-channel increases as we increase the slurry concentration.

From the higher magnification of BSE images, the diameter of the channel in the specimen obtained from 15 vol% slurry is about 10 μm and the diameter of the channels in the specimen obtained from the 25 vol% slurry reduces to 5 μm. The diameter of the channels in the specimen obtained from the 20 vol % slurry is about 7 μm, which is between the size of the channels in the specimens obtained from 15 and 25 vol % slurries.

The results show that the diameter of the micro-channel decreases as we increase the slurry concentration. Therefore we can fabricate different sizes of the micro-channels for different purposes by manipulating the slurry concentration.
Figure 4.41 (a), (b) Green specimen; (c), (d) channels in the specimen obtained from 15 vol% initial slurry concentration; (e), (f) channels in the specimen obtained from 20 vol% initial slurry concentration; (g), (h) channels in the specimen obtained from 25 vol% initial slurry concentration.
Mechanical test: compression test

The top and bottom side of cylindrical sintered specimens produced from different slurry concentrations were polished to flat and paralleled surface for compression tests. The INSTRON 3342 machine equipped with a strain tensile meter was used to measure the compression strength and Young’s modulus of both the conventional sintered and FPSPS specimens. The strain rate we used was $10^{-4}$ s$^{-1}$, which corresponded to a load
head speed of about 0.03 mm/min depending on the different height of the specimen.

Three samples for each sintering process including conventional sintering and FPSPS at 1000°C were tested and the results are shown in Figure 4.42.

Both the compressive strength and compressive modulus are higher in specimens processed by FPSPS than in specimens processed by conventional sintering. The influence of the porosity on the mechanical resistance of HAP under compressive stress has been investigated. The previous results all show the decrease in compressive strength with increased porosity.[126,127] The higher initial vol % of the slurry renders higher mechanical strength of the consolidated specimens which is reasonable because the specimens based on the higher initial vol % of the slurry have lower porosity. The compressive strength of the specimens made from 15, 20, 25 vol % slurries by pressureless SPS are 0.65, 1.5, and 1.86 MPa, respectively. For the conventional sintering the compressive strengths are only 0.285, 0.815, and 0.685 MPa, respectively. The compressive modulus of the specimens made from 15, 20, and 25 vol % slurry processed by conventional sintering are 8.09, 15.06, and 22.24 MPa, respectively. However the compressive modulus of the specimen made from 15, 20, and 25 vol% slurry processed by pressureless SPS are 13.59, 54.04, and 80.81 MPa, respectively, which are higher than
those of the specimens processed by conventional sintering for every different slurry concentration. The improvement of the compressive modulus of the specimens processed by free pressureless spark plasma sintering over the specimens processed by conventional sintering also increases as the slurry concentration increases.

Figure 4.42 (a)

Figure 4.42  (a) Plot of compressive modulus vs. initial vol% slurry concentration for specimens processed by conventional sintering and free pressureless spark plasma sintering at 1000°C; (b) Plot of compressive strength vs. initial vol% slurry concentration for specimens processed by conventional sintering and free pressureless spark plasma sintering at 1000°C.
X-ray diffraction

Figure 4.43 is the XRD pattern of the specimens processed by different sintering approaches including conventional sintering and free pressureless SPS ranged from 2 theta of 20° to 60°. The red lines is the XRD pattern of the HAP powders as received, the blue one is for the specimens processed by conventional sintering and the green one is for the specimens processed by free pressureless SPS. The red line represent the typical peaks of HAP which are 25.8°, 31.8°, 39.6°, 47.2°, 49.3°, 53.1°. Compared with the as
received HAP powders, the samples processed by conventional sintering and FPSPS show the same peaks as the original HAP powders, which means that there is no phase change during sintering process. However the intensity of the sintered HAP is higher than that of raw HAP powder due to the high crystallinity.

Figure 4.43 Comparison of X-ray diffraction pattern of HAP processed by different sintering processes showing that there is no phase change via different processes.
4.5.2.3 Micro-channel specimen sintering at 1300°C

According to literature survey, sintering HAP at around 1300°C can achieve fully dense state and good mechanical properties. Sintering temperature of 1000°C is not high enough and results in the porous HAP specimens. Therefore, we increased the maximum sintering temperature to 1300°C to achieve higher mechanical and higher relative density. Both the conventional sintering and free pressureless spark plasma sintering were utilized to consolidate the green specimens for comparison. Because the sintering temperature exceeded 1100°C, the radiation thermometer was used to measure the temperature instead of using thermal couple. Double carbon felts wrap the graphite die for the radiation thermometer to precisely detect the sintering temperature. The temperature profile was 100 °C/min to 600°C and holding at 600°C for 3 minutes to stabilize the temperature at 600°C because the radiation thermometer can only detect the temperature about 600°C. Then the temperature continued to increase at the rate of 100 °C/min to 1100°C, 50 °C/min to 1300°C, and hold at 1300°C for 1 hour was followed by 50 °C/min cooling rate down to 600°C. The total sintering time was 92 minutes.

The relative density was measured by the Archimede’s method and was plotted with respect to the slurry concentration (Figure 4.44). The relative density
increases from 52% to 63% when the slurry concentration increases from 15 to 25 vol %.

On the contrary, the porosity decreases with the increase of the slurry concentration.

Therefore, the higher the concentration of the slurry, the lower porosity specimens with smaller diameter of the micro-channels can be fabricated.

Figure 4.44  Plot of relative density and porosity vs. initial vol% slurry showing that higher relative density and lower porosity results from higher initial vol% slurry.

4.5.2.3.1 Mechanical tests

Compression test was conducted on the specimen prepared by pressureless SPS at maximum sintering temperature of 1300°C. Specimens sintered at 1300°C were
consolidated better than those processed at 1000°C. The compressive strength of the specimens made from 15, 20, and 25 vol % slurry were 4.66, 5.97, and 6.85 MPa, respectively. The graph (Figure 4.45 (a)) of the compressive strength versus the vol % of slurry shows that the compressive strength increases as the vol% slurry increases. Figure 4.45 (b) is the plot of compressive strength vs. porosity of specimens processed by free pressureless SPS at 1300°C. The compressive strengths decrease with the increase of the final porosity of the specimen. Zhang et al. [128] also used pressureless spark plasma sintering to consolidate calcium phosphate scaffolds; the compressive strength of the specimens processed by them ranged from 2 and 7 MPa, which is comparable to our results. Fu et al. [129,130] reported that the compressive strength for loading parallel to the channel direction was about 10 MPa which was higher than the compressive strength in the direction perpendicular to the channels (4 MPa). Both reported compressive strengths are of the same order as the compressive strength we obtained. The reason that the very high compressive strength 131 could not be achieved should be attributed to the difficulty in pre-pressing the green specimen before sintering process. The pre-pressing process before sintering can destroy the aligned porous structure. The green specimens obtained after directional freeze-drying are much more fragile than pre-pressed
specimens commonly produced by consolidation in rigid dies.

Figure 4.46 includes the SEM images of the fracture surface of the specimens processed from different slurry concentrations after compression tests. The grain sizes of the specimens made from three different slurry concentrations are almost the same, which is about 2-3 µm for specimens processed at 1300°C of pressureless SPS temperature. A lot of pores can be seen in the fracture surface showing that the specimen has very low relative density.
Figure 4.45 (a) Plot of compressive strength vs. initial vol% slurry; (b) plot of compressive strength vs. porosity.
Figure 4.46  Compression fracture surface for specimens processed from different initial vol% slurry: (a), (b) 15 vol% initial slurry; (c), (d) 20 vol% initial slurry; (e), (f) 25 vol% initial slurry.
Figure 4.47 are two of the typical stress-strain curves obtained from the compression tests conducted for the specimens produced from two different initial HAP slurry concentrations. The results show higher compressive strength in the specimens produced from 25 vol%HAP slurry than in the specimens produced from 20 vol%HAP slurry. Unlike common brittle behavior of ceramics, the stress-strain curves do not only show elastic response and failure at low strain. The curves exhibit up and down trend, which reveals that the specimens are not fully dense.
The green specimens with micro-channel structure were also sintered by conventional sintering with a dilatometer. The temperature profile for the conventional sintering was $10^\circ\text{C/min}$ heating rate to $1300^\circ\text{C}$ and hold for 1h followed by $10^\circ\text{C/min}$ cooling down to room temperature. The difference between free pressureless SPS and
conventional sintering are the heating rate and the environment. The heating rate for FPSPS was 100 °C/min which was much higher than that of conventional sintering, 10 °C/min.

Figure 4.48 is the plot of porosity and relative density vs. initial slurry concentration. The relative densities of 15, 20, 25 vol% slurry are 40%, 56.5%, and 61.8%, respectively. Compared with specimens prepared by free pressureless SPS, the specimens processed by conventional sintering show lower relative density in each different initial slurry concentration. These lower relative densities by conventional sintering should lead to lower compressive strength compared with the compressive strength of the specimens processed by free pressureless SPS.
Figure 4.48  Plot of relative density and porosity vs. initial vol% slurry of specimen by conventional sintering showing that higher relative density and lower porosity result for the specimens produced from higher initial vol% slurry.

Figure 4.49 shows a typical stress-strain curve the specimen prepared by conventional sintering. Compared with the specimen prepared by FPSPS, the compressive strength of the conventional sintered specimen is only about 4 MPa. The
low mechanical strength may be attributed to the insufficient holding time. In order to make the conditions more similar in the compared FPSPS and conventional sintering, 1hr holding time was applied to conventional sintering. According to other literature sources [39], the holding time for conventional sintering of HAP is usually several hours instead of 1 hr.
Figure 4.49  Compression stress-strain curves for specimens produced from 20 vol% initial slurry by conventional sintering at 1300°C.

All of the obtained results indicate that the free pressureless spark plasma sintering provides a better approach than conventional sintering to consolidate the specimens with micro-channel structure. The advantages of higher heating rate and shorter sintering time result in higher relative density and mechanical strength of the specimens prepared by
free pressureless spark plasma sintering.

### 4.5.2.3.2 Compression tests in different directions

Because of the anisotropic structure of the micro-channeled materials, compressive tests in different loading directions were conducted on the specimens to verify how the compressive strength depends on the loading orientation. The utilized orientations included the loading direction perpendicular to the channels and parallel to the channels. In the case of the loading direction perpendicular to the freezing direction, the cylindrical specimens were grinded into rectangular shapes. The results show that the compressive strength in the loading direction perpendicular to the channel is about 3 MPa which is smaller than that of the strength along the loading direction parallel to the channel, 6 MPa.

Figure 4.50 shows the stress-strain curve for the loading direction perpendicular to the channel. The first local maximum stress is 3 MPa, the peak stress is 3.5 MPa and then the specimen starts to fail at a strain of about 3%. Unlike most of the typical results of tests conducted for brittle ceramic materials, the stress-strain curve for the fabricated micro-channelled material does not drop immediately, instead, the compression stress decreases gradually.
Figure 4.50 Stress-strain curve of the compression test with loading direction perpendicular to channels

4.5.3 Fabrication of a complex-shape dental implant prototype

The obtained results on the characterization of natural dental materials show that the hardness of human dentin is about 0.5 GPa and the hardness of enamel region is about 1.7 GPa. However, the pure HAP fabricated by spark plasma sintering process can reach
hardness of 3.9 to 4.1 GPa under different sintering temperatures (see section 4.3.2.1) which is much higher than hardness of natural teeth. With an optimized dispersion-mixing process of HAP and CNTs powders, the addition of CNTs can even improve the mechanical properties of HAP-based specimens.

The obtained results on the processing of HAP-CNT composites, including the processing of functionally graded and special topology structures render impetus to the ultimate applied goal of the present research project: the fabrication of a dental implant prototype. The real-world prototype should possess a complex shape, which creates and additional challenge of net-shape powder processing.

In our project, net shape SPS is explored by employing a punch with a special geometry. Instead of the 15.4 mm diameter die and 15 mm diameter punch used for the fabrication of cylindrical specimens, a 10.4mm diameter die and 10 mm diameter punch were utilized to approximate the size of a real human tooth.

Figure 4.51 shows a realistic tooth shape design which mimics a real molar human tooth. In order to reduce the complexity of the geometry, only the top punch was machined. Thereby the tooth root was ignored, so that the bottom punch remained of the same traditional cylindrical shape. Figure 4.52 is the design of the upper punch showing
the machined pressing surface of the punch. The surface of the processed dental implant prototype is a mirror reflection of the machined punch end-face.

Two types of sintering processes were explored for the dental implant prototype fabrication: the conventional spark plasma sintering (SPS) and the free pressureless spark plasma sintering (FPSPS). FPSPS is a modified SPS process applying the T shape punch instead of typical cylindrical punch. (Figure 4.53) This way the FPSPS is an alternative way to fabricate the porous structure materials.

![Figure 4.51 3D image of the prototype of dental implant](image)
4.5.3.3 Fabrication of a complex-shape dental implant prototype: Conventional SPS

Hydroxyapatite with particle size of 0.09µm was used in the fabrication of a complex-shape dental implant prototype. Graphite paper was inserted into the 10 mm die around the inside die wall. Two round graphite paper pieces were used between the punches and the specimen. Differently from the original flat punch, the carbon oil was also applied on the textured surface of the upper punch to prevent the powder adhesion to the punch in the case of the fracture of the graphite paper. The amount of the used powder was about 0.8g. The whole set was pre-pressed under ambient temperature. Then
the set was placed into the SPS machine vacuum chamber and a constant pressure of 50 MPa was applied before and during the entire SPS process. The temperature increased to 700°C by 100°C/min, 50 °C/min to 800°C and holding time was 5 minutes at 800°C followed by 50 °C/min cooling to 600°C.

The specimen did not consolidate and turned into several pieces with fracture along the radial deep ridge. This was apparently caused by a non-uniform electric current density and non-uniform pressure distributions in the specimen’s volume.

4.5.3.4 Fabrication of a complex-shape dental implant prototype: Free pressureless spark plasma sintering (FPSPS)

Since the conventional spark plasma sintering could not successfully consolidate the complex-shape dental implant prototype, a novel free-pressureless spark plasma sintering (FPSPS) [132,133] was utilized to fabricate the complex shape specimen. The difference between SPS and FPSPS is that there is no pressure applied to the specimen in FPSPS. The T-shape upper punch is not in contact with the specimen. Figure 4.53 is the sketch of the modified design of the die assembly for the FPSPS. The two red parts are the T-shape punches which were fabricated from the same graphite material used for the die. The blue part represents the sample which is not in contact with the top T-shape punch but is in contact with the bottom punch. In order to have a high contact resistance
through the die wall and exterior punch, zirconia felt and graphite paper were inserted between the exterior punch and the die wall for the purpose of isolation. Therefore, the current was directed through the punch to the inner side wall, which was the same current path as in a regular SPS. The white parts in Figure 4.53 represent the isolation layers made from zirconia felt. The outer diameter of the T-shape punch was 30 mm which was same as the outer diameter of the die and the inner diameter of the punch and die were 10 mm and 10.4 mm, respectively.

![Figure 4.53 The sketch of FPSPS setup.](image)

Hydroxyapatite powder with particle of 0.09\(\mu\)m was used in the experiment. A conventional SPS 10.4 mm die and a machined punch were used to pre-press the sample by the SPS machine under 50 MPa. And then the punches were replaced by the T-shape
punches without contacting the specimen to conduct the experiment. The minimum pressure of the machine, 4.2 MPa was applied between the punches and the die; no external pressure was applied to the specimen. The sintering temperature increased to 800°C which was followed by 0, 15min holding time. The tooth shape specimen was successfully consolidated.

SEM images (Figure 4.54) are the typical fracture surfaces of the complex shape dental prototype consolidated by SPS and FPSPS at 800°C. The microstructure of the specimen processed by conventional SPS exhibits larger grain size than that of the specimen processed by FPSPS. On the other hand, the fracture surface of the specimen processed by FPSPS exhibits less faceted structure. The final relative density of the specimen processed by FPSPS is 73.8% which is much lower than that of the (fractured) specimen processed by SPS (94.9%).
Figure 4.54 (a-c) SEM micrograph of complex shape dental prototype by FPSPS; (d-f) SME micrograph of complex shape dental prototype by SPS

Chapter 4, in part, has been submitted for publication of the material as it may appear in “Micro-channel hydroxyapatite components by sequential freeze drying and free pressureless spark plasma sintering”, in Advanced in Applied Ceramics, 2011, Lin,
Yen-Shan; Meyers, Marc A.; and Olevsky, Eugene A. The dissertation author was the primary investigator and author of this paper.
Chapter 5  Conclusions

The natural dental materials have been investigated and studied. All the studied animal and human dental structures have outside hard enamel and inside tougher dentin. The dentin-enamel junction plays an important role in toughening the tooth, which inspired the fabrication of artificial functionally graded materials. The higher mineral content such as calcium in enamel than in dentin results in the higher hardness value of enamel. The results of nano-indentation show that the hydration content in the shark and piranha teeth has a significant effect on the mechanical properties of dentin, but no significant difference is observed in the highly mineralized enameloid. The important feature of dentin structure the tubules which affect the mechanical properties of dentin, have been studied.

The mechanical properties and structure of Arapaima scales have been studied. The scales have a laminate structure with approximate 50 µm thick in each layer. The basic building blocks to construct the scale are Type-I collagen. The collagen fibers are oriented in the same direction in each layer but in different directions in the adjacent layers forming the twisted plywood or plywood structure. The hydration content and strain rate play an important role in biological materials. Both the tensile strength and
Young’s modulus of arapaima scales in dry conditions are higher than those in wet conditions. The Young’s modulus and tensile strength of arapaima scale both increase as the strain rate increases, which is in agreement with the results obtained for other biological material such as bovine bone. However, the scale is more strain rate sensitive than bovine bone because of the lower mineral content in the scale. Both micro and nano-hardness are higher in the external region of scale than those in the internal region, which indicates the high mineral content in the external region of the arapaima gigas scale.

The SPS processing of pure HAP and HAP-based composites was carried out. The experiments indicate that the samples are fully densified between 800°C to 900°C. The obtained relative densities of the samples are all above 90%. Compared with pure HAP, the CNT-HAP composites show slight increase in Vickers hardness. The micro-hardness values increase from 3.51 to 4.26 GPa when the CNT vol% increases from 0 to 2vol% in CNT-HAP composite. However, the CNT-HAP composites show higher nanohardness and elastic modulus than that of HAP. The addition of CNT does not increase much the hardness of CNT-HAP composites. But it may increase the fracture toughness of the composite. The potential advantage of adding CNT may result in toughening the HAP.
HAP specimens consolidated by FPSPS and conventional sintering were compared. The results show that HAP specimens prepared by FPSPS have higher relative density than the specimens processed by conventional sintering and micro hardness values are also higher in HAP specimens prepared by FPSPS than by conventional sintering. The differences between the specimens prepared by FPSPS and by conventional sintering are larger at lower sintering temperatures. The improvement of the mechanical properties of the specimens processed by FPSPS at higher sintering temperatures is not obvious as for those of the specimens processed at lower sintering temperatures.

The functionally graded material with different CNT concentration ranged from 0 to 4vol% was successfully fabricated by spark plasma sintering. The SEM images confirm that the CNT concentrations gradually increase in one direction in different layers. The graphite punch can be machined into a complex geometry to create a green specimen prior to the sintering process. In the study, a complex-shape dental implant green specimen was created by a modified punch with geometry similar to tooth surface and then successfully consolidated by FPSPS.

The combination of the unidirectional freeze drying, which creates micro-channel green structure, and FPSPS, which consolidates the green specimen, can fabricate
materials with micro-channel structure. The porosities, relative densities and micro-channel size can be manipulated by changing the initial slurry concentration. The specimens prepared with higher initial slurry concentrations show high relative density and smaller micro-channel size. On the other hand, the lower initial slurry concentration results in higher porosity and larger micro-channel diameter of the consolidated specimens. The pore alignment along the freeze direction results in the anisotropic structure of the materials. Therefore the compressive strength of the produced specimens shows different values in different compressive loading orientations. Specimens exhibit higher compressive strength in the loading direction parallel to the channel axis (~6 MPa) than in the loading direction perpendicular to the channel axis (~3 MPa).
References


